Thesis submitted for the degree of

Philosophiae Doctor (PhD)

The hidden biodiversity of pollen

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Replace us with the things

That do the job better

Hot Chip – Huarache Lights

Een mens lijdt 't meest

Door 't lijden dat hij vreest

Dutch saying

Contents

List of manuscripts

This thesis is based on the following five manuscripts, which are referred to in the main text using Roman numerals (I-V).

I. **Polling, M.**, Li, C., Cao, L., Verbeek, F., de Weger, L.A., Belmonte, J., De Linares, C., Willemse, J., de Boer, H., Gravendeel, B. 2021. Neural networks for increased accuracy of allergenic pollen monitoring. *Scientific Reports* https://doi.org/10.1038/s41598-021-90433-x

II. **Polling, M.** DNA from pollen. *In: Molecular Identification of Plants: From Sequence to Species (Pensoft)*. Peer-reviewed book chapter.

III. **Polling, M.** and Chua, P., Lynggaard, C., Ariza, M., Bohmann, K. Amplicon Metabarcoding. *In: Molecular Identification of Plants: From Sequence to Species (Pensoft)*. Peer-reviewed book chapter.

IV. **Polling, M.**, Sin, M., de Weger, L.A., Speksnijder, A., Koenders, M., Gravendeel, B., de Boer, H. In review. DNA metabarcoding using nrITS2 provides highly qualitative and quantitative results for airborne pollen monitoring. *Submitted to Science of The Total Environment*

V. **Polling, M.**, ter Schure, A.T.M., van Geel, B., van Bokhoven, T., Boessenkool, S., MacKay, G., Langeveld, B.W., Ariza, M., van der Plicht, H., Protopopov, A.V., Tikhonov, A., de Boer, H.J., Gravendeel B. In press. Multiproxy analysis of permafrost preserved faeces provides an unprecedented insight into the diets and habitats of extinct and extant megafauna. *Accepted with minor revisions at Quaternary Science Reviews*

Summary

In an age of rapid digitization, the study of pollen grains (palynology) has not seen much change. Pollen is traditionally studied using a microscope and different pollen types can be distinguished based on their unique morphology. Information from pollen is used in a multitude of fields including allergology, taxonomy, forensics, biostratigraphy, apiology, paleoecology and aerobiology. However, the expertise needed to perform the laborious and specialized task of pollen analysis is rapidly disappearing. Moreover, many plant taxa produce highly similar pollen that cannot be distinguished beyond genus, family or even order level. This prevents detailed information to be gained from pollen analysis, as different species may have diverse ecological preferences or allergenic profiles. Therefore, there is a high need for new techniques to help transform palynology. In this thesis, innovative microscopic and molecular techniques are used to improve pollen analysis. The aim is to unravel hidden pollen biodiversity.

In Manuscript I of the thesis, using a case study, it is shown that sufficiently trained deep learning algorithms can differentiate visually similar pollen that cannot be distinguished by palynologists. This distinction is of medical importance as pollen from one of the studied genera is allergenically unimportant while the other is highly allergenic. For species that produce pollen grains that are too similar to each other to be morphologically distinguished, other techniques are required. Therefore, Manuscript II presents a literature review on the extraction and amplification of DNA from pollen, while Manuscript III reviews the molecular method DNA amplicon metabarcoding. Insights gained from these chapters are applied in Manuscript IV, where DNA metabarcoding is used on airborne pollen collected for allergenic pollen monitoring. It is shown that this technique not only highly increases the taxonomic resolution, but can also provide reliable semi-quantitative results of pollen grains. In Manuscript V, DNA metabarcoding is used as a complementary tool to pollen and macrofossil analyses in a case study on faeces from extinct megafauna. By integrating results from all proxies, an accurate reconstruction of the last meals and habitats of these megafauna could be made. The techniques applied in this thesis show high potential in uncovering hidden biodiversity of pollen grains, and the results and implications for future research are discussed in the light of other innovative methods to study pollen.

List of abbreviations and terms used in this thesis

ASV	Amplicon Sequence Variant
bp	Base pair
CNN	Convolutional Neural Network
DNA	Deoxyribonucleic Acid
ICTA-UAB	Institute of Environmental Science and Technology (Barcelona, Catalonia, Spain)
LM	Light Microscopy
LUMC	Leiden University Medical Center (Leiden, the Netherlands)
MS	Manuscript
NGS	Next Generation Sequencing
NMDS	Nonmetric Multi-Dimensional Scaling
nrITS	nuclear ribosomal Internal Transcribed Spacer
OTU	Operational Taxonomic Unit
perMANOVA	Permutational Multivariate Analysis of Variance
PCR	Polymerase Chain Reaction
*rbc*L	Ribulose-1,5-bisphosphate carboxylase
rRNA	Ribosomal Ribonucleic Acid
RRA	Relative Read Abundance
SEM	Scanning Electron Microscopy
*trn*L	transfer RNA gene for Leucine
UNINETT Sigma2	the National Infrastructure for High Performance Computing and Data Storage in Norway
Yr BP	Years before present

1. Introduction

1.1 Pollen

Pollen grains represent the male gametes or sperm cells in the plant kingdom. Within a single grain of pollen, all genetic information required to specify an entire plant is contained (Knox, 1984). To protect this genetic material from environmental factors and ensure safe transfer to the female ovule, pollen grains have an extremely strong outer layer. This layer consists of an inner layer of polysaccharides (intine) and a chemically inert outer layer (exine) made of sporopollenin that is highly resistant to biodegradation (Li et al., 2019). The exine is characterized by a taxon-specific shape, sculpture and structure that allows palynologists to distinguish pollen from different plant taxa (Figure 1; Beug, 2004; Erdtman, 1943; Wodehouse, 1935). Knowledge obtained by studying pollen is used in various fields, including taxonomy, archaeology, apiology, allergology, forensics, biostratigraphy, paleoecology and aerobiology.

While some plant taxa can be distinguished by their unique pollen or spores (i.e., eurypalynous taxa), many plant taxa produce highly similar pollen that cannot be morphologically distinguished beyond genus, family or even order level (stenopalynous taxa, e.g., Pteridophyte, Poaceae, Asteroideae, *Quercus*). Studying pollen grains of eurypalynous taxa allows investigation at high 'taxonomic resolution', while this resolution is low for stenopalynous taxa (Mander and Punyasena, 2014). Since pollen grains are generally in the size range of ~10 – 100 µm (Hesse et al., 2009), palynologists rely on visual inspection of pollen using a microscope. This laborious task relies on highly trained specialists that manually count hundreds of pollen grains to get a reliable estimate of pollen diversity in a sample. Furthermore, as many stenopalynous plant taxa exist, often no information can be obtained

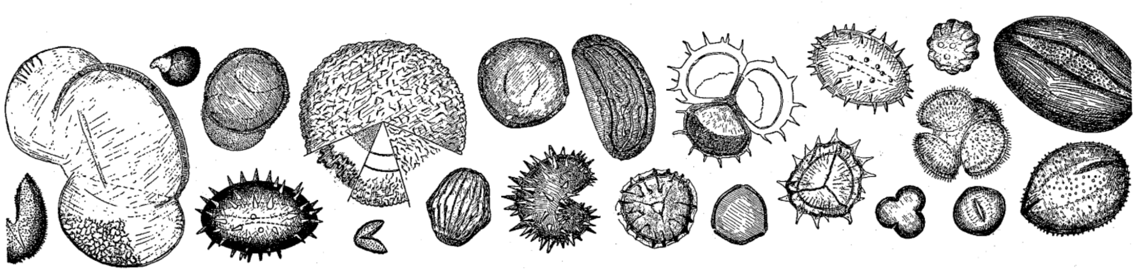

Figure 1. Variations in pollen shapes and exine sculpture. Adapted from Erdtman, 1943.

of the specific species of pollen present in a sample. Therefore, pollen of highly allergenic plants may not be distinguishable from those produced by non-allergenic plants, and pollen of invasive species may not be differentiated from pollen of native plants. Similarly, pollen of species from wetlands may not be differentiated from pollen of species typical for more arid conditions, preventing detailed (paleo)ecological reconstructions. Therefore, much research effort has been put into finding methods of revealing the hidden biodiversity of pollen grains.

1.2 Automating palynology

Developments in computing power and imaging software have paved the way to what is by some considered the "holy grail" of palynology: automatic counting and classification of pollen (Holt and Bennett, 2014). Automatic machines have the potential to speed up the process, while also being more consistent and accurate than human analysts (Mander et al., 2014). Early works on automating the pollen identification process can be traced back to the

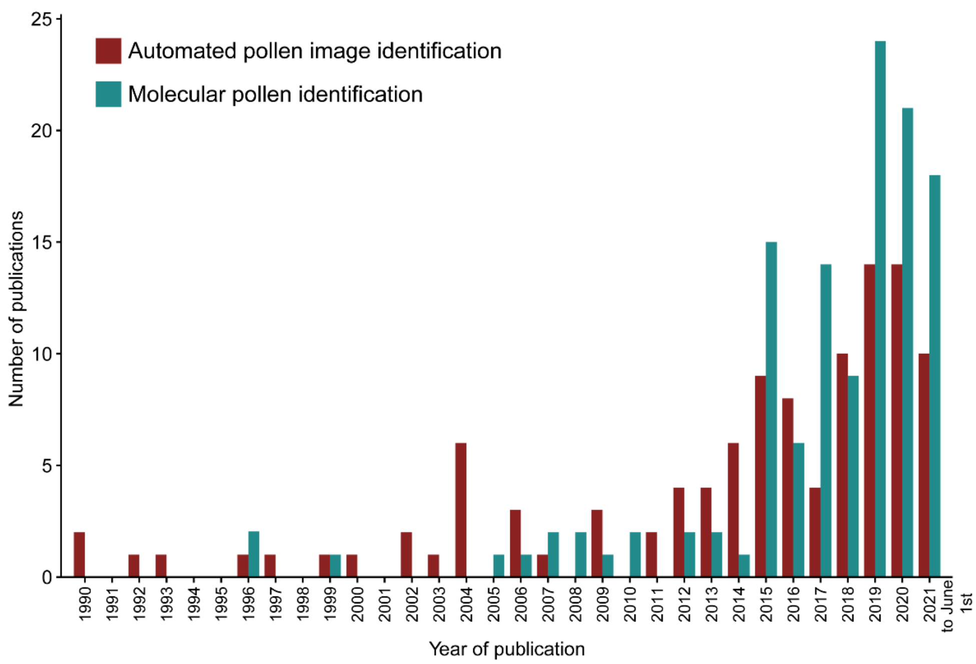

Figure 2. The number of papers published from 1990 until June 1st 2021 on the two main methods used in this thesis to study pollen. Data was retrieved from Web of Science (https://www.webofknowledge.com), based for automated pollen image identification on the search string 'TS=(pollen AND ("neural netw" OR "deep learn*" OR *CNN OR "machine learn*" OR "automatic image recognition" OR "automatic image analysis" OR automated) NOT forecast)' and supplemented with references cited in Holt and Bennett (2014) and (Sevillano and Aznarte, 2018). For molecular pollen identification the string 'TS=(pollen AND (metabar* OR *barcod* OR metagen* OR qPCR OR shotgun))' was used, supplemented with references cited in Bell et al. (2016) and Manuscript II of this thesis.*

early nineties, when Vezey and Skvarla (1990) presented a method of automatically detecting features on SEM images using statistical classifiers (Figure 2). The field progressed as new technologies became available, including machine learning and early neural networks (e.g., France et al., 2000; Holt et al., 2011). However, it was not until recent incorporation of deep learning that studies have shown successful automatic segmentation and identification of pollen from large numbers of pollen taxa (Olsson et al., 2021; Sevillano et al., 2020).

While these studies have the potential to automate pollen analysis, they do not generally increase taxonomic resolution of pollen identifications. Automatic image recognition can, however, also be used to differentiate highly similar pollen by combining it with high resolution imaging. This is because subtle taxon-specific variations that are not readily apparent through manual investigation may be consistently detected by sufficiently trained classifiers. Machine learning has, for example, been successfully applied to distinguish similar pollen of species of *Picea* L. (93% accuracy; Punyasena et al., 2012) and deep learning was used to differentiate fossilised pollen taxa in the Fabaceae family (~85% accuracy; Romero et al., 2020). Spatiotemporal knowledge of the species distribution gained in these studies have highly improved (paleo)ecological reconstructions. The methods used, however, do require relatively extensive sample preparation for microscopes that are not readily available. Many routine palynological studies rely on light microscopy (LM) images instead, where visualizing the distinguishing features is much harder. Nevertheless, several studies have shown that increasing the taxonomic resolution of LM pollen images for, e.g., hay fever monitoring is possible, notably in the family Urticaceae (De Sá-otero et al., 2004; Rodriguez-Damian et al., 2006). This family forms an excellent case study because of the subtle difference in morphology between common genera that have highly different allergenic profiles.

1.2.1 Case study: Urticaceae

The nettle family (Urticaceae) contains two genera that are common in Europe, *Urtica* L. (stinging nettles) and *Parietaria* L. (pellitory), of which pollen is very hard to distinguish using light microscopy (Figure 3). It is important to separate these genera because pollen grains from species of *Urtica* are allergenically unimportant while those from several species of *Parietaria* are one of the main causes of hay fever in the Mediterranean (D’Amato et al., 1991). These *Parietaria* species are currently undergoing a range expansion as a result of

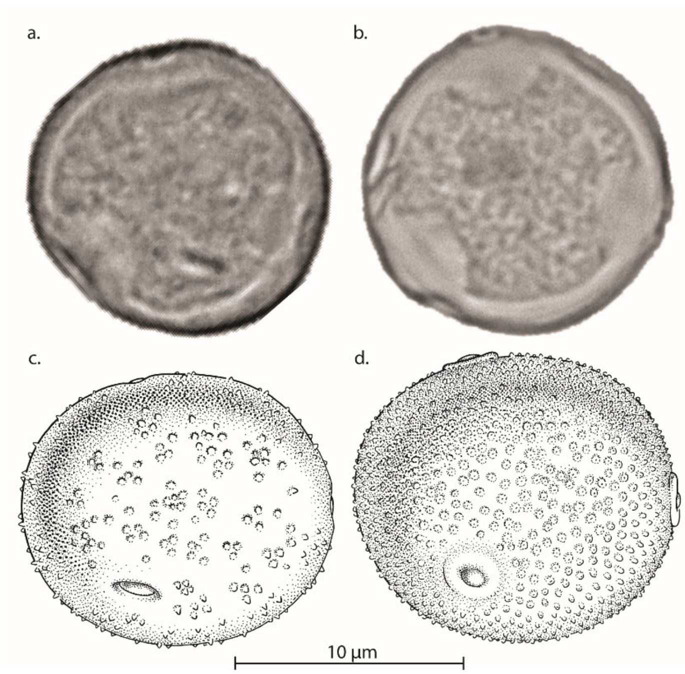

Figure 3. Pollen grains of Urticaceae species at high magnification (100X) a) LM image of Parietaria judaica, b) LM image of Urtica dioica c) drawing of Parietaria judaica pollen grain emphasizing distinctive features including lack of annulus around pores and irregular (micro)echinate surface ornament d) drawing of Urtica dioica pollen grain highlighting annulus around pore and regular scabrate surface ornament. Images a and b were obtained from PalDat (Halbritter et al., 2020). Images c and d were drawn by Esmée Winkel.

increased urbanization and climate change, but their impact on the total pollen load is currently not monitored in either native or expanded range. While previous studies cited above have shown relatively high accuracy scores in distinguishing these genera using machine learning, the models have not been applied to all species of Urticaceae and have not been tested on for the model unseen images. It is expected that higher accuracies may be achieved by incorporating the latest deep learning models trained with variable input images. However, this technique does not work for all stenopalynous taxa and other techniques may be required to distinguish pollen from species that are too similar to each other to be morphologically distinguished.

1.3 Molecular palynology

DNA barcoding has made it feasible to identify species by extracting and amplifying DNA from barcoding regions that have specificity within a species and variability between species (Hebert et al., 2003). Early attempts at identifying pollen using DNA were based on PCR approaches on single pollen grains (Figure 2; Longhi et al., 2009; Petersen et al., 1996; Suyama et al., 1996; West et al., 2008). However, with the development of Next-Generation Sequencing (NGS), all species within mixed bulk samples could be identified using DNA metabarcoding (Taberlet et al., 2012). With cost-reductions and further improvements, the application of DNA metabarcoding in pollen analysis has shown a sharp increase, most notably since 2015 (Figure 2). During this time, DNA metabarcoding was successfully applied to identify pollen collected by pollinators (e.g., Hawkins et al., 2015; Richardson et al., 2015), but also of aerobiological samples (Kraaijeveld et al., 2015).

While in the animal kingdom the mitochondrial marker COI can be used as a universal barcode for identifying species (Hebert et al., 2003), no such universal barcode has been identified for plants. This may be the result of plants having greater levels of paraphyly and hybridization (Fazekas et al., 2009). Plants contain nuclear, as well as mitochondrial and chloroplast genomes. A combination of markers has been advised for plants, including a biparentally inherited nuclear marker and a uniparentally inherited plastid marker (CBOL Plant Working group, 2011). Pollen contains several nuclei (large vegetative and several generative cells) and cytoplasm containing plastids and mitochondria (Bennett and Parducci, 2006). Some nuclear and chloroplast genes in pollen DNA are present in multiple copies, allowing amplification of both types of DNA, although much is unknown about, e.g., copy number variability between species (Bell et al., 2016). In molecular palynology, plastid *rbc*L has been used extensively (e.g., Bell et al., 2017; Brennan et al., 2019; Campbell et al., 2020; Uetake et al., 2021) but, unfortunately the taxonomic resolution of this marker is mostly restricted to genus level, unless tailored local reference databases are used.

1.3.1 *trn*L and nrITS2

The P6 loop of the chloroplast *trn*L intron represents a short and highly variable region that has been shown to work well even on samples with highly degraded DNA (Taberlet et al., 2006). For this reason the marker is popular with ancient DNA studies, but is has also been successfully used on airborne pollen (Kraaijeveld et al., 2015) as well as honey samples (Milla et al., 2021), although its short length may prevent detailed taxonomic inferences in some families (e.g. in Poaceae, Asteraceae, Cyperaceae; De Barba et al., 2014).

The nuclear ribosomal Internal Transcribed Spacer (nrITS) region has been proposed as a potential barcode for plants (Kress et al., 2005), but it was not commonly used until promising high species-resolution results were obtained from large datasets of plants (Chase and Fay, 2009). This nuclear marker is shared between eukaryotes, and is often used for taxonomic studies on plants and fungi, both of which can be explicitly targeted using primers designed for fungi (Ihrmark et al., 2012) or plants (Table 1; Cheng et al., 2016; Moorhouse-Gann et al., 2018). In plants and fungi, the nrITS region consists of two highly variable regions: nrITS1 located between 18S and 5.8S, and nrITS2 located between 5.8S and 26S rRNA genes. The easier amplifiable nrITS2 was identified as having high discriminatory power (Chen et al., 2010) and it has been used successfully in molecular palynological studies (e.g., Brennan et al., 2019; Núñez et al., 2017; Richardson et al., 2015). However, nrITS2 has been shown to perform less well for gymnosperms (CBOL Plant Working Group et al., 2011) and due to the relatively long expected amplicon length of nrITS2 (350 - 500 bp), successful amplification relies on DNA to be well preserved. Applying these two markers in combination can thus account for degraded DNA using *trn*L, while providing extended taxonomic resolution in well preserved DNA with nrITS2 (Table 1).

*Table 1. Comparison of trnL P6 loop versus nrITS2. *depending on primers used **specific groups like fungi or plants can be targeted using specific primers*

	***trn*L P6 loop**	**nrITS2**
Marker	Chloroplast	Nuclear ribosomal
Length (bp)	8 - 152	~350 – 500*
Works well on degraded DNA	yes	no
Taxonomic resolution	Relatively low	Relatively high
Targets	Only plants	Eukaryotes**

1.3.2 Pollen quantification

Apart from identifying which pollen species are present in a particular sample, pollen grain quantification is of equal, if not higher, importance. For example, in airborne pollen monitoring it will not suffice to know whether certain allergenic pollen is present in the air, but more so how much there is of it at a given point in time. While DNA-based methods for pollen quantification are less developed than methods for identification, recent studies have shown promising results. Some studies have shown correlations between absolute DNA read counts and pollen counts (Baksay et al., 2020; Pornon et al., 2016), but most studies have shown the best correlations between relative abundance of DNA reads and microscopic pollen counts (Bänsch et al., 2020; Keller et al., 2015; Richardson et al., 2021). The aforementioned studies have focussed on pollen from honey samples or from bee-collected pollen. This correlation has not been sufficiently tested for aerobiological samples.

1.3.3 (Paleo)-ecological information of pollen

Beside obtaining DNA directly from pollen, DNA metabarcoding has the potential of providing additional information on plant species in bulk samples that may remain hidden if only pollen is studied. One example is the reconstruction of the non-analogous Pleistocene paleo-environment. Analyses of vegetation changes and megafaunal diets during this time interval have been based mainly on fossil pollen and plant remains (Anderson et al., 2003). These suggest that the landscape was dominated by grasses and sedges, a landscape often referred to as the 'Mammoth Steppe' (Guthrie, 1990). There are several problems with these techniques though, since pollen analyses from these samples are biased towards plants that produce high amounts of pollen (e.g., grasses), while plant fossils often preserve poorly. Moreover, as the taxonomic resolution of visual pollen analysis is limited, no information of the specific composition of plant species can be obtained. Studies have shown that incorporating DNA metabarcoding in the study of megafaunal faecal samples can provide a significantly more refined reconstruction of last meals and habitats (e.g., Hofreiter et al., 2000; Van Geel et al., 2014; Willerslev et al., 2014). Most studies have, however, relied on short chloroplast markers, including *rbc*L minibarcodes and the *trn*L P6 loop, while the nrITS marker has never been applied. Since megafaunal faecal samples are often conserved in permafrost, their DNA can be excellently preserved. Therefore, inclusion of the relatively long nrITS has the potential to provide an unprecedented insight into the diets and habitats of megafauna.

2. Aims and objectives of the thesis

The main aim of this thesis is to unravel the hidden biodiversity of pollen by utilizing innovative microscopic and molecular techniques.

The aim of **Manuscript I** is to investigate the limits of morphological pollen identification by incorporating deep learning algorithms. The main questions to answer are (1) can a CNN distinguish morphologically similar pollen of taxa in the Urticaceae family that cannot be distinguished by palynologists, even though they have highly differing allergenic profiles? and (2) can models trained using reference pollen grains be successfully applied on pollen from aerobiological samples?

Molecular pollen analysis is a promising tool to increase taxonomic resolution of pollen identifications. **Manuscripts II** and **III** present literature reviews in the form of educational book chapters, aimed at obtaining the most up to date knowledge in current methodology and trends in molecular pollen identification. **Manuscript II** aims to give an overview of how DNA can be extracted from pollen grains and what knowledge can be obtained by doing so, while **Manuscript III** aims at giving an overview of the molecular method amplicon metabarcoding.

The aim of **Manuscript IV** is to incorporate the knowledge obtained from the literature reviews and apply this to airborne pollen monitoring. The questions to be answered are whether DNA metabarcoding using chloroplast *trn*L P6 loop and nrITS2 can (1) increase the taxonomic resolution of pollen identifications, (2) be used as a semi-quantitative tool for pollen monitoring and (3) reveal fine scale spatiotemporal patterns between pollen monitoring locations.

Information from pollen lies at the foundation of many ecological reconstructions. **Manuscript V** aims at comparing plant identifications from pollen and macrofossils to multiproxy DNA results. This includes the nrITS marker, which has never been used before in the study of extinct megafauna, alongside the *trn*L P6 loop. The aim is to test whether DNA metabarcoding can increase taxonomic resolution of plant identifications in order to reconstruct the last diets and habitats of extinct and extant megafauna.

3. Material and methods

An overview of all materials and methods used in this thesis will be outlined in this section. For more detailed information on the material and methods for each individual project, please refer to the manuscripts.

3.1 Material and sample collection

3.1.1 Pollen samples

For Manuscript I, pollen was collected from all five species of the nettle family (Urticaceae) present in the Netherlands (*Parietaria judaica* L., *P. officinalis* L., *Urtica dioica* L., *U. membranacea* Poir. ex Savigny, *U. urens* L.). Pollen collected from plants in the Netherlands was supplemented with pollen from herbarium plant specimens of the Naturalis Biodiversity Center, including material from Spain, Portugal and the Netherlands (Fig 4a-c). Thecae of flowers were opened using tweezers and mounted on microscopic slides using a glycerin:water:gelatin (7:6:1) solution with 2% phenol and stained with Safranin (0.002% w/v). Pollen was not acetolyzed (i.e. method to remove organic material) since pollen on aerobiological slides is unacetolyzed as well. The pollen images were used to train the CNNs, which were subsequently tested on Urticaceae pollen grains collected by pollen monitoring stations in Leiden, the Netherlands and Vielha and Lleida (Barcelona, Spain; Figure 4a) for validation.

A total of 58 samples with airborne pollen was collected for Manuscript IV using Burkard pollen samplers located in Leiden and Helmond, the Netherlands (Figure 4a). Airborne pollen was captured on Melinex adhesive tapes and mounted on microscopic slides using the same protocol as described for Manuscript I. In this study both unmounted tapes from 2020 as well as tapes from microscopic slides from 2019 were used. Samples with high pollen counts in three target taxa that flower abundantly in the Netherlands during either spring (*Alnus* sp., Cupressaceae/Taxaceae) or fall (Urticaceae) were selected for DNA extraction.

3.1.2 Faecal samples

For Manuscript V, eleven permafrost and ice-preserved faecal samples from four mammal species (woolly mammoth, steppe bison, horse and caribou) were included. Samples were derived from Sakha Republic (Russia), Alaska (USA), Yukon and Northwest Territories (Canada; Figure 4d) and ranged in age from 28,000 yr BP to modern.

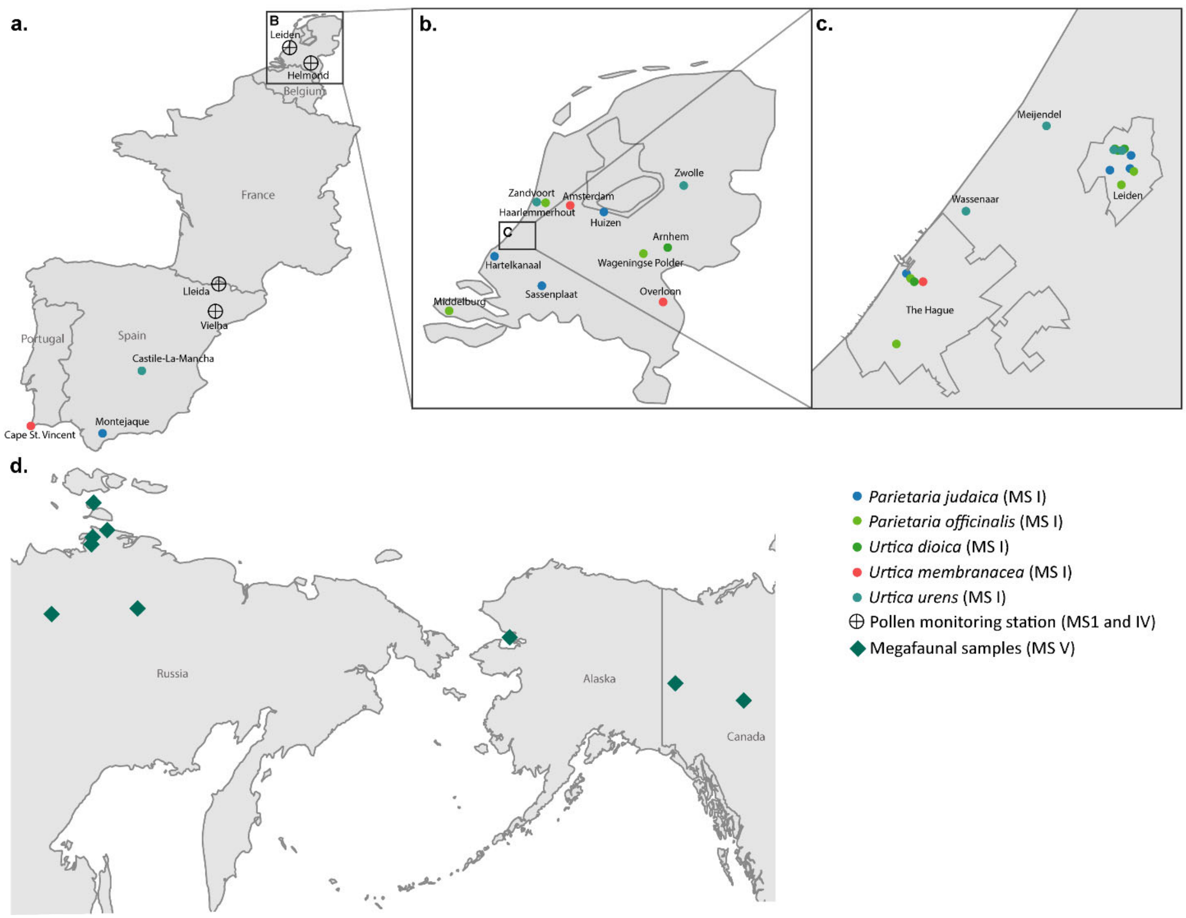

Figure 4. Sampling locations, a) locations of pollen monitoring stations and Urticaceae pollen reference material from Spain and Portugal (herbarium Naturalis Biodiversity Center) for Manuscript I, b) Urticaceae samples from the Netherlands, c) Urticaceae samples from around and within Leiden and The Hague (the Netherlands), d) locations of permafrost and ice-preserved megafaunal faecal samples in Sakha Republic (Russia), Alaska (USA), Yukon and Northwest Territories (Canada). MS = Manuscript

3.2 Analytical methods

3.2.1 High resolution pollen imaging and CNN

To sufficiently train CNNs to distinguish the highly similar pollen of Urticaceae in Manuscript I, a total of 6,472 individual pollen grains were imaged at high resolution (100X magnification) and using 20 focus levels (z-stacks). For each of the five species, a minimum of 1,000 images was captured from at least four different plant specimens. Pollen grains were imaged using a microscope with an automatic stage and post-processed in ImageJ (Rasband, 1997) using a custom script ("Pollen_Projector"). Briefly, the script identified free lying pollen grains and cropped them out of the stack of images. CNNs need three-channel input images (commonly RGB in colour images) but as grayscale images were used in this study, three different Z-stack projections were chosen to represent the three different channels.

The pollen reference images were used to train three CNNs using different settings for splitting the dataset into training and testing sets (80/20 and 90/10). A transfer learning approach using data augmentation was adopted as it is important to increase the variability of images in this relatively small dataset. Pre-trained models on large open-source image databases were compared to models trained from scratch. Finally, the best performing model was tested using unknown Urticaceae pollen collected at pollen monitoring stations from the Netherlands and Spain.

3.2.2 Morphological identification

In Manuscripts IV and V, pollen was manually counted under the microscope using 40X magnification. For Manuscript IV, pollen from microscopic slides collected by Burkard samplers was counted in three longitudinal bands, an area corresponding to 1m^3 of filtered ambient air over 24 hours (Galán et al., 2017).

For Manuscript V, microscopic pollen slides as well as plant macrofossil samples were made by taking subsamples from the core of the faecal samples. Pollen identifications were made using a reference pollen collection and following Moore et al. (1991) and Beug (2004). For the preparation of macrofossils, the procedure of Mauquoy and Van Geel (2007) was followed.

3.2.3 Molecular identification

3.2.3.1 DNA extraction and amplification

An overview of DNA extraction protocols and molecular techniques to study pollen grains is given in Manuscript II. Following insights gained from this book chapter, pollen DNA was extracted from airborne pollen in Manuscript IV using the commercially available QIAamp

DNA Mini kit (Qiagen). This extraction protocol is based on spin-columns and silica-membrane purification. Prior to extraction, pollen cell walls were disrupted using a bead beating protocol to break the exines and release the DNA from inside the pollen. For Manuscript V, the DNA of plants in the faecal samples was extracted using the silica-based protocol of Rohland and Hofreiter (2007) adjusted to smaller volumes of material described in Stech et al. (2011).

Considering results from Manuscript II and III, DNA metabarcoding was applied for the airborne pollen samples in Manuscripts IV and on megafaunal faeces in Manuscript V. In both studies, DNA was amplified using primers *g* and *h* to amplify the chloroplast *trn*L P6 loop (Taberlet et al., 2006) and plant-specific primer ITS-p3 (Cheng et al., 2016) and ITS4 (White et al., 1990) to amplify nrITS2. Furthermore, in Manuscript V, nrITS1 was amplified using plant-specific primers ITS-p5 / ITS-u2 (Cheng et al., 2016), while fungal DNA was amplified using fungal-specific primers fITS7 / ITS4 for the nrITS2 region (Ihrmark et al., 2012; White et al., 1990). In Manuscript IV a two-step PCR approach was adopted to create indexed amplicon libraries, while in Manuscript V this was performed using a dual-indexing approach, with tagged primers. For both studies, extraction and negative blanks as well as positive controls were incorporated, and three PCR replicates were used per sample. Sequencing was performed on an Illumina MiSeq.

3.2.3.2 Bioinformatics and filtering

To get from DNA sequences to species information for Manuscripts IV and V, bioinformatic pipelines were used on a Galaxy instance (Afgan et al., 2018) or using OBITools (Boyer et al., 2016) on the Oslo computing server (UNINETT Sigma2). In short, the steps included quality checking of raw sequences, assembling forward and reverse reads, removal of adapters and primers, demultiplexing, dereplication, clustering and taxonomic assignment. Clustering was performed using strictly identical sequences (often referred to as ASVs) or assigning sequences directly to taxa. Taxonomic assignment was performed using local reference databases where available, and also compared to global reference libraries.

Sequence filtering was performed in R, using strict protocols aimed at removing as many false positives as possible. For both manuscripts, steps included removal of sequences with (a) low identity scores, (b) below a threshold of reads per PCR replicate, (c) higher abundance in PCR controls than in samples and, only for Manuscript IV, (d) taxonomic assignments other that green plants. Potential leakage of sequences was accounted for using a custom R script to detect which filtering threshold resulted in removal of all reads from negative controls. Since the aim of Manuscript IV was to get reliable quantification results, only OTUs present in at least two PCR replicates per sample were kept. This strategy was different in Manuscript V (keeping all taxonomic identifications, regardless of the amount of PCR replicates in which they were present) because here the aim was to discover as much diversity as possible. For both studies, a final manual filtering step was performed to remove common lab contaminants (e.g., *Solanum lycopersicum, Musa* spp., *Glycine max*) and other suspected food contaminants.

3.2.4 Ecological inferences

For Manuscripts IV and V, all sequencing reads were converted to relative read abundances for semi-quantitative comparison with pollen counts and, for Manuscript V, macrofossil abundance. In order to reconstruct the last diets of the megafauna studied in Manuscript V, the average relative abundance values of macrofossils and all available DNA results were taken, since pollen represents a regional signal. Megafaunal habitats were reconstructed by taking all species level as well as some genus level taxonomic assignments from the three proxies (pollen, macrofossils and DNA). Taxa were divided into habitat types ranging from very dry (steppe) to very wet (wetlands).

In Manuscript IV, least squares regression was used to compare molecular quantification results with those made by morphological identification of pollen. Furthermore, to test whether DNA metabarcoding results could be used to distinguish samples from the different pollen monitoring stations and seasons, Bray-Curtis dissimilarities were calculated in *vegan* (Jari Oksanen et al., 2018) between all sample pairs. Results for both *trn*L and nrITS2 were ordinated using NMDS, and grouped per pollen monitoring site and per season. The statistical significance of these groupings was calculated using a perMANOVA.

4. Main results of manuscripts I-V

The results of this thesis are presented in one published manuscript (Manuscript I), two peer-reviewed book chapters (Manuscript II and III) and two submitted manuscripts currently under review (Manuscript IV and V) and will be briefly outlined here.

4.1 Manuscript I

Neural networks for increased accuracy of allergenic pollen monitoring

This manuscript demonstrates incorporating neural networks to increase the taxonomic resolution of pollen grain identifications in aerobiological samples. Using a case study from the nettle family (Urticaceae), it is shown that sufficiently trained CNNs can successfully distinguish pollen genera that cannot currently be separated under the microscope by specialists. Two genera and one species of Urticaceae were distinguished by trained CNNs with >98% accuracy. Not all species could be recognized because the distinguishing features of pollen from these species (exine ornamentation) could not be resolved in the unacetolyzed pollen grains. Various settings were tested for the CNNs and the best result was obtained using 80% for training images and 20% for validation, using either the very deep VGG16 (98.61%) or the faster MobileNetV2 (98.76%). The models consistently learned features such as pollen edges in the first convolutional layers, while finer features such as pores and annuli were learned in deeper layers. Models were trained on pollen collected from various plant samples in the field and from an herbarium, but it was also shown to work very well on for the model before unseen pollen collected directly from the air. In Leiden (the Netherlands), *Urtica* of low allergenicity was shown to be the dominant source of pollen, while for Lleida (Catalunya, Spain) severely allergenic pollen of *Parietaria* was most abundant. A low amount of *Parietaria* pollen was found in Leiden. Since *Parietaria* is recently showing a large range expansion, these numbers are expected to rise in the near future and this can now be studied using the presented method. Furthermore, this can be more broadly applied to distinguish pollen from similarly challenging allergenic plant families and can help in producing more accurate pollen monitoring for allergy sufferers.

4.2 Manuscript II

Book chapter - DNA from pollen

This educational book chapter highlights the latest trends in molecular research on pollen. An overview is provided of recent literature (since 2017) showing that DNA metabarcoding is the most commonly used method for plant-pollinator and airborne pollen identifications. While most earlier studies relied on plastid DNA markers (e.g., *rbc*L and *trn*L P6 loop), increasingly, the nuclear marker nrITS2 is being incorporated because of the high taxonomic accuracy it provides, as well as promising (semi-)quantitative results. Studies adopt varying strategies for pollen DNA extraction, including different pollen lysis and extraction protocols. Pollen lysis was identified as a crucial step to increase the yield of pollen DNA. Lastly, several recent studies show the potential of metagenomic approaches to quantify pollen samples, although this is currently hampered by the lack of reference genomes and the high costs compared to amplicon metabarcoding.

4.3 Manuscript III

Book chapter - Amplicon Metabarcoding

In this educational book chapter, the main advantages and disadvantages of plant DNA metabarcoding are discussed. Plant metabarcoding is currently hampered by the lack of a universal plant marker, PCR amplification / binding biases and dependency on (local) reference libraries. However, it is one of the most cost-efficient methods for molecular identification as the amplicon tagging system allows high throughput of samples, even if they have relatively low quality and quantity of DNA. Furthermore, with the right choice of marker, nucleotide tagging strategy, PCR replication, clean laboratory setting and inclusion of PCR controls, highly meaningful information on the taxonomic composition of plant bulk samples can be obtained. These samples can include water, soil, sediment, snow, faeces and air.

4.4 Manuscript IV

DNA metabarcoding using nrITS2 provides highly qualitative and quantitative results for airborne pollen monitoring

This study shows that DNA metabarcoding using plant markers *trn*L and nrITS2 is able to provide highly improved taxonomic resolution of airborne pollen. From the 58 samples collected over two consecutive years at two pollen monitoring stations in the West and Southeast of the Netherlands, manual pollen identification detected 23 plant genera and 22 families. In contrast, DNA metabarcoding using both markers resulted in 168 species from 143 genera and 56 plant families, most of which uniquely found by nrITS2. At the family level, all pollen identified by microscope was also found with metabarcoding. Both markers identified plant taxa that were not detected using manual pollen counts, including several taxa of potential allergenic importance (e.g., *Mercurialis* spp. and *Parietaria* spp.).

Regressing the relative read abundances from both DNA markers against the relative abundances of manual pollen counts, highly significant positive correlations were identified (R^2 for all taxa = 0.821 for nrITS2 and 0.620 for *trn*L). These correlations were found to be species-dependent, with *Alnus* showing nearly a one-to-one relation for both markers, while this relationship was slightly weaker, though still statistically significant, for Cupressaceae and Urticaceae. Plotting the relative abundance of species detected by nrITS2 through time, it is shown that pollen spectra from three common taxa in the Netherlands (*Alnus*, Cupressaceae, Urticaceae), are dominated by single species (*Alnus glutinosa/incana*, *Taxus baccata* and *Urtica dioica*). For *Alnus*, cultivated non-native species were identified that significantly prolong the hay fever season. Lastly, finer-scaled spatiotemporal patterns were distinguished between the two pollen monitoring stations using nrITS2 than using *trn*L. This was mainly the result of the higher taxonomic resolution of nrITS2, identifying species that were either typically found in the West or the Southeast of the Netherlands. All results indicate that nrITS2 should be the preferred marker of choice for molecular airborne pollen monitoring.

4.5 Manuscript V

Multiproxy analysis of permafrost preserved faeces provides an unprecedented insight into the diets and habitats of extinct and extant megafauna

In this study, results of pollen and macrofossil analysis on eleven megafaunal faecal samples were compared to plant DNA metabarcoding results from the chloroplast *trn*L P6 loop and nrITS marker. The results show that it is important to incorporate a multiproxy approach in studying megafaunal faeces, since unique plants were identified using pollen, macrofossils and DNA. However, most unique plant identifications were found using DNA, likely because the studied faeces contained many vegetative remains that could not be identified using macrofossils or pollen. The *trn*L P6 loop showed the highest number of plant identifications, partly because the reference library was more complete and partly because DNA may have been degraded. Nevertheless, for the first time it is shown that the relatively long nrITS marker can be successfully amplified from samples as old as 28,610 yr BP. This allowed plants to be identified to the species level where other proxies only found family or genus level identifications in e.g. Asteraceae, Poaceae and bryophytes.

By integrating results from all proxies, an accurate reconstruction of the last meals and habitats of modern and extant caribou could be made. These showed, as expected, that that the caribou were mainly foraging on shrubs and low amounts of lichen in alpine/arctic tundra. Extending this approach, the Holocene mammals studied here (horse and steppe bison) could be reconstructed as mixed feeders living in a marshy environment. For the woolly mammoths, highly variable diets were identified from a range of habitats. Some of the mammoth fed exclusively on grasses, while others showed abundant shrubs or forbs. This result shows that mammoths may have been more flexible in their food choice than previously thought, and that they made full use of the various habitats present in the landscape mosaic often referred to as the 'mammoth steppe'.

5. Discussion and concluding remarks

This thesis shows the added value of incorporating novel techniques, including automatic image recognition and DNA metabarcoding into pollen analysis. Using a case study from the family Urticaceae, Manuscript I demonstrates that neural networks are able to differentiate highly similar pollen from genera that cannot be distinguished by palynologists. Without prior knowledge of the morphological differences between pollen of these genera, the neural networks correctly focused on the distinguishing features of each genus. To improve robustness of the CNNs, increasing variability of the pollen training images was found to be of high importance. This is because pollen from different plant samples of the same species were found to show subtle, but distinct variability. This naturally occurring intra-specific variability was also recognized in a recent study on automatic image recognition of bee-collected pollen (Olsson et al., 2021). Here, the authors collect pollen from at least two, but for most species over four samples. Deep and sensitive CNNs may recognize sample-specific, instead of species-specific patterns if not trained correctly. While Olsson et al. (2021) deal with a much larger number of species, including some that are quite similar, the magnification used in that study (40X) would not allow visualization of distinguishing features in very small pollen such as those from Urticaceae. Furthermore, several species from genera were distinguished at 40X magnification that are very hard to discriminate even using SEM images (e.g., *Acer campestre*, *A. platanoides, A. pseudoplatanus*; Biesboer, 1975, Beug, 2004). This raises the question whether the CNN used by Olsson et al. (2021) really identified the different species, or that potentially due to the different uptake of the fuchsin staining used, artificial differences may have been introduced that were picked up by the CNN. Nevertheless, at the genus level, very high accuracies were found and this approach can be extended to airborne pollen identification. However, for high-resolution species differentiation, the method of Manuscript I of this thesis may be more suitable.

For distinction of pollen and plants that cannot be morphologically distinguished, DNA metabarcoding has been successfully applied in this thesis. DNA was obtained directly from pollen to refine allergenic pollen monitoring in Manuscript IV, and used as a complementary method to pollen-based paleoecological reconstructions in Manuscript V. Taxonomic resolution using *trn*L and nrITS2 was found to be much higher than using microscopic pollen analysis (Figure 5). A multilocus approach was crucial for identifying plant diversity, as unique families, genera and species were found using both *trn*L as nrITS2 in both studies. However, the nrITS marker was found to be harder to amplify, with less samples successfully amplified, likely resulting from the relatively long amplicon size (~350 – 500 bp). In the Pleistocene and Holocene samples in Manuscript V, where DNA was expected to be more degraded, the very short and stable *trn*L P6 loop was found to perform better than nrITS2 in terms of taxa recovery (Figure 5). On the other hand, this thesis shows for the first time that nrITS can be successfully amplified from samples preserved over 28,000 years in permafrost, providing highly valuable insights into hitherto hidden diversity in megafaunal diets and habitats. nrITS results showed much higher percentages of taxa recovered to the species level for both studies (~80% of OTUs, versus ~25-40% for *trn*L). For Manuscript IV, where recent

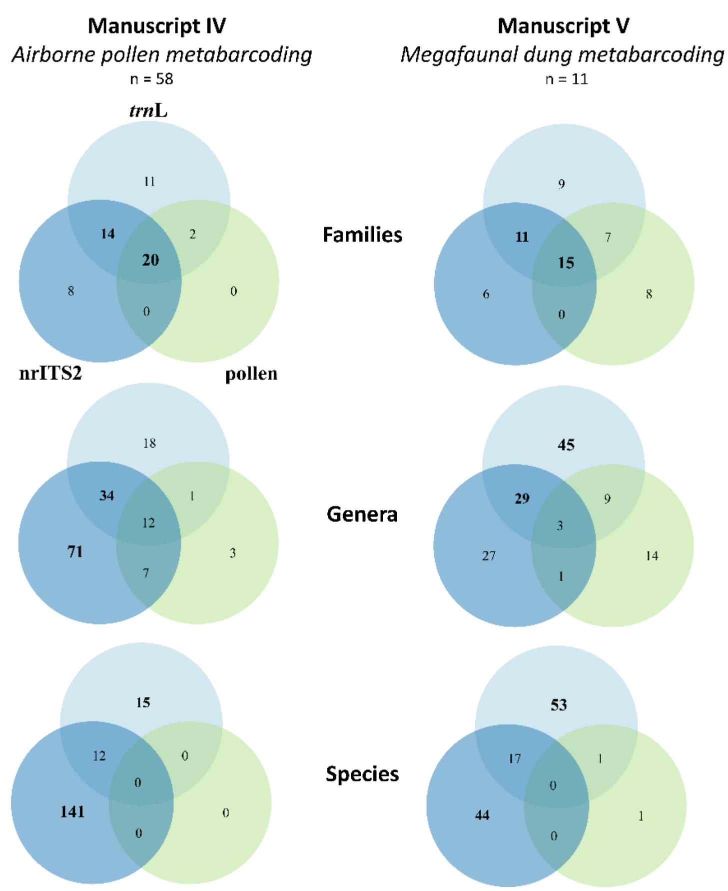

Figure 5. Venn diagrams for manuscripts IV and V. The number of taxa detected using trnL*, nrITS2 and microscopic pollen analysis is shown at family, genus and species level. n = number of samples studied*

samples were used, DNA was of high quality, allowing nrITS2 to show its full potential in identifying hidden diversity of pollen grains (Figure 5).

Since quantifying relative abundance is essential for answering many ecological questions, the main arguments against DNA metabarcoding have often been that the results cannot be reliably used in a quantitative way (Bell et al., 2019; Deagle et al., 2019; Pawluczyk et al., 2015; Piñol et al., 2019). However, in Manuscript IV it is shown that for allergenic pollen monitoring, nrITS2 results can be used to infer statistically significant species-level semi-quantitative results. This result is corroborated in pollen quantification studies using nrITS2 for bee-collected pollen, also finding similar correlation values (R2 ~ 0.8; Bänsch et al., 2020; Keller et al., 2015; Richardson et al., 2021). Despite initial good results of quantifying pollen using chloroplast *trn*L (Kraaijeveld et al., 2015), the results of Manuscript IV indicate that it performs less well than nrITS2, similar to results in Richardson et al. (2021). DNA from pollen may be easier amplifiable using nuclear markers because of the high number of nuclear ribosomal ITS tandem copies inside the multiple nuclei in pollen grains (Long and Dawid, 1980). Furthermore, in most angiosperm species chloroplast DNA is inherited maternally, which is why it is either increased or reduced in pollen from different taxa (Nagata et al., 1999; Sakamoto et al., 2008). In Manuscript V, the relative abundance of nrITS2 reads showed high overlap with plant macrofossil abundance for some samples, while this correlation was higher for *trn*L in others. This is most likely related to differences in DNA preservation for the different samples. Quantifying pollen using absolute DNA metabarcoding reads has been shown to be possible by some studies, but because of biases originating from PCR as well as library preparation steps (e.g., equimolar pooling), this needs further research and standardization (Baksay et al., 2020; Bell et al., 2019; Pornon et al., 2016). PCR-free approaches including genome skimming have recently shown promising results in quantifying polling quantification (Lang et al., 2019; Peel et al., 2019), but are currently hindered by the high costs associated with the analysis, as well as a lack of reference genomes. Therefore, amplicon metabarcoding using nrITS2 is currently the most feasible method for semi-quantitative molecular pollen analysis.

Several alternative methods for identifying pollen have been introduced in recent years that will be briefly discussed here. Among these, the most promising results have been obtained using multispectral imaging flow cytometry, in combination with deep learning (Dunker et al., 2021), digital holography with supervised learning techniques (Sauvageat et al., 2020) and qPCR barcoding (Rowney et al., 2021). The first two morphological methods have high potential for automated and accelerated pollen counting, but have as yet not been tested on fresh pollen mixtures of unknown pollen types. Furthermore, the main aim of these methods is not to increase the taxonomic resolution (although this may partly be achieved with sufficient training), but rather to automate and accelerate palynological investigations. The method of Rowney et al. (2021) links particular Poaceae species prevalence, measured using qPCR, with respiratory disease incidence. A subset of species was particularly targeted in this study, while DNA metabarcoding has the potential to capture all species from bulk samples. Other techniques have been developed that do focus on increasing taxonomic resolution in pollen, including Raman spectroscopy (Pereira et al., 2021) and FTIR chemotaxonomy of pollen (Jardine et al., 2019). However, these techniques currently require further developments to overcome specific technical issues and have not yet been tested on real samples. Therefore, the techniques applied in this study, including automatic image recognition and DNA metabarcoding currently have the highest potential to increase both taxonomic resolution and quantify pollen.

In future research, the automatic image recognition method of Manuscript I has the potential to uncover currently hidden species distribution patterns back in time by utilizing historical microscopic pollen slides. This is harder to achieve using DNA metabarcoding, since historical samples may have highly degraded DNA. The method can also be applied to other case studies where distinction is desirable due to differences in allergenicity (e.g., family Oleaceae, allergenic *Olea* versus non-allergenic *Fraxinus* and *Ligustrum* pollen) or to detect invasive taxa (e.g., family Polygonaceae, invasive *Reynoutria* versus native *Polygonum* pollen). For pollen DNA metabarcoding, identifying and correcting for species-specific amplification biases will help in creating robust species-level allergenic pollen monitoring. This method has the potential in showing spatiotemporal patterns in more species and over prolonged periods of times, and could also be used as an early detection system for pollen from invasive plants.

Further contributions to manuscripts not included in this thesis

Li, C., **Polling, M.**, Cao, L. and Verbeek, F.J. Analysis of Automatic Image Classification Methods for Urticaceae Pollen Classification. *Manuscript to be submitted to BMC Bioinformatics*

Mota de Oliveira, S., Duijm, E., Ruijgrok, J., Stech, M., **Polling, M.**, Barbosa, C.G.G, Cerqueira, G.R., Nascimento, A.H.M., Godoi, R.H.M., Wolf, S., Pöhlker, C., Weber, B., Kesselmeier, J. Life is in the Air: A Botanical Expedition into the Amazonian Atmosphere. *Manuscript in preparation*

Veltman, M. and Garrett, S., Anthoons, B., Ariza, M., Chua, P. **Polling, M.**, de Boer, H. and Hollingsworth, P. Trends and Developments in Molecular Plant Identification for Science and Society. *Manuscript in preparation*

Ariza, M., Alsos, I.G., **Polling, M.**, Lammers, Y., Garcés Pastor, S., de Boer, H., Halvorsen, R., Plant detectability with soil eDNA. *Manuscript in preparation*

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And then I suddenly found myself on a huge 250 m long drilling ship, way out offshore in the US Gulf of Mexico… sweltering heat, dressed up in orange coveralls and wearing a hard hat.. Thinking: what happened? How did I end up here? I was really interested in the sharks, tuna, dolphins and migrating hummingbirds on and around the ship, but what I was there for was actually helping to find oil, over 10 km below the deck of the ship. True, I was there as a palynologist looking down a microscope at fascinating micro-organisms, but it was not quite the right place for a biologist to be... for many different reasons. It was time for a change, but how do you find a job as a biologist if you are actually trained as a geologist? Dilemma. Luckily, I did not stand alone in this problem. After many doubts and some very random job applications, me and my girlfriend Yvonne drew out a roadmap: what do I want? A job outside oil and gas. What should the job be about? Biology, biodiversity. What requirements should the job have? Applied, analytical. I still have the piece of paper with the whole diagram. Amazingly, one of the routes we laid out in 2018 is exactly the one I ended up following: do biology PhD in the Netherlands, finish PhD, get job with new skills.

Sounds easy-peasy, but I think many of you reading this can attest to it definitely not having been easy-peasy over the course of the three years. I don't think I would have been able to finish it without the support of all of you, so here goes: first I want to thank the people who gave me this opportunity in the first place. To Barbara for her unbridled support and can-do attitude, and Hugo for keeping me in check. To all the people in the labs introducing me to the world of DNA, Marcel E., Arjen, Elza, Roland, Frank.. I will never be king of the pipette, but thanks to you I got it done. Rob and Bertie-Joan for always being there for an informal chat over coffee. My fellow group members, Dewi and Richa, your positivity is an inspiration. Thanks to all the fellow Naturalis PhDs and postdocs for the mental support, Lisette, Andres, Kasper, Hector, Kevin, Eka, Esther, Anita, Le Qin, Deyi and all the others. To Ozan, because suffering together is so much better than doing it alone. Thanks to the amazing students for their help during their internships; Tom, Marit, Charissa, Melati – you made my life so much easier. To the many co-authors helping me out in my projects, in particular Lu, Chen, Bas, Anneke, Sanne, Letty, Physilia, Fons, Joost – I highly value your input and support throughout. To the Plant.ID team, Brecht and Marcella for managing the whole project and organizing great trips to Barcelona, Scotland and Oslo for the wicked bunch of ESRs - thanks to Bastien, Margret, Nataly, Mehrdad, Yannick, Ntwai, Maurizio, Feli, Stephen, Tibo, Anne-Sophie for getting through together in this rollercoaster journey. Special thanks to Corillo, fjord diving and lightning were very welcome distractions. María, you got me through some bad times – te amo.

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Publications and Manuscripts

Manuscript 1

Neural networks for increased accuracy of allergenic pollen monitoring

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Neural networks for increased accuracy of allergenic pollen monitoring

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Monitoring of airborne pollen concentrations provides an important source of information for the globally increasing number of hay fever patients. Airborne pollen is traditionally counted under the microscope, but with the latest developments in image recognition methods, automating this process has become feasible. A challenge that persists, however, is that many pollen grains cannot be distinguished beyond the genus or family level using a microscope. Here, we assess the use of Convolutional Neural Networks (CNNs) to increase taxonomic accuracy for airborne pollen. As a case study we use the nettle family (Urticaceae), which contains two main genera (*Urtica* and *Parietaria*) common in European landscapes which pollen cannot be separated by trained specialists. While pollen from *Urtica* species has very low allergenic relevance, pollen from several species of *Parietaria* is severely allergenic. We collect pollen from both fresh as well as from herbarium specimens and use these without the often used acetolysis step to train the CNN model. The models show that unacetolyzed Urticaceae pollen grains can be distinguished with > 98% accuracy. We then apply our model on before unseen Urticaceae pollen collected from aerobiological samples and show that the genera can be confidently distinguished, despite the more challenging input images that are often overlain by debris. Our method can also be applied to other pollen families in the future and will thus help to make allergenic pollen monitoring more specific.

Pollen allergies are on the rise globally, with worldwide approximately 10–30% of adults and 40% of children affected[1,2]. For patients the symptoms include a runny nose, sneezing and itchy eyes, mouth or skin. Control measures and medication are readily available, but to alleviate the symptoms most efficiently, exposure to allergens should be kept to a minimum[3]. Therefore, for more and more people, fast and accurate monitoring of airborne pollen provides an essential early warning system[4,5]. Pollen concentrations in the air are monitored using samplers that collect airborne pollen on sticky tape, e.g. Hirst type samplers[6]. These tapes are microscopically inspected for their pollen content, a process that requires highly trained specialists. Moreover, although the allergenic pollen from some plants can be monitored at the species level (e.g. species of plantain, *Plantago* L.[7]), many other pollen grains cannot be accurately identified to this level. In many taxa, only a genus- or family-level identification is possible using current microscopic methods[8]. This is problematic since different species and even genera within the same family can possess very different allergenic profiles. An extra challenging factor in airborne pollen identification from Hirst samples is that they are collected directly from the air. In contrast to pollen grains that have been acetolyzed [9], these pollen grains still contain all organic material, and defining features are less apparent[10].

This identification challenge is exemplified in the case of the nettle family (Urticaceae). Pollen grains produced by all species from the genus *Urtica* L. (stinging nettles) have a low allergenic profile[11], while pollen from several species of *Parietaria* L. (pellitory) is a major cause of hay fever and asthma, in particular *P. judaica* L. and *P. officinalis* L.[12,13]. These pellitory species are native to the Mediterranean, but throughout the second half of the twentieth century, a range expansion occurred through north-eastern Europe, the Americas and Australia

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as a result of anthropogenic distribution and climate change[14,15]. *Parietaria* sensitization is highly different per geographic area, but has been reported to reach 80% in southern Italy while a value of 13% was found in the United Kingdom[16]. Species of *Parietaria* flower throughout the year but their main flowering peaks occur in May–June and August-October, which overlaps with the flowering season of *Urtica* species (June–October)[17]. Cross-reactivity is present between species of *Parietaria*, but is absent between the genera *Urtica* and *Parietaria*[11,18,19]. *Parietaria* pollen is microscopically indistinguishable from that of *Urtica* and their contribution to the total airborne pollen load is currently not assessed in either native or expanded range[20].

Pollen grains from *Urtica* and *Parietaria* species have a simple morphology: they are small (~ 11–20 μm), rounded to slightly ellipsoidal tri-, tetra- or zonoporate with a psilate to scabrate surface ornament and small pores. Most species have an annulus around the pore, i.e. a thickening of the otherwise very thin exine and a germination area called the oncus (lens-shaped body located in the apertural region)[7]. The only species of Urticaceae that can be distinguished in aerobiological samples is *Urtica membranacea* due to its small size (~ 10–12 μm) and a high number of pores (usually more than six[21]. The main difference between the pollen of *Urtica* and *Parietaria* are the slightly smaller size and coarser surface ornamentation of *Parietaria*, and a more angular outline and more pronounced annulus of *Urtica*[22].

Despite recent advances in innovative technologies, palynology is still largely an image-based discipline[23]. Therefore, automating this process currently receives a lot of attention. Automatic classification using manually selected pollen-specific features has typically resulted in relatively low classification success (see e.g.[24,25]). However, recent studies applying advances using deep learning have been very promising[26–29]. Neural networks have been used successfully to manage both the tasks of differentiating pollen from non-pollen debris as well as correctly identifying different taxa (for an overview please refer to [23]). Automatic image recognition can, however, also be used to improve identification of pollen taxa that are difficult to distinguish using traditional methods. Subtle variations in morphology that are not readily apparent through microscopic investigation may be consistently detected by neural networks. This has for example been shown for the highly similar pollen of black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) and white spruce (*Picea glauca* (Moench) Voss) using machine learning [30] and for pollen of ten species of the thistle genus *Onopordum* L. using an artificial neural network[31]. Recent advances have also been made in the field of aerobiological samples with for example the distinction of anomalous from normal pollen grains of common hazel (*Corylus avellana* L.) [32]. However, neural networks have so far not been tested for improvement of taxonomic resolution in unacetolyzed pollen in aerobiological samples.

Here we use Convolutional Neural Networks (CNNs) to distinguish morphologically similar, unacetolyzed pollen from the nettle family. We collect pollen from all species of Urticaceae present in the Netherlands (*Urtica dioica, U. membranacea, U. urens, Parietaria judaica* and *P. officinalis*). The pollen was collected from several sources for each species, freshly collected as well as from herbaria, and used to create a pollen image reference dataset. We compare the results of CNNs trained from scratch with those from pre-trained CNNs using transfer learning. Because of the limited size of the pollen image dataset, pre-training the CNN on a publicly available image database can help to recognize the distinguishing features of pollen grains such as pores, texture and shape.

We test both the deep CNN VGG16 and the faster CNNs MobileNetV1 and V2, and optimize the performance using data augmentation. The model is then applied to unknown Urticaceae pollen from three aerobiological samples with high Urticaceae pollen counts. We use one sample from the Leiden University Medical Centre (LUMC), Leiden, the Netherlands as well as one sample each from Lleida and Vielha, Catalonia, Spain (ICTA-UAB). In the Netherlands, stinging nettles (*Urtica*) are highly abundant and therefore it is expected that most Urticaceae pollen will be from this genus. *Urtica* is also expected to be dominant in Vielha, while in the direct surroundings of Lleida, *Parietaria* is very abundant.

The main objectives of this study are (1) to see whether a CNN model can distinguish morphologically similar unacetolyzed pollen of two common genera and a species in the Urticaceae family that have highly differing allergenic profiles; (2) to test whether the trained model can be successfully applied on aerobiological samples containing more complex and for the model before unseen input images.

Results

Model performance. In this study three different CNNs were tested on unacetolyzed pollen of Urticaceae which cannot currently be separated by specialists. The highest accuracy of the models using the three classes *Urtica, Parietaria* and the species *Urtica membranacea* was obtained using fivefold cross-validation (i.e. 80% training, 20% validation) with either VGG16 (98.61%) or MobileNetV2 (98.76%) (Table 1). Since VGG16 and MobileNetV2 had very similar performance, we trained these two models two more times to see which model performed more consistently. The mean accuracy after three repetitions was 98.50% for VGG16 with 0.145% standard deviation and 98.45% for MobileNetV2 with relatively higher standard deviation (0.289%). The models trained from scratch showed significant lower accuracy for MobileNetV1 and V2 (both < 89%) while this value was 96.29% for VGG16.

As the CNNs showed equally high accuracies with the pre-trained method (> 98%), we applied the more consistent VGG16 model using fivefold cross-validation and show the results here. The model accurately identified pollen to the genus level for 97.8% of the test images for *Urtica* and 99.0% for *Parietaria* (Fig. 1). For *Parietaria* three images were misclassified, while five were misclassified for *Urtica* (all to *Parietaria*). The species *Urtica membranacea* was confidently distinguished from all other Urticaceae species (99.2%), but distinction at the species-level was not possible for any of the other *Urtica* and *Parietaria* species. This is because the distinguishing features of pollen from these species (e.g. exine ornamentation) could not be resolved in the used image projections.

For all species, pollen grains were collected from a minimum of four different plants. Looking at the raw pollen images from the different plants, we identified intra-specific differences that result from natural variability

CNN	Method	Cross-validation	Accuracy (%)	Precision	Recall	F1-score
VGG16	From scratch	Fivefold	96.29	0.9632	0.9629	0.9629
		Tenfold	96.14	0.9616	0.9614	0.9614
	Pre-trained	Fivefold	**98.61**	0.9861	0.9861	0.9861
		Tenfold	**98.30**	0.9831	0.9830	0.9830
MobileNetV1	From scratch	Fivefold	84.54	0.8454	0.8454	0.8454
		Tenfold	86.40	0.8640	0.8640	0.8641
	Pre-trained	Fivefold	**98.15**	0.9815	0.9815	0.9816
		Tenfold	**98.15**	0.9815	0.9815	0.9815
MobileNetV2	From scratch	Fivefold	87.64	0.8769	0.8764	0.8763
		Tenfold	88.56	0.8857	0.8856	0.8856
	Pre-trained	Fivefold	**98.76**	0.9877	0.9876	0.9876
		Tenfold	**98.45**	0.9849	0.9845	0.9846

Table 1. Performance comparisons of VGG16, MobileNetV1 and MobileNetV2, comparing models trained from scratch with pre-trained models as well as fivefold versus tenfold cross-validation.0 . Values in bold represent the highest accuracy scores obtained for each of the three models.

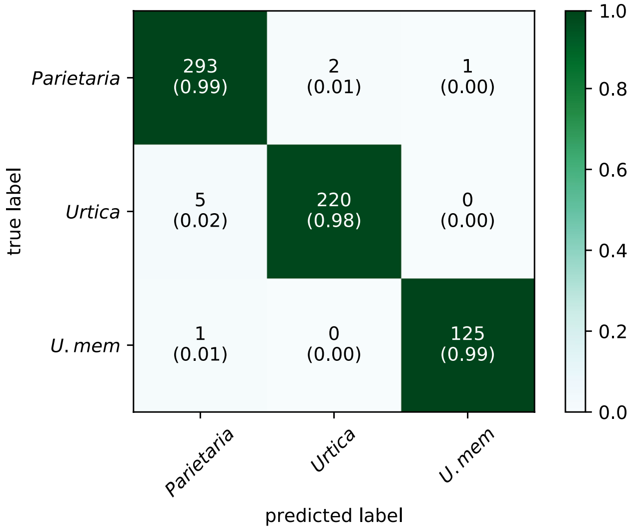

Figure 1. Confusion matrix of results of pre-trained VGG16 using 80% of the images for training and 20% for testing. Numbers represent the actual number of correctly recognized images while those between brackets represent the ratio of correctly classified images. *U.mem* = *Urtica membranacea*.

within each species. To test whether the CNNs learned the pollen-specific distinguishing features rather than sample-specific details, we produced feature maps for the VGG16 model (Fig. 2b–d). Despite the highly variable input images of unacetolyzed pollen from different plants, the model consistently learned features such as edges in the first convolutional layers, while finer features such as pores and annuli were learned in deeper layers.

Application to test cases. Table 2 shows the results of the CNN on unknown and before unseen Urticaceae pollen from an aerobiological sample from Leiden, the Netherlands, as well as from Lleida and Vielha, Catalonia, Spain. We set the identification threshold at a value of 60% as derived from the model test images, and therefore the CNN also returned unknown images (see Supplementary Table S1 for the full results). For the sample from Leiden, 85.7% of the Urticaceae pollen was identified as *Urtica*, with only a minor presence of *Parietaria* (4.5%). The sample from Lleida shows dominance of *Parietaria* pollen grains (81.0%) while 14.3% of the Urticaceae pollen grains were classified as *Urtica*. Finally, for Vielha we find a mixture of ~ 70% *Urtica* and ~ 20% *Parietaria*. No *Urtica membranacea* pollen grains were identified in any of the samples. On average, unknown images account for 8.7% of the total images when using 60% identity threshold. When using a stricter identity threshold (e.g. 70%, see Table 2), the unknown image category increases to an average value of 13.5%.

Discussion

This study demonstrates incorporating neural networks to increase the taxonomic resolution of pollen grain identifications in aerobiological samples. The feature maps in Fig. 2 show that the trained deep learning model VGG16 looks at the traditionally used morphological features to distinguish *Urtica* from *Parietaria* pollen grains.

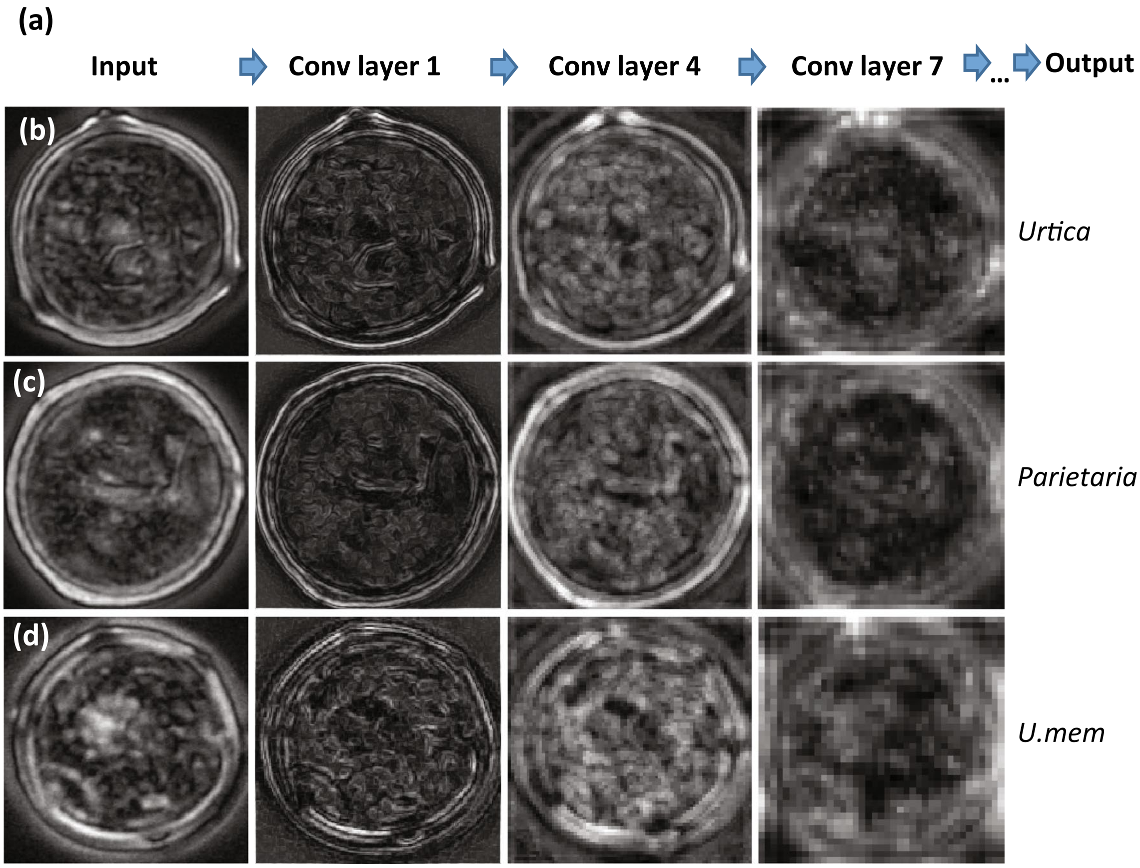

Figure 2. Feature maps. (a) simplified view of the VGG16 model showing three convolutional layers. (**b–d**) Feature maps of Urticaceae pollen grains from the standard deviation projection created using ImageJ, that were confidently distinguished by the CNNs. (**b**) *Urtica urens*, (**c**) *Parietaria judaica* and (**d**) *Urtica membranacea*. Activation levels are indicated with white indicating high activation and black very low/no activation.

Sample location	Date collected	No. Pollen	% *Urtica*	% *Parietaria*	% *U. mem*	% Unknown	Identity threshold
Leiden, NL	23/08/2019	112	**85.7**	4.5	0	9.8	60%
Lleida, SP	16/06/2019	63	14.3	**81.0**	0	4.8	60%
Vielha, SP	09/08/2019	26	**69.2**	19.2	0	11.5	60%
Leiden, NL	23/08/2019	112	**83.0**	3.6	0	13.4	70%
Lleida, SP	16/06/2019	63	12.7	**79.4**	0	7.9	70%
Vielha, SP	09/08/2019	26	**69.2**	11.5	0	19.2	70%

Table 2. Results of the deep learning model VGG16 on Urticaceae pollen from an area representing 10% of the total deposition area of Hirst-type aerobiological samples from Leiden (the Netherlands), Lleida and Vielha (both Catalonia, Spain). Values in bold represent the highest accuracy scores obtained for each of the three classes. The threshold for identification was tested at 60% and 70%. Images that were classified below this level were classified as unknown. *U.mem* = *Urtica membranacea*.

The characteristic thickening of the exine around the pores of *Urtica* shows the highest activation in the deeper convolutional layers. The distinct thickening is missing in *Parietaria* pollen, and the model instead focuses on the pollen outline. As expected, the only species to be distinguished by our model is *Urtica membranacea* which shows a slightly angular outline due to the larger numbers of pores (Fig. 2d). For the other species used in this study, no distinction was possible even though it has been shown that pollen from species of *Urtica* (*U. dioica* and *U. urens*) (Fig. 2b) and *Parietaria* (*P. judaica* and *P. officinalis*) (Fig. 2c) can be separated based on differences in their exine ornamentation[22]. These differences can, however, only be imaged using specialized microscopy methods such as SEM or phase-contrast imaging, and are very hard to visualize using brightfield microscopy. Furthermore, these features are obscured when pollen grains are not acetolyzed. For our purposes, this species level distinction is not relevant as no known differences in allergenicity are known between either the species of *Urtica*[11] or *Parietaria*[18].

Similar to a recent study comparing pollen image classification methods, we found that using a pre-trained CNN consistently outperforms the models trained from scratch[33]. This transfer learning approach is also used by many other recent studies on deep learning of pollen images, mainly because of the limited amount of training images[26–29,34,35]. Still, we find that the VGG16 model trained from scratch achieves a high accuracy of 96.29%. This is because compared to the MobileNets, VGG16 architecture has more and deeper parameters. The MobileNets have less training parameters making them much lighter and faster, and the high accuracies found here indicate that they can be used as a light-weight alternative. In our models the amount of False Positives (FP) is nearly equal to the amount of False Negatives (FN) which is why recall, precision and F1-score were very similar.

This is the first time deep learning has been used to increase the taxonomic accuracy of unacetolyzed pollen identifications. The models represent a significant improvement of earlier attempts in distinguishing Urticaceae pollen using automatic image classification. In a previous study using hand-designed shape and texture features, pollen from three Urticaceae species could be distinguished from another with an 89% accuracy[36], though only a small image dataset was used to train the model (i.e. 100 images per species). Similar results were obtained by [24] where shape features were used with a minimum distance classifier to obtain a 86% accuracy between three species of Urticaceae. Because not all species of Urticaceae were included and a low amount of training images was used, these studies have limited applicability to the highly diverse pollen encountered in aerobiological slides. Furthermore, for both studies the trained model was tested on real case examples and only *Urtica membranacea* was successfully identified (> 98%). The other two classes (*Urtica*) and (*Parietaria*) showed very high error rates (up to 44.4%)[24]. This could be because the model was not trained with sufficient variability. Because we trained the models with pollen from various sources and used data augmentation, they had a better generalizing capability.

Deep learning models have shown similar accuracy rates to ours on larger and more varied pollen datasets as well, but these either focussed on the family level[37–39] or on insect-collected pollen for honey analysis[26–28]. Increasing the taxonomic resolution of pollen grains has been achieved by incorporating an extensively trained deep learning model with super-resolution microscopy on a case study of fossil pollen[35]. Similarly, incorporating SEM images has been found to allow for highly accurate distinction of pollen types[29]. These microscopy methods, however, are often much more expensive than using light microscopy and require extensive sample preparation. Moreover, nearly all of these studies work with acetolyzed pollen that allow easier recognition of distinguishing features, and used pollen collected from a single location.

To validate our model, we tested it on Urticaceae pollen from aerobiological samples collected from different locations in Spain and the Netherlands. Most of the pollen grains from the sample from Leiden, the Netherlands were identified by the deep learning model as *Urtica*, with only a low number of images identified as *Parietaria*. While *Parietaria* plants are relatively abundant around the sampling location in Leiden and were flowering on the chosen date, its pollen is most likely simply outnumbered by the much larger number of nettles in the area. For Lleida (Catalonia), where pellitory plants are abundantly present, *Parietaria* pollen grains dominated the assemblage, while the sample from Vielha showed a mixed assemblage. The number of unknown images was the highest for the sample from Vielha (11.5%), which is most likely the result of the presence of more debris on the pollen grains making a certain identification impossible. In all aerobiological slides, debris on top of or below the pollen grains was observed in different focal plains. Nevertheless, the model still successfully classified most of the pollen grains, and in most cases with high confidence (Supplementary Table S1). This shows the potential broad application of this method and opens up opportunities to study both seasonal as well as long-term yearly dynamics of *Parietaria* versus *Urtica* abundance of airborne pollen, as well as using this method to distinguish other morphologically similar species of allergenic importance from different families (e.g. Betulaceae, Amaranthaceae, Oleaceae). To further improve the generalization of this classification system, future work will focus on increasing the amount of training images from variable sources. Furthermore, more elaborate techniques like regularization will be considered to improve the variability in the image dataset [40]. Since for allergenic pollen monitoring reducing the amount of false negatives (i.e. increasing recall) is particularly important, more models will be tested to identify the best recall values.

A limitation of our method is that currently pollen from aerobiological slides have to be located manually. It has already been shown that automating this process is feasible, e.g. using a deep learning approach[41]. In other systems like the commercially available Classifynder system, pollen are automatically located and imaged using darkfield imaging after which a simple neural network classifies the pollen[42]. This is also the case for the BAA500 system used by, e.g. Oteros et al.[43], that was particularly developed for recognizing and classifying unacetolyzed airborne pollen for hay fever predictions. Lastly, using a CNN and digital holography on pollen grains directly from the air (i.e. unacetolyzed) showed great promise in quantifying pollen automatically to the family level[44]. While these systems achieve automated and accelerated pollen counting, our method instead particularly increases the accuracy of information useful for allergy prevention by making it more specific.

Conclusions

In conclusion, using a combination of an image-processing workflow and a sufficiently trained deep learning model, we were able to differentiate unacetolyzed pollen grains from two genera and one species in the nettle family. These are genera that are indistinguishable with current microscopic methods but possess different allergenic profiles, and thus the ability to differentiate them is of medical significance. Our method can be more broadly applied to distinguish pollen from similarly challenging allergenic plant families and can help in producing more accurate pollen spectra to improve the forecasts for allergy sufferers.

Material and methods

A flowchart has been constructed to visualize all the steps in the Urticaceae pollen image classification process (Fig. 3). Details on the individual steps are described in this section.

Collection of pollen. Pollen grains were collected from all five species of Urticaceae found in the Netherlands. In the genus *Urtica*, the native species *U. dioica* L. (common nettle) and *U. urens* L. (small nettle) are ubiquitous in nitrogen rich moist areas, ditches, woodlands, disturbed sites and roadsides. The exotic Mediterranean species *U. membranacea* is rarely encountered, though is included in this study since its range is expected to increase due to the effects of global warming. The genus *Parietaria* is represented in the Netherlands by the species *P. judaica* L. (pellitory of the wall) and *P. officinalis* L. (upright pellitory) that both occupy rocky substrates, mainly in the urban environment[15]. Moreover, *P. judaica* has shown a big increase in abundance over the past decades, e.g. in the Netherlands (Supplementary Fig. S1), but also in many other parts of the world.

Pollen from all Urticaceae species was either freshly obtained or collected from herbarium specimens (Naturalis Biodiversity Center). Fresh material was collected with the help of an experienced botanist (Barbara Gravendeel) in the direct surroundings of Leiden and The Hague during the nettle flowering seasons of 2018 and 2019. All newly collected plant specimens have been vouchered and were deposited in the herbarium of the Naturalis Biodiversity Center (L.3993376–L.3993387) (Supplementary Table S2). Original taxonomic assignments for the herbarium specimens were verified using identification keys and descriptions[45]. A minimum of four different plants were sampled per species, from different geographical locations to cover as much of the phenotypic plasticity in the pollen grains as possible and reflect the diversity found on aerobiological slides.

To produce palynological reference slides, thecae of open flowers were carefully opened on a microscopic slide using tweezers. A stereo microscope was mounted in a fume hood to avoid inhalation of the severely allergenic pollen of *Parietaria* species. Non-pollen material was manually removed to obtain a clean slide. The pollen grains were mounted using a glycerin:water:gelatin (7:6:1) solution with 2% phenol and stained with Safranin (0.002% w/v). These represent the same conditions as used in airborne pollen analysis on pollen collected with a Hirst type sampler. Cover slips were secured with paraffin.

Pollen image capture. A total of 6472 individual pollen grains were scanned from the five different species of Urticaceae. The number of images for each species varied between 1055 and 1670 (Supplementary Table S2). The images were divided into three classes, namely *Urtica* (*U. dioica* + *U. urens*), *Parietaria* (*P. judaica* + *P. officinalis*) and *U. membranacea*. The system used for imaging was a Zeiss Observer Z1 (inverted microscope) linked to a Hamamatsu EM-CCD Digital Camera (C9100), located at the Institute of Biology Leiden (IBL). Grayscale images were used, since the pollen was stained to increase contrast and not for species recognition.

The imaging procedure was as follows: on each microscope reference slide containing only pollen of one species of Urticaceae, an area rich in pollen was identified by eye and this area was automatically scanned using multidimensional acquisition with the Zeiss software Zen BLUE. For areas that were very rich in pollen, a user-defined mosaic was created consisting of all the tiles to be scanned (e.g. 20 × 20 tiles), while a list of XY positions was used for microscopic slides less rich in pollen. Because pollen grains are 3-D shapes, catching all important features can only be achieved using different focal levels, so-called 'Z-stacks'. A total of 20 Z-stacks were used in this study with a step size of 1.8 µm. The settings used for scanning were a Plan Apochromat 100× (oil) objective and numerical aperture 0.55 with a brightfield contrast manager. To maintain similar conditions in the image collection process, the condenser was always set to 3.3 V with an exposure time of 28 ms.

Reference pollen image library. All images were post-processed in ImageJ v1.52a (Fiji)[46] using the script Pollen_Projector (https://github.com/pollingmarcel/Pollen_Projector). The input for this script is a folder containing all raw pollen images (including all Z-stacks), and the output is a set of projections for each individual pollen grain that are subsequently used as input for the deep learning model.

Pollen_Projector identifies all complete, non-overlapping pollen grains and extracts them as stacks from the raw Z-stack. This is achieved using binarization on the raw images to detect only those rounded objects with a circularity > 0.3 and a size larger than 5 µm. Out-of-focus images within each group of 20 Z-stack slices were removed using a threshold for minimum and maximum pixel values. The conventional input of a convolutional neural network is a three-channel image. In colour images RGB channels are commonly used, but since we use grayscale images, three different Z-stack projections were chosen to represent the three different channels. The projections used are Standard Deviation, Minimum Intensity and Extended Focus. Standard Deviation creates an image containing the standard deviation of the pixel intensities through the stack, where positions with large differences appear brighter in the final projection. Minimum intensity takes the minimum pixel value through the stack and uses that for the projection. Finally, the Extended Focus projection was created using the 'Extended_Depth_of_Field' ImageJ macro of Richard Wheeler (www.richardwheeler.net)[47]. This macro takes a stack of images with a range of focal depths and builds a 2D image from it using only in focus regions of the images. A schematic overview of the processes behind the Pollen_Projector script is shown in Supplementary Fig. S2. Finally, to keep the original size information of the pollen grains they were inserted into a 276 × 276 frame.

Convolutional neural networks. Convolutional Neural Networks (CNNs) are widely used in the field of computer vision for image classification, object detection, facial recognition, autonomous driving, etc. For this study we used the VGG16 network[48], MobileNetV1[49] and MobileNetV2[50] in Keras[51]. Compared with traditional neural networks and shallow convolutional neural networks, VGG16 has deeper layers that extract more representative features from images (Fig. 2a). In contrast, MobileNets are small low-power models that offer a time-efficient alternative. A feature extractor and classifier are two key structural parts of the CNN that perform the classification task. The VGG16 network contains 13 convolutional layers that form five blocks, which generate features from images in the feature extraction phase. Subsequently, three fully connected (FC) layers were built and added to the convolutional layers to classify the different classes (Supplementary Fig. S3). The MobileNetV1 uses depth-wise separable convolutions to build light weight deep neural networks. It has 28 lay-

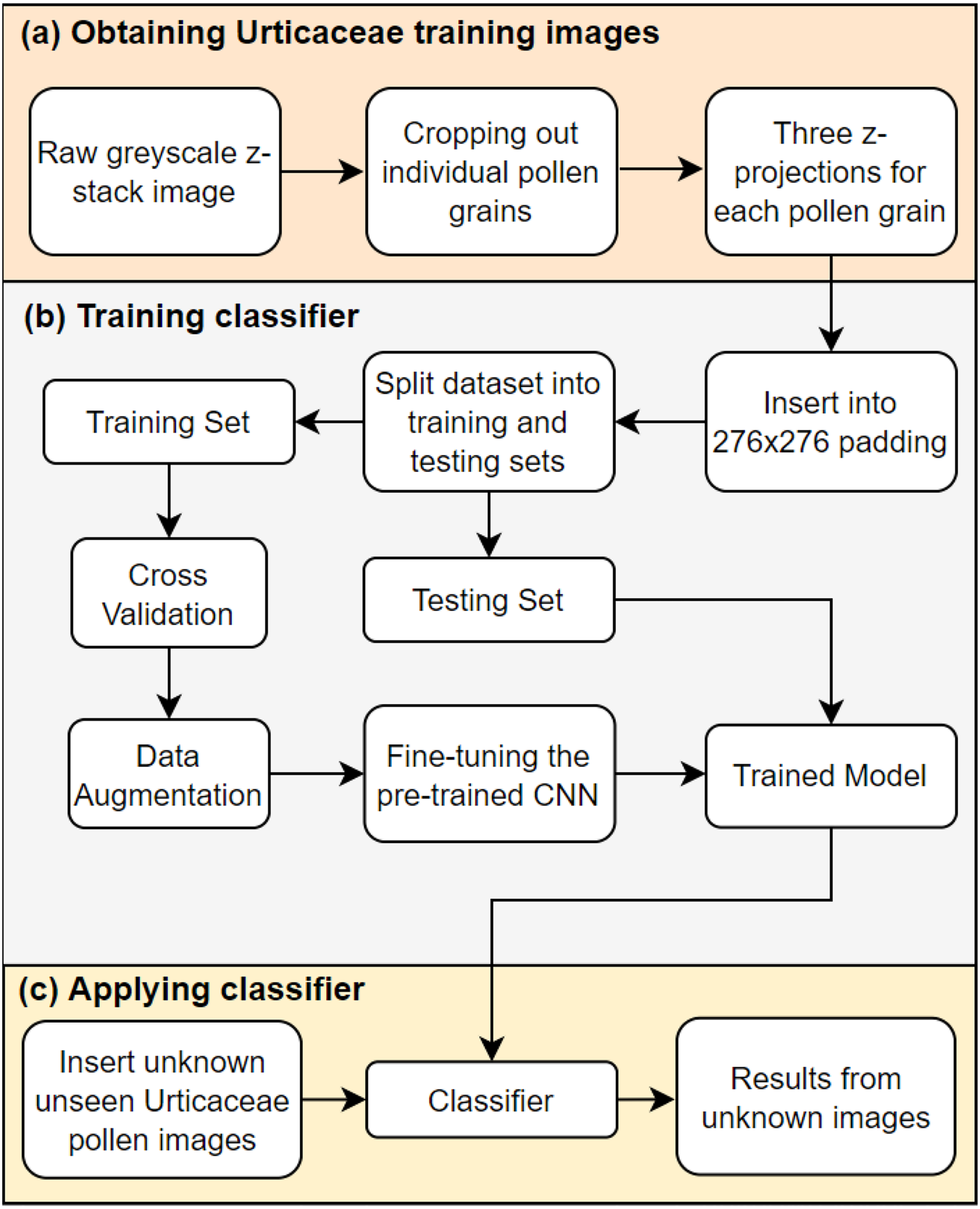

Figure 3. Flowchart showing the pollen image classification process. (**a**) Reference pollen image capture using the custom Fiji macro Pollen_Projector. (**b**) Images were inserted into a fixed frame and split into training and testing sets. The training set was used for cross-validation and data augmentation (flip, brightness) so as to train the CNNs VGG16, MobileNetv1 and MobileNetv2. Results from the models trained from scratch are compared to results from transfer learning on pre-trained models. (**c**) Images from before unseen unknown Urticaceae pollen grains are fed to the resulting classifier. Created using https://app.diagrams.net/.

ers in total. A final average pooling reduces the spatial resolution to 1 and connected with FC and Softmax layer for classification[49]. MobileNetV2, which has 53 layers, is an improved version of MobileNetV1 by introducing inverted residual structure and linear bottleneck layers[50]. MobileNetV2 is more accurate than MobileNetV1 and can be much faster. We trained classification models based on aforementioned CNNs using our pollen image dataset.

During the training process, the initial parameters of convolutional layers were derived from the pre-trained network on the ImageNet dataset. Subsequently, the convolutional layers and the following fully connected layers were further fine-turned based on our own image dataset so as to classify the different classes. The pre-trained models were compared to models trained from scratch. In order to avoid overfitting, we compared the results of five- and tenfold cross-validation in the training process. For fivefold cross-validation the pollen image dataset is split into a training and validation data set in the ratio 80/20 while this is 90/10 for tenfold cross-validation. For each fold, the number of epochs was set to 30. The accuracy of the model converged at this point and the model is therefore found not to be overfitting (Supplementary Fig. S4).

In order to quantify model accuracy, several commonly used performance measures were used:

$$precision = \frac{TP}{TP + FP}$$

$$recall = \frac{TP}{TP + FN}$$

$$F1score = 2 * \frac{precision * recall}{precision + recall}$$

$$CCR = \frac{TP + TN}{TP + TN + FP + FN}$$

where *TP* refers to True Positives, *TN* to True Negatives, *FP* to False Positives and *FN* to False Negatives. Recall is the number of True Positives divided by the total number of elements that belong to the correct class, which is the sum of the True Positives and False Negatives. The F1-score is the weighted average of the precision and recall. The correct classification rate (CCR) reflects the accuracy of the model. The values represent the average weighted by the number of images in each class.

Data augmentation. A large number of images for each class is required to train a deep learning model, as the performance will increase when more variation is fed to the model. Due to the nature of the images investigated in this study, the model was sensitive to small changes, since the differences between the pollen grains are very subtle. Therefore, data augmentation was used to increase the variety of pollen images used as input. We selected the augmentation options brightness and flip. These options were used since size and shape of pollen are key features for their identification, and using other augmentation options would artificially change the original morphology of the pollen grains. Brightness range was set from 0.1 to 2, with <1 corresponding to a darker image and >1 to a brighter image. Horizontal- and vertical flip were also applied randomly (Supplementary Fig. S5). In addition, we applied L2 regularization and dropout in our neural network structures to prevent overfitting.

Test cases. For each aerobiological sample an area representing 10% of the total deposition area was scanned manually for Urticaceae pollen grains (i.e. eight full transects at 100× magnification) resulting in 112 pollen grains from the sample from Leiden (LUMC, the Netherlands), 63 from Lleida and 26 from Vielha (both ICTA-UAB, Catalonia, Spain). One aspect of the Catalonian aerobiological samples was the presence of pollen from families that produce pollen similar to Urticaceae, that are rarely encountered in the Netherlands. These included *Humulus lupulus* L. (Cannabaceae) and *Morus* sp. (Moraceae) which were not included in our training dataset. These can be distinguished from Urticaceae, however, in the case of *H. lupulus* by their much larger size (up to 35 μm) and the very large onci and, in the case of *Morus* by the more ellipsoidal shape. These pollen grains were removed from the dataset before they were fed to the CNN for classification.

Data availability

All data generated or analyzed during this study are included in this published article (and its "Supplementary Information" files). Raw pollen images can be made available upon request.

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M.P.: conceptualization, methodology, visualization, formal analysis, writing—original draft. C.L.: methodology, software, formal analysis, data curation, investigation. L.C.: resources, formal analysis, software, supervision. F.V.: validation, supervision, software. L.W., J.B.: resources, validation, writing—review and editing. C.D.L.: resources, validation. J.W.: software, methodology. H.B.: funding acquisition, writing—review and editing. B.G.: conceptualization, supervision, project administration, funding acquisition.

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Competing interests

The authors declare no competing interests.

Additional information

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Supplementary Information

Neural networks for increased accuracy of allergenic pollen monitoring

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Supplementary Table S1

Probability scores for Urticaceae pollen grains scanned from aerobiological samples using the pre-trained VGG16 model with 5-fold cross-validation. *U. mem* = *Urtica membranacea*

Lleida (16-06-2019), n = 63

Image No.	Probability *Parietaria*	Probability *Urtica*	Probability *U. mem*	Final ID (threshold 0.6)	Final ID (threshold 0.7)
1	0.95	0.05	0.00	*Parietaria*	*Parietaria*
2	0.98	0.02	0.00	*Parietaria*	*Parietaria*
3	0.29	0.70	0.01	*Urtica*	*Urtica*
4	0.98	0.02	0.00	*Parietaria*	*Parietaria*
5	0.24	0.76	0.00	*Urtica*	*Urtica*
6	1.00	0.00	0.00	*Parietaria*	*Parietaria*
7	0.94	0.06	0.00	*Parietaria*	*Parietaria*
8	0.99	0.01	0.00	*Parietaria*	*Parietaria*
9	0.12	0.88	0.00	*Urtica*	*Urtica*
10	0.99	0.01	0.00	*Parietaria*	*Parietaria*
11	0.99	0.01	0.00	*Parietaria*	*Parietaria*
12	0.96	0.04	0.00	*Parietaria*	*Parietaria*
13	1.00	0.00	0.00	*Parietaria*	*Parietaria*
14	0.96	0.04	0.00	*Parietaria*	*Parietaria*
15	1.00	0.00	0.00	*Parietaria*	*Parietaria*
16	0.90	0.09	0.01	*Parietaria*	*Parietaria*
17	0.90	0.01	0.09	*Parietaria*	*Parietaria*
18	0.73	0.16	0.10	*Parietaria*	*Parietaria*
19	0.95	0.04	0.00	*Parietaria*	*Parietaria*
20	0.98	0.00	0.02	*Parietaria*	*Parietaria*
21	0.16	0.83	0.00	*Urtica*	*Urtica*
22	0.67	0.31	0.02	*Parietaria*	unknown
23	0.02	0.98	0.00	*Urtica*	*Urtica*
24	0.95	0.04	0.00	*Parietaria*	*Parietaria*
25	0.99	0.01	0.00	*Parietaria*	*Parietaria*
26	0.99	0.01	0.00	*Parietaria*	*Parietaria*
27	0.95	0.05	0.00	*Parietaria*	*Parietaria*
28	0.02	0.98	0.00	*Urtica*	*Urtica*
29	0.34	0.66	0.00	*Urtica*	unknown
30	1.00	0.00	0.00	*Parietaria*	*Parietaria*

31	0.98	0.02	0.00	*Parietaria*	*Parietaria*
32	1.00	0.00	0.00	*Parietaria*	*Parietaria*
33	0.99	0.01	0.00	*Parietaria*	*Parietaria*
34	0.92	0.02	0.06	*Parietaria*	*Parietaria*
35	0.57	0.41	0.02	unknown	unknown
36	1.00	0.00	0.00	*Parietaria*	*Parietaria*
37	0.87	0.13	0.00	*Parietaria*	*Parietaria*
38	0.99	0.00	0.00	*Parietaria*	*Parietaria*
39	0.97	0.03	0.00	*Parietaria*	*Parietaria*
40	0.58	0.41	0.01	unknown	unknown
41	0.98	0.02	0.00	*Parietaria*	*Parietaria*
42	0.70	0.29	0.00	*Parietaria*	*Parietaria*
43	0.84	0.16	0.00	*Parietaria*	*Parietaria*
44	0.97	0.02	0.01	*Parietaria*	*Parietaria*
45	0.83	0.17	0.00	*Parietaria*	*Parietaria*
46	0.99	0.00	0.00	*Parietaria*	*Parietaria*
47	1.00	0.00	0.00	*Parietaria*	*Parietaria*
48	0.99	0.00	0.00	*Parietaria*	*Parietaria*
49	0.96	0.04	0.01	*Parietaria*	*Parietaria*
50	0.00	1.00	0.00	*Urtica*	*Urtica*
51	0.99	0.01	0.00	*Parietaria*	*Parietaria*
52	0.99	0.00	0.01	*Parietaria*	*Parietaria*
53	0.91	0.04	0.05	*Parietaria*	*Parietaria*
54	0.95	0.04	0.00	*Parietaria*	*Parietaria*
55	0.90	0.10	0.00	*Parietaria*	*Parietaria*
56	0.95	0.05	0.00	*Parietaria*	*Parietaria*
57	0.99	0.01	0.00	*Parietaria*	*Parietaria*
58	1.00	0.00	0.00	*Parietaria*	*Parietaria*
59	0.99	0.01	0.00	*Parietaria*	*Parietaria*
60	0.17	0.82	0.00	*Urtica*	*Urtica*
61	0.41	0.56	0.02	unknown	unknown
62	0.98	0.02	0.00	*Parietaria*	*Parietaria*
63	0.76	0.21	0.03	*Parietaria*	*Parietaria*

Vielha, 09-08-2019, n = 26

Image No.	Probability *Parietaria*	Probability *Urtica*	Probability *U. mem*	Final ID (threshold 0.6)	Final ID (threshold 0.7)
1	0.03	0.97	0.00	*Urtica*	*Urtica*
2	0.07	0.86	0.07	*Urtica*	*Urtica*
3	0.10	0.90	0.00	*Urtica*	*Urtica*
4	0.02	0.98	0.00	*Urtica*	*Urtica*
5	0.09	0.91	0.00	*Urtica*	*Urtica*
6	0.26	0.74	0.00	*Urtica*	*Urtica*
7	0.00	1.00	0.00	*Urtica*	*Urtica*
8	0.41	0.04	0.55	unknown	unknown
9	0.61	0.39	0.01	*Parietaria*	unknown
10	0.81	0.10	0.09	*Parietaria*	*Parietaria*
11	0.02	0.98	0.00	*Urtica*	*Urtica*
12	0.01	0.99	0.00	*Urtica*	*Urtica*

13	0.49	0.13	0.38	unknown	unknown
14	0.00	1.00	0.00	*Urtica*	*Urtica*
15	0.14	0.84	0.02	*Urtica*	*Urtica*
16	0.63	0.10	0.27	*Parietaria*	unknown
17	0.12	0.88	0.00	*Urtica*	*Urtica*
18	0.09	0.90	0.00	*Urtica*	*Urtica*
19	0.24	0.76	0.00	*Urtica*	*Urtica*
20	0.04	0.96	0.00	*Urtica*	*Urtica*
21	0.85	0.12	0.03	*Parietaria*	*Parietaria*
22	0.80	0.14	0.07	*Parietaria*	*Parietaria*
23	0.00	1.00	0.00	*Urtica*	*Urtica*
24	0.17	0.83	0.00	*Urtica*	*Urtica*
25	0.02	0.98	0.00	*Urtica*	*Urtica*
26	0.57	0.43	0.00	unknown	unknown

Leiden (23-08-2019), n = 112

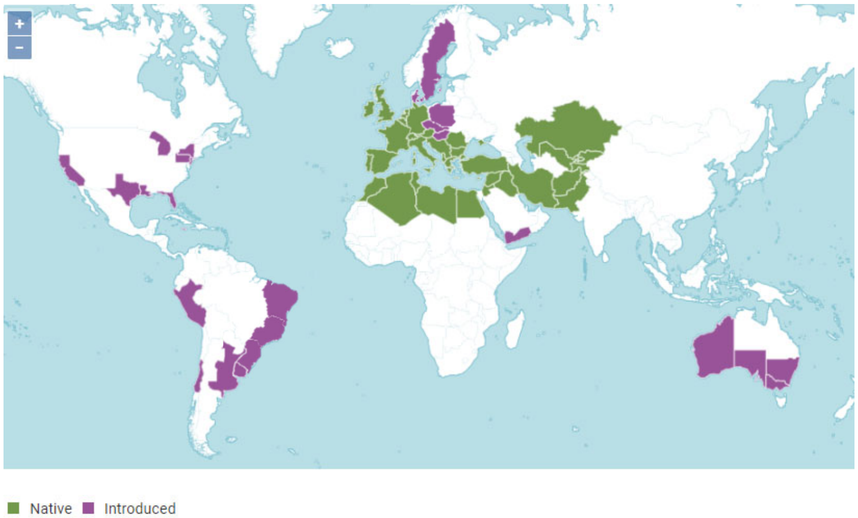
Image No.	Probability *Parietaria*	Probability *Urtica*	Probability *U. mem*	Final ID (threshold 0.6)	Final ID (threshold 0.7)
1	0.04	0.96	0.00	*Urtica*	*Urtica*
2	0.01	0.99	0.00	*Urtica*	*Urtica*
3	0.07	0.93	0.00	*Urtica*	*Urtica*
4	0.16	0.83	0.00	*Urtica*	*Urtica*
5	0.19	0.81	0.00	*Urtica*	*Urtica*
6	0.02	0.98	0.00	*Urtica*	*Urtica*
7	0.00	1.00	0.00	*Urtica*	*Urtica*
8	0.28	0.72	0.00	*Urtica*	*Urtica*
9	0.11	0.89	0.00	*Urtica*	*Urtica*
10	0.34	0.66	0.00	*Urtica*	unknown
11	0.04	0.96	0.00	*Urtica*	*Urtica*
12	0.18	0.81	0.00	*Urtica*	*Urtica*
13	0.00	1.00	0.00	*Urtica*	*Urtica*
14	0.47	0.53	0.00	unknown	unknown
15	0.11	0.89	0.00	*Urtica*	*Urtica*
16	0.01	0.99	0.00	*Urtica*	*Urtica*
17	0.20	0.80	0.00	*Urtica*	*Urtica*
18	0.00	1.00	0.00	*Urtica*	*Urtica*
19	0.00	1.00	0.00	*Urtica*	*Urtica*
20	0.01	0.99	0.00	*Urtica*	*Urtica*
21	0.75	0.25	0.00	*Parietaria*	*Parietaria*
22	0.00	1.00	0.00	*Urtica*	*Urtica*
23	0.03	0.97	0.00	*Urtica*	*Urtica*
24	0.01	0.99	0.00	*Urtica*	*Urtica*
25	0.69	0.31	0.00	*Parietaria*	unknown
26	0.11	0.89	0.00	*Urtica*	*Urtica*
27	0.12	0.88	0.00	*Urtica*	*Urtica*
28	0.17	0.83	0.00	*Urtica*	*Urtica*
29	0.09	0.91	0.00	*Urtica*	*Urtica*
30	0.00	1.00	0.00	*Urtica*	*Urtica*
31	0.48	0.52	0.00	unknown	unknown

32	0.24	0.76	0.00	*Urtica*	*Urtica*
33	0.06	0.94	0.00	*Urtica*	*Urtica*
34	0.29	0.71	0.00	*Urtica*	*Urtica*
35	0.14	0.86	0.00	*Urtica*	*Urtica*
36	0.38	0.62	0.00	*Urtica*	unknown
37	0.06	0.94	0.00	*Urtica*	*Urtica*
38	0.55	0.45	0.00	unknown	unknown
39	0.01	0.99	0.00	*Urtica*	*Urtica*
40	0.00	1.00	0.00	*Urtica*	*Urtica*
41	0.00	1.00	0.00	*Urtica*	*Urtica*
42	0.02	0.98	0.00	*Urtica*	*Urtica*
43	0.03	0.97	0.00	*Urtica*	*Urtica*
44	0.21	0.79	0.00	*Urtica*	*Urtica*
45	0.02	0.98	0.00	*Urtica*	*Urtica*
46	0.00	1.00	0.00	*Urtica*	*Urtica*
47	0.01	0.99	0.00	*Urtica*	*Urtica*
48	0.79	0.20	0.00	*Parietaria*	*Parietaria*
49	0.54	0.46	0.00	unknown	unknown
50	0.01	0.99	0.00	*Urtica*	*Urtica*
51	0.00	1.00	0.00	*Urtica*	*Urtica*
52	0.01	0.99	0.00	*Urtica*	*Urtica*
53	0.01	0.99	0.00	*Urtica*	*Urtica*
54	0.00	1.00	0.00	*Urtica*	*Urtica*
55	0.00	1.00	0.00	*Urtica*	*Urtica*
56	0.00	1.00	0.00	*Urtica*	*Urtica*
57	0.02	0.98	0.00	*Urtica*	*Urtica*
58	0.00	1.00	0.00	*Urtica*	*Urtica*
59	0.54	0.46	0.00	unknown	unknown
60	0.45	0.55	0.00	unknown	unknown
61	0.09	0.91	0.00	*Urtica*	*Urtica*
62	0.00	1.00	0.00	*Urtica*	*Urtica*
63	0.00	1.00	0.00	*Urtica*	*Urtica*
64	0.00	1.00	0.00	*Urtica*	*Urtica*
65	0.06	0.94	0.00	*Urtica*	*Urtica*
66	0.05	0.95	0.00	*Urtica*	*Urtica*
67	0.01	0.99	0.00	*Urtica*	*Urtica*
68	0.23	0.77	0.00	*Urtica*	*Urtica*
69	0.21	0.79	0.00	*Urtica*	*Urtica*
70	0.72	0.28	0.00	*Parietaria*	*Parietaria*
71	0.49	0.51	0.00	unknown	unknown
72	0.06	0.94	0.00	*Urtica*	*Urtica*
73	0.33	0.67	0.00	*Urtica*	unknown
74	0.00	1.00	0.00	*Urtica*	*Urtica*
75	0.28	0.72	0.00	*Urtica*	*Urtica*
76	0.00	1.00	0.00	*Urtica*	*Urtica*
77	0.03	0.97	0.00	*Urtica*	*Urtica*
78	0.05	0.95	0.00	*Urtica*	*Urtica*
79	0.21	0.79	0.00	*Urtica*	*Urtica*
80	0.00	1.00	0.00	*Urtica*	*Urtica*
81	0.00	1.00	0.00	*Urtica*	*Urtica*

82	0.03	0.97	0.00	*Urtica*	*Urtica*
83	0.02	0.98	0.00	*Urtica*	*Urtica*
84	0.12	0.88	0.00	*Urtica*	*Urtica*
85	0.17	0.83	0.00	*Urtica*	*Urtica*
86	0.01	0.99	0.00	*Urtica*	*Urtica*
87	0.90	0.10	0.00	*Parietaria*	*Parietaria*
88	0.11	0.89	0.00	*Urtica*	*Urtica*
89	0.02	0.98	0.00	*Urtica*	*Urtica*
90	0.00	1.00	0.00	*Urtica*	*Urtica*
91	0.29	0.71	0.00	*Urtica*	*Urtica*
92	0.11	0.89	0.00	*Urtica*	*Urtica*
93	0.12	0.88	0.00	*Urtica*	*Urtica*
94	0.01	0.99	0.00	*Urtica*	*Urtica*
95	0.01	0.99	0.00	*Urtica*	*Urtica*
96	0.00	1.00	0.00	*Urtica*	*Urtica*
97	0.00	1.00	0.00	*Urtica*	*Urtica*
98	0.02	0.98	0.00	*Urtica*	*Urtica*
99	0.00	1.00	0.00	*Urtica*	*Urtica*
100	0.21	0.79	0.00	*Urtica*	*Urtica*
101	0.55	0.45	0.00	unknown	unknown
102	0.00	1.00	0.00	*Urtica*	*Urtica*
103	0.48	0.52	0.00	unknown	unknown
104	0.00	1.00	0.00	*Urtica*	*Urtica*
105	0.00	1.00	0.00	*Urtica*	*Urtica*
106	0.57	0.43	0.00	unknown	unknown
107	0.11	0.89	0.00	*Urtica*	*Urtica*
108	0.23	0.77	0.00	*Urtica*	*Urtica*
109	0.26	0.74	0.00	*Urtica*	*Urtica*
110	0.12	0.88	0.00	*Urtica*	*Urtica*
111	0.58	0.42	0.00	unknown	unknown
112	0.00	1.00	0.00	*Urtica*	*Urtica*

Supplementary Figure S1

(a)



(b)

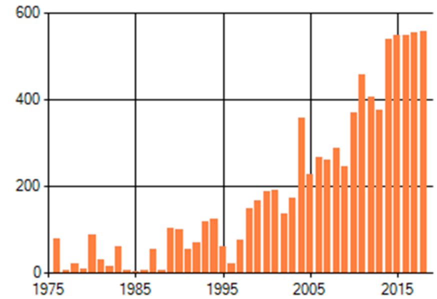


Figure S1. **(a)** Global native (green) and introduced (purple) distribution of *Parietaria judaica* and *P. officinalis* (POWO (2019). "Plants of the World Online. Map taken from the Royal Botanic Gardens, Kew. http://www.plantsoftheworldonline.org/ Retrieved 05 October 2020"). **(b)** Trend in Pellitory of the wall (*Parietaria judaica*) plant sightings per square kilometre in the Netherlands over the past 45 years. Index number = 100 for 1990 © NEM (CBS & FLORON) 2019.

Supplementary Table S2

Locations of all Urticaceae specimens and number of images. NL = the Netherlands, SP = Spain and PO = Portugal. *collected in 2018 and 2019, deposited in the Naturalis Biodiversity Center herbarium.

Species (n = total images)	**Geographical origin**	**Collection date**	**No. of images used**	**Deposition number**
Parietaria judaica L. (n = 1670)	Montejaque (SP)	17/10/2011	54	WAG.1186948
	Leiden, Stationsweg (NL)	19/11/2019	168	L.3993376*
	Huizen (NL)	20/09/2014	174	L.4303913
	Leiden, Robijnstraat (NL)	23/07/2012	139	L.2071680
	Den Haag (NL)	05/10/2018	392	L.3993377*
	Leiden, Paterstraatje	09/10/2018	250	L.3993378*
	Sassenplaat (NL)	03/07/2013	233	L.4304093
	Rotterdam, Hartelkanaal (NL)	27/09/2014	260	L.4304136
Parietaria officinalis L. (n = 1359)	Middelburg (NL)	26/06/2014	234	L.3974371
	Haarlem (NL)	13/07/2013	191	L.2073373
	Wageningse Polder (NL)	19/07/2012	64	WAG.1186992
	Leiden (NL)	07/2012	369	L.3963901
	Den Haag, Escamplaan (NL)	12/10/2018	383	L.3993379*
	Den Haag, Bosjes van Poot (NL)	01/08/2012	248	L.2071818
Urtica dioica L. (n = 1055)	Leiden, Hogeschool 1 (NL)	06/11/2019	316	L.3993380*
	Leiden, Hogeschool 2 (NL)	07/11/2019	299	L.3993381*
	Den Haag (NL)	17/11/2019	182	L.3993382*
	Leiden, Sandifortdreef (NL)	15/11/2019	191	L.3993383*
	Arnhem (NL)	29/05/2001	67	WAG.1188104
Urtica membranacea Poir. ex Savigny (n = 1118)	Amsterdam (NL)	11/2018	521	L.3993384*
	Overloon (NL)	17/06/2014	135	L.3959964
	Cape st. Vincent (PO)	03/1995	87	L.1629741
	Den Haag (NL)	06/03/2019	375	L.3993385*
Urtica urens L. (n = 1270)	Leiden (NL)	01/11/2019	128	L.3993386*
	Castilla-la-Mancha (SP)	27/05/2016	165	WAG.1962413
	Zandvoort (NL)	05/08/2012	201	L.2071917
	Meijendel (NL)	12/08/2011	140	L.2074446
	Zwolle (NL)	29/04/2005	134	L.4271105
	Wassenaar (NL)	15/09/2002	219	L.4233917
	Den Haag (NL)	13/03/2020	283	L.3993387*

Supplementary Figure S2

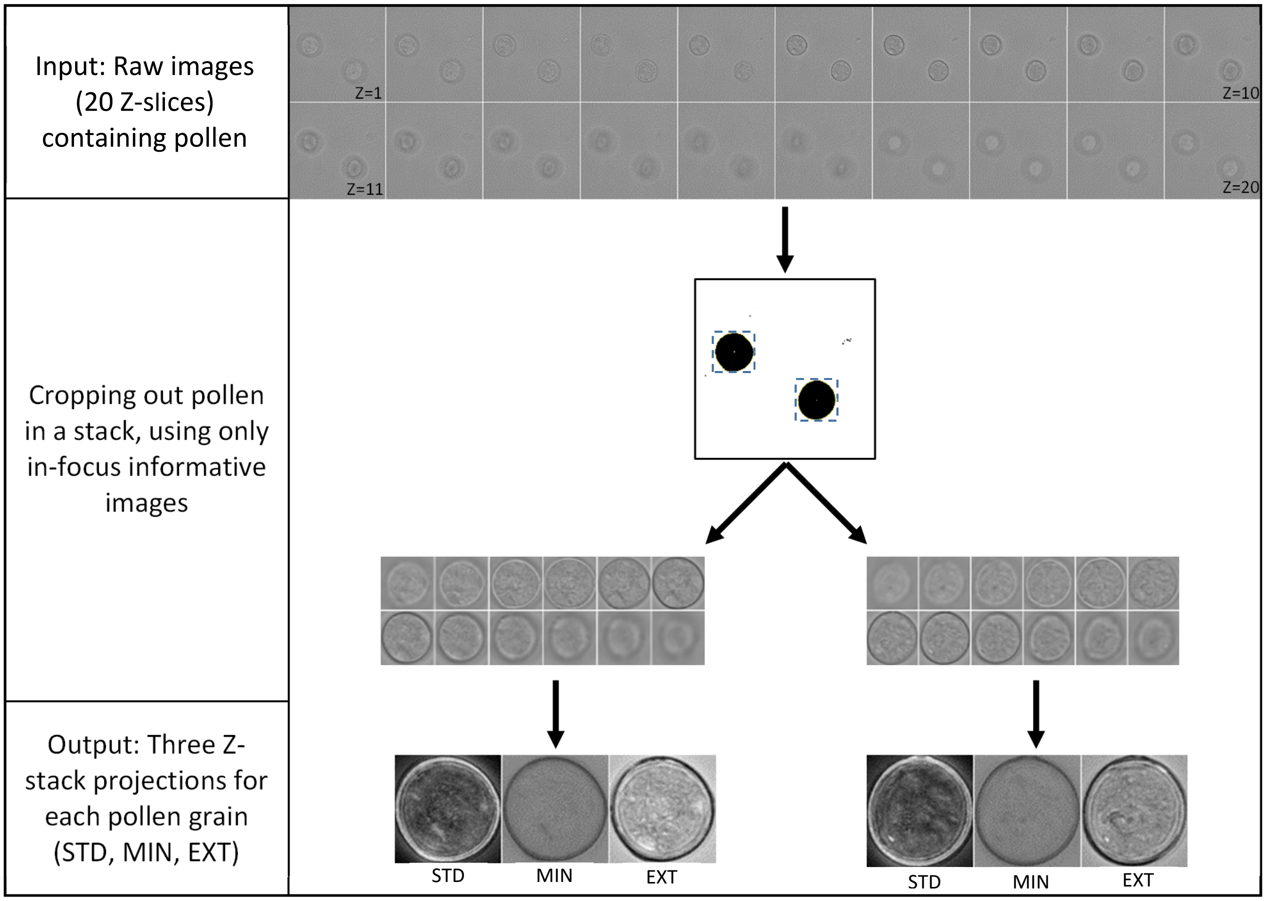


Figure S2. Pollen image acquisition and processing workflow carried out with in-house designed Pollen_Projector script. Once raw images are obtained at 20 different focal levels ('Z-slices'), subsequent steps involve cropping of whole individual pollen grains and producing three different projections from the Z-stacks. Abbreviations of projections: STD = Standard Deviation, MIN = Minimum Intensity and EXT = Extended Focus.

Supplementary Figure S3

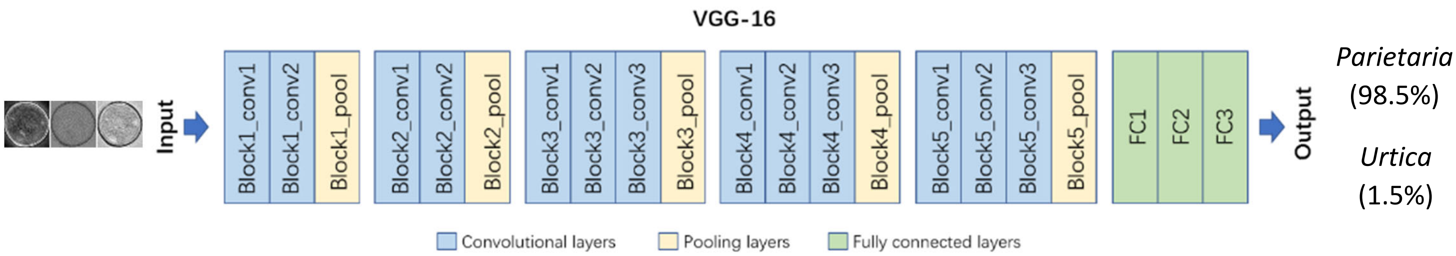


Figure S3. Schematic overview of the structure of VGG-16 with an example of three-channel input image of a *Parietaria judaica* pollen grain (known label) and the output generated, where it confidently identifies the images as *Parietaria* (98% probability). Adapted from Simonyan et al., (2014)[1]

Supplementary Figure S4

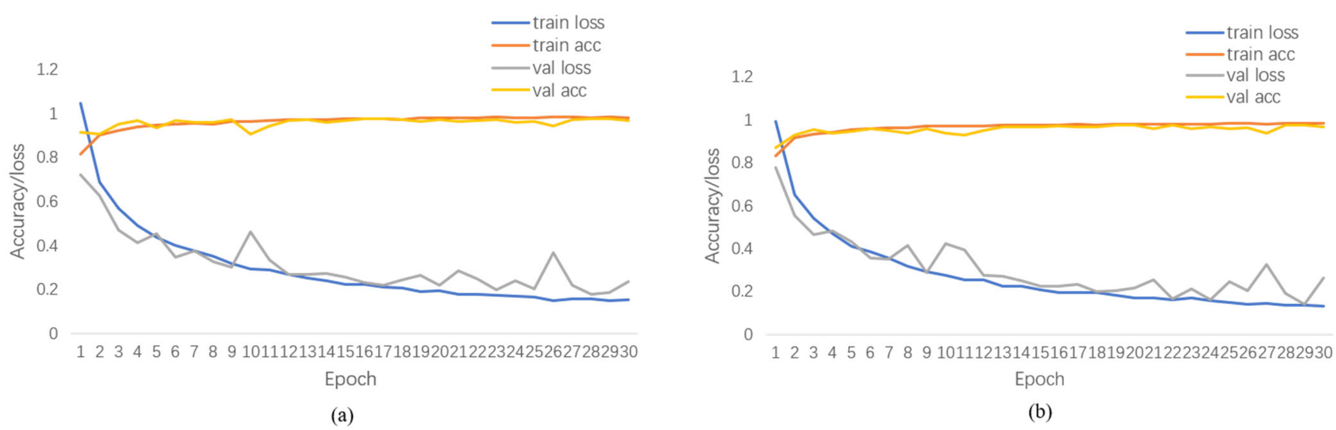


Figure S4. Figures showing the accuracy/loss plots for the VGG16 model with 5- and 10-fold cross-validation.

Supplementary Figure S5

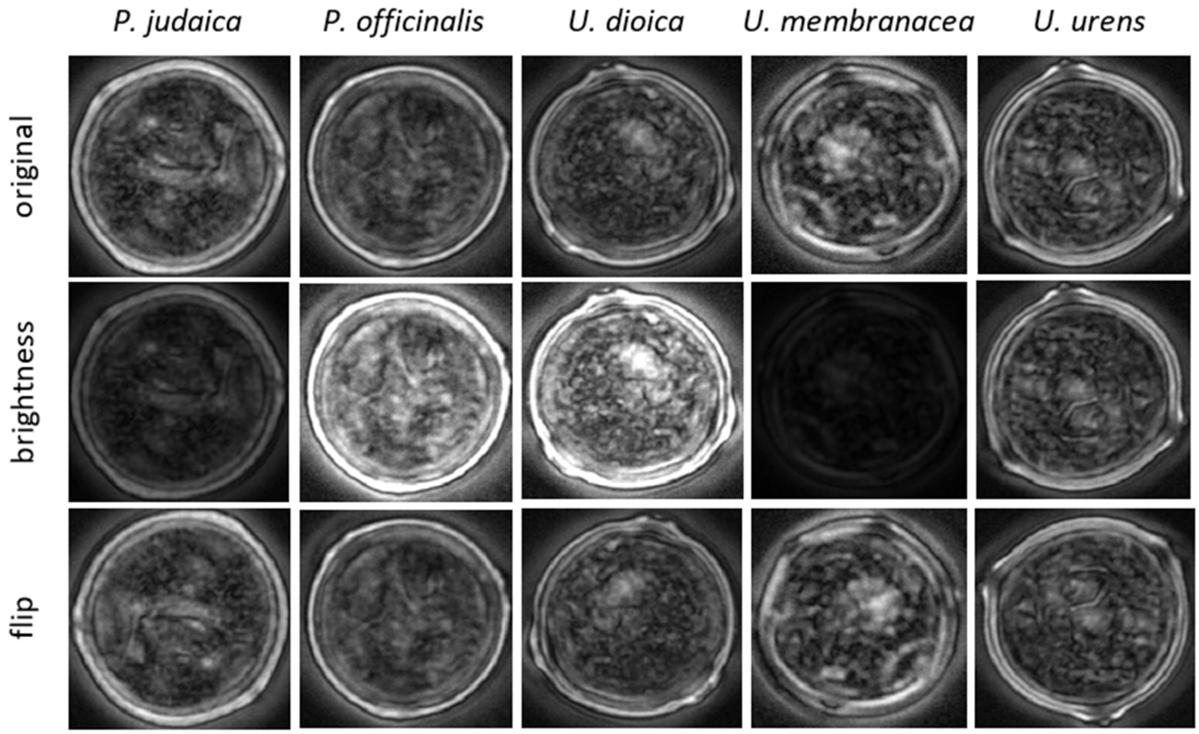


Figure S5. Examples of data augmentation on the Standard Deviation Projection (STD) of selected pollen grains of all Urticaceae pollen species used in this study.

Supplementary reference

1 Simonyan, K. & Zisserman, A. Very deep convolutional networks for large-scale image recognition. *arXiv preprint arXiv:1409.1556* (2014).

Manuscript 2

DNA from pollen

Molecular Identification of Plants: From Sequences to Species

Book Chapter 5 DNA from pollen

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BACKGROUND

Why use DNA from pollen instead of morphology?

To identify pollen, spores, and other plant-related micro remains, the field of palynology has traditionally relied on microscope-based analyses. This is a time-consuming process that requires highly trained specialists. Additionally, pollen grains from many plant families are morphologically indistinguishable using light microscopy (Beug 2004). Therefore, pollen can often not be distinguished beyond the genus- or family-level. Using more advanced microscopy techniques, the finer and potentially species-specific details on the pollen surface (i.e., exine) can be visualized (e.g., scanning electron microscope (SEM) and super-resolution microscopy (see e.g. Sivaguru et al. (2018)). However, these techniques often require extensive sample preparation, highly trained palynologists, and require costly microscopes. Moreover, some pollen grain features are so fine (less than 500 nm) that not even these sophisticated imaging techniques can visualize them. A combination of high-resolution imaging and automatic image detection using sufficiently trained neural networks is another emerging method to increase taxonomic resolution with pollen morphology (Romero et al. 2020; Polling et al. 2021). This technique, however, requires an extensively trained network with a large and varied pollen image reference database.

These challenges highlight the necessity for innovative methods within the field of palynology, to increase both the speed and accuracy of pollen identifications. DNA-based methods for the molecular identification of pollen grains have the potential to be of complementary value. However, the extraction of DNA from pollen is non-trivial. This chapter therefore focuses on how DNA can be extracted from pollen, the common problems encountered, and the qualitative and quantitative molecular possibilities for analyses.

Applications of DNA-based methods for pollen identification

Using pollen grain DNA for identification has shown promising results in a number of applications, including the study of provenance and authentication of honey (Hawkins et al. 2015; Prosser and Hebert 2017; Utzeri et al. 2018), plant-pollinator networks (Pornon et al. 2017; Richardson et al. 2019), hay fever predictions (Kraaijeveld et al. 2015; Leontidou et al. 2017; Campbell et al. 2020), forensic science (Bell et al. 2016a, and references therein), and environmental reconstructions from pollen in soil (Parducci et al. 2017) (see Section 3 for full information on applications). Ancient DNA can be extracted from pollen grains as old as 150 kyr (Suyama et al. 1996), and has also been used for reconstructing ancient plant-pollinator networks (Gous et al. 2019) (see Chapter 21 Palaeobotany).

Collecting pollen for DNA analysis

Collecting pollen for DNA analysis is mostly similar to collecting pollen for microscopic analysis, though more care should be taken to avoid contamination from other potential sources of DNA. This is because pollen generally contains low quantities of DNA and is therefore prone to contamination. Pollen grains can either be collected directly from the environment (air, water, soil, etc.) or from pollinators (pollen baskets, honey). Pollen collected from the environment will most often (though not always) be derived from anemophilous (wind pollinated) plants, while pollinators collect the majority of pollen from so-called entomophilous (insect pollinated) plants. Pollinators may, however, also have anemophilous pollen sticking to their bodies. For studies looking at pollen from pollinators, either all pollen grains on the animal's body are collected by washing off the pollen or, when present, only the corbicular pollen baskets are collected (Bell et al. 2017; Richardson et al. 2015). Pollinators can either be collected from the field using aerial netting or collected from natural history collections (Gous et al. 2019). Insect-collected pollen baskets contain many hundreds of thousands of pollen grains, and collecting even a small subset of this basket is sufficient for molecular analysis. Honey also contains huge numbers of pollen grains, but it can be more challenging to work with for DNA analyses. This is because there are many compounds in honey such as polyphenols and flavonoids that can chemically inhibit methods used for DNA sequencing (Prosser and Hebert 2017). In contrast, while airborne pollen grains lack these inhibitors, it is present in only relatively low concentrations in the ambient air. Therefore, to collect sufficient amounts of pollen for molecular analyses, most of the sampling methods focus on air filtration methods. These include both volumetric (e.g., Hirst type; Hirst 1952) and gravimetric methods (Levetin 2004; for an overview please see Banchi et al. 2019).

POLLEN DNA EXTRACTION

Pollen lysis

Pollen grains can be referred to as "natural plastic": they have a very hard outer cell wall called an exine, which is made of sporopollenin (Brooks and Shaw 1968). Pollen exine is very resistant to non-oxidative physical, biological, and chemical degradation. This is evidenced by their ubiquitous presence in the fossil record and some fossil pollen exines have been found preserved for over 243 million years

(Hochuli and Feist-Burkhardt 2013). Extracting DNA from pollen grains is thus not trivial, since the exine must be broken to release the inner DNA. Entomophilous pollen grains also contain DNA-rich pollenkitt outside the exine, but this DNA is usually heavily degraded, and it is the DNA inside the pollen grains that remains intact (Pornon et al. 2017; Pacini and Hesse 2005). A lysis step using mechanical bead-beating and a lysis buffer is often used before DNA extraction of pollen grains, and has been shown to improve DNA quantity (Swenson and Gemeinholzer 2021). However, if lysis time is too long, or bead beating too vigorous, DNA yield may actually decrease. (Swenson and Gemeinholzer 2021) found that best results can be obtained at 33 to 67% exine rupture, instead of 100% exine rupture and using 2 hours of lysis incubation instead of 24 hours. Various different bead-beating strategies have been adopted (Table 1), including using a single relatively large bead (5 mm) or different mixtures of large and small beads. Many different types of material have also been used, including stainless steel, tungsten carbide, glass and zirconium beads, but the choice of material does not seem to influence the extraction. It is always recommended to test the lysis efficiency, which can be done by checking the fraction of broken (i.e., lysed) pollen grains under the microscope after the bead beating process (e.g., Kraaijeveld et al. 2015).

It should be noted that other methods for DNA extraction from pollen exist in which the pollen grains are not destroyed, and in some specific cases, excluding the bead-beating step has even given better results (Ghitarrini et al. 2018; Gous et al. 2019).

DNA extraction

Several commercially available DNA extraction protocols have been used for DNA extraction from pollen grains after the lysis step. Table 1 gives an overview of protocols used in recent literature (for a full overview see Bell et al. 2016b). DNA is most commonly extracted from pollen using the DNeasy Plant Mini Kit (Qiagen) due to its ease of use and high success rate. However, while this is the most commonly used method, recent papers comparing different methods suggest that the best DNA extraction protocol should be empirically found. In one recent paper, several extraction protocols were compared for airborne pollen collected using air samplers (Leontidou et al. 2017). The highest DNA yield was obtained by using a DNA lysis step with steel beads and the Nucleomag Kit. For bee-collected pollen grains, however, the DNeasy Mini Kit gave the best results amongst several different protocols (Gous et al. 2019). Thus, it is always recommended to test several different DNA extraction methods for optimal DNA yield within the chosen study system.

The quality of DNA that can be extracted from pollen samples is critical for any molecularly-based identification method, and particularly when working with very small amounts of DNA. Therefore, avoiding contamination is critical and it is essential to work in a clean lab, keeping windows closed and using sterilized tools in a laminar flow cabinet, and to keep the DNA extraction lab separated from the post-PCR environment.

MOLECULAR METHODS FOR POLLEN IDENTIFICATION

Molecular methods can contribute to the analysis of pollen both by identifying which species are present (qualitative) as well as by giving a measure of the abundance of different pollen species (quantification). While DNA metabarcoding methods are currently most often used (Table 1), DNA barcoding techniques have also been applied to target specific species from a mixture, while metagenomics now allows for pollen quantification. For a review of these different sequencing methods, please see Chapter 10 DNA barcoding, Chapter 11 Amplicon metabarcoding, and Chapter 12 Metagenomics.

Qualitative pollen analysis

DNA barcoding

Species-resolution in pollen grain identifications is critical for studies that try to answer specific research questions including: what particular species of flower does a common carder bee prefer? What grass species is responsible for most of the pollen in the ambient air in early May? Species-specific markers and qPCR techniques can be used for the identification of specific species within a mixture of different pollen types (see Chapter 10 DNA barcoding). One study used custom-made primers for the nuclear Internal Transcribed Spacer (ITS) to differentiate between mugwort (*Artemisia vulgaris*) and ragweed (*Ambrosia artemisiifolia*), two notoriously allergenic species from the Asteraceae family (Müller-Germann et al. 2017). These newly constructed primers were then applied on aerobiological samples to show that ragweed pollen can travel long distances, since it was detected outside of the local pollination period. Barcoding was also used to show that allergenic *Juniperus ashei* pollen grains could be found in Canada, even if the closest plants that they could have originated from were located in Texas and Oklahoma, USA (Mohanty et al. 2017). These are two studies that illustrate the potential to identify pollen grains at the species level using DNA-based methods, though this level of resolution is not always necessary. In the grass family (Poaceae) for example, all species from certain subfamilies are known to have much higher allergenic prevalence than other subfamilies, and therefore subfamily resolution is sufficient for hay fever predictions (Frenguelli et al. 2010). Ghitarrini et al. (2018), for example, used species- but also subfamily-specific primers with real-time PCR to target the most allergenic types of grasses. Pooideae (a subfamily of grasses with many allergenic species) and individual species within this subfamily were detected in aerobiological samples on a presence/absence basis.

DNA metabarcoding

DNA barcoding can be used to target specific species, yet it is rare that a pollen sample contains only a single pollen species. DNA metabarcoding is therefore the most-often used method for the molecular identification of the different species of pollen grains from mixed samples (see Chapter 11 Amplicon metabarcoding). Both nuclear and chloroplast DNA can be amplified in pollen DNA (Bell et al. 2016b), and amongst the many different markers that have been tested, *rbc*L, *trn*L, *matK*, and *trnH-psbA* from the chloroplast, as well as nuclear ribosomal ITS2 (nrITS2), have so far shown the most promise for the molecular identification of pollen grains. Since no universal barcode exists that would allow detection of all plant lineages, a combination of a nuclear and chloroplast marker has been advised (Hollingsworth 2011). nrITS2 (~450 bp) is particularly relevant for the identification of pollen grains when relatively fresh (and non-degraded) DNA is available. In one example, pollen was collected from the bodies of the migratory butterfly species *Vanessa cardui* and identified based on nrITS2, providing geographical information on where the butterflies were migrating from (Suchan et al. 2019). Because several Saharan endemic plants were identified to the species level, this provided excellent evidence for the butterflies originating from the Sahara region.

While research into targeting different barcoding regions and primers is ongoing (*trnT-F*, Alan et al. 2019; and nrITS1, Baksay et al. 2020), another development is the use of more specific reference databases. The commonly used NCBI GenBank returns many untrustworthy hits since it is not curated (see e.g. Meiklejohn et al. 2019). Brennan et al. (2019) designed a metabarcoding study with two common markers (*rbcL* and nrITS2), but using a strictly curated reference library containing sequences only from those grass species that occurred locally. They further customized this database to include all other invasive as well as cultivated species in the UK. Using their customized database, the authors showed signals in temporally restricted grass genera throughout the grass pollen season, with minimal background from unexpected species that often results from mismatches when using a more generic reference database. Furthermore, they identified that while some genera of grass may flower early in summer in one location, it could be months later for flowering to occur in other locations. This information can be used by hay fever patients to figure out what specific grass genus they are allergic to, and additionally illustrates the relationship between flowering phenology and airborne pollen incidence.

It is important to use positive controls with known concentrations of different pollen species in any DNA metabarcoding study. This is because the amount of DNA that can be extracted from different pollen types has been shown to vary. For example, it can be easier to extract DNA from pollen with a thinner exine and from plant species that are richer in chloroplast DNA than from those having a more ‘sturdy’ exine (Leontidou et al. 2017). Furthermore, in-silico testing of the chosen primers on target plant species, and making sure reference sequences are available can help to improve the efficiency of the study.

Quantitative pollen analysis

Beyond identifying which pollen species are present in a particular sample, pollen grain quantification is equally important. For example, for hay fever forecasts, it is not just important to know *if* there are certain allergenic pollen in the air, but also how many pollen grains there are at a given point in time. The golden standard for palynology has been to count a certain number of pollen grains under the microscope (e.g. 200 to 500) to obtain a semi-quantitative measure of the pollen types in a sample. While DNA-based methods for pollen quantification are less developed than DNA-based methods for identification, DNA-based pollen quantification using metagenomics (reviewed in Chapter 12) seems feasible, while there is still strong debate about using DNA metabarcoding reads for this purpose.

DNA metabarcoding reads

In a recent study on the use of DNA to quantify pollen grains, Bell and colleagues found a very weak correlation between pollen counts recorded by palynologists and the proportion of metabarcoding reads (Bell et al. 2019). They constructed different mixtures of known pollen species, and then amplified the marker regions *rbcL* and nrITS2. The authors showed that it depends not only on the species studied, but also on the presence of other species in the mock mixture whether or not this correlation was higher or lower. They identified four metabarcoding related factors that influenced this quantitative bias: copy number, preservation, DNA isolation technique, and amplification bias. Indeed, in many other studies that explore quantification using metabarcoding reads, these factors are often identified as major problems, and DNA metabarcoding reads are therefore mostly used only for relative read abundances in other fields of science (Pawluczyk et al. 2015; Deagle et al. 2019; Lamb et al. 2019).

Another group of scholars, however, are finding more promising results in using DNA metabarcoding to quantify pollen grains. Baksay et al. (2020) for example studied the influence of several factors on quantifying species abundance using mock pollen mixtures, with two commonly found bee-collected pollen species (Baksay et al. 2020). First, the marker regions nrITS1 and *trn*L were chosen and the amplification results were compared to the number of pollen grains counted using flow cytometry. They found the best results using *trn*L and 30 PCR cycles, or with a high-fidelity PCR polymerase and nrITS1 to circumvent the high GC content in the nuclear ribosomal ITS region. It is important to note that while *trn*L overall gave the best results for quantification, species-level resolution was only possible with the nrITS1 marker region. Similarly promising results were obtained by Richardson et al. (2019) where a multi-locus approach was used to quantify bee-collected pollen. The amplification results for *trn*L and *rbc*L matched well with the microscopy results, while nrITS2 showed a weak correlation. The authors therefore recommended using the median or mean abundance from several loci to improve the quantification accuracy. Bänsch et al. (2020) in contrast found a high correlation between read count and microscopy count using the nrITS2 region on pollen collected by honey bees and bumblebees. The authors suggested that the correlation depends on the specific type pollen species studied.

Metagenomic approaches

Since using DNA metabarcoding approaches for pollen abundance may not give quantitative results with complex, multi-species samples, other molecular methods such as genome skimming and shotgun sequencing are being used to circumvent some of the drawbacks. The major advantage of these two methods is that they do not include a PCR-step and therefore do not introduce amplification bias (see Chapter 12 Metagenomics). Genome skimming has already been used to show that quantification is feasible, even for pollen from species that are very rare in mock mixtures (Lang et al. 2019). Because full genomes are only available for less than 1% of all plant species, Peel et al. (2019) developed a method where only partial genome skims are used (0.5x coverage). They found a high correlation between their partial genome skimming results and the expected relative abundance for each pollen type in the mixture. Moreover, the authors indicate that while genome skimming a single pollen sample is still relatively expensive (€70), the advancements made in sequencers technology will help to reduce this price significantly in the near future.

Table 1. Overview of selected studies since 2017 that have used molecular techniques to identify pollen, including the research aim, strategy for pollen lysis, extraction protocol, amount of PCR cycles, marker choice, and sequencing method used.

Study	Aim	Pollen Lysis Step	Extraction Method	PCR cycles	Molecular Method	Markers
(Leontidou et al. 2017)	Airborne pollen identification	Bead beating (one 5 mm stainless steel bead), two 1-min cycles at 30 Hz	DNeasy Plant Mini Kit (Qiagen) and Nucleomag kit (Macherey–Nagel)	30	Sanger sequencing	*trn*L
(Lang et al. 2019)	Pollen quantification	Bead beating (mix of 0.5 and 1 mm silica beads), 2 min	Wizard (Promega)	N/A	Genome skimming	N/A
(Bell et al. 2019)	Pollen quantification	Bead beating (mini-bead beater), 3 min	FastDNA SPIN Kit for Soil (MP Biomedicals)	30	Metabarcoding	nrITS2, *rbc*L
(Peel et al. 2019)	Pollen quantification	Bead beating (five 1 mm stainless steel beads), 2 min at 22.5 Hz	Adapted CTAB	N/A	Genome skimming	N/A
(Gous et al. 2019)	Plant pollinator interactions over time	Bead beating (one 3 mm stainless steel bead + lysis buffer), 2 min at 25 Hz	QIAamp DNA Micro Kit and DNeasy Plant Mini Kit (Qiagen), Nucleospin DNA Trace Kit (Macherey-Nagel)	30	Metabarcoding	nrITS1, nrITS2, *rbc*L
(Brennan et al. 2019)	Airborne pollen identification	Bead beating (3 mm tungsten beads), 4 min at 30 Hz	DNeasy Plant Mini Kit (Qiagen)	35	Metabarcoding	nrITS2, *rbc*L
(Richardson et al. 2019)	Bee pollen diet	Bead beating (3.355 mg 0.7 mm zirconia beads), 5 min	DNeasy Plant Mini kit (Qiagen)	Three steps (55 cycles in total)	Metabarcoding	nrITS2, *rbc*L, *trn*L, *trnH*
(Suchan et al. 2019)	Insect migration analysis	Bead beating (five zirconium beads), 1 min at 30 Hz	No extraction, using Phire Plant Direct Polymerase	Two steps (32 cycles in total)	Metabarcoding	nrITS2

(Baksay et al. 2020)	Pollen quantification	CF lysis buffer (Nucleospin Food Kit)	DNeasy Plant Mini Kit (Qiagen)	25, 30, 35	Metabarcoding	nrITS1, *trn*L
(Campbell et al. 2020)	Airborne pollen identification	Bead beating (0.2 g 425-600 µm glass beads + lysis buffer), two 1-min cycles (3450 oscillations/min)	Adapted CTAB	40	Metabarcoding	*rbc*L
(Leidenfrost et al. 2020; Bänsch et al. 2020)	Bee pollen diet	Bead beating (150 g mix of 1.4 mm ceramic and 3 mm tungsten beads + lysis buffer), two 45 second cycles at 6.5 m/s	DNeasy Plant Mini Kit (Qiagen)	37	Metabarcoding	nrITS2

GLOSSARY

- **Anemophilous** - Wind-pollinated.
- **Bead beating** - The application of beads to break open the outer cell wall of pollen grains.
- **Hirst-type pollen trap** - Volumetric air sampler that is one of the standard devices for monitoring airborne pollen and spores.
- **cpDNA** - Chloroplast DNA.
- **Entomophilous** - Insect-pollinated.
- **Exine** - Outer wall of pollen grains. Composed mainly of sporopollenin that is extremely resistant to degradation. The exine of pollen grains has to be broken to release the DNA from the organic material within the grains.
- **Palynology** - The science that studies both living and fossil spores, pollen grains and other microscopic structures (including, e.g., chironomids, dinocysts, acritarchs, chitinozoans, scolecodonts) .
- **Pollen grains** - The male gametophyte of seed plants; source and carrier for the male gametes (spermatozoids or sperm cells)
- **Pollenkitt** - The outermost hydrophobic lipid layer mostly present on entomophilous pollen grains
- **Sporopollenin** - A chemically inert biological polymer that is a component of the outer wall (see Exine) of a pollen grain.

- **Super-resolution microscopy** - Technique in optical microscopy that allows visualization of images with resolutions up to 140 nm, much higher than those imposed by the diffraction limit. This technique allows visualization of internal structures.

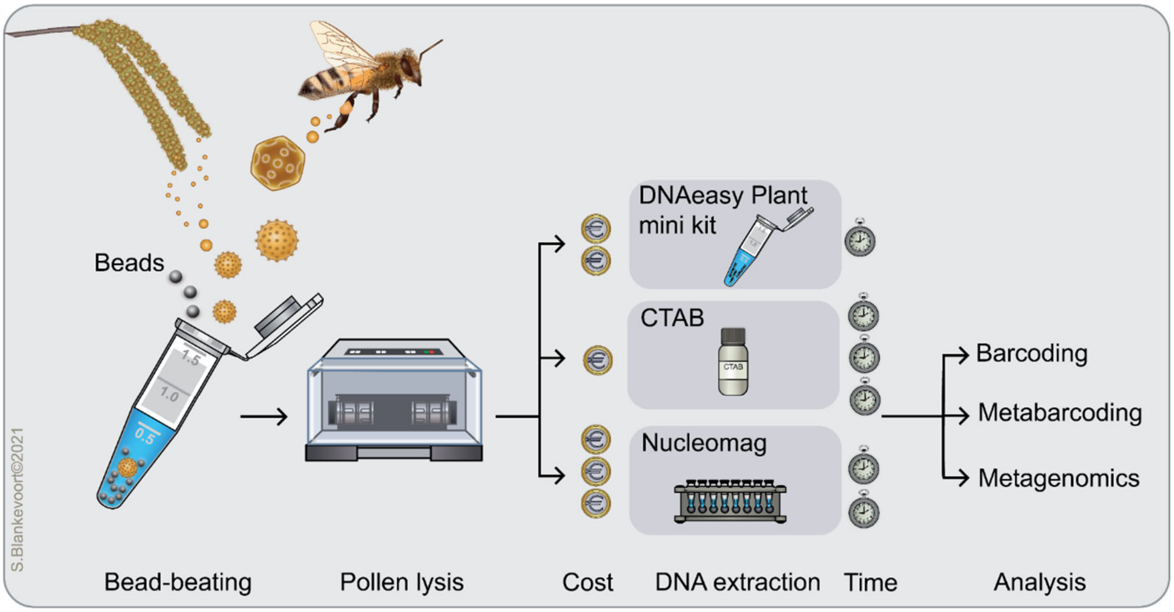
QUESTIONS

1. What are the main advantages of molecular pollen identification over traditional (microscopic) methods? Justify your answer.
2. Pollen is dispersed by various vectors. There are two main types of pollination strategies in land plants, please name them and also explain the importance of the difference between the two in terms of DNA yield.
3. Which four factors make the quantification of pollen grains using metabarcoding problematic?

ANSWERS

1. A higher taxonomic resolution can be achieved using molecular methods such as metabarcoding. Furthermore, pollen analysis requires highly trained experts that have to spend considerable time to analyze a single sample and therefore molecular techniques are faster, especially with a large number of samples.
2. Entomophilous (insect collected) and anemophilous (wind dispersed) pollen. The presence of pollenkitt on entomophilous pollen grains influences the amount of DNA that can be obtained per pollen grain.
3. Copy number, DNA preservation, DNA isolation technique, and amplification bias.

Figures



Infographic 1. Overview of pollen sources, DNA extraction and downstream analytical methods for the molecular identification of plants from pollen DNA**.**

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Manuscript 3

Amplicon metabarcoding

Molecular Identification of Plants: From Sequences to Species

Book Chapter 11 Amplicon metabarcoding

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BACKGROUND

What is metabarcoding?

DNA metabarcoding is a targeted approach where taxonomically informative regions in the DNA are amplified from mixed-template samples containing DNA from different taxa for identification (Pompanon et al., 2012; Riaz et al., 2011). These taxonomically informative regions, also referred to as DNA barcodes or markers, ideally have low intraspecific variability and high interspecific variability to be able to discriminate between species, and conservative regions for universal amplification of the targeted community (Coissac et al., 2016). To target these DNA barcode regions, some prior knowledge is required for the design of primers that are complementary to flanking conservative regions of barcodes. Additionally, dependent on the metabarcoding approach used, primers can contain unique nucleotide tags to discern between samples during downstream bioinformatics processes (Binladen et al., 2007; Valentini et al., 2009b). After PCR amplification, amplicons are built into libraries where library indexes are added to allow for multiple amplicon libraries to be sequenced in one flow cell (Elbrecht et al., 2017; Elbrecht and Leese, 2015). Adapters specific to the sequencing platforms are added to the PCR products (amplicons) and sequenced on a high-throughput sequencing (HTS) platform. The resulting sequences can be taxonomically identified by matching them to a reference database (De Barba et al., 2014; Kress and Erickson, 2008; Taberlet et al., 2018, 2012). This method is useful for identifying different taxa from bulk samples of organismal DNA (Yu et al., 2012), and specifically to detect plants from environmental DNA (eDNA) samples including water, soil, sediment, air, and organic remains such as faeces (Deiner et al., 2017; Taberlet et al., 2012).

Plant metabarcoding

Metabarcoding is based on the DNA barcoding concept (see Chapter 10 DNA barcoding). However, for metabarcoding, samples containing DNA from a mix of different taxa are typically used. One of the first studies that used metabarcoding on a parallel sequencing system (herein referred to as DNA barcoding) to identify plants was by Valentini and colleagues (Valentini et al., 2009a) who analyzed the diet of a variety of animals using their faeces. Earlier attempts at diet analyses were also made using chloroplast (Poinar et al., 2001) and nuclear regions (Bradley et al., 2007), though these are not strictly speaking metabarcoding studies since they did not use high-throughput sequencing. Identification of plants through barcoding has had a turbulent history due to the lack of consensus on which plant barcodes should be used as standard (Pennisi, 2007). In the landmark paper by Hebert and colleagues (Hebert et al., 2003), it was shown that animal species can be confidently identified through a short and highly variable piece of mitochondrial DNA called cytochrome oxidase subunit 1 (*CO1*). This has led many research groups to search for a similar barcode for the identification of plants (Chase et al., 2007; Kress et al., 2005). For plant species identification, the metabarcoding community has heavily relied on short fragments of plastid barcodes *rbc*L, *trnH-psbA*, *mat*K, the P6 loop of the *trn*L intron and the nuclear ribosomal internal transcribed spacers nrITS1 and nrITS2 (China Plant BOL Group et al., 2011; Hollingsworth et al., 2016). There is, however, still no consensus on which plant DNA barcode(s) perform best. Studies that test various DNA barcodes for specific groups of plants find big differences between them (e.g., Braukmann et al., 2017), while others find that none of the available DNA barcodes provides species discrimination in certain plant groups (Zarrei et al., 2015). The search for the universal plant barcode is thus still ongoing.

Sample types and application

Plant metabarcoding is widely used to study the taxonomic composition of mixed template samples such as water (Zimmermann et al., 2015) (see Chapter 3 DNA from water), soil and sediments (Yoccoz et al., 2012) (see Chapter 4 DNA from soil and sediments), bryophyte spores (Stech et al. 2011) bee-collected pollen or pollen from ambient air (Sickel et al. 2015; Kraaijeveld et al. 2015) (see Chapter 5 DNA from pollen), honey, food and medicine (Hawkins et al., 2015; Raclariu et al., 2018) (see Chapter 6 DNA from food and medicine), faeces (Valentini et al., 2009a) (see Chapter 7: DNA from faeces), ancient sediments (Alsos et al., 2016) (see Chapter 8 DNA from ancient sediments), ice and snow (Thomsen and Willerslev, 2015; Varotto et al., 2021) plant macrofossils (Murray et al., 2012), whole insects (Kajtoch, 2014), gut contents (McClenaghan et al., 2015), and epilithic samples (Apothéloz-Perret-Gentil et al., 2017). DNA extraction methods are highly dependent on the type of material used and this is covered separately in the chapters of Section 1 of the book.

Plant metabarcoding has been used in various types of applications including species delimitation (see Chapter 17 Species delimitation), archaeo- and palaeo-botany (Parducci et al., 2017) (see Chapter 21 Palaeobotany), healthcare (Reese et al., 2019) (see Chapter 23 Healthcare), food safety (Raclariu et al., 2017) (see Chapter 24 Food safety), environmental and biodiversity assessments (Fahner et al., 2016) (see Chapter 25 Environment and biodiversity assessments), wildlife trade (de Boer et al., 2017) (see Chapter 26 Wildlife trade), hay fever forecasts (Kraaijeveld et al., 2015) (see Chapter 5 DNA from pollen), water quality assessments (Smucker et al., 2020; Zimmermann et al., 2015) (see Chapter 3 DNA from water), and documenting environmental change (Jørgensen et al., 2012). These are some examples of plant-specific applications where metabarcoding has proven its value, though further detailed information can be found in the chapters referred to here as well as in Veltman et al. (2021).

Advantages and limitations of metabarcoding

DNA metabarcoding is a cost-effective method as compared to metagenomics (see Chapter 12: Metagenomics) or target capture (see Chapter 14: Target capture) as only DNA from targeted taxa is amplified and sequenced (Taberlet et al., 2012; Chua et al., 2021). The tagging system makes it possible to process large numbers of samples simultaneously, further decreasing the sequencing costs and increasing the total sample throughput. DNA present in low quantities (e.g. from rare species) can be targeted and amplified using specific primers and PCR-amplified. It is also a useful method for samples with low-quality DNA (i.e., degraded DNA) since it targets small barcodes that are relatively stable through time (Goldberg et al., 2016; Deiner et al., 2017). For example, plant DNA can be sequenced from ice core samples as old as 500 000 years old (Willerslev et al., 2007).

However, DNA metabarcoding also has its limitations, and the PCR amplification step has previously proven to be particularly problematic (Taberlet et al., 2012). This step can cause stochasticity (Murray et al., 2015) and create false positives (Ficetola et al., 2015), which stresses the need for both PCR and extraction replicates. However, depending on the specific research question, it may also be advisable to limit the number of PCR replicates and instead focus on sequencing depth (Smith and Peay, 2014), although this would decrease species richness estimates (Dopheide et al, 2018).

Another drawback of DNA metabarcoding is primer binding bias due to mismatches between the primer and the template DNA. This can result in discrepancies between the proportion of the original taxa in the DNA extract and the amplified DNA sequences (Bista et al., 2018; Elbrecht and Leese, 2015). Although quantitative results can be obtained from some primers using certain laboratory and bioinformatic controls (Ji et al., 2020; Piñol et al., 2019), this is still taxa-dependent and therefore not commonly used. Depending on the metabarcoding strategy, tag jumps during library building should also be taken into consideration as they can cause false sequence-to-sample assignments (Carøe and Bohmann, 2020; Schnell et al., 2015).

Finally, the taxonomic assignment of sequences to species is heavily dependent on the DNA reference database used for sequence matching. When the reference database to which the resulting sequences are compared to is incomplete and/or consists of inaccurately identified species, this results in erroneously identified species and/or false negatives (Banchi et al., 2020; Meiklejohn et al., 2019). This also affects the species resolution of the results. For example, a reference database based on the *trnL* barcode region may give a resolution of 33% species identification on a large circum-arctic scale, but within a localised area, this resolution may increase to 77-93% (Alsos et al., 2018; Sønstebø et al., 2010). Thus, both the plant marker of choice as well as the reference database used are important and often limiting factors in metabarcoding studies for species identification. Lastly, taxonomic assignments between different species can have the same highest identity scores, but this can be handled by using a Last Common Ancestor approach (e.g. using MEGAN Huson et al, 2006 or OBITools Boyer et al, 2016).

SETTING UP A METABARCODING STUDY

At the start of any (plant) metabarcoding study lies a clearly defined research question. A study design should furthermore encompass a clear sampling strategy, and identification of suitable DNA extraction techniques for the sample type used before carrying out downstream analysis (Zinger et al., 2019). As the chapters in Section 1 already details DNA extraction methods based on specific starting materials, this section will cover the subsequent steps, starting with selecting the plant barcodes to best answer the research question, choosing a nucleotide tagging strategy, sequencing and finally analyzing the sequence output using bioinformatics pipelines.

Barcode choice

Barcode choice is one of the most important aspects of metabarcoding studies as it will determine which taxa are identified and to what resolution. Considerable efforts have gone into constructing libraries for these plant barcodes and in assessing their limitations (CBOL Plant Working Group, 2009; Cowan et al., 2006; Fazekas et al., 2012; Hollingsworth et al., 2011; Kress, 2017). Metabarcoding studies are often heavily dependent on reducing the potentially identifiable species, e.g., using *trnL* P6 loop one can make species-specific identifications of the Greenland flora, but family level identification in a tropical rainforest. The objective of the study determines the level of taxonomic resolution needed, and thus the approach (marker, replicates, etc.), if only relative abundances at the family level are desired or if specific species in a vegetation plot need to be identified from soil. Different research groups use different 'preferred' barcodes that they consider best suited for their specific target plants. Despite this lack of consensus, the efficacy of metabarcoding for identifying the majority of plant species from plant mixtures still makes this a very useful tool. When choosing barcodes for metabarcoding studies, three factors must be considered: 1) sequence availability and presence in a reference library, 2) discriminatory power / taxonomic resolution, and 3) degree of DNA degradation in the sample (Hollingsworth et al., 2011). These three steps will be briefly explained below.

1) The first step is to check whether or not reference libraries exist for the sequences of the targeted organism(s). This is because barcodes are only useful if the sequences for the targeted organism(s) are available in sequence repositories or reference libraries (Weigand et al., 2019). For some barcodes and specific geographic regions, optimized plant reference libraries exist that minimize inaccurate identification of sequences. One such example is the arctic boreal vascular plant and bryophyte database that is based on the P6 loop of *trnL* (Sønstebø et al., 2010). A curated global plant database is also available for nrITS2 (Banchi et al., 2020). Premade reference databases are not complete and it is therefore recommended to compare several databases to obtain the best resolution. Another option is to construct a tailored reference database, for example using the BOLD data portal or in GenBank using the e-utilities tool kit. The use of the publicly available GenBank database is generally discouraged as it contains many erroneous sequences (e.g., Steinegger and Salzberg, 2020). If the target organisms are not present in any public sources, then one would opt for constructing de novo reference libraries. The idea behind it is to sequence barcodes from specimens collected in the study site, which are then assigned taxonomical annotations/identification (see Chapter 10 DNA barcoding). The construction of regional reference libraries usually employs a combination of both strategies described

above. Last, one would opt for blasting the obtained sequences to a public source. This strategy would incur multiple taxonomic assignments to one single sequence and thus a threshold of blasting similarity would have to be arbitrarily designed.

2) Discriminatory power refers to how effectively the barcodes can discriminate between closely related species and is linked to the variability of the locus. Typically, barcodes can only identify plants up to a certain taxonomic level (resolution) depending on the barcode used and the group of plants targeted. Moreover, because reference libraries are incomplete for all DNA barcodes, some species may only be detected using one DNA barcode while others may only be detected by another. Therefore, using a single primer set will most often not result in the recovery of all species present in a sample. We recommend adopting a multilocus approach to gain highly resolved taxonomic coverage for complex samples (e.g., Arulandhu et al., 2017).

3) DNA is relatively unstable in the environment and can degrade quickly depending on certain factors such as age, transport, and abiotic factors (Deiner et al., 2017). In highly degraded and/or old materials, the use of very short, highly distinctive barcodes is recommended (e.g. P6 loop of *trnL* intron). Although this can provide a good indication of the plant community from mixed samples, some taxa cannot be identified beyond the family level (e.g., Asteraceae and Poaceae). Therefore, when possible, it is recommended to use the longer and in some cases more distinctive nuclear ribosomal barcodes ITS1 (De Barba et al., 2014; Omelchenko et al., 2019) and/or ITS2 (Yao et al., 2010). However, the nuclear ITS region is also present in fungi and in order to avoid amplification of fungal DNA, plant-specific primers should be used (Cheng et al., 2016; Chen et al., 2010; Moorhouse-Gann et al., 2018; Omelchenko et al., 2019; Timpano et al., 2020).

Metabarcoding nucleotide tagging strategies

In the metabarcoding laboratory workflow, unique nucleotide tags are added to amplicons, and these tags are used to assign sequences to the sample they originate from (Binladen et al., 2007). This allows for the pooling of many labelled PCR replicates for sequencing, and dramatically increases the throughput. Labelling amplicons with unique nucleotide tags can be done at two stages during a metabarcoding workflow: prior to library building as 5’ nucleotide tags added to the amplicons, and/or after library completion as library indexes. The strategies to achieve this labelling can be condensed into three main approaches: the ‘one-step PCR’ approach, the ‘two-step PCR’ approach, and the ‘tagged PCR approach’.

In the ‘one-step PCR’ approach, the metabarcoding barcode is amplified and built into libraries during one PCR. This is achieved through the use of metabarcoding primers that carry both adapters and library indexes (Elbrecht et al., 2017; Elbrecht and Leese, 2015), though unique nucleotide tags instead of library indexes can also be added in the one-step PCR approach (Elbrecht and Steinke, 2018). In this approach, each PCR replicate is a library.

In the ‘two-step PCR’ approach, sample extracts are PCR-amplified with metabarcoding primers that only carry 5’ tails. These are added to act as templates for the following second PCR and do not include any labelling. The second PCR is carried out on each PCR product with primers that carry adapters and indexes (Galan et al., 2018; Miya et al., 2015; Swift et al., 2018), although unique nucleotide tags can also be added in the first PCR (Kitson et al., 2019). In the two-step PCR approach, each PCR replicate is also a library.

In the 'tagged PCR' approach, DNA extracts are PCR amplified with metabarcoding primers that carry 5' unique nucleotide tags. Next, the individually 5' tagged PCR products are pooled and library preparation is carried out on the pools (first demonstrated by Binladen et al. (2007) on the 454 FLX platform). Library preparation can be with (Drinkwater et al., 2019; Hibert et al., 2013) or without (e.g., Carøe and Bohmann, 2020; Sigsgaard et al., 2017) an indexing PCR step. Care should be taken with using this approach, as several studies have shown it to be prone to so-called tag-jumping where amplicon sequences carry false combinations of nucleotide tags after amplification (Schnell et al, 2015). This can be avoided using specific library preparation protocols (e.g. Carøe and Bohmann, 2020). Finally, indexes can also be ligated to the amplicons with the primers, a technique used for example in Nanopore sequencing.

With the cost of sequencing decreasing exponentially, more effort can be put into applying technical PCR replicates to circumvent sequencing errors and other PCR related issues. When using PCR replicates they should be sequenced in separate locations on the same 96-well plate or, ideally, with replicates in separate plates. Taxa identification lies at the core of any ecological research question. Thus, it is crucial to perform a reliable and reproducible identification workflow to ensure correct identification. In general, care should be taken to avoid cross-contamination between samples by working in clean laboratories with filter-tipped pipettes and separate pre- and post-PCR labs. Normalization of the amplicons prior to library construction is crucial to avoid overamplification of the most represented taxa in the sample. Since some often-used plant-specific marker regions are very short (e.g. *trn*L P6 loop, 8 to152 bp), they are prone to picking up the slightest contaminants from the environment. It is therefore recommended to work in a clean environment, e.g. an ancient DNA laboratory with protective clothing.

Sequencing platforms

The preferred platforms for sequencing are currently IonTorrent and Illumina. Both platforms require an additional post-ligation PCR-step or PCR-free ligation of platform-specific adapters to the amplicons before sequencing. However, due to the different technologies behind both platforms, both the error rates and error types can differ. For Illumina (optical sequencing), a substitution error rate of 0.1% has been identified, while IonTorrent (based on detection of hydrogen ions) can show up to 1% indel errors (Quail et al., 2012; Shin et al., 2017). The IonTorrent platform has a slightly higher error rate when the material contains high amounts of homopolymers because no good correlation exists between the number of identical bases incorporated and the observed voltage change (Bragg et al., 2013). Illumina is the most often used platform in metabarcoding studies due to its lower error rates, and the generation of relatively long reads by paired-ending (Forin-Wiart et al., 2018). Since IonTorrent and Illumina are limited in the maximum length of amplicons that can be generated (up to 600 bp), more recent sequencing platforms like Nanopore and PacBio are increasingly being used. These long read technologies have the advantage of being able to retrieve for example the whole nuclear ITS or plastid *mat*K regions. For more information on sequencing platforms, please refer to Chapter 9 Sequencing platforms and data types.

Bioinformatics tools

Several different bioinformatic tools can be used to analyze the sequence output. Some commonly used packages are OBITools (Boyer et al., 2016), BEGUM (Yang et al., 2020), MOTHUR (Schloss et al., 2009), QIIME (Caporaso et al., 2010) and DADA2 (Callahan et al., 2016). The bioinformatics workflow includes these common steps: quality check of raw reads, removal of adapter sequences, demultiplexing, filtering of erroneous sequences, sequence dereplication, removal of singletons and PCR/sequencing errors, clustering/denoising, and taxonomic annotations using reference databases (most commonly using BLASTn). Depending on the pipelines used, sequences are either clustered into OTUs based on sequence similarity level (often 97%) such as in QIIME, MOTHUR, VSEARCH, or denoised into strictly unique sequences called ASVs such as in DADA2 or USEARCH (unoise). The choice to cluster sequences into OTUs or denoise into ASVs is dependent on the research question. Clustering sequences into OTUs reduces sequencing errors, but increases false negatives as multiple similar species are clustered into a single OTU. In datasets where it is expected that closely related species are present, such as species with homopolymers (e.g. *Vaccinium* spp.), denoising sequences into ASVs would be preferred since these homopolymers can be sorted out into separate sequence variants. However, using this technique may also result in artificially inflating diversity as species may have more than one sequence variant, especially if the reference database used is incomplete. Alternatively, sequences can be assigned directly to taxa such as in OBITools, one of the most frequently used open-source programs for plant metabarcoding studies. OBITools was specifically designed for the analysis of metabarcoding data generated from HTS. It relies on filtering and sorting algorithms, which allows users to customize their pipelines tailored to their needs. A distinct feature of OBITools is its ability to account for taxonomic annotations, which allows the sorting of sequences based on taxonomy instead of OTUs/ASVs.

FUTURE OF METABARCODING

Currently, metabarcoding is the dominant technique used in the identification of plants from mixed samples. Developments and improvements in addressing methodological challenges such as PCR bias may one day allow for unbiased quantitative inferences from metabarcoding datasets. This would be a huge step forward for the metabarcoding community since it is still controversial to use read counts as an indication for biomass (Deagle et al., 2019). With the continued advances in HTS technologies coupled with the inherent limitations of metabarcoding, there is also a possibility that alternative HTS techniques can be used in the future. For example, the development of more regional DNA reference databases based on whole organelle genomes instead of single barcode regions (Coissac et al., 2016) (see Chapter 10 DNA barcoding) would encourage the use of HTS techniques that rely on whole genomes or multiple non-standard barcode regions for taxonomic identification. Particularly, if sequencing becomes cheaper and if the limitations of metagenomics (see Chapter 12 Metagenomics) or target capture (see Chapter 14 Target capture) are addressed, we may see an increase in other types of methods used to identify plants in mixed templates. However, metabarcoding has the advantage of being a cheaper option, where large numbers of samples can be processed for meaningful statistical analysis. Bioinformatics pipelines are also well-established and better reference databases are available for mini barcodes as compared to whole organelles. This makes metabarcoding the preferred technique for many applications. In addition, ongoing efforts to build curated reference databases, design better primers, and detect potential plant-specific barcode regions might increase species resolution and circumvent many of the drawbacks associated with metabarcoding.

Metabarcoding could potentially be used to determine plant composition in a landscape from bulk arthropod samples. Bulk arthropod samples have been used for biodiversity monitoring of vertebrates (Lynggaard et al., 2019), but it has not been used for any plant-related studies. Another potential application of metabarcoding is in forensic genetics (see Chapter 28 Forensic genetics, botany and palynology), where plants are used as evidence in criminal investigations (Bryant, 2013). For example, morphological identification of pollen grains has been used to solve murders and determine marijuana distribution locations (Alotaibi et al., 2020; Bryant and Jones, 2006). However, metabarcoding is underutilized in these applications where morphological identification is still the main technique. One possible limiting factor for this lack of utilization could be that pollen DNA extraction destroys the samples and therefore cannot be stored as evidence (Bell et al., 2016). Metabarcoding could also potentially be used in meta-phylogeographic studies to simultaneously study the phylogeographic features and intraspecies patterns of many species (Turon et al., 2019).

GLOSSARY

- **Adapters** - Specific nucleotide sequences unique to different types of sequencing platforms that are added to amplicon libraries to allow for the attachment of library fragments to the flow cell for sequencing.
- **Amplicons** - Products of PCR amplification.
- **ASVs -** Amplicon sequence variants, also known as exact sequence variants or zero-radius OTUs. Although sometimes considered synonymous to OTUs, they correspond to all the unique reads in a dataset and do not require clustering used in creating OTUs.
- **Barcode** - Targeted gene region, see Locus.
- **Demultiplexing** - Bioinformatics step of assigning sequences to samples based on assigned nucleotide tags and/or library indexes.
- **Epilithic** - Plant growing on surfaces of rocks, e.g., seaweeds.
- **Homopolymers** - Nucleotide repetition, usually in tandem of more than 7 nucleotides.
- **Indel errors** - Insertions or deletions in sequences resulting from mutations.
- **ITS** - The internal transcribed spacer is a nuclear ribosomal region found between the small subunit ribosomal RNA (rRNA) and large-subunit rRNA genes.
- **Library indexes** - Nucleotide index added to amplicon libraries to allow for the parallel sequencing of multiple libraries, which can be used bioinformatically to assign reads to the correct amplicon libraries.
- **Locus** - Section and position in a chromosome where a particular DNA sequence is located. It can also be referred to as a barcode.
- **Macrofossils** - Preserved plant remains large enough to be seen without a microscope.
- ***mat*K** - Maturase K is a gene found in the chloroplast genome.
- **Meta-phylogeography** - Study of phylogeographic features and intraspecies variation.

- **Multiplexing** - Parallel amplification of barcodes in one PCR reaction.
- **OTU** - Operational taxonomic unit. The term is used to categorize clusters of similar sequences.
- **Overhangs** - Stretch of unpaired nucleotides at the end of DNA fragments.
- **PCR** - Polymerase chain reaction.
- **PCR stochasticity** - Uneven amplification of molecules during PCR that can be a result of some sequences being present in lower copy numbers than others.
- **Phylogeography** - Investigate the origin of genetic variation within closely related species across a landscape.
- **Primers** - A short single-stranded nucleic acid sequence that serves as a starting point for the DNA replication in the PCR.
- **Primer set** - Nucleic acid sequences explained above complementary to the 5' end and 3' end of the flanking regions of a locus.
- **Primer bias** - Differences in DNA amplification due to a primer inefficiently binding to the target template. This can result from sequence divergence in the primer binding sites.
- **qPCR** - Polymerase chain reaction used for quantifying DNA.
- ***rbc*L** - The ribulose-1,5-bisphosphate carboxylase large subunit gene is found in the chloroplast genome.
- **Singletons** - A sequence only present in one copy.
- **Nucleotide tags** - Short nucleotide sequences added at the 5' end of the primer in metabarcoding studies.
- **Tag jumps** - Generation of amplicons with different tags than originally used, resulting in false positives in the data. For more detail see Schnell et al. (2015).
- **Taxa** - Plural of taxon. A taxon is a group of organisms that form a taxonomic group.
- **Taxonomic assignment** - Matching the obtained sequences to taxa names.
- ***trnH-psbA*** - An intergenic spacer region found in the chloroplast genome.
- ***trn*L** - The *trn*L gene is part of the *trn*L-F region of the chloroplast genome.

QUESTIONS

1. How can overamplification of the most represented taxa in a single sequencing run of multiple complex mixtures be avoided?
2. Which DNA barcode region is most suitable for dealing with plant DNA from samples where DNA is expected to be degraded?
3. The nuclear ribosomal ITS region is shared between plants and fungi. How can undesirable fungal DNA amplification be avoided?

ANSWERS

1. By using equimolar pooling of individual samples.
2. The highly stable P6 loop can best be targeted in this case, using *trn*L primers.
3. By using plant-specific ITS primers that minimize the amplification of fungal DNA.

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Manuscript 4

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DNA metabarcoding using nrITS2 provides highly qualitative and quantitative results for airborne pollen monitoring

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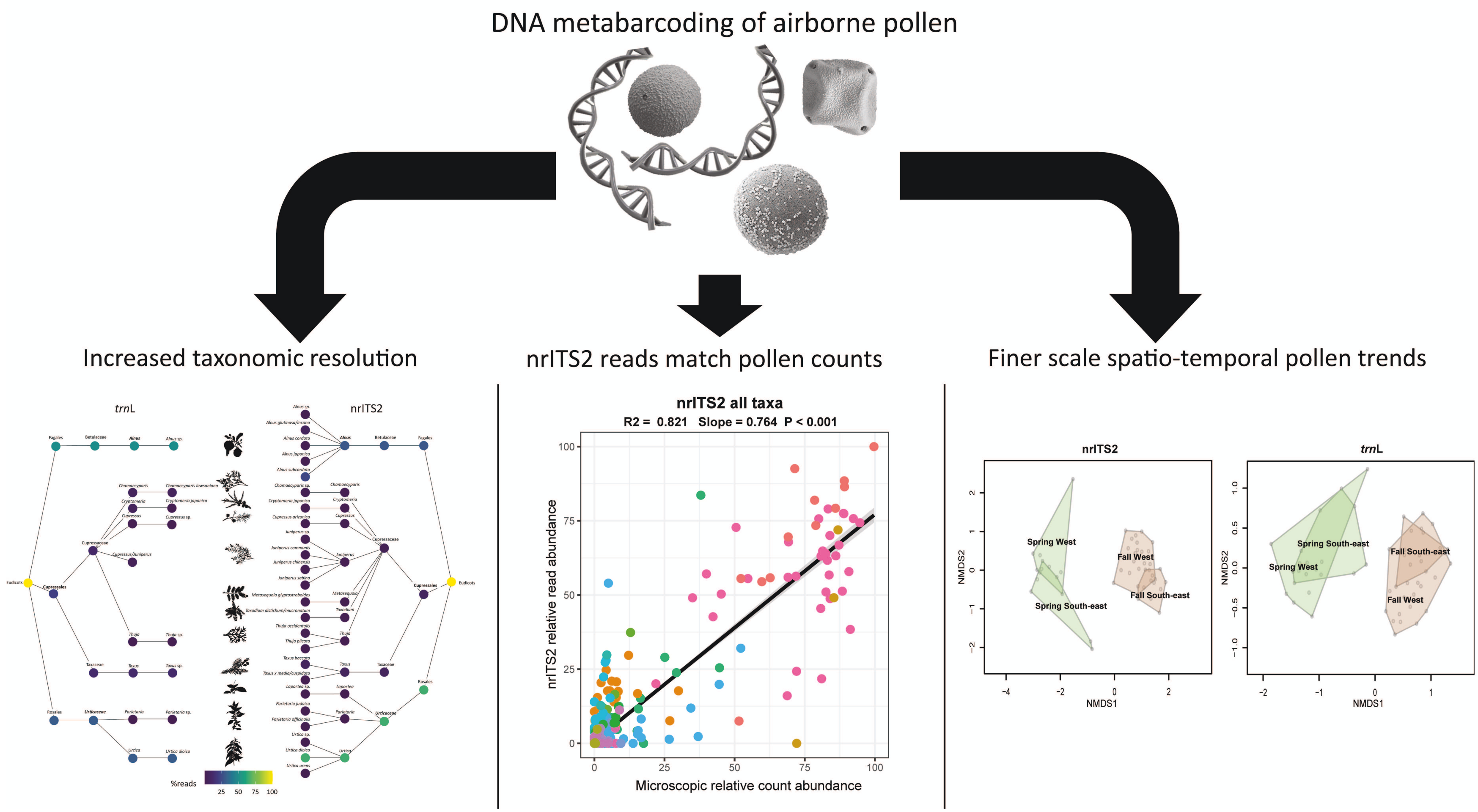
Graphical Abstract



DNA metabarcoding using nrITS2 provides highly qualitative and quantitative results for airborne pollen monitoring

Highlights:

- DNA successfully extracted from microscopic pollen slides and Burkard-collected tapes
- nrITS2 and *trn*L DNA metabarcoding improves taxonomic resolution of airborne pollen
- Relative read abundance nrITS2 shows higher correlation with pollen counts than *trn*L
- Finer scale spatiotemporal patterns in pollen trends detected using nrITS2
- Detection of artificial hybrid that significantly prolongs hay fever season

Abstract

Airborne pollen monitoring is of global socio-economic importance as it provides information on presence and prevalence of allergenic pollen in ambient air. Traditionally, this task has been performed by microscopic investigation, but novel techniques are being developed to automate this process. Among these, DNA metabarcoding has the highest potential of increasing the taxonomic resolution, but uncertainty exists about whether the results can be used to quantify pollen abundance. In this study, it is shown that DNA metabarcoding using *trn*L and nrITS2 provides highly improved taxonomic resolution in airborne pollen samples from the Netherlands. A total of 168 species from 143 genera and 56 plant families were detected, while microscopic pollen counts identified 23 genera and 22 plant families. NrITS2 produced almost double the number of OTUs and a much higher percentage of identifications to species level (80.1%) than *trn*L (27.6%). Furthermore, regressing relative read abundances against the relative abundances of microscopic pollen counts showed a better correlation for nrITS2 ($R^2 = 0.821$) than for *trn*L ($R^2 = 0.620$). Using three target taxa commonly encountered in early spring and fall in the Netherlands (*Alnus* sp., Cupressaceae/Taxaceae and Urticaceae) the nrITS2 results showed that all three taxa were dominated by single species (*Alnus glutinosa/incana*, *Taxus baccata* and *Urtica dioica*). Highly allergenic species were found using nrITS2 that could not be identified using *trn*L or microscopic investigation (*Alnus* x *spaethii*, *Cupressus arizonica*, *Parietaria* spp.). Furthermore, perMANOVA analysis indicated spatiotemporal patterns in airborne pollen trends that could be more clearly distinguished for all taxa using nrITS2 rather than *trn*L. All results indicate that nrITS2 should be the preferred marker of choice for molecular airborne pollen monitoring.

1. Introduction

With hay fever incidence on the rise in the 21st century, monitoring of pollen in ambient air is of high socio-economic relevance to both health care and research (Anderegg et al., 2021; Suanno et al., 2021). The diversity of pollen in ambient air is typically monitored using pollen traps and microscopic identification. This information is important for hay fever patients, but it is a time-consuming process that requires highly trained specialists. Automating pollen counting and identification using new technologies (Dunker et al., 2021; Sauvageat et al., 2020) or by using deep learning algorithms on pollen images (Holt and Bennett, 2014; Olsson et al., 2021; Sevillano et al., 2020) has been shown to increase speed and accuracy. However, these methods do not generally improve the taxonomic resolution of pollen identifications. Neural networks have in some cases been shown to increase taxonomic resolution for pollen that cannot be separated by specialists by their morphology (Polling et al., 2021; Romero et al., 2020). This technique, however, requires an extensively trained network with varied pollen images and high-resolution microscopes, and does not work for all pollen types. Since many important allergenic plant families like Poaceae, Urticaceae and Cupressaceae / Taxaceae are stenopalynous (i.e. produce morphologically identical pollen), much information on the relative abundance and spatial patterns of individual species is lost (Erdtman, 1986; Kurmann, 1994). This information is important as different species may possess different allergenic profiles and ecological preferences. Moreover, it is currently impossible to obtain information on airborne pollen from many cultivated and exotic species versus native plant species.

As an alternative to morphological pollen identification, DNA metabarcoding has been shown to provide increased taxonomic resolution and it has been used successfully on bee-collected pollen (Bänsch et al., 2020; Elliott et al., 2021; Gous et al., 2021; Richardson et al., 2019) as well as airborne pollen (Banchi et al., 2020; Brennan et al., 2019; Campbell et al., 2020; Kraaijeveld et al., 2015; Uetake et al., 2021). For example for grasses (Poaceae), a recent study has shown that pollen of a small subset of all species present in the UK is likely to have a disproportionate influence on human health (Rowney et al., 2021). However, such highly detailed information is not yet available for other plant families.

Increasingly, studies are demonstrating that the relative abundance of metabarcoding read counts shows a good correlation with relative abundances of microscopically counted pollen grains (e.g., Bänsch et al., 2020; Kraaijeveld et al., 2015; Richardson et al., 2021; Richardson et al., 2019), although this correlation may depend on both the species studied as well as the other species present in the mixture (Bell et al., 2019). Furthermore, since pollen from different species possesses different copy numbers of plastid and nuclear DNA, this correlation may be highly dependent on the marker choice (Bell et al., 2016a; Rogers and Bendich, 1987). Commonly used DNA marker regions in pollen metabarcoding include plastid *rbc*L and *trn*L as well as the nuclear ribosomal Internal Transcribed Spacer (nrITS) regions ITS1 and ITS2. For complex aerobiological samples containing pollen from various species as well as fungal spores, bacteria and viruses, the correlation between microscopically counted pollen and DNA reads has been found to be relatively low using the *rbc*L plastid marker (Campbell et al., 2020; Uetake et al., 2021). While *trn*L has shown promising results in quantifying pollen (Kraaijeveld et al., 2015), it has not yet been tested on a large dataset and nrITS2 has not been sufficiently tested for aerobiological samples.

In this study we first test whether DNA metabarcoding using plastid *trn*L and nuclear ribosomal ITS2 loci can be used to increase taxonomic resolution of airborne pollen identifications. Pollen samples were collected from two pollen monitoring in the Netherlands, with a focus on three commonly encountered pollen types in the Netherlands in early spring and fall (*Alnus* sp., Cupressaceae/Taxaceae and Urticaceae). The alders (*Alnus*) can be identified to the genus level under a microscope, while nettles (Urticaceae) can only be recognized to the family level. Cypress (Cupressaceae) pollen cannot be distinguished from pollen of the yew family (Taxaceae) and is therefore counted together. Using the three target taxa, the quantitative performances of the two DNA markers are compared to microscopic pollen counts. The quantitative results are used to visualize trends in species that could hitherto not be distinguished using traditional methods. We also investigate whether DNA metabarcoding shows significant differences between the two pollen monitoring sites in early spring and fall.

2. Material and methods

2.1 Material

Samples used in this study were collected in 2019 and 2020 at two airborne pollen monitoring stations in the Netherlands, including the Leiden University Medical Center (LUMC), Leiden, West of the Netherlands and Elkerliek Hospital in Helmond, South-east of the Netherlands (Figure 1a). These stations routinely collect airborne pollen from ambient air for allergenic pollen monitoring using a Burkard spore trap (Burkard Manufacturing, Rickmansworh, UK) (Figure 1b). This device has been placed on top of the roof of LUMC since 1969 and the Elkerliek Hospital since 1975. The Burkard trap sucks in air continuously using a vacuum pump and impacting any particles >3.7 µm on a Melinex adhesive tape mounted on a drum that rotates behind the inlet in 7 days. Since the drum rotates at a constant speed, a given section of tape corresponds to a known length of time. This tape is cut into seven pieces of 48 mm, each corresponding to 24 hours, from which a microscopic slide is prepared. Pollen slides are made by placing the Melinex tapes on a microscopic glass slide and mounted using a glycerin:water:gelatin (7:6:1) solution with 2% phenol and stained with Safranin (0.002% w/v). A cover glass is placed over the tape which is sealed with nail polish.

This study focuses on three taxonomic groups in particular (*Alnus* sp., Cupressaceae/Taxaceae and Urticaceae), and samples with high pollen counts in these taxa were selected from either late winter – early spring (February to May) for *Alnus* and Cupressaceae/Taxaceae or summer – early fall for the Urticaceae (Figure 1e-f, h-i). When referring to these time periods from now on in this manuscript the terms 'spring' and 'fall' will be used, and 'Cupressaceae' is used from now on when referring to Cupressaceae/Taxaceae. The 20-year pollen count averages from the two pollen monitoring sites show broadly similar patterns for *Alnus* sp., although a peak in late December – early January is only observed in the West of the Netherlands (Fig. 1d). Cupressaceae are notably more abundant in the South-east of the Netherlands, while Urticaceae show a similar 'twin-peak' abundance pattern (early July and late August; Fig. 1d,g). For metabarcoding analysis in this study, we had access to 20 tapes mounted on microscopic slides from the South-east of the Netherlands. From the West of the Netherlands we obtained 6 mounted tapes as well as 32 unmounted tapes (Fig. 1c). The unmounted tapes from the West of the Netherlands were obtained from a second (backup) Burkard device placed two meters away from the first. Mounted tapes were stained with safranin and preserved in glycerol, both of which are potential inhibitors for DNA amplification.

2.2. Pollen counts

To obtain daily pollen concentrations from the microscopic slides collected using the Burkard pollen samplers in the South-east and West of the Netherlands, pollen on microscopic slides were counted under the microscope in three longitudinal bands at 40X magnification. This is an area that corresponds to 1 m^3 of ambient air over a time period of 24 h (Galán et al., 2017).

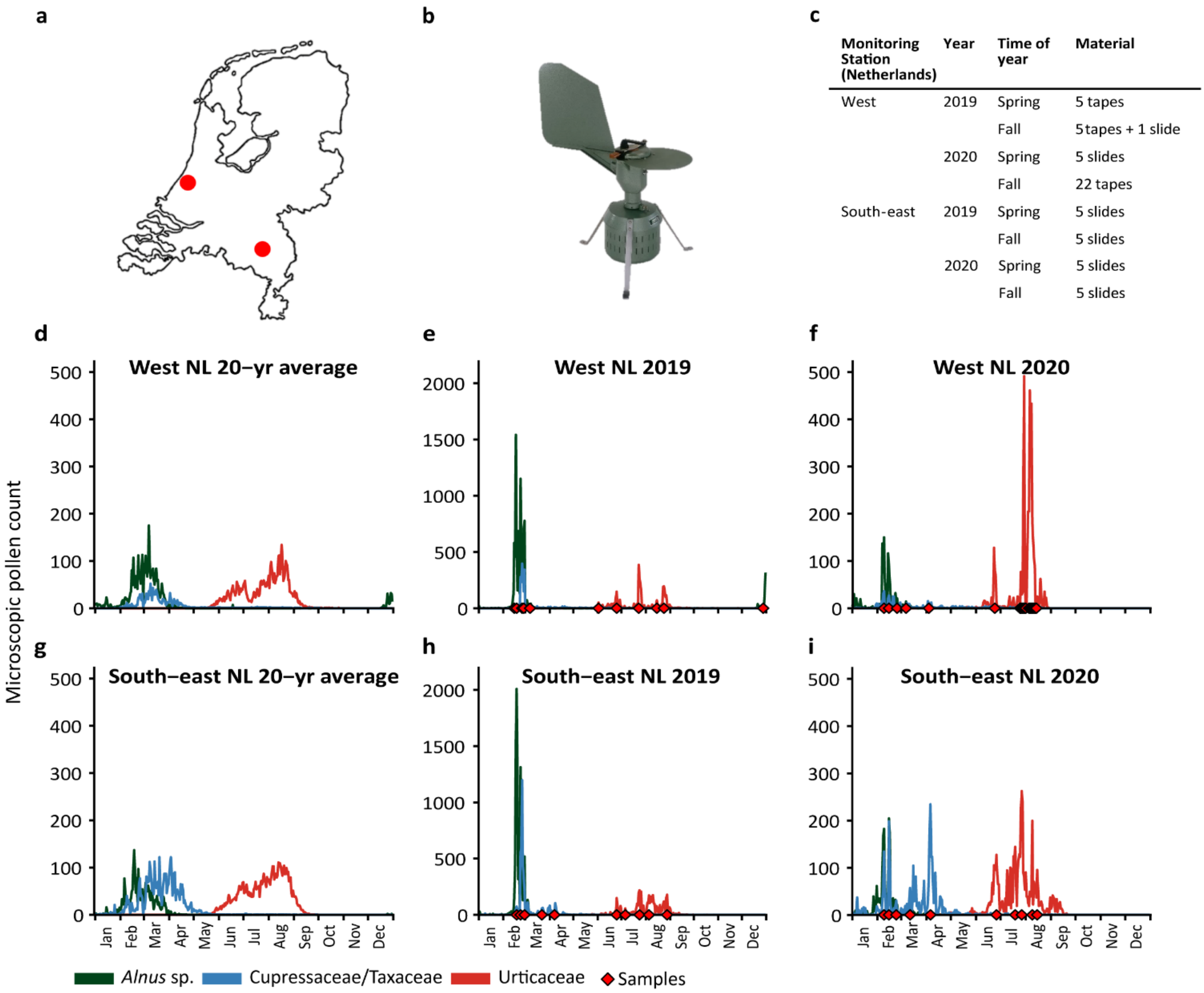


Figure 1. Pollen collection in the Netherlands a) locations of pollen monitoring sites, the West (Leiden) and South-east of the Netherlands (Helmond) b) Hirst-type Burkard pollen sampler c) sample selection of Melinex tapes and microscopic slides with mounted tapes d,g) 20-year average pollen counts of Alnus, Cupressaceae and Urticaceae at both pollen monitoring stations e,h) 2019 pollen counts of the three target taxa and f,i) 2020 pollen counts. Sampling dates are shown with red diamonds on the x-axis. Note scale change for figure e and h. NL = the Netherlands, yr = year.

2.3 Methods

2.3.1 DNA extraction and amplification

All the next steps were performed in a flow cabinet in a dedicated DNA clean room laboratory of Naturalis Biodiversity Center (Leiden, the Netherlands). To extract the Melinex tape from the microscopic slide, the outside surface of the slide was cleaned sequentially with 70% EtOH and 1:100 Chlorine solution to remove potential contamination. Slides were then placed on a heating plate for several seconds to dissolve the nail polish that was used to seal the cover glass, and the cover glass was carefully lifted with UV-cleaned tweezers to remove the tape. From here, the procedure was the same as that used for the tape directly obtained from the backup Burkard sampler. Half of the Melinex tape was cut for DNA analysis while the other half was preserved for future analysis. The tape for DNA extraction was cut in small pieces and placed in a 2 ml tube. Prior to DNA extraction, pollen cell walls were disrupted using the pollen lysis protocol described in Kraaijeveld et al. (2015), adjusted by using four 2.3 mm stainless steel and ten 0.5 mm glass beads, and disrupting the pollen in a Retsch Mixer Mill MM 400 for 3 x 2 min at 30 Hz. After bead beating, 100 µl of 5% SDS was added to the samples and these were incubated at 65°C for 30 min. DNA was extracted using the QIAamp DNA Mini kit according to the manufacturers' protocol (Qiagen). Extraction blanks (Melinex tape without pollen) were included in each round of extractions and these were pooled per three during the PCR step resulting in two sets of extraction blanks in the final dataset.

A two-step PCR protocol was used to create a dual index amplicon library, using the *trn*L primers *g* and *h* to amplify the chloroplast *trn*L intron P6 loop (Taberlet et al., 2006) and the plant-specific primers ITS-p3 (Cheng et al., 2016) and ITS4 (White et al., 1990) to amplify nuclear ribosomal Internal Transcribed Spacer region nrITS2. We used three PCR replicates per sample (giving each a unique tag combination). All extraction blanks, PCR negative blanks (seven) and positive controls (two; pollen from non-native *Citrus japonica*) were included in both rounds of PCRs and sequencing. First round PCRs were carried out in 25 µl reactions containing 14.75 µl nuclease-free ultrapure water, 1x Phire Green Reaction Buffer (Thermo Scientific), 1.0 µl of each 10 mM primer, 0.5 µl of 1.25 mM dNTP's, 0.5 µl Phire Hotstart II DNA Polymerase and 1.0 µl of sample DNA extract. This mixture was denatured at 98°C for 30 sec, followed by 35 cycles including 5 sec at 98°C, 5 sec annealing at 55°C for *trn*L or 58°C for nrITS2, extension at 72°C for 15 sec and a final extension at 72°C for 5 min. PCR success was checked on an agarose gel. All PCR products were cleaned using one-sided size selection with Agencourt AMPure XP beads (Beckman Coulter), at a 1:0.9 (nrITS2) or 1:1 ratio (*trn*L).

To add individual P5 and P7 Illumina labels to all samples (Nextera XT Index Kit; Illumina, San Diego, CA, USA), a second round of PCRs was performed in a final volume of 20 µl using 3.0 µl of the cleaned PCR product from the first round, 5.0 µl ultrapure water, 10.0 µl KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Boston, Massachusetts, USA) and 0.5 µM of each Illumina label. The PCR program included an initial denaturation at 95°C for 3 min followed by eight cycles of 20 sec at 98°C, 30 sec at 55°C and 30 sec at 72°C, followed by a final extension at 72°C for 5 min. The resulting PCR products were pooled into two pools based on amplicon length: a pool containing the shorter *trn*L fragments and one containing the longer nrITS2 fragments. For each marker a library was constructed by equimolar pooling of the PCR products after measuring amplicon concentrations on a QIAXcel (Qiagen). The pools were purified using Agencourt AMPure XP beads (Beckman Coulter), with a 1:0.9 ratio for nrITS2 and 1:1 for *trn*L, and quantified using an Agilent 2100 Bioanalyzer DNA High sensitivity chip (Agilent Technologies, Santa Clara, CA, USA). The pools were sequenced in separate runs on an Illumina MiSeq (v3 Kit, 2x300 paired-end) at Baseclear (Leiden, the Netherlands). Raw sequence data is available at ENA project nr PRJEB45538.

2.3.2 Bioinformatics and filtering

The sequences were analysed on a custom pipeline on the OpenStack environment of Naturalis Biodiversity Center through a Galaxy instance (Afgan et al., 2018). Raw sequences were merged using FLASH v1.2.11 (Magoč and Salzberg, 2011) with a minimum overlap of 10 bp and maximum mismatch ratio of 0.25, discarding all non-merged reads. Primers were trimmed from both ends of the merged reads using Cutadapt v2.8 (Martin, 2011). Any reads without both primers present (allowing a maximum mismatch of 0.2) or shorter than 8 bp (*trn*L) or 150 bp (nrITS2) were discarded. Sequences were dereplicated and sorted by size in VSEARCH v2.14.2 (Rognes et al., 2016) and clustered into “zero-noise” Operational Taxonomic Units (OTUs) using the *unoise3* algorithm from USEARCH v11.0.667 (Edgar, 2016) with default settings and a minimum abundance of 10 reads before clustering, removing singletons and potential chimeras. The resulting OTU sequences were compared to two taxonomic reference libraries for both markers. In order to avoid false BLAST hits, custom reference databases were constructed for both markers consisting of all native and introduced plants from the Netherlands (obtained from https://www.verspreidingsatlas.nl/soortenlijst/vaatplanten and including recent arrivals from Denters (2020)). This list was further supplemented with a list of all cultured plants in the Netherlands, obtained from the 'Standard list of Dutch culture plants 2020' (Marco Hoffman, pers. comm.) resulting in a list of 19,561 green plant taxa. All available *trn*L and nrITS2 sequences belonging to species on this list were downloaded from NCBI GenBank on 21 April 2021, resulting in a reference library of taxa occurring in the Netherlands consisting of 8,391 sequences for *trnL* and 10,015 for nrITS2. To mitigate erroneous or missing taxonomic assignment due to references potentially missing in the Dutch custom databases, a second reference library was constructed for both markers, consisting of worldwide *trn*L and nrITS2 plant sequences, downloaded from NCBI GenBank on 21 April 2021. Priority was given to the local database and if multiple blast hits were found with the same maximum BIT-score, the lowest common ancestor of these hits was chosen. A minimum of 97% identity was used for species level identification, 90% for genus and 80% for family. For *trn*L only sequences with a 100% cover were accepted, while this value was 90% for nrITS2 to account for incomplete reference sequences in the database (partial ITS2 records). Finally, OTUs with the same taxonomic assignment were aggregated.

The resulting sequences were further filtered in R (version 3.5.2; R Core Team, 2020) to remove a) OTUs that were more abundant in negative or extraction blanks than in samples, b) sequences present with <10 reads per PCR repeat, c) potential leakage, using a custom R script to determine the filtering threshold that would result in removal of all reads from negative controls (0.0035% (nrITS2) and 0.05% (*trn*L) of each sequence read count per sample) d) PCR repeats with fewer than 3,000 reads, e) OTUs from fungi, bryophytes or green algae, f) any OTUs that were present in only one of the three PCR repeats (see Table S1 for all filtering steps and read counts). Several samples (12 for nrITS2 and one for *trn*L) had only one PCR replicate left after these filtering steps. Since these samples could not be cleaned using the minimum threshold of two PCR repeats, they were carefully checked for potential contaminations.

Several suspicious OTUs of potential food contaminants still remained in both datasets after these filtering steps. The microscopic slides that we analysed were not made with DNA metabarcoding in mind, and no particular precautions were taken to avoid contamination. This may explain the presence of, e.g., *Arachis hypogaea* (peanut), *Glycine max* (soj), *Ananas comosus* (pineapple) and *Persea americana* (avocado) in the *trn*L results (Figure S2). However, we also found DNA from *Solanum lycopersicum* (tomato), *Secale* *cereale* (rye), *Pisum sativum* (pea) and *Phaseolus vulgaris* (bean) (among others) that grow naturally and are commonly cultivated in the Netherlands. However, since DNA from many of these species was found in samples from both spring and fall, they were conservatively assumed to be derived from contamination. This approach was adopted across all OTUs, and OTUs from potential food contamination were removed (see Figure S1-2 for all removed taxa).

2.3.3 Data analysis

The reads from the remaining replicates were averaged and converted to relative read abundances (RRA) using the *decostand* function of the *vegan* package in R (Jari Oksanen et al., 2018) in order to compare them to the relative abundances of the microscopic pollen counts. The RRA represents the proportion of reads for each taxon present in a sample out of the total reads for a sample. To visualize the taxonomic diversity and RRA distribution of *trn*L and nrITS2 in the three target taxa studied here, we used the *metabaR* package in R from Zinger et al. (2021).

To determine which marker performed best in quantifying pollen, the RRA values were regressed against relative abundance of pollen counts using least squares regression of the *lm* function in R base (R Core Team, 2020). Since this relationship has been shown to be taxon dependant (Bell et al., 2019), independent statistical analyses were performed for each of the three target taxa (*Alnus*, Cupressaceae and Urticaceae) and DNA marker combination (*trn*L or nrITS2). Another regression model was made using RRA values from any taxon in the entire dataset that had >5 % relative abundance in the microscopic pollen count. For these regressions all molecular taxonomic assignments were adjusted to the maximum taxonomic resolution obtained using microscopic pollen identification (e.g., RRA values from all OTUs of Cupressaceae and Taxaceae and for *Alnus* all species were summed up). For the nrITS2 results, the RRA values were plotted for all species identified within the three target taxa.

Finally, to visualize the (dis)similarity of the pollen identifications in samples from the different pollen monitoring stations (South-east and West of the Netherlands) and the different seasons, the Bray-Curtis dissimilarity index was calculated using the RRA values of nrITS2 and *trn*L between each pair of samples using the *vegdist* function of the *vegan* package in R (Jari Oksanen et al., 2018). These values were ordinated using nonmetric multidimensional scaling (NMDS) and visualized with the *ordiplot* function in *vegan*, grouped per pollen monitoring site and per season. The statistical significance of the differences between these variables were tested using a permutational multivariate analysis of variance (perMANOVA) with 999 permutations, using the *adonis* function in *vegan*.

3. Results

3.1 Sequence run statistics

DNA was obtained from both the unmounted tapes and the microscopic slides that contained safranin and glycerin. For nrITS2, seven samples were discarded before sequencing because they did not yield any amplicons after two rounds of PCR. Illumina sequencing resulted in 7.5 M read pairs for nrITS2 and 8.6 M for *trn*L. After quality filtering and merging, 6.4 M reads remained for nrITS2 and 6.8 M for *trn*L. Respectively three and five samples were discarded because they had <3,000 reads in all PCR replicates for nrITS2 and *trn*L. Forty-eight out of the 58 analyzed samples were retained for nrITS2 and 53 for *trn*L (Table S1). Per sample read abundance was 52,775 ± 4,671 for nrITS2 and 48,784 ± 4,241 for *trn*L. Mean GC-content for nrITS2 amplicons was 58.4 ± 2.7 %.

3.2 Taxonomic resolution

Across all samples and markers, 56 plant families, 143 genera and 168 different plant species were identified (Figures S1, S2; Tables S2 – S5). At the family level, all pollen identified by microscope was also found with metabarcoding. The total number of OTUs identified using nrITS2 was almost twice as high (191) than for *trn*L (98), and was also higher per sample for nrITS2 (14.4 ± 1.7) than for *trn*L (12.0 ± 1.0) (Table S1). For nrITS2, 80.1% of all OTUs could be identified to the species level, while this was 27.6% for *trn*L. Most species were uniquely identified using nrITS2 (141), while 15 species were only found using *trn*L and 12 were shared between the two markers (Figure S3). Several families were identified using DNA that were not detected using microscopic counting. Families including Araliaceae, Equisetaceae, Myricaceae and Cornaceae were additionally identified by *trn*L, while Euphorbiaceae, Boraginaceae, Scrophulariaceae and Papaveraceae were additionally identified by nrITS2

(Figure S1, S2). The Euphorbiaceae were represented by *Mercurialis annua* and *M. perennis*, species of potential allergenic importance (Ariano et al., 1993). Within the three target taxa *Alnus*, Cupressaceae and Urticaceae, nrITS2 identified four families, nine genera and 16 species while *trn*L identified four families, six genera and three species (Figure 2).

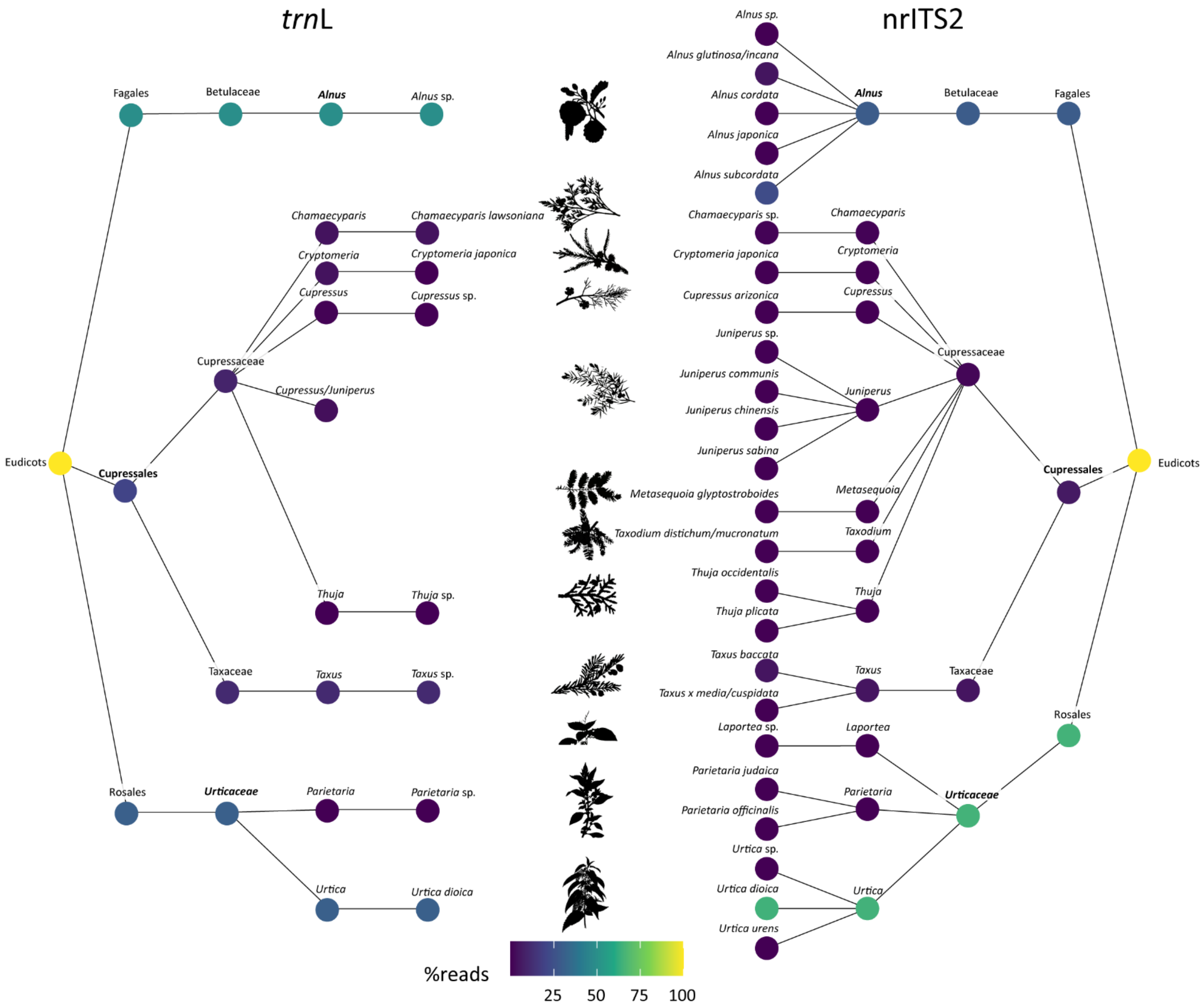


Figure 2. Taxonomic resolution for Alnus*, Cupressaceae and Urticaceae achieved using trn*L *and nrITS2 metabarcoding of pollen grains collected with a Burkard sampler at two pollen monitoring sites in the Netherlands. Result from trn*L *are on the left side while nrITS2 is shown on the right. Colours of the circles represent percentage of identified reads. The maximum taxonomic resolution achieved using microscopic pollen identification for the three target taxa is noted in bold.*

For *Alnus*, no taxa could be identified at species-level using *trn*L, while *Alnus cordata*, *A. japonica* and *A. subcordata* were identified by nrITS2. The latter two species are the parental species of the commonly planted artificial hybrid *Alnus x spaethii* (Spaeth Alder). The native species *Alnus glutinosa* and *A. incana* could not be distinguished from each other using nrITS2. For Cupressaceae, *trn*L identified five genera and two species, with some genera that could not be distinguished (*Cupressus*/*Juniperus*). nrITS2 could distinguish eight genera within the Cupressaceae, with most identifications at the species level (nine). Within the Urticaceae, two taxa were distinguished by *trn*L (*Urtica dioica* and *Parietaria* sp.) while three genera (*Urtica, Parietaria* and *Laportea*) were distinguished using nrITS2, with two species in both *Urtica* and *Parietaria*.

3.3 Pollen quantification using metabarcoding

Highly significant positive relationships between the relative abundance of sequencing reads (RRA) and relative abundance of microscopically counted pollen grains were found for all studied taxa using *trn*L and nrITS2 ($p < 0.001$ for all correlations; Figure 3). For *Alnus* the highest correlation was found using *trn*L ($R^2 = 0.969$) and nrITS2 ($R^2 = 0.952$). For the other two target taxa a lower correlation was found using *trn*L ($R^2 = 0.525$ and 0.664 for Cupressaceae and Urticaceae respectively) compared to nrITS2 ($R^2 = 0.637$ and 0.773). The regression line slopes also had lower values using *trn*L (0.589 and 0.416 for Cupressaceae and Urticaceae respectively) compared to nrITS2 (1.066 and 0.693), while a slope of ~0.97 was found for *Alnus* in both markers. The relationships were not affected by the material used (microscopic slide or unmounted tape). When combining the RRA values from all taxa in the dataset with >5 % relative abundance in the microscopic pollen counts, corresponding results were found with an R^2 value of 0.620 and slope of 0.588 for all *trn*L data, while the R^2 value was 0.821 for nrITS2, with a slope of 0.764 (Figure S4).

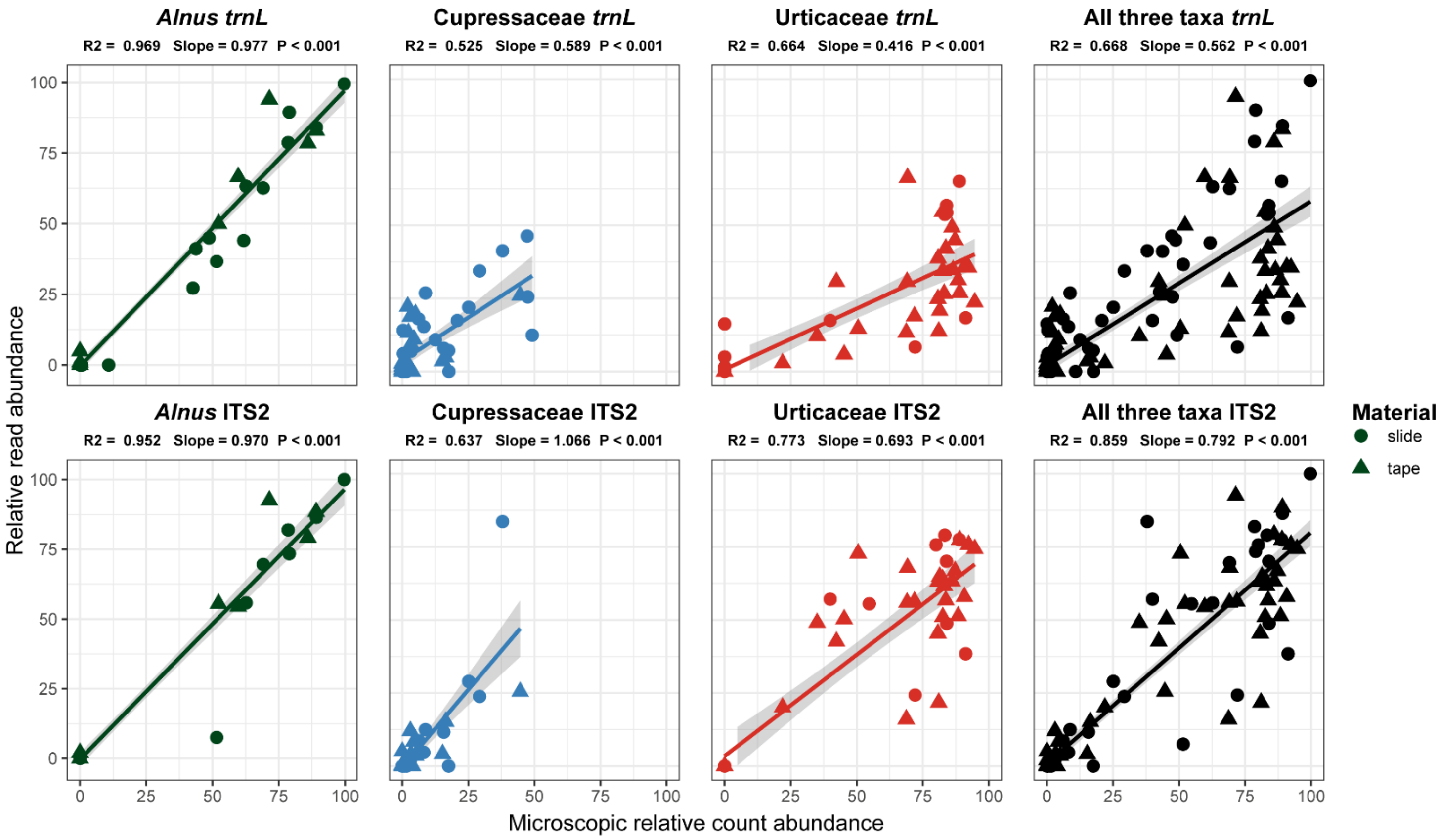


Figure 3. Correlations of microscopic pollen counts and sequencing read abundances. Regressions for Alnus *sp., Cupressaceae, Urticaceae as well as all three combined are shown. The top panels show the results of trn*L *and the bottom panels nrITS2. Comparisons are at the maximum taxonomic levels these taxa can be identified with a microscope. Pollen counts were converted to relative abundances for comparison to DNA relative read abundances.*

3.4 Trends in plant species abundance

Since nrITS2 results showed the highest taxonomic resolution and correlation between RRA and microscopically counted abundances, prevalence and presence of different plant species through time was only plotted for nrITS2 (Figure 4). In spring, the genus *Alnus* was dominated by native *Alnus glutinosa and A. incana* for both studied pollen monitoring sites. DNA from pollen of non-native *Alnus cordata* was most abundantly identified in samples from late February 2019 in the West of the Netherlands (up to 26.6%), while only very low abundances of this species were found in the South-east of the Netherlands. Non-native *Alnus japonica* and *A. subcordata* were found in high abundance in the sample from late December 2019 in the West of the Netherlands. Cupressaceae show highly diverse species recovery in spring, but the pollen spectra are almost entirely dominated by *Taxus baccata* at both pollen monitoring stations. In April, for the South-east of the Netherlands non-native *Chamaecyparis* sp. was found, while this taxon was absent in the West of the Netherlands.

Here, *Cupressus arizonica* was identified in the sample from April 2020. Native *Juniperus communis* was only found in very low abundance in April 2020 in the South-east of the Netherlands. In fall, Urticaceae pollen spectra are almost entirely dominated by *Urtica dioica* for both monitoring stations. *Urtica urens* was only found in low abundances in the fall of 2020 at both monitoring sites. Highly allergenic *Parietaria* species were detected in low abundances only in the West of the Netherlands in 2020. Finally, non-native *Laportea* was identified in the samples from the West of the Netherlands in 2020.

3.5 Comparison of monitoring sites and seasons

A perMANOVA of Bray-Curtis dissimilarities using RRA data of *trn*L and nrITS2 results showed significant discrimination between samples from spring and fall collected at the two Dutch pollen monitoring stations ($p < 0.001$ for both markers; Figure 5). For nrITS2 a slightly higher R^2 was found of 0.532 versus 0.440 for *trn*L. Spring and fall samples clearly fell within two

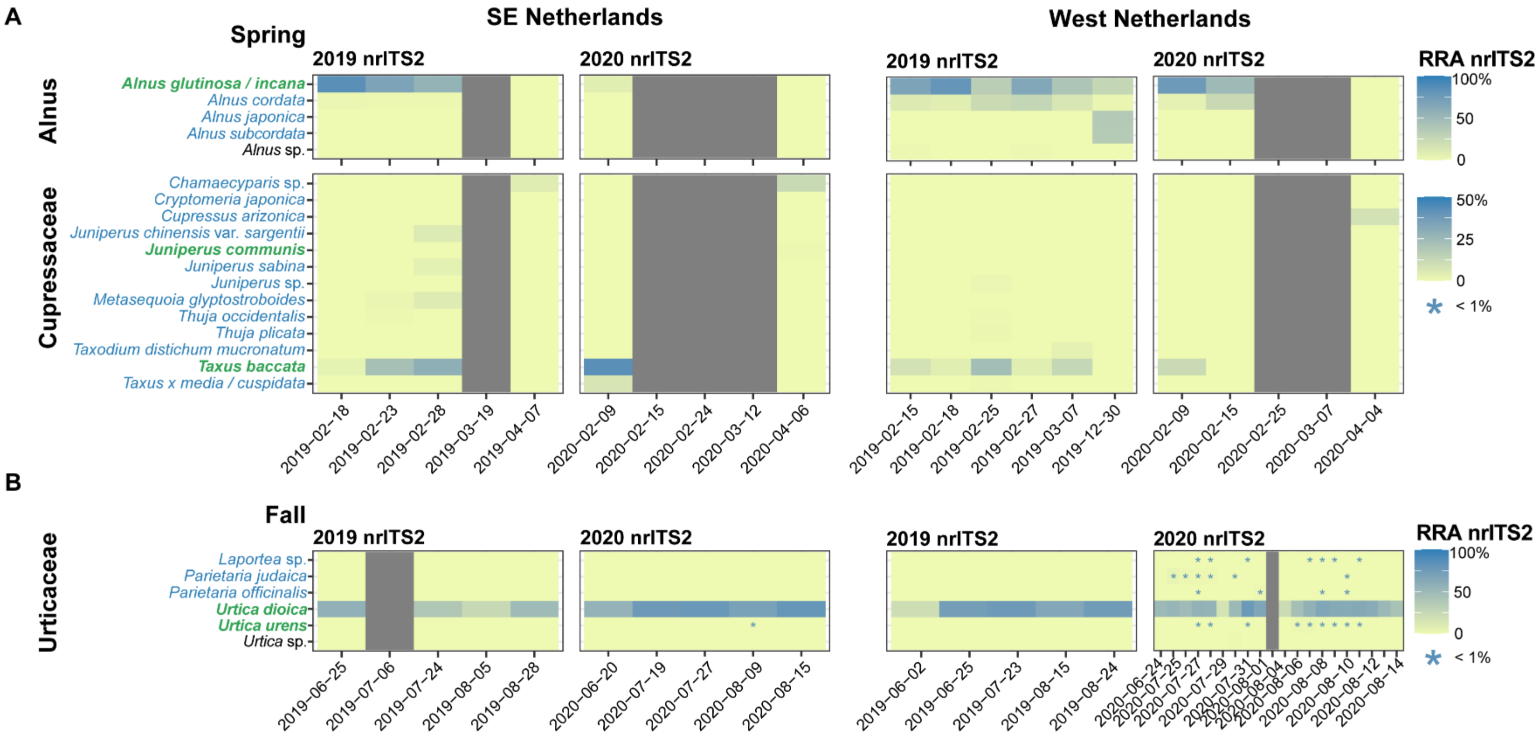


Figure 4. Relative nrITS2 molecular read abundance of species of Alnus, *Cupressaceae in spring and Urticaceae in fall of the 2019 and 2020 seasons of two pollen monitoring sites in the Netherlands (West and South-east of the Netherlands). The x-axis represents the material collection dates (see Figure 1). * presence at low relative abundance (< 1%). Taxa in green are native to the Netherlands, taxa in blue are either cultivated or introduced, and for taxa in black this is unknown. Grey bars indicate samples for which amplification failed.*

separated groups for both markers, and within these groupings the samples from both stations also clustered together. For *trn*L a higher overlap was identified, especially between the samples from the fall for the two stations, while these were more separated in nrITS2.

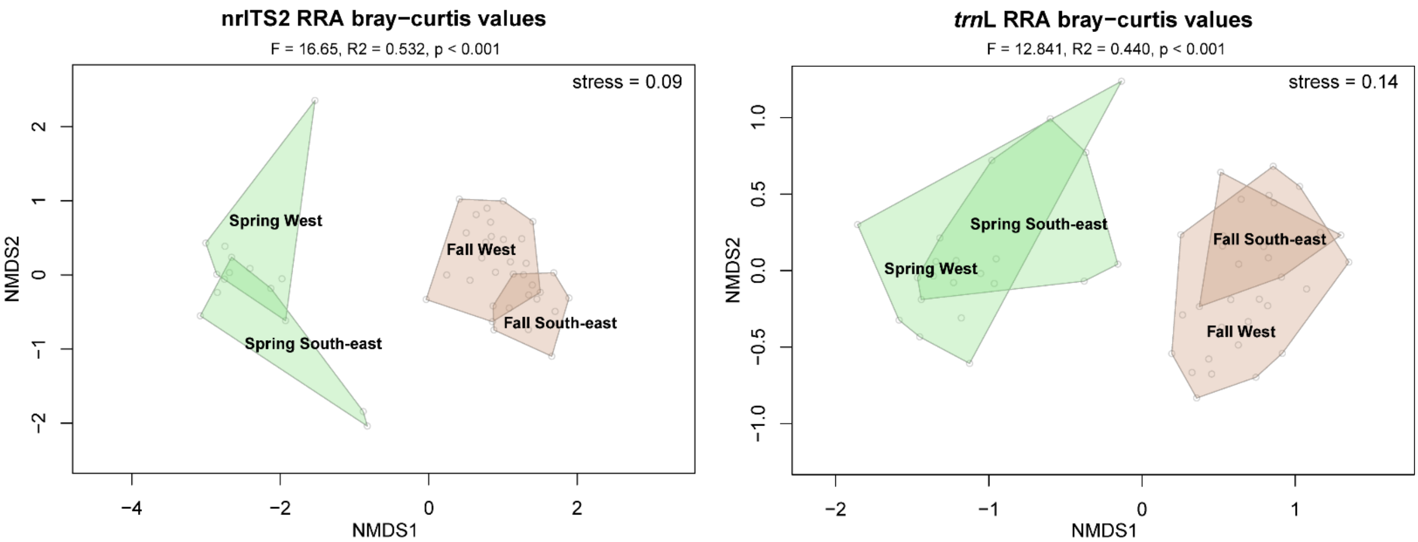


*Figure 5. Two-dimensional NMDS plots on RRA-based Bray-Curtis dissimilarities of trn*L *and nrITS2 results from spring and fall at the West and South-east of the Netherlands. Polygons in green represent samples from spring while those in brown represent fall.*

4. Discussion

While previous studies have shown that DNA can be amplified from pollen collected by Hirst-type samplers (Banchi et al., 2020; Campbell et al., 2020; Kraaijeveld et al., 2015; Leontidou et al., 2018), our study presents the first successful amplification of DNA from pollen that have been stained and mounted on microscopic slides. This opens up opportunities of utilizing the vast historic resources of daily microscopic slides that have been collected for decades at pollen monitoring stations all over the world (see Buters et al., 2018 for an overview of pollen monitoring stations). DNA studies on historical pollen species dynamics in ambient air can potentially be reconstructed back in time using our methodology.

4.1 Molecular airborne pollen monitoring

Previous studies on aerobiological samples have mostly relied on plastid *rbc*L which has limitations in taxonomic resolution (mostly to the genus level) and relatively poor quantitative performances (Bell et al., 2017; Uetake et al., 2021). Although less samples were successfully amplified with nrITS2 than using *trn*L in our study (48 versus 53), the qualitative performance of nrITS2 was significantly better than plastid *trn*L with double the amount of OTUs and >80% identified to the species level. Using *trn*L, several plant families were exclusively found that were also identified by microscopic pollen identification (Juncaceae and Pinaceae). However, these taxa were only present in <5% maximum relative abundance in the selected samples and do not represent important hay fever plants. In a recent study, Milla et al. (2021) found instead that *trn*L performed better than nrITS2 in Australian honey samples. However, as the authors indicate, DNA in these honey samples was degraded by long storage, causing the more stable and much shorter *trn*L P6 loop to be better preserved than nrITS2.

Our study adds to the growing body of evidence that nuclear markers are well suited for quantitative molecular pollen research (Banchi et al., 2020; Bänsch et al., 2020; Núñez et al., 2017; Richardson et al., 2021; Rowney et al., 2021). The correlation values for all taxa using *trn*L and nrITS2 in this study are very similar to those found in a recent study on bee-collected pollen quantification (Richardson et al., 2021). Here, at the genus level a relatively low correlation was found between *trn*L read proportions and microscopic proportions (R^2 = 0.456, $P < 0.001$) while these values were much higher for nrITS2 (R^2 = 0.846, $P < 0.001$). Furthermore, and similar to previous studies, the relationships in our study were taxon dependent and showed differences in the correlation slope (e.g., Baksay et al., 2020; Bell et al., 2019). The slope for the genus *Alnus* was very close to 1 in *trn*L and nrITS2, indicating that for this taxon the relative abundance of reads is almost exactly equal to the relative abundance of pollen in microscopic counts. For the family Urticaceae, however, a low slope value was found in the *trn*L results (0.416) and this underrepresentation of *trn*L RRA was also found for Urticaceae by Kraaijeveld et al. (2015). For plants, plastid and nuclear ribosomal ITS copy numbers per cell vary widely (Prokopowich et al., 2003). From our and previous quantification results it seems that plastid numbers per cell may be more variable than nuclear ribosomal copies, which may explain the better performance of nrITS2 versus *trn*L. Furthermore, plastid DNA is somewhat reduced in the paternal germ line, a feature that has led previous researchers to believe pollen did not contain any plastid DNA, although this has been disproven since (Bell et al., 2016b; Kraaijeveld et al., 2015). On the other hand, nrITS markers may be harder to amplify in plants as this marker has a relatively high GC content (Bell et al., 2016b; Mamedov et al., 2008; Richardson et al., 2019). This has led other researchers to find better quantification results using *trn*L compared to nrITS based on absolute read abundances (e.g., Baksay et al., 2020). However, in our study we find that very few taxa counted using a microscope were missed by nrITS2, and the ones that were missed (Juncaceae, Pinaceae) did not have a very high GC content but were more likely missed due to primer mismatches. When expected species contain high GC contents (>70%), amplification can be improved by adding DMSO additive to the PCR mix and/or lowering annealing temperatures (Varadharajan and Parani, 2021). Because of the highly increased taxonomic resolution and better semi-quantitative performance, we argue that nrITS2 should be the preferred marker of choice in molecular airborne pollen monitoring.

4.2 Pollen species dynamics

Using three case studies, we identified fine scale dynamics in species distribution patterns that could hitherto not be revealed. Within the allergenic genus *Alnus*, we find evidence that in late February a relatively large portion of the *Alnus* pollen is derived from non-native cultivated *Alnus cordata* (Italian alder), while in December the peak is mainly caused by *Alnus* x *spaethii* (Figure 4). The flowering periods of these alders prolong the alder hay fever season in the Netherlands. Traditionally, this was considered to last from February – early March (native *Alnus glutinosa* and *A. incana* flowering seasons), but *A. cordata* flowers from late February into early June (peak in April) and *A.* x *spaethii* from late December into early February (Duistermaat, 2020). These flowering periods correspond well with the dates in which we identified these species using nrITS2. *Alnus* x *spaethii* is of increasing interest to epidemiologists as it starts flowering significantly earlier than the native alders (Gehrig et al., 2015).

*Trn*L and nrITS2 could identify several genera within the Cupressaceae including many that are not native to the Netherlands (e.g. *Cryptomeria, Chamaecyparis, Cupressus, Taxodium, Thuja*). Plants from these genera are popular ornamentals in gardens and city parks in the Netherlands. Some species are well-known causal agents of pollinosis in their native range (including *Cryptomeria japonica* in Japan and *Cupressus arizonica* in the Mediterranean; D'Amato et al., 2007; Yasueda et al., 1983). However, our results show that pollen from these species is relatively insignificant as compared to highly abundant *Taxus baccata* pollen (common yew; Figure 4). Common yew is native to the Netherlands but is also often used as ornamental in hedges and gardens, which could explain its abundance in aerobiological samples. Even though yews are known to produce high amounts of pollen, their pollen is considered of low allergenic importance in Europe, as sensitization levels are very low (Puc et al., 2019). High cross-reactivity has been found, however, between Cupressaceae and Taxaceae (D'Amato et al., 2007).

For the Urticaceae pollen in fall, *Urtica dioica* plants are ubiquitous and highly abundant in the direct surroundings of both pollen monitoring stations, which explains the dominance of this species in the DNA results. Species of *Urtica* are of low allergenic relevance, but highly allergenic *Parietaria* spp. was additionally identified using both DNA markers. Species of *Parietaria* are one of the main causes of allergic rhinitis in the Mediterranean and they are currently undergoing a range expansion as a result of anthropogenic distribution and climate change (D'Amato et al., 2007; Fotiou et al., 2011). Although these genera can be distinguished using high resolution imaging and neural networks (Polling et al., 2021), they are not distinguishable using manual microscopic analysis. One unexpected element in the nrITS2 results for Urticaceae was the presence of the genus *Laportea* in samples from the fall of 2020 in the West of the Netherlands, as species of this genus are native to the Americas, Africa and Australasia (Jiarui et al., 2003). *Laportea* is not native or in cultivation in the Netherlands, so either pollen arrived from long-distance transport or the sequences are the result of a sequencing error. The last option seems unlikely since the differences in the sequence to those of native *Urtica* and *Parietaria* were large (maximum identification of 80% to *Urtica dioica* while this was 95% for *Laportea*). Therefore, the first option seems more likely. Pollen has been found before to be able to travel long distances (de Weger et al., 2016), and even to the Arctic (Campbell et al., 1999). Unfortunately, the species of *Laportea* could not be distinguished due to <97% identity, but the closest match was *L. canadensis* (native to North America) with 95% identity.

4.3 Pollen monitoring sites and seasons

The two pollen monitoring sites could be distinguished based on the taxonomic compositions of fall and spring samples (Figure 5). This was more clearly seen in the nrITS2 results than in *trn*L, likely because of the increased taxonomic resolution of nrITS2. The site-specific variation could be explained by native species that grow more or less exclusively in either the West of the Netherlands (e.g., *Spergularia media*, *Hippophae rhamnoides, Parietaria* spp.) versus the South-east of the Netherlands (e.g., *Juniperus communis*, *Quercus rubra* and *Mercurialis perennis*). Furthermore, several cultivated species were either only identified in the West of the Netherlands (e.g., *Phedimus* spp.*, Panicum virgatum, Alnus x spaethii*) or the South-east of the Netherlands (e.g., *Chamaecyparis* sp., *Cryptomeria japonica, Acer negundo*) indicating differences in the local environment surrounding the pollen monitoring sites. Lastly, some of the variance may be explained by a sampling effect, as more samples were used from the West of the Netherlands from the fall of 2020 (20) than from the South-east of the Netherlands (5). Nevertheless, both *trn*L and nrITS2 results could be used to infer statistically significant differences between the seasons and two pollen monitoring sites.

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Credit Author Statement

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Conflict of Interest

The authors declare no conflict of interest

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DNA metabarcoding using nrITS2 provides highly qualitative and quantitative results for airborne pollen monitoring

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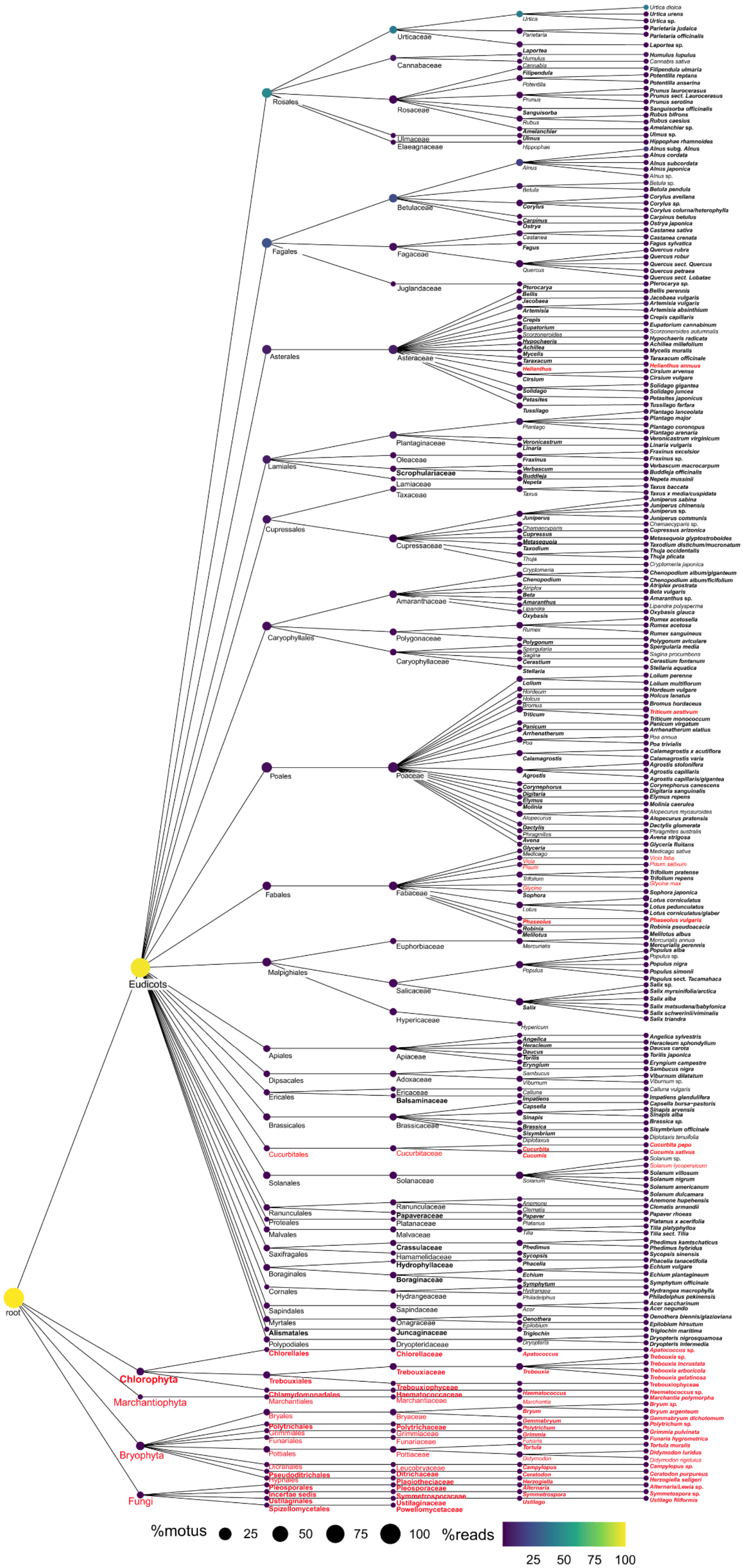
Supplementary Information (1/2)

Contents

Figure S1. All nrITS2 results

Taxa in red have been filtered out, either because it wasn't an eudicot, or because it was interpreted as contamination. Taxa in bold was uniquely found in the DNA results of nrITS2.


root
Eudicots
Chlorophyta
Marchantiophyta
Bryophyta
Fungi
Rosales
Urticaceae
Cannabaceae
Rosaceae
Ulmaceae
Elaeagnaceae
Fagales
Betulaceae
Fagaceae
Juglandaceae
Asterales
Asteraceae
Lamiales
Plantaginaceae
Oleaceae
Scrophulariaceae
Lamiaceae
Taxaceae
Cupressales
Cupressaceae
Caryophyllales
Amaranthaceae
Polygonaceae
Caryophyllaceae
Poales
Poaceae
Fabales
Fabaceae
Malpighiales
Euphorbiaceae
Salicaceae
Hypericaceae
Apiales
Apiaceae
Dipsacales
Adoxaceae
Ericales
Ericaceae
Balsaminaceae
Brassicales
Brassicaceae
Cucurbitales
Cucurbitaceae
Solanales
Solanaceae
Ranunculales
Ranunculaceae
Papaveraceae
Proteales
Platanaceae
Malvales
Malvaceae
Saxifragales
Crassulaceae
Hamamelidaceae
Hydrophyllaceae
Boraginales
Boraginaceae
Cornales
Hydrangeaceae
Sapindales
Sapindaceae
Myrtales
Onagraceae
Alismatales
Juncaginaceae
Polypodiales
Dryopteridaceae
Chlorellales
Chlorellaceae
Trebouxiales
Trebouxiaceae
Trebouxiophyceae
Chlamydomonadales
Haematococcaceae
Marchantiales
Marchantiaceae
Bryales
Bryaceae
Polytrichales
Polytrichaceae
Grimmiales
Grimmiaceae
Funariales
Funariaceae
Pottiales
Pottiaceae
Dicranales
Leucobryaceae
Pseudoditrichales
Ditrichaceae
Hypnales
Plagiotheciaceae
Pleosporales
Pleosporaceae
Incertae sedis
Symmetrosporaceae
Ustilaginales
Ustilaginaceae
Spizellomycetales
Powellomycetaceae
%motus 25 50 75 100
%reads
25 50 75 100

2

Figure S2. All *trn*L results

Taxa in red has been filtered out, either because it wasn't an eudicot, or because it was interpreted as contamination. Taxa in bold was uniquely found in the DNA results of *trn*L.

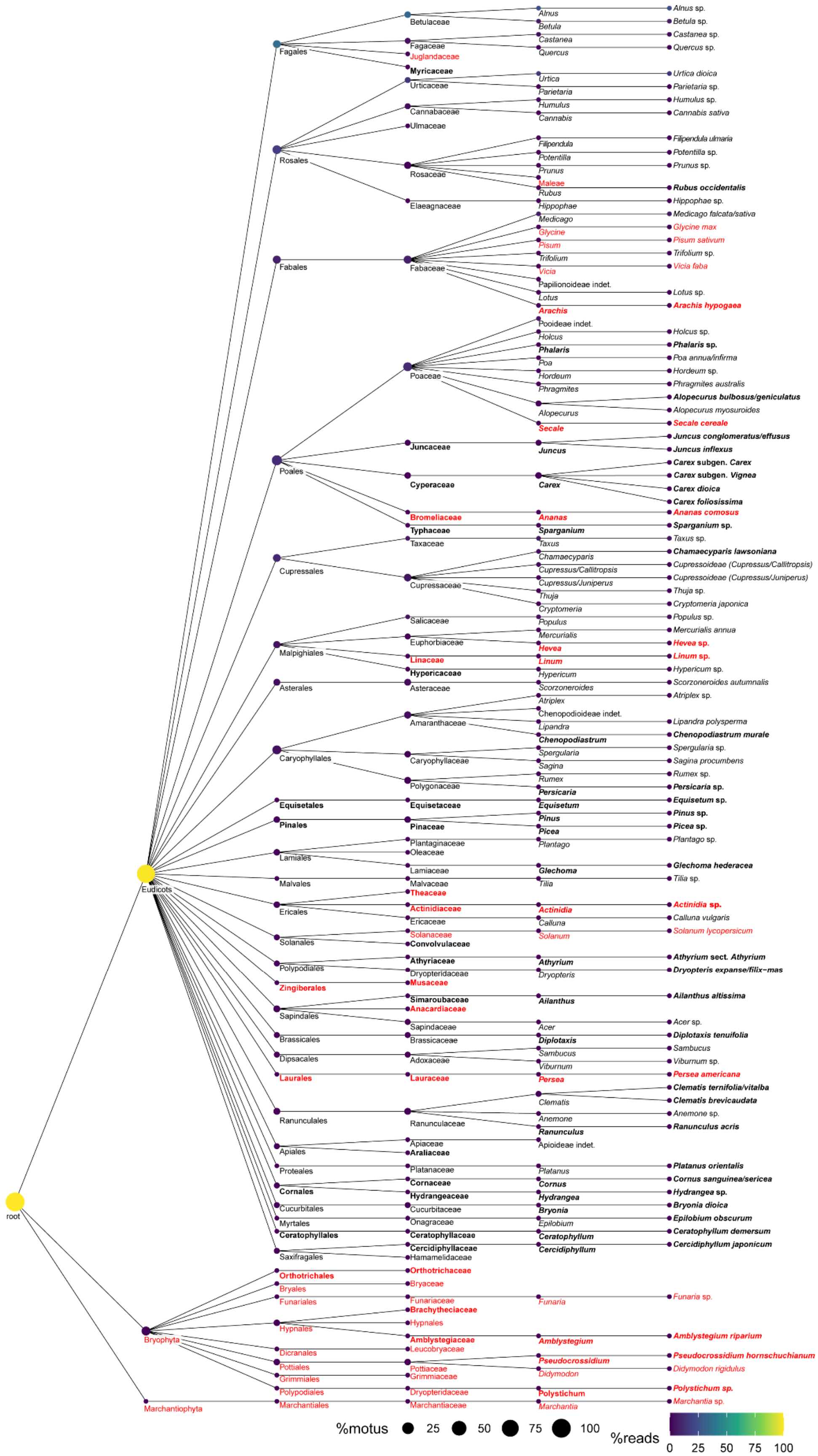


Figure S3. Venn diagrams all data

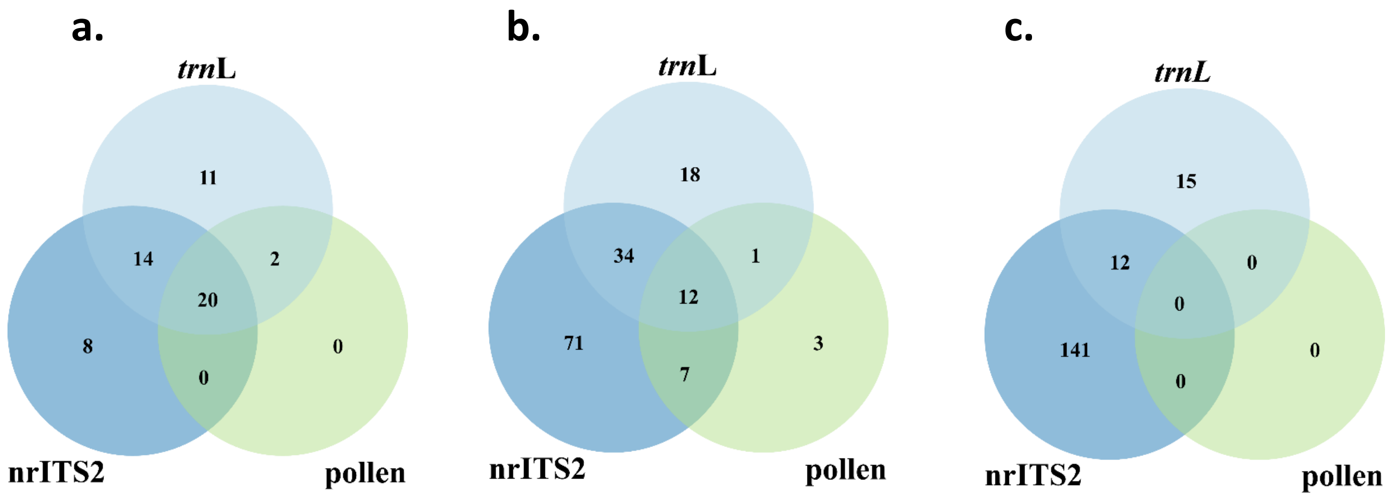


Figure S3. Venn diagrams of all recovered taxa at different taxonomic levels a) family, b) genus and c) species level

Figure S4. RRA Correlations

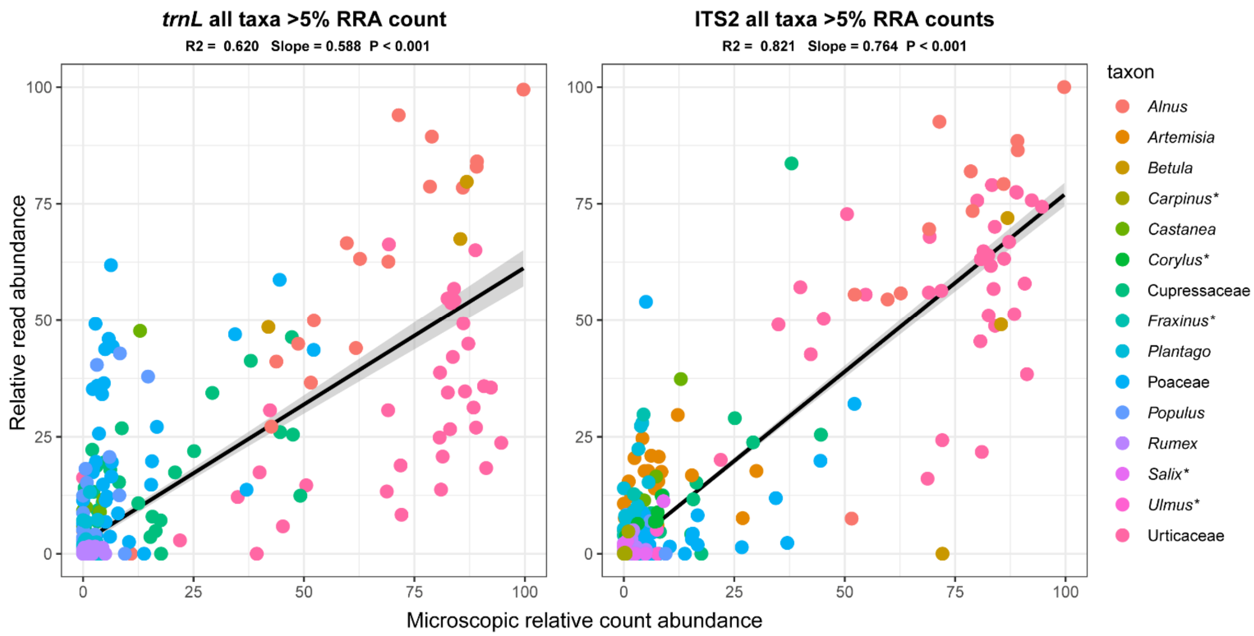


*Figure S4. Molecular relative read abundance regressed against relative abundance of microscopic pollen counts for all taxa recovered using trn*L *and nrITS2 in the 58 studied aerobiological samples. Taxa are indicated using unique colors, showing only those that were present in >5% relative abundance in the microscopic pollen counts. Comparisons are performed at the maximum taxonomic level that can be achieved using microscopic pollen identification. Taxa denoted with a * were only identified using nrITS2.*

DNA metabarcoding using nrITS2 provides highly qualitative and quantitative results for airborne pollen monitoring

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Supporting Information (2/2)

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Table S1. Read, OTU and sample counts after filtering

	nrITS2			*trnL*		
Filtering step	**reads**	**OTUs**	**Samples**	**reads**	**OTUs**	**Samples**
Raw data	7,533,375		51*	8,584,785		58
Merged and demultiplexed	7,409,641			7,855,880		
Clustering		1329			626	
Removal of OTUs with ID <90%		1106			271	
Leakage removal*	7,392,223			7,717,079		
Removal OTUs with <10 reads, aggregating OTUs with same taxonomic assignment	7,391,979	393		7,715,474	214	
Removal of PCR replicates with <3,000 reads	7,389,579	390	36 + 12 single PCR rep	7,704,427		52 + 1 single PCR rep
Removal of OTUs only present in one PCR replicate per sample and samples with <2 PCR reps	6,576,188	231		7,044,944	146	
Removal of suspected food contaminants	6,422,503	219		6,810,278	129	
Removal of OTUs from algae, mosses, fungi	6,395,889	191		6,809,072	98	
FINAL	**6,395,889**	**191**	**36 + 12†**	**6,787,445**	**98**	**52 + 1†**
Per sample	**52,775 ± 4,671**	**14.4 ± 1.7**		**46,784 ± 4,241**	**11.8 ± 1.0**	

* for nrITS2, 7 samples did not produce any DNA after the two PCR steps and these were not used for sequencing

† For nrITS2, 12 samples only had one PCR replicate left after the filtering steps, while this was one sample in the trnL dataset

Table S2. Idenitity and abundance of OTU's *trn* L

OTU	Total read (	best_id	best_id	cover	order	family	subfamily	tribe	genus/subg.	species	maxid	scientific rank	OTU sequence	length of sequence
Otu085	2207	1.00	1.00	100	Apiales	Apiaceae	Apioideae				Apioideae	subfamily	atcctattttccaaaaacaaacaaaggcccagaaggtgaaaaaag	45
Otu125	264	1.00	1.00	100	Apiales	Araliaceae					Araliaceae	family	atcctgttttccgaaaacaaacaaaggttcagaaggcgaaaaaagg	46
Otu011	144351	1.00	1.00	100	Asterales	Asteraceae	Asteroideae	Anthemideae			Anthemideae	tribe	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaagaaaaaaaa	51
Otu081	2823	1.00	1.00	100	Asterales	Asteraceae	Cichorioideae	Cichorieae			Crepidinae	tribe	atcacgttttccgaaaacaaacaaagattcagaaagcgaaaatcaaaaag	50
Otu100	2521	1.00	1.00	100	Asterales	Asteraceae	Cichorioideae		*Scorzoneroides*	*Scorzoneroides autumnalis*	*Scorzoneroides autumnalis*	species	atcacgttttccgaaaacacacaaaggttcagaaagcgaaaatcaaaaag	50
Otu032	31082	1.00	1.00	100	Asterales	Asteraceae					Asteraceae	family	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaataaaaaag	50
Otu123	1170	1.00	1.00	100	Brassicales	Brassicaceae			*Diplotaxis*	*Diplotaxis tenuifolia*	*Diplotaxis tenuifolia*	species	atcctgggttacgcaaacaaaccagagtttagaaagcgg	39
Otu053	9974	1.00	1.00	100	Brassicales	Brassicaceae					Brassicaceae	family	atcctgggttacgcgaacaaaccagagtttagaaagcgg	39
Otu190	331	1.00	1.00	100	Caryophyllales	Caryophyllaceae		Alsineae			Alsineae	tribe	ctccttaattgtctcctaaaaaaaacggattcaaaaagcaagaataaaaaag	52
Otu074	4428	1.00	1.00	100	Caryophyllales	Caryophyllaceae		Sagineae	*Sagina*	*Sagina procumbens*	*Sagina procumbens*	species	ctcctttttttgtctccctttttcaaaagacaaaaaggattcagaaagcaagaataaaacag	62
Otu046	15387	1.00	1.00	100	Caryophyllales	Caryophyllaceae		Sperguleae	*Spergularia*		*Spergularia*	genus	ctccttttgtctcctttttttcaaaagcaaaaataaaaaag	40
Otu026	31442	1.00	1.00	100	Caryophyllales	Chenopodiaceae	Chenopodioideae	Atripliceae	*Atriplex*		*Atriplex*	genus	ctccttttgtcaaaagcaaaaaagaaagattcagaaagcaagaataaaaaaag	53
Otu135	1655	1.00	1.00	100	Caryophyllales	Chenopodiaceae	Chenopodioideae	Atripliceae	*Chenopodiastrum*	*Chenopodiastrum murale*	*Chenopodiastrum murale*	species	ctccttttgtcaaaagcaaaaaatactcaaaagaaaaaaataataaaaaagcaagaaaaaaaaaag	65
Otu124	1855	1.00	1.00	100	Caryophyllales	Chenopodiaceae	Chenopodioideae	Atripliceae	*Lipandra*	*Lipandra polysperma*	*Lipandra polysperma*	species	ctcctttttttcaaaagcaaaaaataaggattcagaaagcaagaataaaaaaaag	54
Otu013	137845	0.00	1.00	100	Caryophyllales	Chenopodiaceae	Chenopodioideae	Atripliceae			Atripliceae	tribe	ctccttttgtcaaaagcaaaaaataaagattcagaaagcaacaataaaaaaag	53
Otu071	6529	1.00	1.00	100	Caryophyllales	Chenopodiaceae	Chenopodioideae				Chenopodioideae	subfamily	ctcctttttttcaaaagcaagaataaaaaaaaaag	33
Otu153	441	1.00	1.00	100	Caryophyllales	Polygonaceae	Polygonoideae	Persicarieae	*Persicaria*		*Persicaria*	genus	ctcctgctttcaaaaaggaaagaaaaaagg	30
Otu115	1705	1.00	1.00	100	Caryophyllales	Polygonaceae	Polygonoideae	Polygoneae			Polygoneae	tribe	ctccttctttccaaaaggaagacaaaag	28
Otu050	10692	1.00	1.00	100	Caryophyllales	Polygonaceae	Polygonoideae	Rumiceae	*Rumex*		*Rumex*	genus	ctcctcctttccaaaaggaagaataaaaaag	31
Otu096	1830	1.00	1.00	100	Caryophyllales	Polygonaceae					Polygonaceae	family	ctccttctttccaaaaggaagaaaaag	27
Otu200	614	0.00	0.96	100	Ceratophyllales	Ceratophyllaceae			*Ceratophyllum*	*Ceratophyllum demersum*	*Ceratophyllum demersum*	species	atccttgatttgagaaaacaagggttccgaaatcgagaatcaaaaaatag	50
Otu177	418	1.00	1.00	100	Cornales	Hydrangeaceae			*Hydrangea*		*Hydrangea*	genus	atcctgttttccgaaaacaaacaaaagttcagaaagcgaaaatcaaaaaag	51
Otu120	1061	1.00	1.00	100	Cornales	Cornaceae			*Cornus*		*Cornus sanguinea/sericea*	genus	atcctgttttccgaaaacaaacaaaagttcagaaagcgaaaataaaaaaag	51
Otu294	89	1.00	1.00	100	Cucurbitales	Cucurbitaceae			*Bryonia*	*Bryonia dioica*	*Bryonia dioica*	species	atcctttttccgaaaaaaaaaaaagg	26
Otu012	135406	1.00	1.00	100	Cupressales	Cupressaceae	Cupressoideae		*Chamaecyparis*	*Chamaecyparis lawsoniana*	*Chamaecyparis lawsoniana*	species	atccgatttctagagacaacagtttcctttccgaaaaagg	40
Otu015	106035	1.00	1.00	100	Cupressales	Cupressaceae	Cupressoideae		*Cupressus/Callitropsis*		Cupressoideae (Cupressus/Callitropsis)	subfamily	atccgatttctagaaacaataggttcctttccgagaacgg	40
Otu041	17384	1.00	1.00	100	Cupressales	Cupressaceae	Cupressoideae		*Cupressus/Juniperus*		Cupressoideae (Cupressus/Juniperus)	subfamily	atccgatttctagagacaatagtttcctttccgagaacgg	40
Otu061	8152	1.00	1.00	100	Cupressales	Cupressaceae	Cupressoideae		*Thuja*		*Thuja*	genus	atctcgatttctagagaaagg	21
Otu107	6069	1.00	1.00	100	Cupressales	Cupressaceae	Taxodioideae		*Cryptomeria*	*Cryptomeria japonica*	*Cryptomeria japonica*	species	atcctatttatagagacaatagtttcctttccgagaaagg	40
Otu029	32494	1.00	1.00	100	Cupressales	Cupressaceae					Cupressaceae (*Chamaecyparis* /Sequoioideae)	family	atccgatttctagagacaatagtttcctttccgagaaagg	40
Otu006	368567	1.00	1.00	100	Cupressales	Taxaceae			*Taxus*		*Taxus*	genus	atccgtattataggaacaataattttattttctagaaaagg	41
Otu066	4755	1.00	1.00	100	Dipsacales	Adoxaceae			*Sambucus*		*Sambucus*	genus	atcctgttttccgaaaacaaacaaaggttcagaaagcgaaaataaaaaag	50
Otu270	679	0.98	0.98	100	Dipsacales	Adoxaceae			*Viburnum*		*Viburnum*	genus	atcctgttttccgaaaacaaaaaaagaagcgaaaaaaaaaaaag	44
Otu020	55202	1.00	1.00	100	Equisetales	Equisetaceae			*Equisetum*		*Equisetum*	genus	attctatttag	11
Otu048	12889	1.00	1.00	100	Ericales	Ericaceae			*Calluna*	*Calluna vulgaris*	*Calluna vulgaris*	species	atcctttttttcgcaaacaaacaaagattccgaaaggtaaaaaaaag	47
Otu072	5085	1.00	1.00	100	Fabales	Fabaceae	Faboideae		*Lotus*		*Lotus*	genus	atcctgctttacgaaaacaagggaaagttcagttaagaaagcgacgagaaaaatg	55
Otu003	468808	1.00	1.00	100	Fabales	Fabaceae	Faboideae		*Medicago*		*Medicago falcata/sativa*	genus	atccttctttccgaaaacaaataaataaaagttcagaaagttaaaatcaaaaaag	55
Otu038	14031	1.00	1.00	100	Fabales	Fabaceae	Faboideae		*Trifolium*		*Trifolium*	genus	atccttctttccgaaaacaaaataaaagttcagaaagttaaaataaaaaagg	52
Otu064	242	1.00	1.00	100	Fabales	Fabaceae	Papilionoideae				Papilionoideae	subfamily	atcctgttttccgaaaacaaagaagagttcagaaagcgagaataaaaaaag	51
Otu001	1768884	1.00	1.00	100	Fagales	Betulaceae			*Alnus*		*Alnus*	genus	atcctgttttccgaaaacaaataaaacaaattgaagggttcataaagcgagactaaaaaag	61
Otu004	446317	1.00	1.00	100	Fagales	Betulaceae			*Betula*		*Betula*	genus	atcctgttttccgaaaacaaataaaacaaatttaagggttcataaagtgagaataaaaaag	61
Otu014	136014	1.00	1.00	100	Fagales	Betulaceae					Betulaceae	family	atcctgttttcccaaaacaaataaaacaaatttaagggttcataaagcgagaataaaaaag	61
Otu008	189420	1.00	1.00	100	Fagales	Fagaceae			*Castanea*		*Castanea*	genus	atcctattttacgaaaacaaataagggttcagaagaaagcgagaataaaaaaaag	55
Otu019	66077	1.00	1.00	100	Fagales	Fagaceae			*Quercus*		*Quercus*	genus	atcctattttacgaaaacaaataagggttcagaagaaagcaagaataaaaaaag	54
Otu128	1782	1.00	1.00	100	Fagales	Myricaceae					Myricaceae	family	atccggttttctgaaaacaaataagggttcataaagcgagaatcaaaaag	50
Otu227	313	1.00	1.00	100	Lamiales	Lamiaceae			*Glechoma*	*Glechoma hederacea*	*Glechoma hederacea*	species	atcctgttttctcaaaaccaaggttcaaaaaacgaaaaaaaaag	44
Otu031	23446	1.00	1.00	100	Lamiales	Oleaceae					Oleaceae	family	atcctgttttcccaaaacaaaggttcagaaagaaaaaag	39
Otu023	48063	1.00	1.00	100	Lamiales	Plantaginaceae			*Plantago*		*Plantago*	genus	atcctgtcttctcaaaataaaggttcagaaagcgagaaaaagg	43
Otu055	925	1.00	1.00	100	Malpighiales	Euphorbiaceae			*Mercurialis*	*Mercurialis annua*	*Mercurialis annua*	species	atccggttttccgaaaacaaacaaaggttcatatcataaagacagaataaaaaag	55
Otu176	177	1.00	1.00	100	Malpighiales	Hypericaceae			*Hypericum*		*Hypericum*	genus	atccggctttccgaaaacaaagcaaagaaagattcataaacagaaaaaaaag	52
Otu007	272264	1.00	1.00	100	Malpighiales	Salicaceae			*Populus*		*Populus*	genus	atcctatttttcgaaaacaaacaaaaaaacaaacaaaggttcataaagacagaataagaatacaaaag	68
Otu028	27575	1.00	1.00	100	Malvales	Malvaceae			*Tilia*		*Tilia*	genus	atcctattattttacgaaaaaaaaacaaaggttcagcaagcgagaataataaaaagaag	59
Otu131	1811	1.00	1.00	100	Myrtales	Onagraceae			*Epilobium*	*Epilobium obscurum*	*Epilobium obscurum*	species	atcctattttacgaaaaccagcaccgggggtttagaaagcgataaaaaaaaag	53
Otu154	1357	1.00	1.00	100	Pinales	Pinaceae			*Picea*		*Picea*	genus	atccggttcatggagacaatagtttcttcttttattctcctaagataggaaggg	54
Otu021	47338	1.00	1.00	100	Pinales	Pinaceae			*Pinus*		*Pinus*	genus	atccggttcatgaagacaatgtttcttctcctaagataggaaggg	45
Otu335	45	1.00	1.00	100	Pinales	Pinaceae					Pinaceae	family	atccggttcatagagacaatggtttctttcctaggataggaaggg	45
Otu134	2639	1.00	1.00	100	Poales	Cyperaceae			*Carex*	*Carex dioica*	*Carex dioica*	species	atcttctttttgagaaaaagaaatatagaaaatatttcttatttcagataagaaagaataatatttcttatctaatattaaa	82
Otu168	983	0.99	0.99	100	Poales	Cyperaceae			*Carex*	*Carex foliosissima*	*Carex foliosissima*	species	atcttcttgttctcaaaaagaaatatataaaatatttcttatttcagataagaaataatattttttcttatctaatattaaa	83
Otu165	969	1.00	1.00	100	Poales	Cyperaceae			*Carex*		*Carex* subgen. *Vignea*	subgenus	atcttcttttgagaaaaagaaatatataaaatatttcttatttcagataagaaagaataatatttcttatctaatattaaa	82
Otu076	5233	1.00	1.00	100	Poales	Cyperaceae			*Carex* subgen. *Carex*		*Carex* subgen. Carex	subgenus	atcttcttttgagaaaaagaaatatataaaatatttcttatttcagataagaaataatatttttttcttatctaatattaaa	82
Otu138	1498	1.00	1.00	100	Poales	Juncaceae			*Juncus*	*Juncus inflexus*	*Juncus inflexus*	species	atctttattttgataaaatttgtttttatagaaaaactcaaatcaaaaaa	50
Otu058	9285	1.00	1.00	100	Poales	Juncaceae			*Juncus*		Juncus conglomeratus/effusus	species	atctttattttgataaaatttgtttttatagaaaaattcaaatcaaaaaa	50
Otu054	1715	1.00	1.00	100	Poales	Poaceae	Arundinoideae	Molinieae	*Phragmites*	*Phragmites australis*	*Phragmites australis*	species	atcccttttttgaaaaaacaggtggttctcaaactagaacccaaaggaaag	52
Otu025	51506	1.00	1.00	100	Poales	Poaceae	Pooideae	Aveneae			Poeae Chloroplast Group 1 (Aveneae type)	tribe	atccgtgttttgagaaaacaaaggggttctcgaatcgaactaataatacaaaggaaaag	58
Otu042	11340	1.00	1.00	100	Poales	Poaceae	Pooideae	Hordeeae	*Hordeum*		*Hordeum*	genus	atccgtgttttgagaagggattctcgaactagaatacaaaggaaaag	47
Otu097	1429	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae	*Alopecurus*	*Alopecurus myosuroides*	*Alopecurus myosuroides*	species	atccgtgttttgagaaaactagggggttcgcaactagaatacaaaggaaaag	53
Otu059	9391	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae	*Alopecurus*		*Alopecurus bulbosus/geniculatus*	genus	atccgtattttgagaaaaccagggggttctcgaactagaatacaaaggaaaag	53
Otu016	100118	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae	*Holcus*		*Holcus*	genus	atccgtgttttgagaaaacaagggggttttccaactagaatacaaaggaaaag	53
Otu022	42618	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae	*Phalaris*		*Phalaris*	genus	atccgtattttgagaaaacaagggggttctcgaactagaatacaaaggaaaag	53
Otu037	16546	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae	*Poa annua/infirma*		Poa annua/infirma	species	atccatgttttgagaaaacaagggggttctcgaactagaatacaaaggaaaag	53
Otu018	152021	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae			Poeae	tribe	atccgtgttttgagaaaacaagggggttctcgaactagaatacaaaggaaaag	53
Otu005	390741	1.00	1.00	100	Poales	Poaceae	Pooideae				Pooideae	subfamily	atccgtgttttgagaaaacaaggaggttctcgaactagaatacaaaggaaaag	53
Otu027	47221	1.00	1.00	100	Poales	Poaceae					PACMAD clade	family	atcccttttttgaaaaaacaagtggttctcaaactagaacccaaaggaaaag	52
Otu276	233	1.00	1.00	100	Poales	Typhaceae			*Sparganium*		*Sparganium*	genus	atcctgatattttgataaaacaagggtttataaaactagaatcaaaaaggaag	53
Otu044	14173	1.00	1.00	100	Polypodiales	Athyriaceae			*Athyrium*		*Athyrium* sect. *Athyrium*	genus	atcttgtattattcggatgaatttcgggcgatgaggcga	39
Otu062	7561	1.00	1.00	100	Polypodiales	Dryopteridaceae			*Dryopteris*		*Dryopteris expanse/filix-mas*	genus	atcttgtcttactcaaatgaatttcgggcgatgaggcga	39
Otu108	2337	1.00	1.00	100	Proteales	Platanaceae			*Platanus*	*Platanus orientalis*	*Platanus orientalis*	species	atcctgttttcagaaaacgagaaaaaaaaggaaag	35
Otu121	3169	1.00	1.00	100	Ranunculales	Ranunculaceae			*Anemone*		*Anemone*	genus	atccttatttcagaaaacaaaaggggggttcagaaagcaagaataaaaaaag	51
Otu162	5941	1.00	1.00	100	Ranunculales	Ranunculaceae			*Clematis*	*Clematis brevicaudata*	*Clematis brevicaudata*	species	atcctttttttcagaaaacaaaagggcttcagaaagcaagaataaaaaaaaaag	52
Otu083	108	1.00	1.00	100	Ranunculales	Ranunculaceae			*Clematis*		*Clematis ternifolia/vitalba*	genus	atcctttttttcagaaaacaaaagggcttcagaaagcaagaataaaaaaaaaaag	53
Otu232	111	1.00	1.00	100	Ranunculales	Ranunculaceae			*Ranunculus*	*Ranunculus acris*	*Ranunculus acris*	species	atcctgctttcagaaagcaaaaaaagg	27
Otu040	485	1.00	1.00	100	Rosales	Cannabaceae			*Cannabis*	*Cannabis sativa*	*Cannabis sativa*	species	atccggttttctgaaaacaaacaaggattcagaaagcaataataaaaaagaatag	55
Otu010	168920	1.00	1.00	100	Rosales	Cannabaceae			*Humulus*		*Humulus*	genus	atccggttttctgaaaacaaacaaggattcagaaagcaataataaaggg	49
Otu109	1483	1.00	1.00	100	Rosales	Elaeagnaceae			*Hippophae*		*Hippophae*	genus	atccagttttctgattcttaaaacaaacaagggttcagaaagcaatacaaag	52
Otu090	1403	1.00	1.00	100	Rosales	Rosaceae	Amygdaloideae		*Prunus*		*Prunus*	genus	atcctgttttattaaaacaaacaagggtttcataaaccgagaataaaaaag	51
Otu079	4923	1.00	1.00	100	Rosales	Rosaceae	Rosoideae		*Potentilla*		*Potentilla*	genus	atcccgttttatgaaaacaaacaaaggtttcagaaaccgagaataaataaag	52
Otu178	880	1.00	1.00	100	Rosales	Rosaceae	Rosoideae		*Rubus*	*Rubus occidentalis*	*Rubus occidentalis*	species	atcccgttttatgaaaacaaacaaaggtttcagaaagcgagaataaataaag	52
Otu170	939	1.00	1.00	100	Rosales	Rosaceae			*Filipendula*	*Filipendula ulmaria*	Filipendula ulmaria	species	atcccgttttctgaaaacaaacacgggtttcagaaatccagaataaataaagtaaag	57
Otu069	3279	1.00	1.00	100	Rosales	Rosaceae					Rosaceae	family	atcccgttttatgaaaacaaacaagggtttcagaaagcgagaataaataaag	52
Otu034	22250	1.00	1.00	100	Rosales	Ulmaceae					Ulmaceae	family	atcctgttttatgaaaataagggttcataaagcgataataaaaaag	46
Otu094	88	1.00	1.00	100	Rosales	Urticaceae			*Parietaria*		*Parietaria*	genus	atccagttttatgaaaacaaacaagggttcagaaagcgataataaaaacagaaag	55
Otu002	1009222	1.00	1.00	100	Rosales	Urticaceae			*Urtica*		Urtica dioica	species	atctggtgttataaaaacaagcgataaaaaaaag	34
Otu130	1467	1.00	1.00	100	Sapindales	Sapindaceae			*Acer*		*Acer*	genus	atcctgttttacgagaataaaacaaagcaaacaagggttcagaaagcgagaaaggg	56
Otu118	2259	1.00	1.00	100	Sapindales	Sapindaceae					Sapindaceae	family	atcctgttttacgagaataaaacaaaacaaacaagggttcagaaagcgagaaaag	55
Otu051	11193	1.00	1.00	100	Sapindales	Simaroubaceae			*Ailanthus*	*Ailanthus altissima*	*Ailanthus altissima*	species	atcctgttttacaagaataaataagggttcagaaagcaaaaaagggg	47
Otu205	355	1.00	1.00	100	Saxifragales	Cercidiphyllaceae			*Cercidiphyllum*	*Cercidiphyllum japonicum*	*Cercidiphyllum japonicum*	species	atcctgttttccgaaaacaaacacaagggttcagaaagcgagaataaaaaaag	53
Otu266	233	1.00	1.00	100	Saxifragales	Hamamelidaceae					Hamamelidaceae	family	atcctgttttccgaaaacaaacacaagggttcagaaagcgagaataaaaatcaaaataaaaaaag	65
Otu101	2768	1.00	1.00	100	Solanales	Convolvulaceae					Convolvulaceae	family	atcctgttttccgaaaacaaacaaaagttcagaaaaaag	39

Table S3. Identity and abundance of OTU's *trn* L

Any OTU with <0.1% relative read abundance is shown as a +

% represents the Relative Read Abundance

Legend	
(green)	**Spring**
(brown)	**Fall**
red text	**Sample failed amplification**

			South-east Netherlands (Elkerliek, Helmond)																																											West Netherlands (LUMC, Leiden)	
			2019-02-18		2019-02-23		2019-02-28		2019-03-22		2019-04-07		2019-06-25		2019-07-06		2019-07-24		2019-08-05		2019-08-28		2020-02-09		2020-02-15		2020-02-24		2020-03-12		2020-04-06		2020-06-26		2020-07-19		2020-07-27		2020-08-09		2020-08-15		2019-02-15		2019-02-18		2019-02-25	
OTU	Total read	maxid	Average read	%	Average read co	%	Average read	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%
Otu001	1768884	*Alnus*	56278.5	84.1	44913.7	62.6	48334.3	63.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	40268.0	36.6	13728.5	44.9	22952.3	27.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	55079.7	78.5	53046.7	82.9	39335.3	50.0
Otu002	1009222	*Urtica dioica*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7607.0	17.4	0.0	0.0	2952.7	18.3	784.3	8.3	8282.5	54.3	0.0	0.0	0.0	0.0	4196.7	5.0	7210.0	16.3	1603.0	1.5	0.0	0.0	0.0	0.0	18932.5	65.1	11466.3	56.8	9571.0	53.7	0.0	0.0	0.0	0.0	0.0	0.0
Otu003	468808	*Medicago falcata/sativa*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1080.0	6.7	0.0	0.0	3138.0	20.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu004	446317	*Betula*	0.0	0.0	0.0	0.0	0.0	0.0	30198.7	48.5	46352.7	79.7	0.0	0.0	0.0	0.0	0.0	0.0	2465.7	26.2	0.0	0.0	0.0	0.0	0.0	0.0	21548.3	25.5	0.0	0.0	70806.5	67.5	0.0	0.0	0.0	0.0	0.0	0.0	279.0	1.4	0.0	0.0	108.0	0.2	18.3	+	0.0	0.0
Otu005	390741	*Pooideae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4590.3	10.5	0.0	0.0	1692.3	10.5	4170.7	44.3	557.5	3.7	0.0	0.0	0.0	0.0	3307.0	3.9	3318.7	7.5	0.0	0.0	0.0	0.0	0.0	0.0	3310.0	11.4	438.3	2.2	1537.7	8.6	0.0	0.0	0.0	0.0	0.0	0.0
Otu006	368567	*Taxus*	1146.5	1.7	20132.0	28.1	12534.7	16.4	238.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	44801.0	40.7	11750.0	38.5	8725.7	10.3	391.7	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7242.3	10.3	3535.7	5.5	18889.0	24.0
Otu007	272264	*Populus*	193.5	0.3	0.0	0.0	9493.0	12.4	2553.0	4.1	190.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16702.0	15.2	2671.5	8.7	10552.0	12.5	19001.3	42.9	629.5	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	221.0	0.3	14307.0	18.2
Otu008	189420	*Castanea*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20843.3	47.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7782.3	9.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu010	168920	*Humulus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4515.0	22.4	4285.7	24.1	0.0	0.0	0.0	0.0	0.0	0.0
Otu011	144351	*Anthemideae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1409.0	15.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	858.0	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu012	135406	*Chamaecyparis lawsoniana*	0.0	0.0	0.0	0.0	0.0	0.0	9723.0	15.6	8894.3	15.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8884.0	20.0	26278.5	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	115.0	0.1
Otu013	137845	Atripliceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1945.3	12.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	79.0	0.1	0.0	0.0	0.0	0.0	2390.0	8.2	1770.7	8.8	2420.3	13.6	0.0	0.0	0.0	0.0	0.0	0.0
Otu014	136014	Betulaceae	4474.5	6.7	1855.7	2.6	1794.7	2.3	14875.7	23.9	1620.3	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6651.5	6.0	0.0	0.0	376.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6687.0	9.5	3067.0	4.8	1129.7	1.4
Otu015	106035	Cupressoideae (Cupressus/Callitrops	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	575.7	6.1	0.0	0.0	0.0	0.0	0.0	0.0	477.0	0.6	1041.7	2.3	573.0	0.5	0.0	0.0	0.0	0.0	4090.5	14.1	0.0	0.0	0.0	0.0	385.0	0.5	493.3	0.8	0.0	0.0
Otu016	100118	*Holcus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1023.7	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu018	152021	*Poeae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4839.7	30.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	382.0	0.5	0.0	0.0	80.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0	290.3	1.4	0.0	0.0	149.0	0.2	0.0	0.0	0.0	0.0
Otu019	66077	*Quercus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	155.0	0.3	1813.3	4.1	0.0	0.0	2739.7	17.0	0.0	0.0	1843.0	12.1	0.0	0.0	0.0	0.0	525.7	0.6	0.0	0.0	287.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	401.3	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu020	55202	*Equisetum*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	322.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1220.3	1.9	0.0	0.0
Otu021	47338	*Pinus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	73.7	0.1	0.0	0.0	94.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu022	42618	*Phalaris*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu023	48063	*Plantago*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	815.3	1.9	0.0	0.0	0.0	0.0	0.0	0.0	1110.0	7.3	0.0	0.0	0.0	0.0	812.7	1.0	570.3	1.3	173.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu025	51506	*Poeae Chloroplast Group 1 (Aveneae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	90.7	0.1	0.0	0.0	0.0	0.0
Otu026	31442	*Atriplex*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu027	47221	PACMAD clade	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1393.0	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	704.3	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	41.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu028	27575	Tilia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	502.7	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu029	32494	*Cupressaceae (Chamaecyparis/Sequ*	1056.5	1.6	3856.0	5.4	2703.0	3.5	741.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	702.5	2.3	736.7	0.9	306.7	0.7	143.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	660.3	1.0	408.7	0.5
Otu031	23446	*Oleaceae*	1356.0	2.0	52.7	0.1	73.3	0.1	2360.3	3.8	332.0	0.6	0.0	0.0	0.0	0.0	346.3	2.1	0.0	0.0	0.0	0.0	990.5	0.9	0.0	0.0	0.0	0.0	686.0	1.5	2119.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	77.3	0.1	151.3	0.2	147.0	0.2
Otu032	31082	*Asteraceae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1403.0	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	470.3	1.1	51.5	+	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.3	+	0.0	0.0
Otu034	22250	*Ulmaceae*	0.0	0.0	116.3	0.2	0.0	0.0	535.3	0.9	17.3	+	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	328.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	211.7	0.3	152.3	0.2	3246.3	4.1
Otu037	16546	Poa annua/infirma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	361.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	144.7	0.2	94.0	0.1
Otu038	14031	*Trifolium*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu040	485	Cannabis sativa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu041	17384	Cupressoideae (Cupressus/Juniperus	1573.5	2.4	0.0	0.0	429.7	0.6	127.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	516.0	0.5	1282.5	4.2	0.0	0.0	672.7	1.5	1177.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1069.7	1.7	463.0	0.6
Otu042	11340	Hordeum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	517.3	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	272.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu044	14173	*Athyrium sect. Athyrium*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu046	15387	*Spergularia*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu048	12889	*Calluna vulgaris*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu050	10692	Rumex	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	685.3	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	52.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu051	11193	*Ailanthus altissima*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3731.0	8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu053	9974	*Brassicaceae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	130.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu054	1715	*Phragmites australis*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu055	925	*Mercurialis annua*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu058	9285	*Juncus conglomeratus/effusus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu059	9391	Alopecurus bulbosus/geniculatus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu061	8152	*Thuja*	99.5	0.1	591.7	0.8	694.3	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	422.0	1.4	536.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	395.7	0.5
Otu062	7561	*Dryopteris expanse/filix-mas*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	323.3	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	357.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	381.5	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu064	242	*Papilionoideae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	80.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu066	4755	*Sambucus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu069	3279	Rosaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu071	6529	*Chenopodioideae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu072	5085	Lotus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu074	4428	*Sagina procumbens*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu076	5233	*Carex subgen. Carex*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu079	4923	*Potentilla*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu081	2823	*Crepidinae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu083	108	*Clematis ternifolia/vitalba*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu085	2207	*Apioideae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu090	1403	*Prunus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	67.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	102.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu094	88	*Parietaria*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu096	1830	*Polygonaceae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	334.0	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu097	1429	*Alopecurus myosuroides*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu100	2521	*Scorzoneroides autumnalis*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu101	2768	*Convolvulaceae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu107	6069	*Cryptomeria japonica*	753.5	1.1	107.0	0.1	423.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	83.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	83.3	0.1	157.0	0.2	199.0	0.3
Otu108	2337	*Platanus orientalis*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	198.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	656.5	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu109	1483	*Hippophae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu115	1705	*Polygoneae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu118	2259	Sapindaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu120	1061	*Cornus sanguinea/sericea*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu121	3169	*Anemone*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu123	1170	*Diplotaxis tenuifolia*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu124	1855	*Lipandra polysperma*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu125	264	Araliaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu128	1782	*Myricaceae*	0.0	0.0	0.0	0.0	0.0	0.0	594.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu130	1467	*Acer*	0.0	0.0	59.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	93.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu131	1811	*Epilobium obscurum*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu134	2639	*Carex dioica*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu135	1655	*Chenopodiastrum murale*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu138	1498	Juncus inflexus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu153	441	*Persicaria*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu154	1357	Picea	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu162	5941	*Clematis brevicaudata*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu165	969	*Carex subgen. Vignea*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu168	983	*Carex foliosissima*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu170	939	*Filipendula ulmaria*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu176	177	*Hypericum*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	59.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu177	418	*Hydrangea*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu178	880	*Rubus occidentalis*	0.0	0.0	0.0	0.0	0.0	0.0	293.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu190	331	Alsineae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu200	614	Ceratophyllum demersum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu205	355	*Cercidiphyllum japonicum*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu227	313	*Glechoma hederacea*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu232	111	*Ranunculus acris*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu266	233	*Hamamelidaceae*	0.0	0.0	77.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu270	679	Viburnum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	89.7	0.1	0.0	0.0	0.0	0.0
Otu276	233	*Sparganium*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu294	89	*Bryonia dioica*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Otu335	45	*Pinaceae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.5	+	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

2019-02-27		2019-03-07		2019-06-02		2019-06-25		2019-07-23		2019-08-15		2019-08-24		2019-12-30		2020-02-09		2020-02-15		2020-02-25		2020-03-07		2020-04-04		2020-06-24		2020-07-25		2020-07-26		2020-07-27		2020-07-28		2020-07-29		2020-07-30		2020-07-31		2020-08-01		2020-08-02		2020-08-03		2020-08-04		2020-08-05	
Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average read	%	Average reac	%	Average reac	%	Average read	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%	Average reac	%
69477.0	94.0	42041.3	66.6	0.0	0.0	0.0	0.0	244.7	0.9	0.0	0.0	0.0	0.0	51601.7	99.5	52290.3	78.7	31728.7	89.4	3605.5	44.0	2646.0	41.1	0.0	0.0	2662.0	4.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	1198.7	2.9	4911.3	14.6	9722.3	35.5	1499.5	20.8	8400.5	23.7	0.0	0.0	449.7	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3188.0	5.9	8257.0	18.9	5895.7	12.1	18579.0	42.0	11519.0	30.7	1160.0	13.3	4786.0	31.3	13655.3	27.0	6386.5	66.3	0.0	0.0	0.0	0.0	8873.0	34.7	21002.7	13.7
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3025.0	11.1	0.0	0.0	10759.5	30.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25725.0	58.8	19663.0	40.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	247.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0	1135.5	4.4	80380.3	52.5
0.0	0.0	597.3	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3522.0	52.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	1237.3	2.9	7597.7	22.7	8111.0	29.6	0.0	0.0	4572.5	12.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12476.7	22.9	6359.0	14.5	0.0	0.0	5542.3	12.5	5657.7	15.1	218.5	2.5	3945.5	25.8	17570.0	34.7	0.0	0.0	0.0	0.0	0.0	0.0	5590.0	21.9	4791.3	3.1
2286.3	3.1	2606.3	4.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5272.7	7.9	2535.7	7.1	0.0	0.0	696.0	10.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	13067.0	20.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3354.0	5.0	1230.0	3.5	3105.0	37.9	2600.0	40.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1043.0	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	2996.3	8.9	2715.3	9.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6333.0	11.6	0.0	0.0	2879.0	5.9	210.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2128.0	29.5	1454.5	4.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	194.7	0.5	0.0	0.0	0.0	0.0	125.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1408.7	0.9
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1805.0	5.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5837.7	12.0	1521.7	3.4	574.3	1.5	3435.0	39.4	1812.0	11.8	758.0	1.5	1168.5	12.1	0.0	0.0	0.0	0.0	0.0	0.0	12974.7	8.5
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	83.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	827.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	975.0	1.8	0.0	0.0	0.0	0.0	730.0	1.7	1676.7	4.5	1962.5	22.5	0.0	0.0	565.3	1.1	0.0	0.0	0.0	0.0	0.0	0.0	1453.5	5.7	8504.0	5.6
917.7	1.2	1577.7	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4018.7	6.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
34.3	+	0.0	0.0	7872.3	18.8	666.7	2.0	574.7	2.1	955.5	13.2	300.5	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1116.0	16.7	112.0	0.2	0.0	0.0	9544.7	19.6	1886.3	4.3	258.0	0.7	1937.5	22.2	198.5	1.3	479.7	0.9	153.0	1.6	0.0	0.0	0.0	0.0	995.0	3.9	3081.0	2.0
0.0	0.0	0.0	0.0	11631.0	27.7	1696.7	5.1	0.0	0.0	0.0	0.0	25.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1369.7	2.5	0.0	0.0	0.0	0.0	719.7	1.6	541.3	1.4	0.0	0.0	0.0	0.0	5690.0	11.2	0.0	0.0	0.0	0.0	0.0	0.0	3012.0	11.8	1234.7	0.8
0.0	0.0	0.0	0.0	4772.3	11.4	3066.0	9.1	1224.3	4.5	2632.5	36.5	1116.5	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1390.3	2.6	2291.7	5.2	0.0	0.0	1115.0	2.5	1510.0	4.0	0.0	0.0	1611.5	10.5	6942.0	13.7	0.0	0.0	0.0	0.0	0.0	0.0	575.5	2.3	807.0	0.5
0.0	0.0	0.0	0.0	806.0	1.9	141.3	0.4	66.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1548.0	2.8	0.0	0.0	30.0	0.1	115.3	0.3	766.3	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3247.5	9.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	+	3421.7	9.1	0.0	0.0	268.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6011.7	3.9
0.0	0.0	1233.7	2.0	11465.7	27.3	2141.0	6.4	717.7	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	84.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14206.0	26.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	1031.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	163.0	0.3	0.0	0.0	917.3	1.9	1020.0	2.3	569.0	1.5	0.0	0.0	1051.0	6.9	327.0	0.6	1271.0	13.2	0.0	0.0	0.0	0.0	795.0	3.1	1155.3	0.8
0.0	0.0	0.0	0.0	0.0	0.0	3389.0	10.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1750.7	3.2	0.0	0.0	0.0	0.0	759.7	1.7	1253.7	3.3	0.0	0.0	1481.0	9.7	892.3	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1299.3	0.8
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1376.3	2.8	403.3	0.9	1534.7	4.1	0.0	0.0	0.0	0.0	0.0	0.0	655.0	6.8	0.0	0.0	0.0	0.0	0.0	0.0	2296.0	1.5
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1257.0	3.5	0.0	0.0	0.0	0.0	0.0	0.0	532.5	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	243.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2310.0	1.5
0.0	0.0	0.0	0.0	0.0	0.0	4026.0	12.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1685.7	3.1	0.0	0.0	2467.7	5.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	279.7	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
150.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
131.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	278.3	0.5	185.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	1952.0	29.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2207.0	6.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	450.3	0.8	0.0	0.0	0.0	0.0	216.7	0.5	733.7	2.0	0.0	0.0	0.0	0.0	440.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
741.0	1.0	576.0	0.9	268.7	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	534.3	0.8	0.0	0.0	950.0	11.6	495.0	7.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	14.7	+	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	767.3	1.4	0.0	0.0	0.0	0.0	155.0	0.4	434.3	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	818.7	1.9	497.3	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	439.7	0.3
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	547.3	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	311.7	0.8	0.0	0.0	0.0	0.0	206.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	514.3	1.2	0.0	0.0	0.0	0.0	0.0	0.0	1866.7	3.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5129.0	11.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	267.5	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	540.7	1.3	514.7	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	387.0	0.7	0.0	0.0	0.0	0.0	359.7	0.8	0.0	0.0	0.0	0.0	153.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1065.7	2.4	301.7	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	334.0	0.2
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	116.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	308.3	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3095.0	5.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	649.7	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
152.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	132.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1148.7	2.1	0.0	0.0	0.0	0.0	98.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	93.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	1585.0	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1093.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2034.7	1.3
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2542.5	10.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	558.3	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1744.3	4.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	174.3	0.4	1125.7	3.0	0.0	0.0	0.0	0.0	341.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	941.0	0.6
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	36.0	+
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	648.0	0.4
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	29.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	39.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	476.3	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	726.7	1.7	0.0	0.0	65.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	130.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31.0	+	464.7	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	494.3	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	604.7	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	148.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	353.7	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	360.3	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	368.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	603.7	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	551.7	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	499.3	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	147.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	206.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	580.0	2.3	1381.0	0.9
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	327.7	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	110.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	118.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	84.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	37.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	77.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	29.7	+

2020-08-06		2020-08-07		2020-08-08		2020-08-09		2020-08-10		2020-08-11		2020-08-12		2020-08-13		2020-08-14	
Average read	%	Average reac	%	Average read	%	Average read	%	Average reac	%	Average read	%	Average read	%	Average reac	%	Average read	%
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2980.5	24.8	18356.5	35.8	42647.0	44.9	24568.0	49.3	37738.7	38.7	28155.0	54.7	19558.5	26.6	4137.7	34.5	12186.7	30.7
224.5	1.9	0.0	0.0	368.5	0.4	0.0	0.0	385.0	0.4	0.0	0.0	23019.5	31.4	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	5122.5	10.0	8021.5	8.5	3997.0	8.0	6315.7	6.5	4507.0	8.8	4128.5	5.6	711.3	5.9	3261.0	8.2
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	209.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
242.5	2.0	1521.0	3.0	10846.5	11.4	1815.0	3.6	11277.3	11.6	9700.0	18.8	4299.0	5.9	894.7	7.5	9034.3	22.8
1055.5	8.8	4093.0	8.0	4650.0	4.9	5928.5	11.9	3705.7	3.8	1375.0	2.7	1660.5	2.3	1213.0	10.1	818.0	2.1
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
602.5	5.0	423.5	0.8	1700.5	1.8	2962.5	5.9	3063.0	3.1	1115.3	2.2	3231.0	4.4	2166.7	18.1	10319.0	26.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
326.0	2.7	0.0	0.0	623.0	0.7	0.0	0.0	756.3	0.8	0.0	0.0	0.0	0.0	338.0	2.8	0.0	0.0
0.0	0.0	2744.0	5.4	1944.5	2.0	0.0	0.0	2122.3	2.2	754.3	1.5	2183.5	3.0	0.0	0.0	0.0	0.0
721.0	6.0	6042.5	11.8	4653.0	4.9	0.0	0.0	6971.3	7.2	838.7	1.6	1784.5	2.4	1272.3	10.6	0.0	0.0
0.0	0.0	465.5	0.9	0.0	0.0	0.0	0.0	3677.3	3.8	58.7	0.1	10047.5	13.7	0.0	0.0	0.0	0.0
0.0	0.0	64.0	0.1	0.0	0.0	0.0	0.0	4737.0	4.9	228.3	0.4	0.0	0.0	53.0	0.4	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
264.0	2.2	1431.0	2.8	1755.5	1.9	774.0	1.6	858.3	0.9	1035.3	2.0	202.5	0.3	861.7	7.2	0.0	0.0
295.0	2.5	2663.5	5.2	2559.5	2.7	0.0	0.0	2622.0	2.7	445.3	0.9	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	650.0	1.3	2916.5	3.1	622.0	1.2	1091.3	1.1	244.3	0.5	458.5	0.6	0.0	0.0	0.0	0.0
431.0	3.6	790.0	1.5	394.5	0.4	4075.5	8.2	5756.0	5.9	344.7	0.7	868.5	1.2	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	34.3	0.1	293.5	0.4	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	25.0	+	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
143.5	1.2	1264.0	2.5	1575.5	1.7	146.5	0.3	677.7	0.7	471.3	0.9	596.5	0.8	0.0	0.0	1491.7	3.8
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	55.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0
777.5	6.5	0.0	0.0	0.0	0.0	383.5	0.8	0.0	0.0	169.3	0.3	0.0	0.0	0.0	0.0	2600.3	6.5
0.0	0.0	1529.5	3.0	1866.0	2.0	0.0	0.0	657.7	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	242.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	670.0	1.3	0.0	0.0	0.0	0.0	1245.3	1.3	233.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	1292.0	1.4	1409.5	2.8	542.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	757.5	1.5	1352.5	1.4	634.0	1.3	1754.7	1.8	0.0	0.0	1068.5	1.5	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	1253.0	1.3	0.0	0.0	0.0	0.0	86.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	499.5	0.5	0.0	0.0	1105.3	1.1	54.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	235.5	0.5	0.0	0.0	298.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3678.5	30.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	28.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	40.7	+	71.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	141.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	1287.5	1.4	0.0	0.0	0.0	0.0	59.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	87.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	498.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	128.0	0.1	0.0	0.0	210.3	0.2	52.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	1187.5	2.4	0.0	0.0	48.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	96.3	0.1	46.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	852.5	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	1055.5	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	352.7	2.9	0.0	0.0
44.5	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	927.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	88.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	1319.5	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	244.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	83.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	212.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	438.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	354.5	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
209.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	307.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.7	+	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	136.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table S4. Idenity and abundance of OTU's nrITS2

OTU	Total read	best_id_N	cover	order	family	genus/subgenus	species	maxid	scientific r	OTU sequence (truncated)	length
Otu0479	417	100	95	Alismatales	Juncaginaceae	*Triglochin*	*Triglochin maritima*	*Triglochin maritima*	species	TCTTGGCCCTTGCATCGATGAAGAACGTA	425
Otu0039	10703	99.504	100	Apiales	Apiaceae	*Angelica*	*Angelica sylvestris*	*Angelica sylvestris*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	403
Otu0088	6588	100	100	Apiales	Apiaceae	*Daucus*	*Daucus carota*	*Daucus carota*	species	TCCCGGCTCTCGCATCGATGAAGAACGTA	405
Otu0620	275	100	99	Apiales	Apiaceae	*Eryngium*	*Eryngium campestre*	*Eryngium campestre*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	402
Otu0085	7066	100	90	Apiales	Apiaceae	*Heracleum*	*Heracleum sphondylium*	*Heracleum sphondylium*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	403
Otu0329	701	100	93	Apiales	Apiaceae	*Torilis*	*Torilis japonica*	*Torilis japonica*	species	TCCCGGCTCTCGCATCGATGAAGAACGTA	400
Otu0211	1534	99.475	100	Asterales	Asteraceae	*Achillea*	*Achillea millefolium*	*Achillea millefolium*	species	TCTCGGCTCATGCATCGATGAAGAACGTA	381
Otu1282	60	100	100	Asterales	Asteraceae	*Artemisia*	*Artemisia absinthium*	*Artemisia absinthium*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	398
Otu0005	236873	99.75	100	Asterales	Asteraceae	*Artemisia*	*Artemisia vulgaris*	*Artemisia vulgaris*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	400
Otu0848	92	99.44	100	Asterales	Asteraceae	*Bellis*	*Bellis perennis*	*Bellis perennis*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	391
Otu0407	579	100	100	Asterales	Asteraceae	*Cirsium*	*Cirsium arvense*	*Cirsium arvense*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	403
Otu0855	119	99.752	100	Asterales	Asteraceae	*Cirsium*	*Cirsium vulgare*	*Cirsium vulgare*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	403
Otu0038	11150	99.751	98	Asterales	Asteraceae	*Crepis*	*Crepis capillaris*	*Crepis capillaris*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	409
Otu0095	4867	96.947	100	Asterales	Asteraceae	*Eupatorium*	*Eupatorium cannabinum*	*Eupatorium*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	392
Otu0122	3145	100	100	Asterales	Asteraceae	*Hypochaeris*	*Hypochaeris radicata*	*Hypochaeris radicata*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	387
Otu0241	2743	99.501	100	Asterales	Asteraceae	*Jacobaea*	*Jacobaea vulgaris*	*Jacobaea vulgaris*	species	TCTTGGCTCACGCATCGATGAAGAACGTA	401
Otu0331	716	100	90	Asterales	Asteraceae	*Mycelis*	*Mycelis muralis*	*Mycelis muralis*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	402
Otu0110	3604	100	100	Asterales	Asteraceae	*Scorzoneroides*	*Scorzoneroides autumnalis*	*Scorzoneroides autumnalis*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	404
Otu0425	376	99.746	100	Asterales	Asteraceae	*Solidago*	*Solidago gigantea*	*Solidago gigantea*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	393
Otu0537	297	100	100	Asterales	Asteraceae	*Solidago*	*Solidago juncea*	*Solidago juncea*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	393
Otu0383	461	100	100	Asterales	Asteraceae	*Taraxacum*	*Taraxacum officinale*	*Taraxacum officinale*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	405
Otu0502	310	99.748	100	Asterales	Asteraceae	*Tussilago*	*Petasites japonicus*	*Petasites japonicus*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	397
Otu0506	275	100	100	Asterales	Asteraceae	*Tussilago*	*Tussilago farfara*	*Tussilago farfara*	species	TCTCGGCTCACGCATCGATGAAGAACGTA	396
Otu0303	771	100	96	Boraginales	Boraginaceae	*Echium*	*Echium plantagineum*	*Echium plantagineum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	401
Otu0201	1826	99.751	100	Boraginales	Boraginaceae	*Echium*	*Echium vulgare*	*Echium vulgare*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	401
Otu0225	1293	99.749	100	Boraginales	Boraginaceae	*Symphytum*	*Symphytum officinale*	*Symphytum officinale*	species	TCTTGGCTCTCGCATCGATGAAGAACGTA	398
Otu0183	2474	99.497	100	Boraginales	Hydrophyllaceae	*Phacelia*	*Phacelia tanacetifolia*	*Phacelia tanacetifolia*	species	TCTAGGCTCTCGCATCGATGAAGAACGTA	398
Otu0118	3000	100	100	Brassicales	Brassicaceae	*Brassica*		*Brassica*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	366
Otu0061	7196	100	100	Brassicales	Brassicaceae	*Capsella*	*Capsella bursa-pastoris*	*Capsella bursa-pastoris*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	369
Otu0319	1155	100	100	Brassicales	Brassicaceae	*Diplotaxis*	*Diplotaxis tenuifolia*	*Diplotaxis tenuifolia*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	366
Otu0142	2646	100	100	Brassicales	Brassicaceae	*Sinapis*	*Sinapis alba*	*Sinapis alba*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	367
Otu0112	3513	100	100	Brassicales	Brassicaceae	*Sinapis*	*Sinapis arvensis*	*Sinapis arvensis*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	368
Otu0352	882	100	100	Brassicales	Brassicaceae	*Sisymbrium*	*Sisymbrium officinale*	*Sisymbrium officinale*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	369
Otu0205	1684	100	100	Caryophyllales	Amaranthaceae	*Amaranthus*		*Amaranthus*	genus	TCTTGGCTCTCGCATCGATGAAGAACGTA	397
Otu0059	9218	100	93	Caryophyllales	Amaranthaceae	*Atriplex*	*Atriplex prostrata*	*Atriplex prostrata*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	405
Otu0086	4938	100	100	Caryophyllales	Amaranthaceae	*Beta*	*Beta vulgaris*	*Beta vulgaris*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	398
Otu0012	67841	100	100	Caryophyllales	Amaranthaceae	*Chenopodium*		*Chenopodium album/giganteum*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	404
Otu0068	7660	100	100	Caryophyllales	Amaranthaceae	*Chenopodium*		*Chenopodium album/ficifolium*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	405
Otu0430	430	100	91	Caryophyllales	Amaranthaceae	*Lipandra*	*Lipandra polysperma*	*Lipandra polysperma*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	402
Otu1021	83	100	100	Caryophyllales	Amaranthaceae	*Oxybasis*	*Oxybasis glauca*	*Oxybasis glauca*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	402
Otu1013	58	99.496	100	Caryophyllales	Caryophyllaceae	*Cerastium*	*Cerastium fontanum*	*Cerastium fontanum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	397
Otu0708	146	99.747	99	Caryophyllales	Caryophyllaceae	*Sagina*	*Sagina procumbens*	*Sagina procumbens*	species	TCTTGGCTCTCGCATCGATGAAGAACGTA	397
Otu0493	358	99.756	99	Caryophyllales	Caryophyllaceae	*Spergularia*	*Spergularia media*	*Spergularia media*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	413
Otu0297	879	100	100	Caryophyllales	Caryophyllaceae	*Stellaria*	*Stellaria aquatica*	*Stellaria aquatica*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	398
Otu0159	1799	100	100	Caryophyllales	Polygonaceae	*Polygonum*	*Polygonum aviculare*	*Polygonum aviculare*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	377
Otu0195	2082	98.526	100	Caryophyllales	Polygonaceae	*Rumex*	*Rumex acetosa*	*Rumex acetosa*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	407
Otu0066	7605	98.966	100	Caryophyllales	Polygonaceae	*Rumex*	*Rumex acetosella*	*Rumex acetosella*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	387
Otu0357	542	100	100	Caryophyllales	Polygonaceae	*Rumex*	*Rumex sanguineus*	*Rumex sanguineus*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	376
Otu0600	463	98.481	100	Cornales	Hydrangeaceae	*Philadelphus*	*Philadelphus pekinensis*	*Philadelphus pekinensis*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	392
Otu0187	2096	99.506	100	Cornales	Hydrangeaceae	*Hydrangea*	*Hydrangea macrophylla*	*Hydrangea macrophylla*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	405
Otu0047	11415	97.975	100	Cupressales	Cupressaceae	*Chamaecyparis*	*Chamaecyparis formosensis*	*Chamaecyparis formosensis*	species	TCTCGGCTCTCGCCACGATGAAGAATGTA	393
Otu0400	404	100	96	Cupressales	Cupressaceae	*Cryptomeria*	*Cryptomeria japonica*	*Cryptomeria japonica*	species	TCTCGGCTCTCGCCACGATGAAGAATGTA	400
Otu0076	5440	99.495	100	Cupressales	Cupressaceae	*Cupressus*	*Cupressus arizonica*	*Cupressus arizonica*	species	TCTCGGCTCTCGCCACGATGAAGAATGTA	396
Otu0534	222	99.745	100	Cupressales	Cupressaceae	*Juniperus*	*Juniperus chinensis* var. *sargentii*	*Juniperus chinensis* var. *sargentii*	species	TCTCGGCTCTCGCCACGATGAAGAATGTA	392
Otu0389	529	100	100	Cupressales	Cupressaceae	*Juniperus*	*Juniperus communis*	*Juniperus communis*	species	TCTCGGCTCTCGCCACGATGAAGAATGTA	392
Otu0612	129	100	100	Cupressales	Cupressaceae	*Juniperus*	*Juniperus sabina*	*Juniperus sabina*	species	TCTCGGCTCTCGCCACGATGAAGAATGTA	392
Otu0041	12958	97.97	100	Cupressales	Cupressaceae	*Juniperus*		*Juniperus*	genus	TCTCGGCTCTCGCCACGATGAAGAATGTA	392
Otu0109	3975	100	92	Cupressales	Cupressaceae	*Metasequoia*	*Metasequoia glyptostroboides*	*Metasequoia glyptostroboides*	species	TCTCGGCTCTCGCCACGATGAAGAATGTA	394
Otu0144	3592	100	100	Cupressales	Cupressaceae	*Taxodium*		*Taxodium distichum/mucronatum*	genus	TCTCGGCTCTCGCCACGATGAAGAATGTA	394
Otu0185	1691	100	100	Cupressales	Cupressaceae	*Thuja*	*Thuja occidentalis*	*Thuja occidentalis*	species	TCTCGGCTCTCGCCACGATGAAGAATGTA	393
Otu0208	1582	100	100	Cupressales	Cupressaceae	*Thuja*	*Thuja plicata*	*Thuja plicata*	species	TCTCGGCTCTCGCCACGATGAAGAATGTA	393
Otu0009	206188	100	100	Cupressales	Taxaceae	*Taxus*		*Taxus canadensis/cuspidata*	genus	TCTCGGCTCTCGCCACGATGAAGAATGTA	403
Otu0423	557	99.504	100	Cupressales	Taxaceae	*Taxus*		*Taxus x media/cuspidata*	genus	TCTCGGCTCTCGCCACGATGAAGAATGTA	403
Otu0044	8787	99.261	100	Dipsacales	Adoxaceae	*Sambucus*	*Sambucus nigra*	*Sambucus nigra*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	406
Otu0426	461	97.037	100	Dipsacales	Adoxaceae	*Viburnum*	*Viburnum dilatatum*	*Viburnum dilatatum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	404
Otu0992	116	96.296	100	Dipsacales	Adoxaceae	*Viburnum*		*Viburnum*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	404
Otu0174	2130	100	100	Ericales	Balsaminaceae	*Impatiens*	*Impatiens glandulifera*	*Impatiens glandulifera*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	397
Otu0050	13600	100	100	Ericales	Ericaceae	*Calluna*	*Calluna vulgaris*	*Calluna vulgaris*	species	TCTCGGCTCTTGCATCGATGAAGAACGTA	405
Otu0165	1739	100	100	Fabales	Fabaceae	*Lotus*	*Lotus corniculatus*	*Lotus corniculatus*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	394
Otu0242	1122	100	100	Fabales	Fabaceae	*Lotus*	*Lotus pedunculatus*	*Lotus pedunculatus*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	394
Otu0530	313	99.746	100	Fabales	Fabaceae	*Lotus*		*Lotus corniculatus/glaber*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	394
Otu0016	37068	100	100	Fabales	Fabaceae	*Medicago*	*Medicago sativa*	*Medicago sativa*	species	TCTAGGCTCTTGCATCGATGAAGAACGTA	395
Otu0298	714	100	100	Fabales	Fabaceae	*Melilotus*	*Melilotus albus*	*Melilotus albus*	species	TCTAGGCTCTTGCATCGATGAAGAACGTA	400
Otu0155	4081	99.75	100	Fabales	Fabaceae	*Melilotus*		*Melilotus*	genus	TCTAGGCTCTTGCATCGATGAAGAACGTA	400
Otu0441	370	99.75	100	Fabales	Fabaceae	*Robinia*	*Robinia pseudoacacia*	*Robinia pseudoacacia*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	400
Otu0119	2614	100	99	Fabales	Fabaceae	*Styphnolobium*	*Sophora japonica*	*Sophora japonica*	species	TCTCGGCTCTTGCATCGATGAAGAACGTA	397
Otu0070	5122	100	100	Fabales	Fabaceae	*Trifolium*	*Trifolium pratense*	*Trifolium pratense*	species	TCTAGGCTCTTGCATCGATGAAGAACGTA	395
Otu0443	513	100	100	Fabales	Fabaceae	*Trifolium*	*Trifolium repens*	*Trifolium repens*	species	TCTAGGCTCTTGCATCGATGAAGAACGTA	402
Otu0008	196913	100	100	Fagales	Betulaceae	*Alnus*	*Alnus cordata*	*Alnus cordata*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	404
Otu0036	13782	100	100	Fagales	Betulaceae	*Alnus*	*Alnus japonica*	*Alnus japonica*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	405
Otu0026	19319	99.752	100	Fagales	Betulaceae	*Alnus*	*Alnus subcordata*	*Alnus subcordata*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	404
Otu0002	967544	100	100	Fagales	Betulaceae	*Alnus*		*Alnus glutinosa/incana*	subgenus	TCTCGGCTCTCGCATCGATGAAGAACGTA	404
Otu0231	1366	93.473	95	Fagales	Betulaceae	*Alnus*		*Alnus*	genus	TCTTGGCTCTCGCATCGATGAAGATCGTA	404
Otu0010	135581	100	100	Fagales	Betulaceae	*Betula*	*Betula pendula*	*Betula pendula*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	402
Otu0007	141000	100	100	Fagales	Betulaceae	*Betula*		*Betula*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	402
Otu0034	12844	100	100	Fagales	Betulaceae	*Carpinus*	*Carpinus betulus*	*Carpinus betulus*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	403
Otu0013	60841	100	100	Fagales	Betulaceae	*Corylus*	*Corylus avellana*	*Corylus avellana*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	403
Otu0202	1890	100	100	Fagales	Betulaceae	*Corylus*		*Corylus colurna/heterophylla*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	404

Otu1075	1314	98.241	100	Fagales	Betulaceae	*Corylus*		*Corylus*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	404
Otu0111	2493	99.246	99	Fagales	Betulaceae	*Ostrya*	*Ostrya japonica*	*Ostrya japonica*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	403
Otu0021	29433	99.223	99	Fagales	Fagaceae	*Castanea*	*Castanea crenata*	*Castanea crenata*	species	TCTAGGCTCTCGCATCGATGAAGAACGTA	387
Otu0018	42526	99.229	99	Fagales	Fagaceae	*Castanea*	*Castanea sativa*	*Castanea sativa*	species	TCTAGGCTCTCGCATCGATGAAGAACGTA	389
Otu0025	19841	100	100	Fagales	Fagaceae	*Fagus*	*Fagus sylvatica*	*Fagus sylvatica*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	407
Otu0250	1078	99.217	99	Fagales	Fagaceae	*Quercus*	*Quercus petraea*	*Quercus petraea*	species	TCTAGGCTCTCGCATCGATGAAGAACGTA	385
Otu0087	4142	99.739	99	Fagales	Fagaceae	*Quercus*	*Quercus robur*	*Quercus robur*	species	TCTAGGCTCTCGCATCGATGAAGAACGTA	386
Otu0055	6485	100	100	Fagales	Fagaceae	*Quercus*	*Quercus rubra*	*Quercus rubra*	species	TCTAGGCTCTCGCATCGATGAAGAACGTA	388
Otu0168	2388	100	99	Fagales	Fagaceae	*Quercus*		*Quercus* sect. *Quercus*	sectio	TCTAGGCTCTCGCATCGATGAAGAACGTA	385
Otu1152	40	98.969	99	Fagales	Fagaceae	*Quercus*		*Quercus* sect. *Lobatae*	sectio	TCTAGGCTCTCGCATCGATGAAGAACGTA	388
Otu0130	2560	99.746	100	Fagales	Juglandaceae	*Pterocarya*		*Pterocarya*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	398
Otu0614	274	99.25	100	Lamiales	Lamiaceae	*Nepeta*	*Nepeta mussinii*	*Nepeta mussinii*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	399
Otu0011	85509	100	100	Lamiales	Oleaceae	*Fraxinus*	*Fraxinus excelsior*	*Fraxinus excelsior*	species	TCTTGGCTCTCGCATCGATGAAGAACGCA	390
Otu1214	388	96.923	100	Lamiales	Oleaceae	*Fraxinus*		*Fraxinus*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	390
Otu0569	199	98.737	100	Lamiales	Plantaginaceae	*Linaria*	*Linaria vulgaris*	*Linaria vulgaris*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	396
Otu0116	3113	100	100	Lamiales	Plantaginaceae	*Plantago*	*Plantago arenaria*	*Plantago arenaria*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	383
Otu0028	15366	100	100	Lamiales	Plantaginaceae	*Plantago*	*Plantago coronopus*	*Plantago coronopus*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	382
Otu0006	200431	100	100	Lamiales	Plantaginaceae	*Plantago*	*Plantago lanceolata*	*Plantago lanceolata*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	384
Otu0015	38764	100	100	Lamiales	Plantaginaceae	*Plantago*	*Plantago major*	*Plantago major*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	374
Otu0374	485	99.745	99	Lamiales	Plantaginaceae	*Veronicastrum*	*Veronicastrum virginicum*	*Veronicastrum virginicum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	393
Otu0782	173	100	100	Lamiales	Scrophulariaceae	*Buddleja*	*Buddleja officinalis*	*Buddleja officinalis*	species	TCTAGGCTCTCGCATCGATGAAGAACGTA	401
Otu0594	250	99.749	100	Lamiales	Scrophulariaceae	*Verbascum*	*Verbascum macrocarpum*	*Verbascum macrocarpum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	403
Otu0024	27646	99.747	100	Malpighiales	Euphorbiaceae	*Mercurialis*	*Mercurialis annua*	*Mercurialis annua*	species	TCTCGGCTCTCGCATCGATGAAGAACGCA	395
Otu0198	1366	99.747	100	Malpighiales	Euphorbiaceae	*Mercurialis*	*Mercurialis perennis*	*Mercurialis perennis*	species	TCTCGGCTCTCGCATCGATGAAGAACGCA	396
Otu0335	670	100	100	Malpighiales	Hypericaceae	*Hypericum*		*Hypericum*	genus	TCTAGGCTCTTGCATCGATGAAGAACGTA	407
Otu0032	16197	100	100	Malpighiales	Salicaceae	*Populus*	*Populus alba*	*Populus alba*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	391
Otu0131	2939	100	100	Malpighiales	Salicaceae	*Populus*	*Populus nigra*	*Populus nigra*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	391
Otu0221	1406	99.745	100	Malpighiales	Salicaceae	*Populus*	*Populus simonii*	*Populus simonii*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	392
Otu0103	7604	100	100	Malpighiales	Salicaceae	*Populus*		*Populus*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	391
Otu0548	213	100	100	Malpighiales	Salicaceae	*Populus*		*Populus balsamifera/trichocarpa*	sectio	TCTCGGCTCTCGCATCGATGAAGAACGTA	391
Otu0148	2285	100	100	Malpighiales	Salicaceae	*Salix*	*Salix alba*	*Salix alba*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	390
Otu0540	444	100	100	Malpighiales	Salicaceae	*Salix*	*Salix triandra*	*Salix triandra*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	391
Otu0035	15656	100	100	Malpighiales	Salicaceae	*Salix*		*Salix*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	391
Otu0082	6406	100	100	Malpighiales	Salicaceae	*Salix*		*Salix myrsinifolia/arctica*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	391
Otu0204	1990	99.744	100	Malpighiales	Salicaceae	*Salix*		*Salix matsudana/babylonica*	genus	TCTCGGCTCTCGCATCGATGAATAACGTA	391
Otu0309	1169	100	100	Malpighiales	Salicaceae	*Salix*		*Salix schwerinii/viminalis*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	391
Otu0113	2755	100	100	Malvales	Malvaceae	*Tilia*	*Tilia platyphyllos*	*Tilia platyphyllos*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	417
Otu0230	1146	99.76	100	Malvales	Malvaceae	*Tilia*		*Tilia* sect. *Tilia*	sectio	TCTCGGCTCTCGCATCGATGAAGAACGTA	417
Otu0660	174	99.744	100	Myrtales	Onagraceae	*Epilobium*	*Epilobium hirsutum*	*Epilobium hirsutum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	391
Otu0251	917	99.744	100	Myrtales	Onagraceae	*Oenothera*		*Oenothera biennis/glazioviana*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	390
Otu0179	2424	100	100	Poales	Poaceae	*Agrostis*	*Agrostis capillaris*	*Agrostis capillaris*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	393
Otu0347	725	100	100	Poales	Poaceae	*Agrostis*	*Agrostis stolonifera*	*Agrostis stolonifera*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	393
Otu0655	174	99.746	100	Poales	Poaceae	*Agrostis*		*Agostis capillaris/gigantea*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	393
Otu0349	702	99.157	90	Poales	Poaceae	*Alopecurus*	*Alopecurus myosuroides*	*Alopecurus myosuroides*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	394
Otu0381	538	100	93	Poales	Poaceae	*Alopecurus*	*Alopecurus pratensis*	*Alopecurus pratensis*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	395
Otu0124	4609	100	99	Poales	Poaceae	*Arrhenatherum*	*Arrhenatherum elatius*	*Arrhenatherum elatius*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	393
Otu0853	135	99.745	100	Poales	Poaceae	*Avena*	*Avena strigosa*	*Avena strigosa*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	392
Otu0731	242	99.235	99	Poales	Poaceae	*Bromus*	*Bromus hordeaceus*	*Bromus hordeaceus*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	395
Otu0810	103	100	100	Poales	Poaceae	*Calamagrostis*	*Calamagrostis varia*	*Calamagrostis varia*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	393
Otu0167	1561	100	100	Poales	Poaceae	*Calamagrostis*	*Calamagrostis x acutiflora*	*Calamagrostis x acutiflora*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	393
Otu0233	1127	100	100	Poales	Poaceae	*Corynephorus*	*Corynephorus canescens*	*Corynephorus canescens*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	394
Otu0487	470	100	100	Poales	Poaceae	*Dactylis*	*Dactylis glomerata*	*Dactylis glomerata*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	393
Otu0263	986	100	100	Poales	Poaceae	*Digitaria*	*Digitaria sanguinalis*	*Digitaria sanguinalis*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	391
Otu0273	1513	100	100	Poales	Poaceae	*Elymus*	*Elymus repens*	*Elymus repens*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	395
Otu1239	63	99.227	99	Poales	Poaceae	*Glyceria*	*Glyceria fluitans*	*Glyceria fluitans*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	393
Otu0071	8475	100	100	Poales	Poaceae	*Holcus*	*Holcus lanatus*	*Holcus lanatus*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	394
Otu0019	27872	100	100	Poales	Poaceae	*Hordeum*	*Hordeum vulgare*	*Hordeum vulgare*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	396
Otu0049	13530	100	100	Poales	Poaceae	*Lolium*	*Lolium multiflorum*	*Lolium multiflorum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	393
Otu0014	70381	100	100	Poales	Poaceae	*Lolium*	*Lolium perenne*	*Lolium perenne*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	393
Otu0280	1268	100	90	Poales	Poaceae	*Molinia*	*Molinia caerulea*	*Molinia caerulea*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	399
Otu0105	2856	99.747	100	Poales	Poaceae	*Panicum*	*Panicum virgatum*	*Panicum virgatum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	396
Otu0525	287	99.725	93	Poales	Poaceae	*Phragmites*	*Phragmites australis*	*Phragmites australis*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	392
Otu0132	3373	100	100	Poales	Poaceae	*Poa*	*Poa annua*	*Poa annua*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	391
Otu0244	1226	99.488	100	Poales	Poaceae	*Poa*	*Poa trivialis*	*Poa trivialis*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	391
Otu0860	122	100	100	Poales	Poaceae	*Triticum*	*Triticum monococcum*	*Triticum monococcum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	395
Otu1042	196	98.246	100	Polypodiales	Dryopteridaceae	*Dryopteris*	*Dryopteris intermedia*	*Dryopteris intermedia*	species	TCTTGGCTCTTGCAACGATGAAGAACGCA	513
Otu1014	118	99.803	99	Polypodiales	Dryopteridaceae	*Dryopteris*	*Dryopteris nigrosquamosa*	*Dryopteris nigrosquamosa*	species	TCTTGGCTCTTGCAACGATGAAGAACGCA	509
Otu0089	4737	100	100	Proteales	Platanaceae	*Platanus*	*Platanus x acerifolia*	*Platanus x acerifolia*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	415
Otu0339	794	99.769	100	Ranunculales	Papaveraceae	*Papaver*	*Papaver rhoeas*	*Papaver rhoeas*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	432
Otu0080	7479	100	100	Ranunculales	Ranunculaceae	*Anemone*	*Anemone hupehensis*	*Anemone hupehensis*	species	TCTCGGCTCTTGCATCGATGAAGAACGTA	388
Otu0146	3787	99.746	100	Ranunculales	Ranunculaceae	*Clematis*	*Clematis armandii*	*Clematis armandii*	species	TCTCGGCTCTTGCATCGATGAAGAACGTA	394
Otu0037	14270	100	100	Rosales	Cannabaceae	*Cannabis*	*Cannabis sativa*	*Cannabis sativa*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	396
Otu0004	300014	100	100	Rosales	Cannabaceae	*Humulus*	*Humulus lupulus*	*Humulus lupulus*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	410
Otu0261	1133	99.747	100	Rosales	Elaeagnaceae	*Hippophae*	*Hippophae rhamnoides*	*Hippophae rhamnoides*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	396
Otu1169	65	100	100	Rosales	Rosaceae	*Amelanchier*		*Amelanchier*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	392
Otu0072	6401	100	100	Rosales	Rosaceae	*Filipendula*	*Filipendula ulmaria*	*Filipendula ulmaria*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	392
Otu0934	173	100	100	Rosales	Rosaceae	*Potentilla*	*Potentilla anserina*	*Potentilla anserina*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	384
Otu0209	2184	100	100	Rosales	Rosaceae	*Potentilla*	*Potentilla reptans*	*Potentilla reptans*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	384
Otu0240	897	99.739	99	Rosales	Rosaceae	*Prunus*	*Prunus laurocerasus*	*Prunus laurocerasus*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	385
Otu1166	50	100	100	Rosales	Rosaceae	*Prunus*	*Prunus serotina*	*Prunus serotina*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	384
Otu1086	44	99.739	99	Rosales	Rosaceae	*Prunus*		*Prunus* sect. *Laurocerasus*	sectio	TCTCGGCTCTCGCATCGATGAAGAACGTA	385
Otu0784	199	100	100	Rosales	Rosaceae	*Rubus*	*Rubus bifrons*	*Rubus bifrons*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	387
Otu0150	1902	100	100	Rosales	Rosaceae	*Rubus*	*Rubus caesius*	*Rubus caesius*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	385
Otu0820	325	100	100	Rosales	Rosaceae	*Rubus*		*Rubus* sect. *Rubus*	sectio	TCTCGGCTCTCGCATCGATGAAGAACGTA	386
Otu0558	348	100	100	Rosales	Rosaceae	*Sanguisorba*	*Sanguisorba officinalis*	*Sanguisorba officinalis*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	382
Otu0115	5764	100	100	Rosales	Ulmaceae	*Ulmus*		*Ulmus*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	389
Otu0284	1151	97.158	96	Rosales	Urticaceae	*Laportea*		*Laportea*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	402
Otu0566	221	95.866	96	Rosales	Urticaceae	*Laportea*		*Laportea*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	402
Otu0097	5608	99.748	95	Rosales	Urticaceae	*Parietaria*	*Parietaria judaica*	*Parietaria judaica*	species	TCTTGGCTCTCGCATCGATGAAGAACGTA	418
Otu0200	2123	99.746	95	Rosales	Urticaceae	*Parietaria*	*Parietaria officinalis*	*Parietaria officinalis*	species	TCTTGGCTCTCGCATCGATGAAGAACGTA	414
Otu0001	2750732	100	100	Rosales	Urticaceae	*Urtica*	*Urtica dioica*	*Urtica dioica*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	421

Otu0090	4807	100	92	Rosales	Urticaceae	*Urtica*	*Urtica urens*	*Urtica urens*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	402
Otu0378	522	96.919	100	Rosales	Urticaceae	*Urtica*		*Urtica*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	422
Otu0572	217	100	100	Sapindales	Sapindaceae	*Acer*	*Acer negundo*	*Acer negundo*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	408
Otu0216	1315	100	100	Sapindales	Sapindaceae	*Acer*	*Acer saccharinum*	*Acer saccharinum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	413
Otu0313	673	97.975	100	Saxifragales	Crassulaceae	*Phedimus*	*Phedimus hybridus*	*Phedimus hybridus*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	394
Otu0140	2520	98.718	99	Saxifragales	Crassulaceae	*Phedimus*	*Phedimus kamtschaticus*	*Phedimus kamtschaticus*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	393
Otu0310	722	99.522	100	Saxifragales	Hamamelidaceae	*Sycopsis*	*Sycopsis sinensis*	*Sycopsis sinensis*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	418
Otu0852	84	100	100	Solanales	Solanaceae	*Solanum*	*Solanum americanum*	*Solanum americanum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	385
Otu1175	48	98.88	90	Solanales	Solanaceae	*Solanum*	*Solanum dulcamara*	*Solanum dulcamara*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	393
Otu0776	117	100	100	Solanales	Solanaceae	*Solanum*	*Solanum nigrum*	*Solanum nigrum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	385
Otu0466	317	100	100	Solanales	Solanaceae	*Solanum*	*Solanum villosum*	*Solanum villosum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTA	385
Otu0064	5714	99.487	100	Solanales	Solanaceae	*Solanum*		*Solanum*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTA	391

Table S5. nrITS2 sequence Avg. rdss and relative read abundance per sample

Any OTU with <0.1% relative read abundance is shown as a +

% represents the Relative Read Abundance

Spring (green) · Fall (brown) · red text: Sample failed amplification

South-east Netherlands (Elkerliek, Helmond): 2019-02-18, 2019-02-23, 2019-02-28, 2019-03-22, 2019-04-07, 2019-06-25, 2019-07-06, 2019-07-24, 2019-08-05, 2019-08-28, 2020-02-09, 2020-02-15, 2020-02-24, 2020-03-12, 2020-04-06, 2020-06-26, 2020-07-19, 2020-07-27, 2020-08-09, 2020-08-15

West Netherlands (LUMC, Leiden): 2019-02-15, 2019-02-18, 2019-02-25, 2019-02-27, 2019-03-07, 2019-06-02, 2019-06-25, 2019-07-23, 2019-08-15, 2019-08-24, 2019-12-30, 2020-02-09, 2020-02-15, 2020-02-25, 2020-03-07, 2020-04-04, 2020-06-24, 2020-07-25, 2020-07-26, 2020-07-27, 2020-07-28, 2020-07-29, 2020-07-30, 2020-07-31

OTU	Total read	maxid	2019-02-18 Avg. rds	%
Otu0002	967544	*Alnus glutinosa/incana*	23865.7	84.6
Otu0009	206188	*Taxus canadensis/cuspidata*	707.3	2.5
Otu0013	60841	*Corylus avellana*	1969.3	7.0
Otu0115	5764	*Ulmus*	0.0	0.0
Otu0109	3975	*Metasequoia glyptostroboides*	0.0	0.0
Otu0008	196913	*Alnus cordata*	531.3	1.9
Otu0216	1315	*Acer saccharinum*	0.0	0.0
Otu0011	85509	*Fraxinus excelsior*	1142.0	4.0
Otu0185	1691	*Thuja occidentalis*	0.0	0.0
Otu0231	1366	*Alnus*	0.0	0.0
Otu0310	722	*Sycopsis sinensis*	0.0	0.0
Otu1075	1314	*Corylus*	0.0	0.0
Otu0400	404	*Cryptomeria japonica*	0.0	0.0
Otu0423	557	*Taxus x media/cuspidata*	0.0	0.0
Otu0024	27646	*Mercurialis annua*	0.0	0.0
Otu0032	16197	*Populus alba*	0.0	0.0
Otu0202	1890	*Corylus colurna/heterophylla*	0.0	0.0
Otu1214	388	*Fraxinus*	0.0	0.0
Otu0001	2750732	*Urtica dioica*	0.0	0.0
Otu0004	300014	*Humulus lupulus*	0.0	0.0
Otu0005	236873	*Artemisia vulgaris*	0.0	0.0
Otu0006	200431	*Plantago lanceolata*	0.0	0.0
Otu0007	141000	*Betula*	0.0	0.0
Otu0010	135581	*Betula pendula*	0.0	0.0
Otu0012	67841	*Chenopodium album/giganteum*	0.0	0.0
Otu0014	70381	*Lolium perenne*	0.0	0.0
Otu0015	38764	*Plantago major*	0.0	0.0
Otu0016	37068	*Medicago sativa*	0.0	0.0
Otu0018	42526	*Castanea sativa*	0.0	0.0
Otu0019	27872	*Hordeum vulgare*	0.0	0.0
Otu0021	29433	*Castanea crenata*	0.0	0.0
Otu0025	19841	*Fagus sylvatica*	0.0	0.0
Otu0026	19319	*Alnus subcordata*	0.0	0.0
Otu0028	15366	*Plantago coronopus*	0.0	0.0
Otu0034	12844	*Carpinus betulus*	0.0	0.0
Otu0035	15656	*Salix*	0.0	0.0
Otu0036	13782	*Alnus japonica*	0.0	0.0
Otu0037	14270	*Cannabis sativa*	0.0	0.0
Otu0038	11150	*Crepis capillaris*	0.0	0.0
Otu0039	10703	*Angelica sylvestris*	0.0	0.0
Otu0041	12958	*Juniperus*	0.0	0.0
Otu0044	8787	*Sambucus nigra*	0.0	0.0
Otu0047	11415	*Chamaecyparis formosensis*	0.0	0.0
Otu0049	13530	*Lolium multiflorum*	0.0	0.0
Otu0050	13600	*Calluna vulgaris*	0.0	0.0
Otu0055	6485	*Quercus rubra*	0.0	0.0
Otu0059	9218	*Atriplex prostrata*	0.0	0.0
Otu0061	7196	*Capsella bursa-pastoris*	0.0	0.0
Otu0064	5714	*Solanum*	0.0	0.0
Otu0066	7605	*Rumex acetosella*	0.0	0.0
Otu0068	7660	*Chenopodium album/ficifolium*	0.0	0.0
Otu0070	5122	*Trifolium pratense*	0.0	0.0
Otu0071	8475	*Holcus lanatus*	0.0	0.0
Otu0072	6401	*Filipendula ulmaria*	0.0	0.0
Otu0076	5440	*Cupressus arizonica*	0.0	0.0
Otu0080	7479	*Anemone hupehensis*	0.0	0.0
Otu0082	6406	*Salix myrsinifolia/arctica*	0.0	0.0
Otu0085	7066	*Heracleum sphondylium*	0.0	0.0
Otu0086	4938	*Beta vulgaris*	0.0	0.0
Otu0087	4142	*Quercus robur*	0.0	0.0
Otu0088	6588	*Daucus carota*	0.0	0.0
Otu0089	4737	*Platanus x acerifolia*	0.0	0.0
Otu0090	4807	*Urtica urens*	0.0	0.0
Otu0095	4867	*Eupatorium*	0.0	0.0
Otu0097	5608	*Parietaria judaica*	0.0	0.0
Otu0103	7604	*Populus*	0.0	0.0
Otu0105	2856	*Panicum virgatum*	0.0	0.0
Otu0110	3604	*Scorzoneroides autumnalis*	0.0	0.0
Otu0111	2493	*Ostrya japonica*	0.0	0.0
Otu0112	3513	*Sinapis arvensis*	0.0	0.0
Otu0113	2755	*Tilia platyphyllos*	0.0	0.0
Otu0116	3113	*Plantago arenaria*	0.0	0.0
Otu0118	3000	*Brassica*	0.0	0.0
Otu0119	2614	*Sophora japonica*	0.0	0.0
Otu0122	3145	*Hypochaeris radicata*	0.0	0.0
Otu0124	4609	*Arrhenatherum elatius*	0.0	0.0
Otu0130	2560	*Pterocarya*	0.0	0.0
Otu0131	2939	*Populus nigra*	0.0	0.0
Otu0132	3373	*Poa annua*	0.0	0.0
Otu0140	2520	*Phedimus kamtschaticus*	0.0	0.0
Otu0142	2646	*Sinapis alba*	0.0	0.0
Otu0144	3592	*Taxodium distichum/mucronatum*	0.0	0.0
Otu0146	3787	*Clematis armandii*	0.0	0.0
Otu0148	2285	*Salix alba*	0.0	0.0
Otu0150	1902	*Rubus caesius*	0.0	0.0
Otu0155	4081	*Melilotus*	0.0	0.0
Otu0159	1799	*Polygonum aviculare*	0.0	0.0
Otu0165	1739	*Lotus corniculatus*	0.0	0.0
Otu0167	1561	*Calamagrostis x acutiflora*	0.0	0.0
Otu0168	2388	*Quercus* sect. *Quercus*	0.0	0.0
Otu0174	2130	*Impatiens glandulifera*	0.0	0.0
Otu0179	2424	*Agrostis capillaris*	0.0	0.0
Otu0183	2474	*Phacelia tanacetifolia*	0.0	0.0
Otu0187	2096	*Hydrangea macrophylla*	0.0	0.0
Otu0195	2082	*Rumex acetosa*	0.0	0.0
Otu0198	1366	*Mercurialis perennis*	0.0	0.0
Otu0200	2123	*Parietaria officinalis*	0.0	0.0
Otu0201	1826	*Echium vulgare*	0.0	0.0
Otu0204	1990	*Salix matsudana/babylonica*	0.0	0.0
Otu0205	1684	*Amaranthus*	0.0	0.0
Otu0208	1582	*Thuja plicata*	0.0	0.0
Otu0209	2184	*Potentilla reptans*	0.0	0.0
Otu0211	1534	*Achillea millefolium*	0.0	0.0
Otu0221	1406	*Populus simonii*	0.0	0.0
Otu0225	1293	*Symphytum officinale*	0.0	0.0
Otu0230	1146	*Tilia* sect. *Tilia*	0.0	0.0
Otu0233	1127	*Corynephorus canescens*	0.0	0.0
Otu0240	897	*Prunus laurocerasus*	0.0	0.0
Otu0241	2743	*Jacobaea vulgaris*	0.0	0.0
Otu0242	1122	*Lotus pedunculatus*	0.0	0.0
Otu0244	1226	*Poa trivialis*	0.0	0.0
Otu0250	1078	*Quercus petraea*	0.0	0.0
Otu0251	917	*Oenothera biennis/glazioviana*	0.0	0.0
Otu0261	1133	*Hippophae rhamnoides*	0.0	0.0
Otu0263	986	*Digitaria sanguinalis*	0.0	0.0
Otu0273	1513	*Elymus repens*	0.0	0.0
Otu0280	1268	*Molinia caerulea*	0.0	0.0
Otu0284	1151	*Laportea*	0.0	0.0
Otu0297	879	*Stellaria aquatica*	0.0	0.0
Otu0298	714	*Melilotus albus*	0.0	0.0
Otu0303	771	*Echium plantagineum*	0.0	0.0
Otu0309	1169	*Salix schwerinii/viminalis*	0.0	0.0
Otu0313	673	*Phedimus hybridus*	0.0	0.0
Otu0319	1155	*Diplotaxis tenuifolia*	0.0	0.0
Otu0329	701	*Torilis japonica*	0.0	0.0
Otu0331	716	*Mycelis muralis*	0.0	0.0
Otu0335	670	*Hypericum*	0.0	0.0
Otu0339	794	*Papaver rhoeas*	0.0	0.0
Otu0347	725	*Agrostis stolonifera*	0.0	0.0
Otu0349	702	*Alopecurus myosuroides*	0.0	0.0
Otu0352	882	*Sisymbrium officinale*	0.0	0.0
Otu0357	542	*Rumex sanguineus*	0.0	0.0
Otu0374	485	*Veronicastrum virginicum*	0.0	0.0
Otu0378	522	*Urtica*	0.0	0.0
Otu0381	538	*Alopecurus pratensis*	0.0	0.0
Otu0383	461	*Taraxacum officinale*	0.0	0.0
Otu0389	529	*Juniperus communis*	0.0	0.0
Otu0407	579	*Cirsium arvense*	0.0	0.0
Otu0425	376	*Solidago gigantea*	0.0	0.0
Otu0426	461	*Viburnum dilatatum*	0.0	0.0
Otu0430	430	*Lipandra polysperma*	0.0	0.0
Otu0441	370	*Robinia pseudoacacia*	0.0	0.0
Otu0443	513	*Trifolium repens*	0.0	0.0
Otu0466	317	*Solanum villosum*	0.0	0.0
Otu0479	417	*Triglochin maritima*	0.0	0.0
Otu0487	470	*Dactylis glomerata*	0.0	0.0
Otu0493	358	*Spergularia media*	0.0	0.0
Otu0502	310	*Petasites japonicus*	0.0	0.0
Otu0506	275	*Tussilago farfara*	0.0	0.0
Otu0525	287	*Phragmites australis*	0.0	0.0
Otu0530	313	*Lotus corniculatus/glaber*	0.0	0.0
Otu0534	222	*Juniperus chinensis* var. *sargentii*	0.0	0.0
Otu0537	297	*Solidago juncea*	0.0	0.0
Otu0540	444	*Salix triandra*	0.0	0.0
Otu0548	213	*Populus balsamifera/trichocarpa*	0.0	0.0
Otu0558	348	*Sanguisorba officinalis*	0.0	0.0
Otu0566	221	*Laportea*	0.0	0.0
Otu0569	199	*Linaria vulgaris*	0.0	0.0
Otu0572	217	*Acer negundo*	0.0	0.0
Otu0594	250	*Verbascum macrocarpum*	0.0	0.0
Otu0600	463	*Philadelphus pekinensis*	0.0	0.0
Otu0612	129	*Juniperus sabina*	0.0	0.0
Otu0614	274	*Nepeta mussinii*	0.0	0.0
Otu0620	275	*Eryngium campestre*	0.0	0.0
Otu0655	174	*Agostis capillaris/gigantea*	0.0	0.0
Otu0660	174	*Epilobium hirsutum*	0.0	0.0
Otu0708	146	*Sagina procumbens*	0.0	0.0
Otu0731	242	*Bromus hordeaceus*	0.0	0.0
Otu0776	117	*Solanum nigrum*	0.0	0.0
Otu0782	173	*Buddleja officinalis*	0.0	0.0
Otu0784	199	*Rubus bifrons*	0.0	0.0
Otu0810	103	*Calamagrostis varia*	0.0	0.0
Otu0820	325	*Rubus* sect. *Rubus*	0.0	0.0
Otu0848	92	*Bellis perennis*	0.0	0.0
Otu0852	84	*Solanum americanum*	0.0	0.0
Otu0853	135	*Avena strigosa*	0.0	0.0
Otu0855	119	*Cirsium vulgare*	0.0	0.0
Otu0860	122	*Triticum monococcum*	0.0	0.0
Otu0934	173	*Potentilla anserina*	0.0	0.0
Otu0992	116	*Viburnum*	0.0	0.0
Otu1013	58	*Cerastium fontanum*	0.0	0.0
Otu1014	118	*Dryopteris nigrosquamosa*	0.0	0.0
Otu1021	83	*Oxybasis glauca*	0.0	0.0
Otu1042	196	*Dryopteris intermedia*	0.0	0.0
Otu1086	44	*Prunus* sect. *Laurocerasus*	0.0	0.0
Otu1152	40	*Quercus* sect. *Lobatae*	0.0	0.0
Otu1166	50	*Prunus serotina*	0.0	0.0
Otu1169	65	*Amelanchier*	0.0	0.0
Otu1175	48	*Solanum dulcamara*	0.0	0.0
Otu1239	63	*Glyceria fluitans*	0.0	0.0
Otu1282	60	*Artemisia absinthium*	0.0	0.0

2020-08-01		2020-08-02		2020-08-03		2020-08-04		2020-08-05		2020-08-06		2020-08-07		2020-08-08		2020-08-09		2020-08-10		2020-08-11		2020-08-12		2020-08-13		2020-08-14	
Avg. rds	%	Avg. rds	%	Avg. rds	%	Avg. rds	%	Avg. rds	%	Avg. rds	%	Avg. rds	%	Avg. rds	%	Avg. rds	%	Avg. rds	%	Avg. rds	%	Avg. rds	%	Avg. rds	%	Avg. rds	%
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
391.7	3.2	0.0	0.0	0.0	0.0	0.0	0.0	291.0	1.9	1033.3	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	197.3	0.3	1300.7	0.8	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8247.0	66.9	0.0	0.0	0.0	0.0	0.0	0.0	3312.0	21.6	27102.0	45.0	43284.3	56.6	81375.3	66.3	31327.3	63.0	31974.3	62.4	48086.3	63.8	97929.0	61.7	29763.3	51.0	23358.5	42.7
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	555.0	3.6	1420.0	2.4	3063.0	4.0	6598.0	5.4	1538.7	3.1	4238.3	8.3	9654.3	12.8	12607.3	7.9	3731.3	6.4	8313.5	15.2
2583.7	21.0	0.0	0.0	0.0	0.0	0.0	0.0	3185.0	20.7	17871.7	29.7	13522.3	17.7	17150.3	14.0	7684.3	15.4	8972.3	17.5	5420.3	7.2	12167.3	7.7	10332.3	17.7	4167.5	7.6
837.3	6.8	0.0	0.0	0.0	0.0	0.0	0.0	697.0	4.5	3071.7	5.1	5520.3	7.2	6541.3	5.3	2456.3	4.9	1284.3	2.5	3011.7	4.0	9031.7	5.7	3121.7	5.4	12473.5	22.8
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	252.0	1.6	2855.0	4.7	208.7	0.3	462.3	0.4	804.3	1.6	269.7	0.5	1194.0	1.6	2463.7	1.6	4430.7	7.6	2231.0	4.1
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	31.0	0.2	122.3	0.2	912.7	1.2	349.0	0.3	318.7	0.6	543.3	1.1	2287.0	3.0	4183.0	2.6	3520.0	6.0	1261.5	2.3
142.3	1.2	0.0	0.0	0.0	0.0	0.0	0.0	236.0	1.5	824.0	1.4	329.7	0.4	3048.0	2.5	252.7	0.5	329.0	0.6	606.7	0.8	3247.0	2.0	0.0	0.0	2485.5	4.5
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5757.0	37.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2015.7	1.3	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	159.3	0.2	0.0	0.0	0.0	0.0	93.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	23.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	101.3	0.1	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	341.3	0.3	347.3	0.7	229.0	0.4	48.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	56.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	265.7	0.4	0.0	0.0	701.3	0.6	455.3	0.9	235.7	0.5	167.7	0.2	2555.3	1.6	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	891.7	0.7	0.0	0.0	0.0	0.0	19.0	0.0	396.7	0.2	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	223.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	38.0	0.1	216.0	0.3	160.0	0.1	159.0	0.3	179.0	0.3	160.0	0.2	599.0	0.4	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	821.0	1.1	351.3	0.3	111.7	0.2	262.7	0.5	0.0	0.0	3080.0	1.9	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	38.0	0.2	486.0	0.8	317.0	0.4	390.7	0.3	99.0	0.2	183.3	0.4	181.7	0.2	257.7	0.2	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	134.7	0.2	47.3	0.0	225.7	0.5	0.0	0.0	132.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	29.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	170.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0	835.3	1.4	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	76.0	0.1	235.0	0.3	42.3	0.0	122.7	0.2	135.3	0.3	386.0	0.5	673.0	0.4	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	213.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	49.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1950.0	2.5	0.0	0.0	167.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	953.0	1.6	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1731.0	2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	206.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	69.3	0.1	0.0	0.0	229.3	0.1	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	382.3	0.6	254.0	0.3	0.0	0.0	292.0	0.6	0.0	0.0	62.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	275.0	0.5	858.0	1.1	183.7	0.1	62.7	0.1	127.7	0.2	89.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	73.0	0.1	0.0	0.0	0.0	0.0	116.3	0.2	493.7	1.0	0.0	0.0	0.0	0.0	543.3	0.9	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	90.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.3	0.0	45.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	277.3	0.4	48.7	0.0	0.0	0.0	28.3	0.1	0.0	0.0	1238.7	0.8	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1296.7	2.6	608.3	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	569.0	0.9	0.0	0.0	184.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	122.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	76.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	154.0	0.2	83.7	0.1	0.0	0.0	50.0	0.1	207.7	0.3	19.0	0.0	849.7	1.5	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	269.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	135.7	0.2	0.0	0.0	53.7	0.1	0.0	0.0	115.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1305.7	0.8	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	104.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	271.7	0.5	0.0	0.0	1266.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	86.0	0.2	0.0	0.0	39.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	903.7	0.6	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	699.3	0.4	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	269.3	0.2	186.3	0.3	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	105.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	69.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	209.7	0.3	998.3	1.3	102.3	0.1	134.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	806.7	1.3	1238.0	1.6	0.0	0.0	0.0	0.0	123.3	0.2	30.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	133.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
119.3	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	350.0	0.3	0.0	0.0	126.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	593.3	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	42.0	0.3	0.0	0.0	0.0	0.0	784.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	357.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	577.7	0.4	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	288.0	0.2	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	437.3	0.7	0.0	0.0	842.0	0.7	0.0	0.0	0.0	0.0	273.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	31.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	455.5	0.8
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	121.3	0.2	427.7	0.3	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	47.3	0.1	43.3	0.0	19.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	35.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	215.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	314.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	34.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	307.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	35.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	160.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	30.0	0.2	0.0	0.0	0.0	0.0	66.0	0.1	11.0	0.0	0.0	0.0	22.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	83.3	0.2	21.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	187.0	0.1	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	435.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	98.0	0.1	173.7	0.1	117.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	255.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	38.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.3	0.1	106.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	182.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	138.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.7	0.0	244.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	53.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	152.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	124.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	64.0	0.1	0.0	0.0	73.0	0.1	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	219.0	1.4	0.0	0.0	91.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	133.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	95.0	0.2	0.0	0.0	0.0	0.0	25.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	217.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	89.0	0.1	42.3	0.0	19.7	0.0	0.0	0.0	26.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	178.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	118.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	117.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	140.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	64.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	93.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	45.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	38.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	32.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	39.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	42.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	73.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	37.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	34.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	27.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	30.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Manuscript V

Multiproxy analysis of permafrost preserved faeces provides an unprecedented insight into the diets and habitats of extinct and extant megafauna

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Multiproxy analysis of permafrost preserved faeces provides an unprecedented insight into the diets and habitats of extinct and extant megafauna

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Abstract

The study of faecal samples to reconstruct the diets and habitats of extinct megafauna has traditionally relied on pollen and macrofossil analysis. DNA metabarcoding has emerged as a valuable tool to complement and refine these proxies. While published studies have compared the results of these three proxies for sediments, this comparison is currently lacking for permafrost preserved mammal faeces. Moreover, most metabarcoding studies have focused on a single plant-specific DNA marker region. In this study, we target both the commonly used chloroplast *trn*L P6 loop as well as nuclear ribosomal ITS (nrITS). The latter can increase taxonomic resolution of plant identifications but requires DNA to be relatively well preserved because of the target length (~300 - 500 bp). We compare DNA results to pollen and macrofossil analyses from permafrost and ice-preserved faeces of Pleistocene and Holocene megafauna. Samples include woolly mammoth, horse, steppe bison as well as Holocene and extant caribou. Most plant identifications were found using DNA, likely because the studied faeces contained many vegetative remains that could not be identified using macrofossils or pollen. Several taxa were, however, identified to lower taxonomic levels uniquely with macrofossil and pollen analysis. The nrITS marker provides species level taxonomic resolution for commonly encountered plant families that are hard to distinguish using the other proxies (e.g. Asteraceae, Cyperaceae and Poaceae). Integrating the results from all proxies, we are able to accurately reconstruct known diets and habitats of the extant caribou. Applying this approach to the extinct mammals, we find that the Holocene horse and steppe bison were not strict grazers but mixed feeders living in a marshy wetland environment. The mammoths showed highly varying diets from different non-analogous habitats. This confirms the presence of a mosaic of habitats in the Pleistocene 'mammoth steppe' that mammoths could fully exploit due to their flexibility in food choice.

Key words: diet – DNA metabarcoding – faecal samples – nrITS – paleoecology – plant macrofossils – Pleistocene – pollen – proxy comparison – *trn*L

Highlights

- The first integrated analysis of DNA, pollen and macrofossils from permafrost faeces
- Successful amplification of up to 28,6 kyr old DNA using long, plant-specific nrITS
- High taxonomic resolutions allow detailed insights in extinct megafaunal habitat
- Macrofossils and DNA show diverse woolly mammoth diet and use of 'mammoth steppe'

1. Introduction

During much of the Late Pleistocene epoch, Siberia, Alaska and northern Canada were connected, forming a dry and largely treeless landmass known as Beringia (Hopkins et al. 1982, Hopkins 1959). The landscape was dominated by emblematic megafauna such as woolly mammoths and steppe bison, and in terms of biomass some authors have compared this period to the current African savannah (Zimov et al. 2012). Mammals had a major role in shaping vegetation community and structure by reducing vegetation density, enhancing nutrient turnover, dispersing seeds and reducing fire potential (Johnson 2009, Hester et al. 2006, Guthrie 2001). Reconstructing the species composition of this former plant community without a modern analogue, as well as the corresponding diets of the mammals that roamed it has been challenging.

According to Guthrie (1990) there were mainly open landscapes with highly productive graminoids and *Artemisia* sp. in a steppe-tundra biome that is often designated the 'mammoth steppe'. Recent studies have changed the view of the mammoth steppe vegetation into a more heterogeneous mosaic of different habitats. This mosaic consisted of areas rich in shrubs combined with permanent moist areas and productive grasslands (Chytrý et al. 2019, Lozhkin et al. 2019, Zazula et al. 2006). Willerslev et al. (2014) further showed that forbs (non-graminoid herbaceous vascular plants) were more abundant in the environment than previously thought, and featured in megafaunal diets to provide important proteins. Relatively little is known, however, about the specific plant species in megafaunal diets.

The shift in appreciation of the Beringian megafaunal habitats has been catalysed by a growing body of research that uses a multidisciplinary approach, combining pollen and plant macrofossils with DNA metabarcoding (Hofreiter et al. 2000, van Geel et al. 2008, Sønstebø et al. 2010, van Geel et al. 2011b, van Geel et al. 2011a, Van Geel et al. 2014, Gravendeel et al. 2014, Willerslev et al. 2014, Haarsma, Siepel and Gravendeel 2016, Boast et al. 2018). By improving taxonomic resolution and finding complementary taxa, DNA metabarcoding can help to resolve vegetation classifications where species resolution is required (e.g. steppe and tundra, partly defined on distinct species of grass; Swanson 2006). Several studies on lake sediments have shown that instead of replacing traditional methods, DNA metabarcoding acts as a complementary proxy by revealing both additional taxa and providing increased taxonomic resolution (Pedersen et al. 2013, see e.g. Boessenkool et al. 2014, Rawlence et al. 2014, Parducci et al. 2019). While pollen grains mostly show a regional signal due to dominant wind-dispersed pollen (grasses and *Artemisia* sp.), DNA may represent a more local signal that is more similar to the spectrum of macrofossil taxa (Boessenkool et al. 2014, Alsos et al. 2018, Jorgensen et al. 2012).

While the studies cited above provide a good overview of the advantages and drawbacks of the different proxies used, all of these studies focussed on lake sediments. So far, there are few studies comparing these proxies in megafaunal faecal samples (e.g. van Geel et al. 2008, Hofreiter et al. 2003, Gravendeel et al. 2014). Strictly speaking, the faecal samples of extinct megafauna are not coprolites since they are not fossilized but perfectly preserved in permafrost. However, the plant macrofossils in these samples are drastically affected by masticatory and digestive processes, which may result in differential preservation of taxa and fragments becoming unidentifiable (van Geel et al. 2008). For pollen recovered from faeces an additional complicating factor is that the faecal samples are often dominated by wind-transported pollen or pollen deriving from ingestion of inflorescences from plants that were flowering at the time of consumption (Van Geel et al. 2014). The advantage of DNA as a proxy for dietary reconstruction is that it does not depend on flowering time or time of fruit setting, as vegetative plant remains are included in the DNA record (Willerslev et al. 2014). However, as in ancient sediments, not all taxa are recorded using DNA metabarcoding due to incomplete reference libraries, PCR bias, primer mismatches and DNA degradation (Jorgensen et al. 2012).

Most studies of ancient DNA from sediments have relied either on the P6 loop of the chloroplast *trn*L (UAA) intron or the *rbc*L gene, and both give good taxonomic resolution for some plant taxa but limited for others (Sønstebø et al. 2010, Taberlet et al. 2006). While in the animal kingdom the mitochondrial marker COI can be used as a universal barcode for identifying species (Hebert et al. 2003) no such universal barcode has been identified for plants. For this reason a combination of markers has been advised for plants, including both a nuclear marker and a plastid marker (CBOL Plant Working Group et al. 2011). Since permafrost acts as an excellent natural freezer, even long DNA fragments (up to 510 bp) have been recovered from sediments as old as 400 kyr (Lydolph et al. 2005, Willerslev et al. 2014). Yet in the study of ancient megafaunal faeces, the relatively long nuclear ribosomal ITS (nrITS) has rarely been used, and only to amplify relatively short amplicons (e.g. 240 bp in the Cape Blossom mammoth; van Geel et al. 2011b). Due to its length, nrITS has the advantage of being able to provide a higher taxonomic resolution, which in turn can give better insight into the paleoenvironmental conditions represented by the taxa in a sample.

In this study, we aim to 1) investigate the potential of using the nrITS marker on megafaunal faeces, 2) compare the nrITS results to *trn*L, pollen and macrofossil records and 3) integrate results of all proxies to obtain a detailed reconstruction of ancient megafaunal diets and habitats. To this end, we applied DNA metabarcoding, pollen and macrofossil analysis on a variety of permafrost and ice-preserved faecal samples from extinct and extant megafauna, specifically woolly mammoth, steppe bison, horse and caribou. In addition to the *trn*L P6 loop, we target the nrITS regions nrITS1 and nrITS2. The wide temporal range of the samples (28,000 to modern) further allows us to capture potential taphonomic effects on the recovery of the different marker regions and read counts, while inclusion of faecal samples from extant caribou with known diets and habitats enables validation of the diet and habitat reconstructions of the extinct megafauna.

2. Materials and Methods

2.1 Material

Eleven faecal samples from four mammal species were included (Table 1; for detailed information about location and dating see Table S1). Several of the samples we used here have been studied previously and DNA from the original material - which was stored at −80°C - was re-extracted and analysed here, except for the Oyogas Yar horse and Yakutian bison of which DNA extracts from previous studies were used (CTAB DNA extraction; Doyle and Doyle 1987). All samples are derived from Russia, Canada and USA (Figure 1) and are briefly discussed below.

2.1.1 Holocene and modern mountain caribou

Three northern mountain caribou (*Rangifer tarandus caribou* (Gmelin, 1788)) faecal samples were collected from cores in ice patch deposits in the Selwyn Mountains, Northwest Territories, Canada. Caribou visit these ice patches during the summer months to escape summer heat and insect harassment and their faeces are subsequently buried by snow creating stratigraphically discrete faecal bands that are very well preserved. The samples include faeces from modern caribou collected from the surface near the ice patch (Selwyn A), and two samples of late Holocene age collected from the ice core, Selwyn B and Selwyn C. From Selwyn A, DNA was retrieved by Galloway et al. (2012) confirming that caribou was indeed the producer of the faeces. For the other samples, the faecal material was identified as being deposited by caribou based on the general shape, size and texture of the pellets, without additional DNA confirmation.

*Table 1. Overview of the samples used in this study including the existing and newly generated data, source of material and their age and collection locality. References from where the existing data was taken are [1] Galloway et al. (2012) [2] Boeskorov et al. (2014) [3] Gravendeel et al. (2014) [4] Van Geel et al. (2014) [5] van Geel et al. (2011b) [6] van Geel et al. (2008) [7] Harington and Eggleston-Stott (1996). * D = DNA, M = plant macrofossils, P = pollen. †DNA extract from previous study used.*

Species	Name	Reference	Existing data*	Newly generated data*	Material	measured ^{14}C age BP	Locality
Caribou	Selwyn A (KfTe-1 surface)	[1]	P	D M	Faeces from ice patch	modern	Selwyn Mountains, NT, Canada
Caribou	Selwyn B (KfTe-1-C2-1)	[1]	M P	D	Faeces from ice patch	1,630 ± 40	Selwyn Mountains, NT, Canada
Caribou	Selwyn C (KfTe-1-C1-3)	[1]	M P	D	Faeces from ice patch	2,840 ± 40	Selwyn Mountains, NT, Canada
Horse	Oyogas Yar	[2,3]	D M P	D†	Faeces from colon	4,630 ± 35	N Sakha, Ust-Yana region, Russia
Bison	Yakutian	[2,4]	D M P	D†	Rumen	9,310 ± 45 9,295 ± 45	N Sakha, Chukchalakh Lake, Yana Mammoth reserve
Woolly mammoth	Cape Blossom	[5]	D M P	D	Faeces	12,300 ± 70	Kotzebue Sound, NW Alaska, USA
Woolly mammoth	Yukagir	[6]	D M P	D	Faeces from colon	18,680 ± 100	N Sakha, oxbow lake near Maxunuokha River, Russia
Woolly mammoth	Adycha	This study	-	D M P	Faeces	21,250 ± 100	N Sakha, Adycha River floodplain, Russia
Horse	Yukon	[7]	D M	D P	Faeces from intestine	26,280 ± 210	Last Chance Creek near Dawson City, Yukon, Canada
Woolly mammoth	Abyland	This study	-	D M P	Faeces	28,460 ± 160	N Sakha, Oguruoha River, Abyysky District, Russia
Woolly mammoth	Maly Lyakhovsky	This study	-	D M P	Faeces from stomach	28,610 ± 110	N Sakha, Maly Lyakhovsky Island, Russia

2.1.2 Holocene bison and horse

A colon sample of a horse (Oyogas Yar or Yukagir horse; *Equus* cf. *lenensis* Russanov, 1968) of middle Holocene age and a rumen sample of a Yakutian steppe bison (*Bison priscus* (Bojanus, 1825)) of early Holocene age were taken directly from permafrost preserved animals from the Sakha Republic, Russia (Boeskorov et al. 2014, Gravendeel et al. 2014, Van Geel et al. 2014) (Table 1). The Oyogas Yar horse was identified as being most closely related to the extinct Lena horse, *Equus lenensis*, based on body size measurements (Boeskorov et al. 2018).

2.1.3 Pleistocene mammoth and horse

Six Pleistocene faecal samples were analysed, including five woolly mammoths (*Mammuthus primigenius* (Blumenbach, 1799)) and one Yukon horse (*Equus lambei* (Hay, 1917)). Four specimens were obtained from the republic of Sakha (Yakutia), Russia, including the Maly Lyakhovsky, Abyland, Adycha and Yukagir mammoths. The Cape Blossom mammoth sample (or Alaskan Late Glacial mammoth) was obtained from Cape Blossom, Alaska, USA, and the Yukon horse was obtained from Dawson City, Yukon, Canada. Faecal samples were taken directly from, or in close vicinity to the permafrost preserved animals, except for the Abyland, Adycha and Cape Blossom samples which were loose faeces. Validation of the faeces as being derived from woolly mammoth for the Yukagir, Maly Lyakhovsky, Cape Blossom and Yukon samples is based on previous studies (Harington and Eggleston-Stott 1996, van Geel et al. 2008, van Geel et al. 2011b, Grigoriev et al. 2017). The identities of the Adycha and Abyland samples were confirmed using Sanger DNA analyses (Supplementary Text S2).

2.2 Radiocarbon dating

Radiocarbon dates of the caribou, horse, bison and Cape Blossom and Yukagir mammoth faeces were reported in previous publications (van Geel et al. 2011b, Galloway et al. 2012, Boeskorov et al. 2014, van Geel et al. 2008, Gravendeel et al. 2014, Harington and Eggleston-Stott 1996). The faecal samples of the Adycha, Abyland and Maly Lyakhovsky mammoths were dated at the AMS facility of the Centre for Isotope Research of the University of Groningen (The Netherlands). The ^{14}C ages are reported in BP, the conventional unit, and includes a correction for isotope fractionation and a defined half-life (Van der Plicht and Hogg 2006). The ^{14}C dates are calibrated into calendar ages using the presently recommended calibration curve IntCal20 (Reimer et al. 2020). The calibrated dates are reported in cal. BP, defined as calendar years relative to AD 1950 (Table S1).

2.3 Pollen and macrofossils

If available, pollen and macrofossil results were taken directly from published records (Table 1). Data was available for the Yukagir and Cape Blossom mammoths, the Yakutian bison, Oyogas Yar horse and two of the Selwyn caribou samples (van Geel et al. 2008, van Geel et al. 2011b, Galloway et al. 2012, Van Geel et al. 2014, Gravendeel et al. 2014). For Selwyn caribou A, only a pollen analysis was available (Galloway et al. 2012). If multiple counts were present from different subsamples, these were averaged to obtain one pollen count per sample. Macrofossil results for the Yukon horse were generated by Paleotec Services, Canada. This sample was previously studied for its plant DNA using *trn*L by Willerslev et al. (2014).

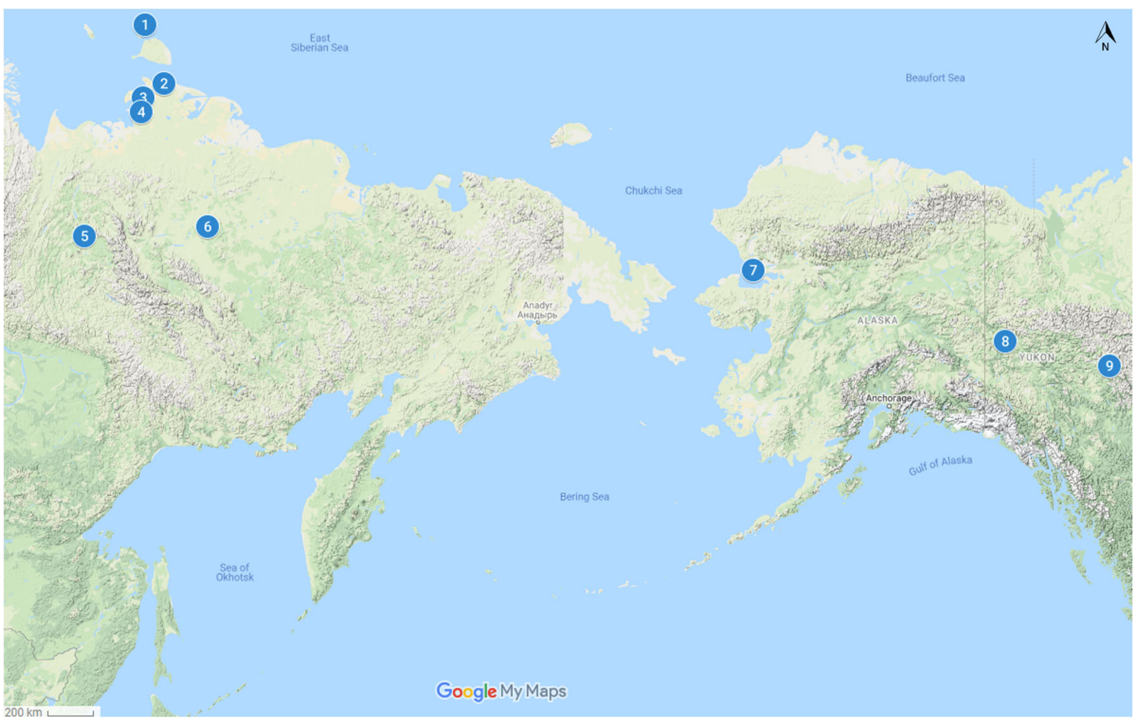

Fig. 1. Sample localities. (1) Maly Lyakhovsky mammoth, (2) Oyogas Yar horse, (3) Yakutian bison, (4) Yukagir mammoth, (5) Adycha mammoth, (6) Abyland mammoth, (7) Cape Blossom mammoth (8) Yukon horse and (9) Selwyn caribou A, B and C.

Pollen and spores (hereafter 'pollen') counts and macrofossil analysis were performed for the faeces of the Abyland, Adycha and Maly Lyakhovsky mammoths, Yukon horse (only pollen) and Selwyn caribou A (only macrofossil). The method for pollen preparation followed Faegri and Iversen (1989). Samples for pollen and macrofossil analyses were taken from the core of the faeces. Microscopic analysis of pollen was done at 400X and 1000X magnification. Pollen identifications were based on Moore, Webb and Collison (1991) and Beug (2004) and a pollen reference collection. For the preparation of macrofossils, Mauquoy and Van Geel (2007) was followed. Bryophyte specimens were identified using Lawton (1971), Crum, Anderson and Anderson (1981) and Vitt and Buck (1992).

In pollen analysis, the use of 'types' is common to denote a group of taxa that produce pollen that cannot be identified to a lower taxonomic level using microscopic analysis. *Potentilla*-type pollen for example includes pollen from species of the genera *Potentilla*, *Comarum*, *Fragaria* and *Sibbaldia* (Reitsma 1966), which are all part of the subtribe Fragariinae of the Rosaceae family. All 'type' identifications were therefore converted to their corresponding maximum taxonomic level so as to better compare them to the DNA and macrofossil data. Similarly, the commonly used Asteraceae pollen subdivision Tubuliflorae and Liguliflorae were converted to Asteraceae subfamilies Asteroideae and Cichorioideae, respectively.

2.4 Molecular analysis: DNA extractions and primer selection

2.4.1 Molecular analysis: DNA extractions

All pre-PCR DNA work (including subsampling) took place in the dedicated ancient DNA laboratory of Naturalis Biodiversity Center (Leiden, The Netherlands). We subsampled the faecal samples following recommendations of Cooper and Poinar (2000) and Wood and Wilmshurst (2016). Samples were UVC-irradiated for 5 min and the outer layer (±2 mm) removed with a clean scalpel. This process was repeated before taking three subsamples (±100 mg each) from the middle of the bisected samples.

The subsamples were ground in a Retsch CryoMill at –196°C, before DNA was extracted separately for each subsample following the silica-based extraction protocol of Rohland and Hofreiter (2007), adjusted to the smaller volume of material used as described in Stech et al.

(2011). DNA extracts from the three subsamples were then pooled together. To control cross-contamination, DNA extractions were carried out in batches of two to three samples with one extraction blank (excluding faecal material) included in each batch (in total five extraction blanks).

2.4.2 Molecular analysis: Primer selection and DNA amplification

Amplification of chloroplast DNA was done using *trn*L intron P6 loop *g* and *h* primers (Taberlet et al. 2006) (Table S3). Nuclear ribosomal Internal Transcribed Spacer regions were amplified using plant-specific primer pairs for nrITS1 (ITS-p5 / ITS-u2; Cheng et al. 2016) and nrITS2 (ITS-p3 / ITS4; Cheng et al. 2016, White et al. 1990) as well as fungi-specific primer pair for nrITS2 (fITS7 / ITS4; White et al. 1990, Ihrmark et al. 2012) to control for amplification of non-target DNA (Table S3).

A dual-indexing approach was applied using a set of unique primer-adapter combinations as described in Fadrosh et al. (2014). All DNA extracts were diluted 1:10, except for the Abyland and Cape Blossom mammoths, for which a 1:50 dilution was used. PCRs were carried out on a Bio-Rad C1000 Touch or Bio-Rad S1000 thermal cycler in 25 µl final volumes consisting of 15.4 µl nuclease-free ultrapure water, 1x Phire Green reaction buffer, 0.52 µM of each primer, 1.25 mM of dNTPs, 1 U Phire Hot Start II DNA Polymerase and 1 µl of the 1:10 or 1:50 diluted DNA sample template. Gradient PCR results were used to determine the optimum annealing temperature for each primer set. The following amplification protocol was used: a 30 sec activation step at 98°C, 40 cycles including 5 sec at 98°C, 5 sec annealing at 55-60°C (depending on primers used; Table S3) and 15 sec elongation at 72°C, plus a final extension step at 72°C for 5 min.

In order to mitigate stochasticity of DNA results, three PCR replicates were used for all samples using a unique tag combination for each replicate. *Coelogyne fimbriata* (Orchidaceae), native to tropical SE Asia, was used as a positive control for each primer set. The resulting PCR products were pooled into two pools based on amplicon length: a pool containing the shorter *trn*L fragments and a pool containing the longer nrITS fragments. Equimolar pools were made after measuring DNA concentrations on a QIAXcel (Qiagen). The pools were purified using Agencourt AMPure XP beads (Beckman Coulter), with a 1:0.9 (nrITS) or 1:1 (*trn*L) ratio and quantified using an Agilent 2100 Bioanalyzer DNA High sensitivity chip. Illumina adapters were ligated onto the amplicons using TruSeq DNA Nano Library Preparation kit (Illumina, USA) and subsequently sequenced at the Norwegian Sequencing Centre on an Illumina MiSeq v2 300 cycles (150 bp x 2) for the *trn*L fragments and an Illumina MiSeq v3 600 cycles (300 bp x 2) for the nrITS fragments.

2.5 Molecular analysis: DNA sequence analysis and filtering

2.5.1 Mammal DNA identification

The mitochondrial Sanger sequencing reads obtained from the Abyland and Adycha faeces were aligned and trimmed using BioEdit version 7.2.5 (Supplementary Text S2; Hall 1999). A MegaBLAST search was performed to check the resulting consensus sequences against the NCBI nucleotide database, and only sequences resulting in percentage ID >98% were kept.

2.5.2 NrITS sequences

The three pools of nrITS sequences (plant nrITS1 and nrITS2, fungal nrITS2) were analysed separately with a custom pipeline on the OpenStack environment of Naturalis Biodiversity Center through a Galaxy instance (Afgan et al. 2018). Paired-end reads were first merged with PEAR (Zhang et al. 2014) using the standard settings and discarding non-merged reads. Amplicons were subsequently demultiplexed using the linked adapters option in Cutadapt version 2.8 (Martin 2011). Only sequences containing both unique sample tags and forward and reverse primers were kept. Primer sequences were subsequently removed from the sequences with Cutadapt, allowing a maximum error rate of 0.15 (i.e. 3 to 4 bases).

The sequences were quality filtered and trimmed using the PRINSEQ sequence filter / converter tool (Schmieder and Edwards 2011), using a minimum mean quality score of 20 and removing any sequences shorter than 150 bp. Sequences were dereplicated and sorted by size in VSEARCH v2.14.2 (Rognes et al. 2016) and clustered into Operational Taxonomic Units (OTUs) using the unoise3 algorithm from USEARCH v11.0.667 (Edgar 2016) with default settings, removing singletons and potential chimeras. OTUs were subsequently identified using a MegaBLAST search against the NCBI Genbank nucleotide database for plant nrITS1 and nrITS2, (Benson et al. 2012) and the UNITE fungal nucleotide database for fungal nrITS2 (Nilsson et al.

2019). OTUs that matched at least 80% in coverage as well as identity to NCBI Genbank were kept. For final taxon identifications, a minimum of 80% identity recognition for family, 90% identity for genus and 97% for the species level was used. Sequences were further filtered in R (version 3.5.2) (R Core Team, 2020) to remove sequences with a lower number of reads from any of the samples than in negative controls (either extraction or PCR). This resulted in removal of suspected food contaminants including *Pisum sativum*, *Brassica rapa/napus* for nrITS1 and *Citrus* sp., *Cucumis sativus* and *Musa* sp. for plant nrITS2. For plant nrITS1 and nrITS2, the positive control was successfully amplified and the presence of *Coelogyne fimbriata* reads in the non-control samples was used to determine an OTU filtering threshold to correct for potential leakage. For nrITS2, this resulted in reduction of each sequence read count per replicate with 0.3%, while this value was 0.35% for nrITS1 and fungal nrITS2 (see Table S5.1 for full steps and read counts). Remaining replicates were merged while averaging the read counts per OTU. Finally, OTUs at species or genus level with the same taxonomic assignment were aggregated.

A curated arctic and boreal vascular plant and bryophyte database exists for *trn*L (see below), but not yet for nrITS. The plant nrITS results have therefore been carefully checked for their presence in the geographical areas where the faeces were collected. To this end, the Panarctic Flora (Elven et al. 2011), database of vascular plants of Canada (VASCAN) (Brouillet et al. 2010), GBIF (www.gbif.org) and the Plants of the World Online (POWO 2019) were used (Cody 2000, Boufford et al. 2016, Brouillet et al. 2010). This resulted in some aberrant records, such as non-boreal/tropical plants (e.g. *Celtis* sp. and *Pteroceltis* sp.) as well as some likely food contaminants (e.g. *Allium cepa*, *Lagenaria siceraria*) and these were manually removed (Supplementary Information S4). When many blast hits from different species with an equal BIT-score were found, the top 20 blast hits were manually checked for likely boreal species. When several species met this criterion, the last common ancestor of these hits was chosen. Fungal OTUs were assigned to functional groups (guilds) using FUNGuild (Nguyen et al. 2016).

2.5.3 Trn*L sequences*

The *trn*L sequences were analysed with the OBITools package (Boyer et al. 2016). OBITools is commonly used in ancient plant DNA studies with *trn*L as it allows direct assignment of sequences to taxa. The forward and reverse reads were assembled using *illuminapairedend* (min quality score of 40) and subsequently assigned to the corresponding samples using *ngsfilter* (only keeping sequences with a 100% tag match and allowing for a maximum of three mismatches with the primers). Using *obiuniq*, strictly identical sequences were merged, after which *obigrep* was used to remove singletons, sequences with ambiguous nucleotides and sequences shorter than 10 bp. Following Bellemain et al. (2013), *obiclean* was used to identify sequencing and amplification errors with a threshold ratio of 5% for reclassification of sequences identified as 'internal' to their corresponding 'head' sequence. The resulting sequences were compared to two taxonomic databases using *ecotag*. The first priority was given to a local taxonomic reference library containing arctic and boreal vascular plant taxa and bryophytes (arctborbryo database; Sønstebø et al. 2010, Willerslev et al. 2014, Soininen et al. 2015). A second reference library based on the global EMBL database (release 137) was used for mitigation of missing taxonomic assignment due to species potentially lacking in the first database (see Table S5.2 for full steps and read counts). The computations were performed on resources provided by UNINETT Sigma2 - the National Infrastructure for High Performance Computing and Data Storage in Norway.

The resulting sequences were further filtered in R to remove sequences that had (a) <100% identity match to the reference libraries, (b) <10 reads per PCR repeat and (c) sequences with higher number of reads in negative controls compared to the samples. This process resulted in the removal of suspected contaminant sequences derived from modern food plants such as *Solanum* subgenus *Lycopersicon* and *Oryza* sp. as well as some potential true positives including the genera *Solidago*, *Trifolium* and *Helictochloa*. No *Coelogyne fimbriata* reads were recorded in the positive control for *trn*L, despite the presence of *C. fimbriata* sequences in the NCBI Genbank database (e.g. MK356212.1). The presence of *C. fimbriata* reads in the non-control samples to determine the MOTU filtering threshold (as was used for nrITS filtering) could therefore not be used. Instead, the maximum number of reads from the most abundant OTU (*Salix* sp.) in control samples was used, and accordingly each sequence read count per replicate was reduced with 1.0%. Remaining replicates were merged while averaging the read counts per OTU. Finally, OTUs at species or genus level with the same taxonomic assignment were aggregated.

Although this filtering resulted in losing potential true positives, these were only present in a low number of reads (<0.1% of the total number of reads). Furthermore, this relatively rigorous filtering allowed for removal of nearly all suspected false positives in the samples, and this was given preference over retaining as many true positives as possible (cf. Alsos et al. 2018). Remaining identifications were manually checked for suspected contaminants or taxa that were known not to occur in the arctic and boreal region. This process resulted in the removal of a few remaining suspected contaminants (Supplementary Information S4). This is a common problem in metabarcoding studies, and the taxa we identify are similar to those found in other studies (Chua et al. 2021, Van Geel et al. 2014, Willerslev et al. 2014).

2.6 Diet analysis and habitat types

The DNA reads were converted to relative read abundances to facilitate comparison with macrofossil and pollen data. When referring to 'diet' in this study from now on, we refer to the composition of the last meal consumed by the animals studied here, as inferred through the multiproxy approach on the faecal samples. The taxon identifications were grouped into the major groups of graminoids (grasses, sedges, rushes), forbs, shrubs/deciduous trees, coniferous trees, mosses and lichens. Since pollen records are biased towards high pollen producers and show primarily a regional signal (Jorgensen et al. 2012), they cannot be used to reliably reconstruct the diet. The record of macrofossils is strongly influenced by the food choice of the animal during its last meal (Mol et al. 2006) and has been shown to largely overlap with DNA results (Parducci et al. 2015). Therefore, to provide a visual representation of the last diets, the average values of the relative abundance of the macrofossil results and all available DNA results were taken.

Plant identifications from DNA, macrofossils and pollen that could be assigned to the species level were used to reconstruct the habitat types of the megafaunal last diets. Some genera that are typically found in specific habitats have also been included (e.g. *Eriophorum*, *Juncus* in wetlands and *Puccinellia* in saline meadows). Habitat types were identified using a combination of sources: efloras (Brach and Song 2006), Kienast et al. (2005), Troeva et al. (2010), Janská et al. (2017), Axmanová et al. (2020) and references therein. Only the presence of taxa and not their abundance was used to reconstruct the habitats, since abundance of certain taxa is highly affected by the selective food choice of the animals and may not reflect the palaeovegetation (Ashastina et al. 2018). The taxa were divided into 13 habitat types, ranging from relatively dry (steppe) to very wet (wetland: marsh, bog, fen, swamp). The modern known habitat preferences for the plant species were used, and the resulting habitat types are compared to modern analogues. For the modern caribou (Selwyn caribou A), the habitat consists of boreal forest in low-elevation areas, found together with arctic-alpine tundra at high altitudes (Galloway et al. 2012).

3. Results

3.1 Mammal sample identity

Genetic analyses confirmed the identity of both the Abyland and Adycha samples as *Mammuthus primigenius* (woolly mammoth), with a 100% match in both cover and identity (Table S2). This was further supported by the shape and size of the faecal pellets.

3.2 Pollen and macrofossil recovery

3.2.1 Pollen

For seven mammals, the pollen records were taken from the published records while four were newly generated in this study (Tables S6.1 – S6.11). The Selwyn caribou samples studied by Galloway et al. (2012) showed a mixed pollen signal with trees (ranging from 25-30%, *Picea* sp., *Pinus* sp., *Alnus* sp. etc.) and forbs (34-40%, mostly *Artemisia* sp.) being the most abundant. Selwyn caribou A further showed 33% shrubs (*Salix* sp. and *Betula* sp.) which were missing in Selwyn B, and rare (6%) in Selwyn C. Low amounts (<10%) of undifferentiated Poaceae as well as insect-dispersed pollen (e.g. Asteraceae, Ericaceae, *Polemonium* sp. and Rosaceae) were identified in all three caribou samples.

The Holocene Yakutian bison and Oyogas Yar horse had high amounts of undifferentiated Poaceae pollen (71% and 92%, respectively; Van Geel et al. 2014, Gravendeel et al. 2014). Cyperaceae was the second most abundant pollen type (4%) in the horse and also accounted for 6% in the bison sample. The bison further had a relatively high amount (9%) of Apiaceae pollen.

Other pollen in both samples was derived from various shrubs (*Betula* sp. and *Salix* sp.) and forbs (e.g. Asteraceae, Plantaginaceae, Rosaceae). Tree-derived pollen (*Abies* sp., *Pinus* sp. and *Alnus* sp.) was present in both samples and made up 3-4% of the total.

The previously studied Yukagir and Cape Blossom mammoths showed abundant wind-dispersed pollen types consisting of Poaceae (both ~70%) and *Artemisia* sp. (16% and 7%, respectively; van Geel et al. 2008, van Geel et al. 2011b). The newly obtained pollen results from the three Pleistocene mammoths (Abyland, Adycha, Maly Lyakhovsky) as well as the Yukon horse were also dominated by Poaceae and *Artemisia* sp. (>85%). The only sample with a low *Artemisia* count (1%) was the Maly Lyakhovsky mammoth, which was for 97% dominated by Poaceae. Insect-dispersed pollen types were rare to very rare in all Pleistocene samples and were derived from many different families, e.g. Apiaceae, Brassicaceae, Caryophyllaceae and Papaveraceae. The only sample with coniferous tree derived pollen was the Adycha mammoth with 1% *Pinus* sp. pollen.

3.2.2 Macrofossils

Macrofossil analyses were taken from published records for eight samples and newly generated for three mammoths (Maly Lyakhovsky, Abyland and Adycha) as well as for Selwyn caribou A (Table S6.1 - S6.11). The macrofossils of the three Selwyn caribou samples showed a mixture of shrubs (genera *Betula* and *Salix*), lichen and mosses as the most dominant taxa, with grasses and forbs (e.g. Asteraceae, Caryophyllaceae) making up the remainder (Galloway et al. 2012). Selwyn C showed 44% lichen fragments.

The Yakutian bison faecal sample was dominated by vegetative remains of Poaceae and Cyperaceae (50%), wetland forbs (e.g. *Comarum palustre* and *Menyanthes trifoliata*) as well as *Salix* sp. and minor moss fragments (Van Geel et al. 2014). The Oyogar Yar horse sample was dominated by unidentified Cyperaceae remains and minor remains of Poaceae and several moss fragments (Gravendeel et al. 2014).

The previously studied macrofossils of the Yukagir mammoth faecal sample showed abundant poaceous remains together with *Salix* sp. and *Carex* sp. (van Geel et al. 2008). The herbaceous component was made up of plant remains from varying families, e.g. Asteraceae, Brassicaceae, Caryophyllaceae, Papaveraceae. Remains from several mosses were also identified, including *Drepanocladus aduncus*, *Bryum* sp., *Entodon concinnus*. The Cape Blossom mammoth macrofossils consisted of over 90% *Carex* sp., followed by Poaceae and a herbaceous component consisting of e.g. *Minuartia rubella*, *Potentilla* sp. and *Cerastium/Silene* sp. (van Geel et al. 2011b). Graminoids dominated the newly obtained data of the three mammoths Abyland, Adycha and Maly Lyakhovsky. This included poaceous vegetative remains, in the case of Abyland combined with one *Carex* sp. fruit and for Maly Lyakhovsky with the remains of a variety of mosses (e.g. *Campylium stellatum*, *Cinclidium stygium*, *Drepanocladus* sp., *Warnstorfia sarmentosa*).

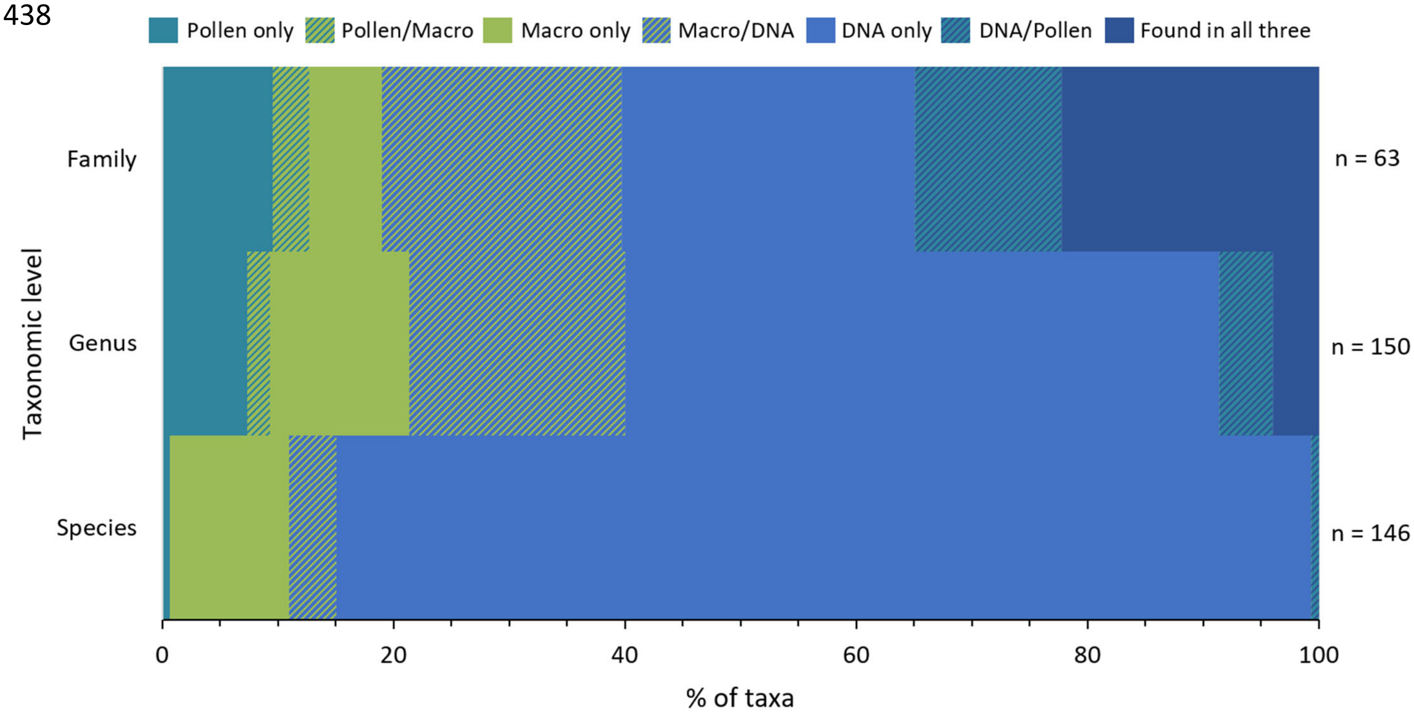

Fig. 2. Percentage of identified plant taxa per proxy (pollen, macrofossil, DNA) at different taxonomic levels across all faecal samples studied here. Hatched areas represent overlap between two proxies. n = total number of taxa that was found in each specific taxonomic level.

3.3 DNA

Illumina sequencing resulted in 20.4 M read pairs for *trn*L and 16.4 M read pairs for nrITS. After quality filtering and clustering, 11.7 M reads were retained for *trn*L, 2.1 M reads for plant nrITS1, 2.2 M reads for plant nrITS2 and 5.0 M reads for fungal nrITS2. *Trn*L and fungal nrITS2 was successfully amplified in all samples while plant nrITS1 and nrITS2 was obtained for all but the Yukon horse, Cape Blossom mammoth and Selwyn caribou C.

The plant specific primers for the nrITS marker effectively amplified plant taxa, where 63.4% (nrITS1) and 70.4% (nrITS2) of the total OTUs were assigned to green plants (Figure S15). Of the total OTUs, 3.8% and 7.3% were assigned to fungi, respectively. The remainder of the OTUs comprised green algae (Chlorophyta) and made up 6.6% of the total OTUs for nrITS1 and 19.4% for nrITS2. Across all samples, *trn*L produced 167 green plant OTUs, while 73 and 71 green plant OTUs were identified using plant nrITS1 and nrITS2, respectively (Tables S7 - S12). Per sample, *trn*L showed the highest number of green plant OTUs with on average 35.2 (range 12 – 74), while nrITS1 recovered on average 10.8 green plant OTUs (0 – 28) and nrITS2 12.5 (0 – 40) (Table S16). For the fungal nrITS2, 88.2% of the total OTUs were assigned to Fungi, 11.6% to Viridiplantae and 0.2% was unidentified, while showing on average 20.2 fungal OTUs per sample (range 7 – 38; Tables S16). Read or OTUs counts were not correlated to the age of the samples for any of the markers.

3.4 Comparison of pollen, macrofossils and DNA data

Across DNA, pollen and macrofossil datasets, 311 plant taxa including 146 species, 150 genera and 63 families were identified (Figure 2; see Table S6.1-S6.11 for full recovered plant taxa information across all samples). With pollen analysis, 65 plant taxa were identified, while 84 plant and 5 lichen macrofossil taxa were found. DNA analysis resulted in 146 (*trn*L), 73 (nrITS1) and 71 (nrITS2) plant taxa. At all taxonomic levels, DNA analysis recovered the most unique plant taxa, with 16 families, 77 genera and 123 species (Figure 2). However, unique taxa were also identified using both macrofossil (four families, 18 genera and 15 species) and pollen analysis (six families, 11 genera and one species). No species were recorded across all three proxies, while six genera (*Androsace, Artemisia*, *Betula*, *Papaver, Rumex* and *Salix*) and 14 families were shared in the DNA, macrofossil and pollen data. The biggest overlap of proxies was found between DNA and macrofossil results at the genus level (29 genera), while there was little overlap between the pollen and macrofossil results (three genera and two families).

Pollen and macrofossils could be identified to species level in 3.1% and 24.7% of the recovered taxa, respectively. For the DNA markers, 44.8% of the OTUs were identified to species level for *trn*L, while this was 70.9% and 78.2% for nrITS1 and nrITS2, respectively (Table S7, S9, S11). To illustrate the differences in taxonomic resolution between the three proxies as well as between the DNA markers, results of three plant families (Poaceae, Asteraceae and Cyperaceae) that were common to abundant in all 11 faecal samples are shown in Table 2. Taxa from these three families were found using all three proxies. For plant families where pollen could only be identified to the family level, macrofossils could in several cases be identified to genera within those families, and in rare cases to species level (e.g., *Carex nardina* and *Carex dioica* in the Cyperaceae family). The nrITS marker could identify species for taxa where *trn*L results were only identifiable to genus or family level. An example of this is the identification of the species *Arctagrostis latifolia* (100% identity) and *Calamagrostis stricta* (99.7%; Poaceae) using nrITS1, while *trn*L identification was only possible to the subtribe level (Agrostidinae). Similarly, where *trn*L identified Asteraceae subfamily Anthemideae, the nrITS marker found the species *Artemisia scoparia* and *A. norvegica* (both 100% identity). Unique Poaceae species (*Koeleria asiatica, Festuca kolymensis*) and Asteraceae species and genera (*Artemisia gmelinii*, *Arnica*, *Saussurea*) were, however, also found using *trn*L and this pattern was found throughout the whole dataset (Table 2 and Table S7).

3.5 Diet analysis

High congruence between the quantitative results of the different DNA markers was found for the Selwyn A and B caribou samples, with a dominance of shrubs (87-98%; *Salix*, *Betula* and various ericaceous taxa) and low abundance of forbs, graminoids and mosses (Figure 3a). In contrast, the macrofossil results indicated high abundance of mosses, graminoids and lichen with only low amounts of shrubs. The combined diet reconstruction - based on DNA and macrofossils only - showed ~75% shrubs with 10-15% mosses (Figure 3b). Fungal nrITS2 results further identified low amounts of lichen, including *Cladonia* spp., *Bryocaulon divergens* and *Stereocaulon saxatile* (Table S13 – S14) that may have formed part of the caribou diet (0.3% of total fungal reads for Selwyn B and 0.1% for Selwyn A). For Selwyn caribou C, *trn*L showed a much higher amount of forbs (72%; mainly Asteraceae tribe Anthemideae and *Sibbaldia procumbens*) than the macrofossils (8%) or pollen (34%). The reconstructed diet differed from the other two caribou samples, consisting of 40% forbs and equal parts (15-20%) of shrubs (*Salix*), lichen and mosses.

Macrofossils of the Oyogas Yar horse were for >95% dominated by graminoids and this was also reflected in the *trn*L (85%) and nrITS1 (69%) data (mainly *Eriophorum* sp. and *Dupontia fisheri* respectively). The plant nrITS2 results, however, were dominated by mosses (73%). The diet reconstruction showed a dominance of graminoids (65%) with 20% mosses and equal amounts of shrubs and forbs (8%). The diet of the other, much older, Yukon horse contained a lower fraction of graminoids (28%) and, instead, was dominated by forbs (on average 60%; consisting of *Braya rosea* and Asteraceae tribe Anthemideae). Tree and shrub taxa were only identified in the macrofossil results for this sample. The Yakutian bison sample consisted on average of 48% forbs (mainly *Cicuta virosa*) and 25% each of graminoids (*Eriophorum, Carex*) and shrubs (*Salix*). The Adycha and Maly Lyakhovsky mammoth samples showed highly similar results from both proxies and the reconstructed diets consisted almost exclusively of graminoids (Figure 3b). Graminoids in the Adycha sample consisted for >75% of *Puccinellia* sp. based on DNA analysis, while many species of Poaceae (including abundant *Deschampsia cespitosa* and *Alopecurus magellanicus*), as well as *Carex* sp. and *Eriophorum* sp. were found in the Maly Lyakhovsky sample. Mosses were found to be relatively abundant in this sample according to nrITS2 results (33%; mainly *Polytrichastrum alpinum*), while much lower percentages of mosses were found in nrITS1, *trn*L or macrofossil results.

Table 2. All taxa recorded of three plant families (Poaceae, Asteraceae and Cyperaceae) that were common to abundant in all 11 faecal samples in DNA (trnL, nrITS1 and nrITS2), macrofossils and pollen analyses. The numbers represent the number of samples in which that specific taxon was found.

Family (subfamily)	Tribe	Subtribe	Genus (subgenus)	Species	*trnL*	nrITS1	nrITS2	Macro	Pollen
Poaceae								11	11
Pooideae	Bromeae		*Bromus*		4				
				B. pumpellianus	5				
	Hordeeae	Hordeinae	*Elymus*					1	
			Hordeum		3			1	
	Meliceae		*Glyceria*					1	
			Pleuropogon	*P. sabinei*	2				
	Poeae				6				
		incertae sedis		*A. fulva/D. fisheri*	2				
			Arctophila	*A. fulva*			4		
			Dupontia	*D. fisheri*		4	1		
		Agrostidinae			5				
			Arctagrostis					2	
				A. latifolia		1	3		
			Calamagrostis				3	2	
				C. stricta		1			
		Alopecurinae	*Alopecurus*					1	
				A. magellanicus		3	2		
		Aristaveninae	*Deschampsia*	*D. cespitosa*		3	3		
		Aveninae	*Koeleria*	*K. asiatica*	2				
		Coleanthinae	*Puccinellia*		2	2	1		
				P. tenuiflora/vahliana			2		
				P. vahliana		2			
		Loliinae	*Festuca*					2	
				F. altaica	3	1			
				F. kolymensis	3				
				F. ovina		1	2		
		Phalaridinae	*Hierochloe*					2	
		Poinae	*Poa*			1		3	
				P. arctica			4		
				P. glauca		2			
	Triticeae				4				
Asteraceae					3				
Asteroideae								1	10
	Anthemideae				6				
		Anthemidinae	*Tripleurospermum*	*T. maritimum*	1				
		Artemisiinae			4				
			Artemisia					2	11
				A. gmelinii	5				
				A. norvegica		2	1		
				A. scoparia		2	3		
	Astereae				3				
	Gnaphalieae				2				
			Antennaria					1	
	Madieae	Arnicinae	*Arnica*		2				
	Senecioneae	Tussilagininae	*Endocellion*	*E. sibiricum*		1	2		
			Tephroseris		1				
Carduoideae	Cardueae								2
		Carduinae	*Saussurea*		3				
Cichorioideae									5
Cyperaceae								4	10
Cyperoideae	Cariceae		*Carex*		5			6	
			Carex subg. *Carex*		1				
				C. aquatilis	3	2	2		
				C. microchaeta	2				
				C. nigra subsp. *juncea*		5			
				C. podocarpa		1			
				C. rostrata		2	1		
				C. vesicaria		1	1		
			Carex subg. *Euthyceras*		1			1	
				C. nardina				1	
			Carex subg. *Vignea*		3				
				C. chordorrhiza		1			
				C. dioica				1	
				C. duriuscula		1	1		
				C. lachenalii	1				
				C. maritima	2				
	Scirpeae		*Eriophorum*		3	1		3	
				E. angustifolium		3	3		

The three other mammoth samples showed a higher contribution of forbs to their diet, often with the DNA results of the different markers showing one species dominating the assemblage. For the Abyland mammoth this dominant species was *Anemone patens*, while in the Yukagir mammoth sample *Myosotis alpestris* was abundant. The Yukagir mammoth was the only one of the mammoth samples showing relatively abundant (on average 34%) shrubs (*Salix*) in its diet. In the Cape Blossom mammoth, graminoids made up >75% of macrofossils, while the *trn*L results showed 28% graminoids, consisting mainly of *Carex*. In the *trn*L results forbs were abundant (71%) and consisted for the largest part of *Chamaenerion angustifolium* and Asteraceae tribe Anthemideae.

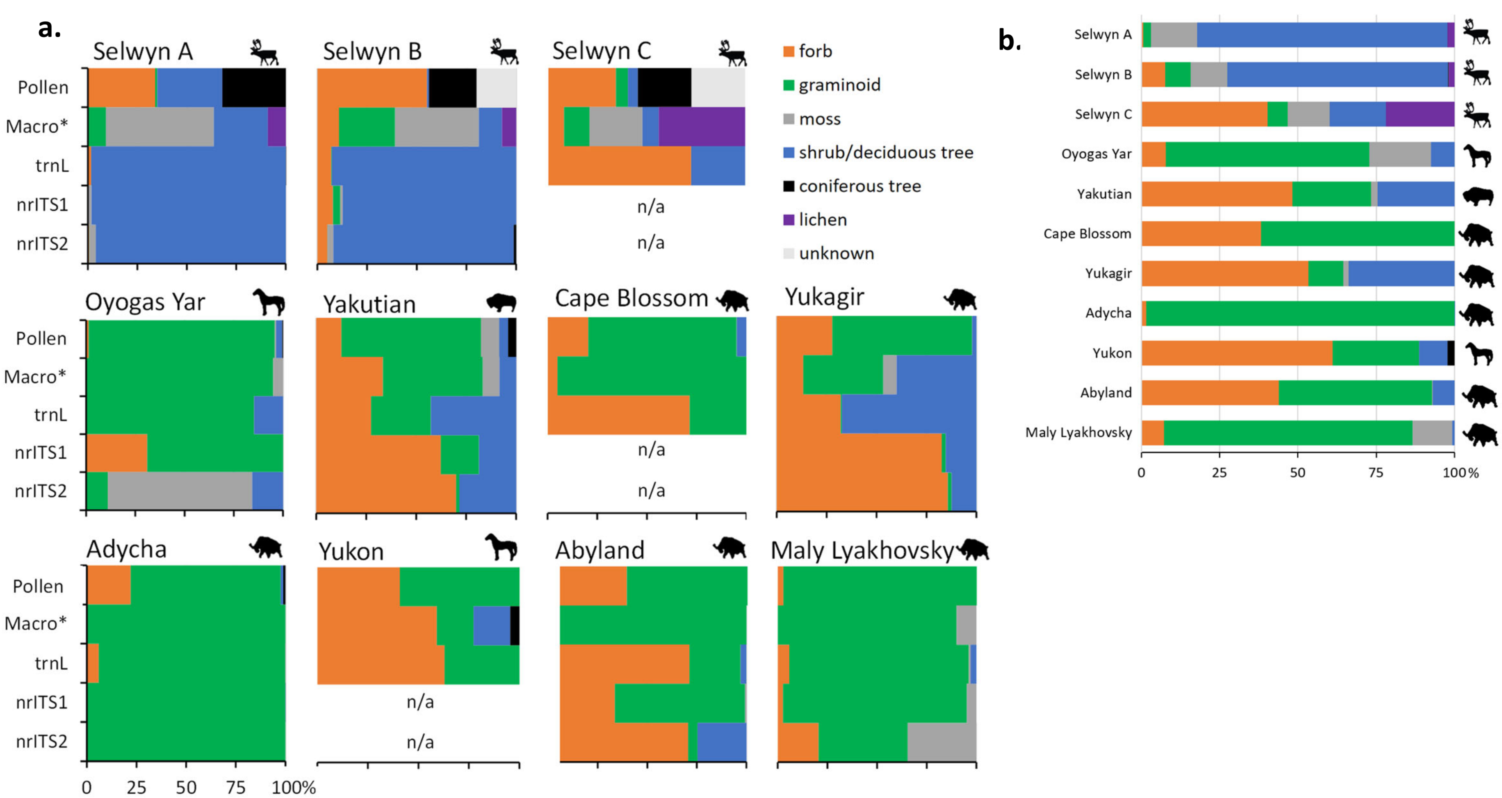

*Fig. 3. Diet reconstructions based on quantitative abundance of plant groups (forbs, graminoids, mosses, shrubs/deciduous trees, coniferous trees and lichens). a) Quantitative comparison of results from the different plant proxies used for all samples in this study. * exact quantitative data from macrofossils was only present for the Selwyn caribou B and C. For all other samples, the semi-quantitative macrofossil results have been converted to quantitative measures for illustrative purposes. b) Reconstruction of the composition of the last diet by taking the average value of the relative abundance of macrofossil and all available DNA results.*

3.6 Habitat types

We combined species and genus-level plant identifications from all proxy results to reconstruct the habitats in which the last meals of the studied megafauna were consumed (Figure 4; Table S17 for all plant species information).

Identified plant species in the Selwyn caribou A and B samples provided a range of habitats including wetland, woods and a large component of arctic-alpine tundra (e.g. *Arctous alpina, Anemone richardsonii, Carex podocarpa* and *Pyrola grandiflora*) along with taxa typical for mountainous/rocky habitats (e.g. *Rhodiola integrifolia*). The Selwyn caribou C sample similarly

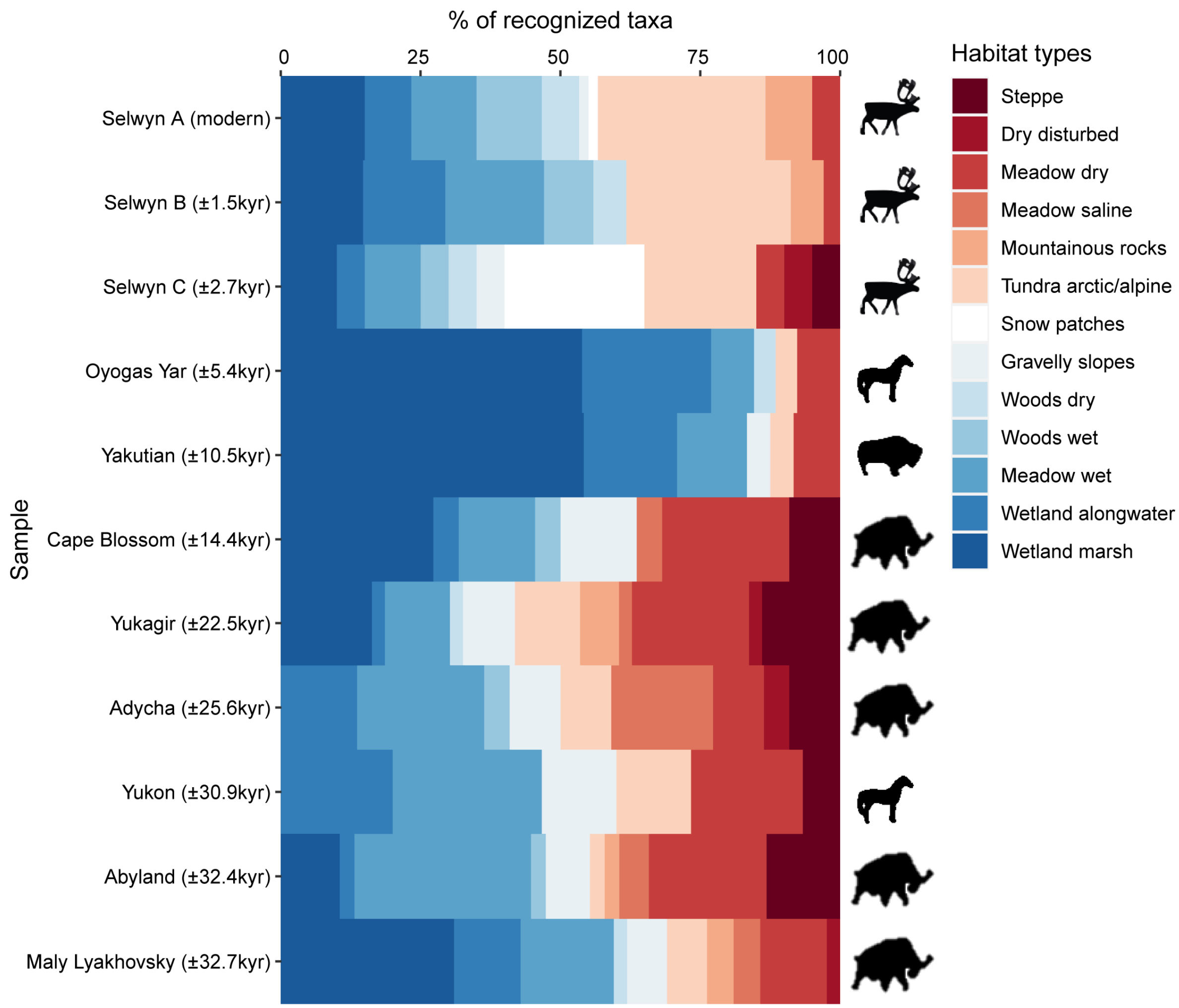

Fig. 4. Habitat reconstruction of megafaunal species based on integrated (pollen, macrofossils, DNA) species and genus resolution data. The samples were sorted according to their age and the average calibrated age of each sample is indicated between brackets.

contained many species typical for arctic-alpine tundra but also included a large component of species typical for snow patches (e.g. *Ranunculus nivalis, Ranunculus pygmaeus, Oxyria digyna*). The reconstructed habitats of the Holocene Oyogas Yar horse and Yakutian bison consisted mainly of wetlands, including marshes and river/lake sides. For the Oyogas Yar horse this included *Eriophorum* sp., *Caltha palustris* and *Comarum palustre* typical for marshes and e.g. *Arctagrostis latifolia* and *Arctophila fulva/Dupontia fisheri* from water sides. The Yakutian bison showed numerous *Carex* species, *Menyanthes trifoliata*, *Epilobium palustre* and *Hippuris* sp., all indicative of marshy wetland conditions as well as e.g. *Endocellion sibiricum* and *Epilobium palustre* typically found along rivers or ponds.

The Cape Blossom and Maly Lyakhovsky mammoth samples also included wetland components, with in the case of Cape Blossom e.g. *Caltha palustris* and species of *Carex* and for Maly Lyakhovsky *Eriophorum* sp., *Caltha palustris* as well as several grass species (*Pleuropogon sabinei*, *Arctophila fulva*). Moss species in the Maly Lyakhovsky mammoth further provided evidence of a wet, marshy environment (e.g. *Drepanocladus sordidus*, *Cratoneuron filicinum*, *Warnstorfia sarmentosa* and *Dicranum bonjeanii*). However, in contrast to the Holocene horse and bison, both these mammoth samples also included species indicative for dry meadows and, in the case of Cape Blossom, steppe (*Festuca kolymensis* and *Artemisia gmelinii*). Several true steppe species were also found in the Abyland mammoth (*Silene samojedorum*, *Carex duriuscula*, *Artemisia scoparia*) and Yukagir mammoth samples (e.g. *Eritrichium sericeum*, *Festuca kolymensis*, *Phlox hoodii*). Other taxa in both samples were indicative for dry meadows (e.g. *Anemone patens* and *Cerastium maximum* for Abyland and *Myosotis alpestris* and *Eremogone capillaris* for Yukagir). Furthermore for the Abyland mammoth, several species typical for wet meadow were identified (e.g. *Sanguisorba officinalis, Stellaria borealis*), while for the Yukagir mammoth a component of gravelly slopes and mountainous/rocky habitat was found (e.g. *Smelowskia alba*, *Oxytropis deflexa, Rhodiola rosea*). The Pleistocene Yukon horse also showed a last meal consisting of a mix of taxa from different habitats with species typically found in wet meadows and wetlands (*Alnus incana*, *Juncus alpinoarticulatus*) as well as dry meadow and steppe (*Bromus pumpellianus, Artemisia gmelinii*). The habitat for the Adycha mammoth consisted of meadows (e.g. *Deschampsia cespitosa*, *Bromus pumpellianus*) as well as a large component of saline meadow (*Puccinellia* sp.).

4. Discussion

4.1 Comparison of proxies

Out of the three proxies used in the present study (DNA, pollen and macrofossils), DNA recovered the highest number of unique taxa at all taxonomic levels (Figure 2). This is likely caused by the large amount of vegetative remains in the faecal samples that could not be identified beyond the family or genus level using macrofossil or pollen analysis. DNA analysis does not depend on the season when plants carry seed, fruit or pollen and allows identification of many taxa to the species level irrespective of their developmental stage. We also used primers for multiple marker regions (*trn*L, nrITS1, nrITS2), each identifying unique taxa and increasing overall taxonomic resolution (Tables S7 - S12).

In comparison to pollen from sediments, pollen spectra from our faecal samples were not very diverse (Jorgensen et al. 2012, Pedersen et al. 2013, Parducci et al. 2015). This could be because lake sediments accumulate pollen over a much larger spatial and temporal scale than faeces do. We took all the samples for our analyses from the middle of the faeces and thus caught only a snapshot of airborne pollen (i.e., sticking on ingested vegetation), mixed with pollen coming from ingestion of inflorescences. The taxonomic overlap between pollen and DNA, as well as between pollen and macrofossils was surprisingly low, and we instead found the highest overlap between DNA and macrofossil results. This is likely because both of these proxies are providing a local signal (showing the food choice of the animal) while the pollen analysis is influenced by accidental intake of pollen sticking to ingested vegetation as well as pollen from species producing high amounts of pollen (e.g. Jorgensen et al. 2012).

4.1.1 Metabarcoding detection gap

We use the term ‘metabarcoding detection gap’ here for taxa that were not retrieved in the DNA results (*trn*L or nrITS) but were present in the macrofossil and/or pollen records. In total, the metabarcoding detection gap consists of 12 families, 32 genera and 16 species (Figure 2). Many of these taxa are very rare in the pollen or macrofossil counts, with most of them found in only one sample and in low abundance. For pollen this includes single identified spores and pollen of *Botrychium* sp. and *Populus* sp. in the Selwyn caribou samples, and *Epipactis* sp., *Persicaria* sp. and *Thalictrum* sp. in the mammoth samples. For such rare pollen grains it seems likely they were only present as pollen while being (very) rare in the consumed vegetation. A lysis step with mechanical bead beating is necessary to break the exine of pollen grains and release the inner DNA (Polling, 2021). Since these steps have not been used here, this could explain the absence of these taxa from the DNA results. On top of this, pollen contains very little DNA that is hard to amplify even if present in high numbers (Parducci et al. 2005). Similar to proxy comparison studies on lake sediments (e.g. Parducci et al. 2019), we find that DNA from pollen contributes very little to the total DNA signal in faeces.

There are also taxa that were found as pollen with high relative abundance, while being very rare or absent in the other proxies. This includes, for example, pollen of the family Pinaceae which account for up to 30% in the caribou samples. Pinaceae pollen is often overrepresented in pollen records from the (sub)Arctic because they are high pollen producers and their pollen is spread over large distances (Aario 1940). The genus *Artemisia* reached up to 40% in some pollen records (Selwyn caribou B; Table S6.2), yet it is very rare in both DNA and macrofossil results. Unfortunately, using *trn*L, the genus *Artemisia* cannot be distinguished from other genera from the subfamily Anthemideae (*Anthemis, Achillea*, *Chrysanthemum*, *Tanacetum* etc.). This subfamily was relatively abundant in Selwyn caribou C, Cape Blossom mammoth and the Yukon horse, and it cannot be resolved whether these reads actually belong to *Artemisia*. Rare fragments of *Artemisia* in the macrofossil records were only recorded in the Yukon horse and Selwyn caribou C samples. Part of this discrepancy can be explained by differential preservation, since macrofossils of *Artemisia* such as seeds or fruits (achenes) deteriorate rapidly and are therefore rarely recovered (Birks 2007, Anderson and Van Devender 1991). Other studies on DNA metabarcoding of Pleistocene megafaunal faeces also found high amounts of *Artemisia* pollen but very low abundance with DNA or macrofossils from the same samples (e.g. Kolyma rhinoceros and Finish Creek mammoth; Willerslev et al. 2014). For caribou, where in all three samples Pinaceae and *Artemisia* pollen is common to abundant, it is furthermore known that they do not actively select *Artemisia* and avoid Pinaceae (Denryter et al. 2017, Jung, Stotyn and Czetwertynski 2015). These records are therefore interpreted as the results of accidental uptake of pollen sticking to selected plant taxa.

In the macrofossil data, we detected many taxa that were represented by one seed or plant fragment (e.g. *Antennaria* sp., *Draba* sp., *Sagina* sp., *Hedysarum* sp., *Lysimachia* sp.) and many of these are part of the metabarcoding detection gap. Furthermore, fragments of various mosses were exclusively found as macrofossils (e.g. *Calliergon* sp., *Plagiomnium* sp., *Rhizomnium* sp., *Thuidium* sp. and the spikemoss *Selaginella* sp.). It should be noted that DNA reference libraries are still far from complete, and this may be especially true for Arctic Russian moss species. Therefore, some of the species found as macrofossils may not be recoverable using DNA at this moment. One such example is the moss *Cinclidium stygium* for which no nrITS sequence is currently available in the NCBI Genbank. Apart from this, the expected amplicon size for bryophytes using the plant-specific nrITS primers in our study is >500 bp (Cheng et al. 2016), which may cause some species to be missed due to the 600 bp restriction using Illumina sequencing. Furthermore, even though we applied a multi-locus approach, DNA primer mismatch in both *trn*L and nrITS could have occurred. Many *Selaginella* species for example show 5 mismatches in their DNA barcodes with the *trn*L-h as well as the ITS4 reverse primers used in this study. Lastly, DNA of plant fragments may have been simply too degraded to be amplified by any of the DNA markers.

4.1.2 Morphology detection gap

A 'morphology detection gap' is designated here as all taxa that are missing in either the pollen or macrofossil record but were found in the DNA results. In total, the morphology detection gap for the studied faecal samples consists of 16 families, 77 genera and 123 species (Figure 2). The biggest factor contributing to many of the taxa only found as DNA is the higher taxonomic resolution that is achieved using DNA (although it depends on the percentage of identity used whether taxa identified by DNA are assignable to either, e.g., genus or species level). There are, however, a number of other factors that may determine the taxa in the morphology detection gap.

First, many taxa only found with DNA were very rare (<0.1% of the relative amount of reads) and only recorded in one sample. These taxa could have either been very minor diet items or taxa that were not targeted (i.e. accidental intake), which were present in such low quantities that they may have been missed with the macrofossil or pollen analyses. Accidental intake could also explain the presence of several species in the DNA results of the caribou samples of which the ingestion of high amounts may be toxic (e.g. Pedicularis capitata, Oxytropis deflexa; Denryter et al. 2017). Secondly, some plant taxa may be more affected by the digestive processes than other plant taxa, causing them to be unrecognizable as macrofossils while still being recoverable using DNA. Lastly, despite extensive reference collections for pollen and macrofossils, identification may still be somewhat subjective with regards to morphologically very similar taxa. This is less the case for DNA using reference libraries that allow more objective identifications.

Taken together, this explains the abundance of some taxa in DNA results even though they were missing in the other proxies. One example is the willowherb family Onagraceae for which *Chamaenerion angustifolium* and *Epilobium palustre* were found in DNA of seven of the samples studied here. Rare Onagraceae pollen were only found in the Cape Blossom mammoth (van Geel et al. 2011b). Although pollen from insect-pollinated plants are always underrepresented in faecal samples, we identified abundant *Chamaenerion angustifolium* in the DNA results of the Cape Blossom sample. No macrofossil remains of Onagraceae were recorded in any of the samples, and this is likely because vegetative Onagraceae remains are very hard to recognize due to their ambiguous morphology (Anderson and Van Devender 1991, Grímsson, Zetter and Leng 2012). Similarly, the forget-me-not family Boraginaceae is only recovered using DNA. It was especially abundant in the last meal of the Yukagir mammoth (*Myosotis alpestris* and *Eritrichium sericeum*). An additional species (*Mertensia paniculata*) was identified in the faecal samples of the caribou and the Cape Blossom mammoth, yet no remains of Boraginaceae were found in either pollen or macrofossil analyses of any sample. Pollen grains of members from this family are particularly small (5-7 µm) and could potentially be overlooked during analysis while vegetative macrofossil remains are hard to identify. Macrofossils of Boraginaceae and Onagraceae have not been recorded in any other mammoth faeces, even though they were recorded in high abundance in DNA data (e.g. Finish and Drevniy Creek mammoths as well as

Yukagir bison; Willerslev et al. 2014). These examples show the added value of DNA analysis and indicate that vegetative plants of these families may likely have formed part of the diets of the studied megafauna.

4.1.3 Comparison of plant DNA markers

Our application of multiple DNA markers on megafaunal faecal samples reveals the added value of a multilocus approach. The three samples for which no plant nrITS results were obtained were of very different ages (±2.7, ±14.4 and ±30.9 kyr BP), while older samples did produce plant nrITS amplicons (Abyland and Maly Lyakhovsky mammoths). While nrITS amplicons were found in all samples, for the three samples where no plant OTUs were found, these were all either derived from contamination, algae or fungi. Fragments of DNA up to 500 bp have been recovered from permafrost preserved sediments as old as 400 kyr (Lydolph et al. 2005). Therefore, it most likely depends on the conditions in which the specimens were preserved over time that determined whether or not these long fragments can be recovered. Some samples may have inadvertently been (partially) thawed at some stage, causing longer DNA fragments to be degraded, while the shorter and more stable *trn*L was not affected.

Most unique taxon identifications of the nrITS marker come from increased taxonomic resolution of several families and genera that show relatively low taxonomic resolution in the other proxies. This includes, for example, the genus *Carex* for which six unique species were found and the family Poaceae for which 11 unique species were identified with nrITS (Table 2). Furthermore, nrITS identified a larger variety of mosses than *trn*L, which is likely the result of the very short sequence length of the bryophyte P6 loop (±22 bp) obtained using the *trn*L *g* and *h* primers. These primers were not designed for bryophytes, and the recovered length often prevents sufficient taxonomic detail (Soininen et al. 2015, Epp et al. 2012). Nevertheless, many unique plant species were found using *trn*L, which could be the result of the more complete reference libraries available for *trn*L compared to nrITS. Many nrITS reference sequences in the NCBI Genbank database do not represent the complete marker region (e.g. *Pleuropogon sabinei* and *Ranunculus nivalis* with partial nrITS2 sequences) or are simply missing altogether because no reference sequences have been deposited yet. This is, for example, seen for species in the genus *Puccinellia* where not all Russian endemics have been sequenced (missing e.g. *Puccinellia manchuriensis*, *P. byrrangensis*, *P. jenisseiensis*), and this might also explain why we find *P. vahliana* (nowadays a western Arctic species) in nrITS results. Apart from that, the shorter and more stable *trn*L P6 loop produced results for the samples that did not produce any results from nrITS, which further explains the number of unique *trn*L identifications.

4.2 Diet analysis

The diet analysis of Selwyn A and B showed that shrubs are highly dominant in the summer diets of caribou, which is in agreement with known diets of summer foraging caribou that consists of deciduous shrubs along with reindeer lichen and fungi (Bergerud 1972, Boertje 1984). Lichen were observed using macrofossil and fungal nrITS2 analysis, and were also indirectly detected with plant DNA by the presence of lichen phycobionts in the plant nrITS2 results (e.g. *Asterochloris, Symbiochloris* and *Trebouxia* spp.), only found in the Selwyn caribou samples (Table S18). *Trebouxia* is the most common phycobiont in extant lichen, while *Asterochloris* is mainly associated with lichen of the families Cladoniaceae and Sterocaulaceae (Pino-Bodas and Stenroos 2020). Both families were also identified using fungal nrITS2 (Table S13 – S14), providing further support that the caribou ate lichen. The diet of modern caribou is well studied and for many Arctic plant species it is known whether they are either “selected”, “neutral” or “avoided” based on observations of foraging caribou (Denryter et al. 2017, Bergerud 1972). An average diet of modern caribou was found to consist of 78% selected, 15% neutral and 7% avoided species (Denryter et al. 2017). For Selwyn A and B, “selected” plant taxa made up >85% of relative abundance of all DNA markers, while “avoided” taxa made up <5% (Table S19). This is in contrast to macrofossil results that showed up to 21% avoided taxa, mainly from mosses. Selwyn caribou C showed a large component of diet items that were of unknown (43%) and neutral diet preference (44%), with only minor (11%) selected plant taxa (Table S19). This points to a somewhat atypical summer diet for this caribou when compared to modern caribou preferences and may suggest a different vegetation composition in its habitat.

The diets of nearest living relatives for Holocene bison and horse are well studied. While horses are typical grazers nowadays with diets consisting >75% of graminoids (Mendoza and

Palmqvist 2008), this has not always been the case. Several studies have shown that prehistoric horses had mixed grass-browse diets, especially in winter when grasses were harder to access (Kaczensky et al. 2017, MacFadden, Solounias and Cerling 1999). The diet of the Holocene Oyogas Yar horse (*Equus* cf. *lenensis*) is typical for a grazer, with the main component being identified as graminoids. The Pleistocene Yukon horse (*Equus lambei*), however, consumed mostly forbs. The season of death could not be determined for the Oyogas Yar horse (although it could be spring to summer due to relatively high amount of Cyperaceae pollen), while for the Yukon horse it was determined as winter (Harington 2002, Harington and Eggleston-Stott 1996). This could explain why grasses made up only 28% of the total diet for the Yukon horse (Figure 3b). It is likely that snow covered much of the grass cover, forcing the horse to focus on other available dietary items or that grasses were simply less abundant or of lower nutritional value (Savage and Heller 1947).

The now extinct steppe bison (*Bison priscus*) was closely related to modern bison (*Bison bison* (Linnaeus, 1758); Marsolier-Kergoat et al. 2015). While modern bison are often thought of as grazers feeding for the majority on graminoids, their summer diets are more variable, consisting on average of 44% grass, 38% forb, 16% shrubs and <2% sedge (Leonard et al. 2017). This is similar to the DNA results of the Yakutian bison studied here, where forbs and shrubs are important components. Pollen of undifferentiated Apiaceae (identified by nrITS as *Cicuta virosa*) were also relatively abundant in this sample (9%) indicating ingestion of inflorescences. This may indicate that the Yakutian bison had its last meal in summer and was a mixed feeder that did not rely solely on grasses. The 'warm season' (spring/summer) was also identified as the most likely season of death for the Yakutian bison by Van Geel et al. (2014) and Boeskorov et al. (2016). The >52 kyr old bison (*Bison* sp.) studied by Willerslev et al. (2014), similarly showed a high abundance of forbs and shrubs (80%), although no season of death was identified for this sample. Lastly, the abundance of poisonous *Cicuta virosa* (water hemlock) in nrITS, and also recognized to lower taxonomic resolution in *trn*L, pollen and macrofossils, possibly indicates that the Yakutian bison died of hemlock poisoning (Jacobson 1915).

The last meals of the Maly Lyakhovsky and Adycha mammoths consisted almost exclusively of graminoids. Some of these grasses can grow to considerable size (75-100 cm) and may have provided sufficient nutritional value for mammoths (e.g. *Bromus pumpellianus*, *Deschampsia* *cespitosa, Dupontia fisheri*). Furthermore, the genus *Puccinellia* which was identified as the main component in the Adycha mammoth last diet, includes several species that are commonly grown for hay making for cattle in modern day Yakutia, Russia (Gavril'eva 2011). The other mammoths studied here had much lower relative amounts of graminoid DNA, or barely any in the case of the Yukagir mammoth. The last diet of the previously studied Mongochen mammoth as reconstructed using macrofossils consisted mainly of mosses, forbs and only minor grasses and shrubs while DNA results showed dominance of 98% graminoids (Willerslev et al. 2014, Kosintsev et al. 2012a). The authors suggested that the underrepresentation of graminoids in the mammoth faeces could be the result of the digestive processes breaking down the poaceous tissues, although this is not supported by our finding of graminoids being dominant in the other mammoth faeces. It does, however, hold for forbs which are underrepresented in macrofossil and pollen results as compared to our DNA data, which has also been found in previous studies (e.g., Willerslev et al. 2014, Kosintsev et al. 2012b). The last meals of the Abyland and Cape Blossom mammoths may not have consisted solely of graminoids as suggested by the macrofossil analysis, but supplemented with *Anemone patens* (Abyland) and various other forbs, while shrubs and *Chamaenerion angustifolium* were consumed by the Cape Blossom mammoth. The abundance of *Salix* sp. and Boraginaceae (Yukagir) provides further evidence for the diversity in mammoth diets.

Another potential explanation for the differing diets may be sought in the different seasons of death, which could be determined for three of the mammoth samples studied here. The season of death of Maly Lyakhovsky mammoth was determined as late summer to early autumn (Grigoriev et al. 2017), while for both Yukagir and Cape Blossom mammoths autumn to early spring was suggested (Mol et al. 2006, van Geel et al. 2011b). A recent study on molar enamel profiles found that mammoths may have had seasonally different diets, shifting between browse and grasses (Uno et al. 2020). Also in the previously published Mongochen mammoth that died mid-summer and for which DNA, pollen and macrofossil results were analysed, the last diet was interpreted to be dominated by graminoids (Kosintsev et al. 2012a, Willerslev et al. 2014). This limited amount of data suggests that warm season diets of mammoth may have been dominated by graminoids (Maly Lyakhovsky, Mongochen), while they relied on various other food sources in the cold season (Cape Blossom, Yukagir). However, more multiproxy data is needed to support this hypothesis.

In some of the faecal samples studied here, mosses were identified in abundance either in the macrofossils (Selwyn caribou A and B) or in DNA results (nrITS2; Oyogas Yar horse and Maly Lyakhovsky mammoth) while being nearly absent in the other proxies. The relative abundance of mosses in the macrofossils of the caribou faeces is probably the result of accidental ingestion when the caribou were foraging low on the ground for dwarf shrubs and lichens. The moss species that was abundantly found with nrITS2 in the Oyogas Yar and Maly Lyakhovsky sample was *Polytrichastrum alpinum* which was detected only as rare fragments in the macrofossil remains of these samples. Potentially the primers used to amplify the nrITS2 region caused preferential amplification of this type of moss. Although abundant moss fragments have been identified in macrofossils from several mammoths (Kosintsev et al. 2012a, Kosintsev et al. 2012b), and are sometimes found in caribou faeces (Denryter et al. 2017), they are unlikely to have formed a major part of the diet for any of the extinct and extant mammals studied here because of their low nutritional value.

4.3 Habitat types

The reconstructed habitat for Selwyn caribou A and B corresponds well with the known current habitat of these animals in the Selwyn Mountains in Northwest Territories, Canada. The habitat for these two samples consists of elements from both downslope boreal forest and its wetlands, along with upslope alpine tundra. It is important to note that the two most dominant diet items as identified by DNA (*Salix* and *Betula*), are not included in the habitat analysis because neither of them could be identified beyond the genus level. Species from these genera have varying habitat preferences and therefore the genus level identifications did not provide enough information to infer the habitat, the only exception being rare *Salix alaxensis* in Selwyn Caribou B which typically grows in forested habitat along streams and lakes (Boufford et al. 2016). The only *Betula* species found in the Selwyn Mountains are *B. glandulosa* (dwarf birch, shrub) and *B. papyrifera* (canoe birch, tree), with the dwarf birch being far more common (Galloway et al.

2012). The habitat reconstructed for Selwyn caribou C may indicate that the faeces in this sample was deposited by caribou that consumed a meal nearer to the ice patch.

When many megafauna species disappeared at the end of the Pleistocene, the Holocene vegetation shifted significantly to become a more waterlogged environment with mossy and shrub-dominated tundra and deciduous forests (Edwards et al. 2005, Guthrie 2001). The habitats reconstructed for the Holocene horse and bison reflect this mesic environment. Previous studies on these samples, however, indicated dry steppe-like conditions based on pollen and macrofossils due to the abundance of Poaceae remains (Gravendeel et al. 2014, Van Geel et al. 2014, Boeskorov et al. 2016). However, here we find that the species composition of Poaceae for both samples included *Dupontia fisheri*, *Arctophila fulva* and *Arctagrostis latifolia*, all species typical for wetland habitats. Similar to the results for the Holocene Yakutian bison, modern bison (*Bison bison*) are known to prefer sedge marshes over other habitat types (Belanger et al. 2020 and references therein). Our results show that both horse and bison are not strictly graminoid grazers, but utilize wetlands in their habitat as well. This is also confirmed by the habitat reconstructed for the Pleistocene Yukon horse studied here, that showed a mixed environment of wetland and dryer meadows and steppe. Furthermore, a recent study on dental micro- and mesowear of horse and steppe bison also found that both were likely mixed feeders, instead of obligate grazers (Kelly et al. 2021).

Mixed environments were also identified for the mammoth samples, although with varying degrees of wetland components. The oldest mammoth studied here, Maly Lyakhovsky, showed many species typical for a marshy environment. This is in contrast to the Abyland mammoth that was collected from the same geographic area (North Sakha republic, Russia) and of similar age, that showed a much larger steppe and dry meadow habitat. This relatively large steppe component was also found for the Yukagir mammoth, although for this mammoth it was mixed with many plants typically found on gravelly slopes and mountainous areas. This may indicate that mammoths may have been versatile in their diets, adapting to the various habitats that were available. This is further supported by the habitat reconstructed for the Adycha mammoth, which shows that saline meadows were present and utilized by mammoths as well. For the Cape Blossom mammoth, no nrITS results were obtained which hampers the habitat reconstruction.

However, with the other proxies a habitat similar to the Maly Lyakhovsky mammoth was reconstructed, with marshy wetland and surrounding wet meadows, intermixed with steppe and dry meadow. The variety of diets obtained from different habitats supports the idea that the 'mammoth steppe' was a mosaic of habitats instead of a homogeneous vegetation type (e.g. Zazula et al. 2007). Furthermore, the specific plant species mixture identified for these mammoths is not found in any modern habitat type, pointing to non-analogue plant communities (Williams and Jackson 2007). Our results also indicate that mammoths were not exclusively grazers, but rather opportunistic mixed-feeders.

5. Conclusions

We integrated multilocus plant DNA, macrofossil and pollen analysis to obtain detailed reconstructions of megafaunal diets and habitats. We found most plant species in faecal samples uniquely using DNA, some of which abundantly so. This could be because of the large number of vegetative plant remains in the faeces which have become unidentifiable for macrofossil analysis due to masticatory and digestive processes. Unique plant taxa were, however, also found using both macrofossil and pollen analysis. We further show that relatively long nrITS fragments can be amplified from faecal samples as old as 28,610 ^{14}C BP and that these help to increase species resolution for many plant families (e.g. Asteraceae, Cyperaceae and Poaceae) as well as mosses that could not be retrieved using *trn*L.

We could accurately reconstruct the known diet and habitat of modern and late Holocene caribou (i.e. abundant shrubs from an arctic alpine tundra) and extended this approach to Holocene and Pleistocene megafauna including horse, steppe bison and woolly mammoth. These reconstructions showed that the Holocene steppe bison and horse were not strict grazers but rather mixed feeders that were foraging in a marshy wetland environment. This result is in sharp contrast with previous reconstructions that suggested dry steppe-like conditions for these samples. We further find that the five Pleistocene mammoths studied here had very different last meals obtained from a variety of habitats including wetland, wet meadow, gravelly slopes, saline meadow and steppe. This confirms the presence of a mosaic of habitats in the Pleistocene landscape often referred to as the ‘mammoth steppe’ that mammoths could fully exploit due to a high flexibility in their diet choice.

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Data availability

All raw read data are available at the European Nucleotide Archive (ENA) with the study accession number PRJEB44352 (sample metadata, including sample names and primer-adapter sequences, is available in Table S20). Processed read data is available in the supporting information (Tables S7 - S14).

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Multiproxy analysis of permafrost preserved faeces provides an unprecedented insight into the diets and habitats of extinct and extant megafauna

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Supporting Information (1/3)

Contents

S1. Sample information

Table S1. Detailed information on age and location of the five woolly mammoth (*Mammuthus primigenius*), steppe bison (*Bison priscus*), horse (Yukon: *Equus lambei* and Oyogas Yar: *Equus* cf. *lenensis*) and northern mountain caribou (*Rangifer tarandus caribou*). Modern and extant caribou samples were collected from cores in ice patches (Galloway et al., 2012).

Species	Name	Reference	Calibrated ^{14}C age calBP	Lab. No. for radiocarbon dating	Coordinates
Caribou	Selwyn A (KfTe-1 surface)	Galloway et al. (2012)	0	modern	62°58'12.4"N 129°27'42.2"W
Caribou	Selwyn B (KfTe-1-C2-1)	Galloway et al. (2012)	1,545 - 1,415	Beta-240104 faeces	62°58'12.4"N 129°27'42.2"W
Caribou	Selwyn C (KfTe-1-C1-3)	Galloway et al. (2012)	2,995 - 2,880	Beta-240102 faeces	62°58'12.4"N 129°27'42.2"W
Horse	Oyogas Yar	Boeskorov et al. (2014); Gravendeel et al. (2014)	5,445 - 5,310	GrA-54020 bone	72°40'49.42"N 142°50'38.33"E
Bison	Yakutian bison	Boeskorov et al. (2014); van Geel et al. (2014)	10,580 - 10,425 10,570 - 10,415	GrA-53290 bone GrA-53292 hair	72°17'30"N 140°54'05"E
Woolly mammoth	Cape Blossom	van Geel et al. (2011)	14,790 - 14,085	AA-77015 faeces	66°44'0''N 162°29'0''W
Woolly mammoth	Yukagir	van Geel et al. (2008)	22,765 - 22,445	GrA-24288 hair	71°52'9.88"N 140°34'8.73"E
Woolly mammoth	Adycha	This study	25,765 - 25,360	GrA-67394 faeces	67°57'3.44''N 135°25'52.39''E
Horse	Yukon Horse	Harington and Eggleston-Stott (1996)	31,225 - 30,560	Beta-67407 bone	64°00'N 139°10'W
Woolly mammoth	Abyland	This study	32,995 - 32,215	GrA-67393 faeces	68°13'1.92''N 146°51'1.88''E
Woolly mammoth	Maly Lyakhovsky	This study	33,165 - 32,260 33,640 - 33,110	GrA-60021 hair GrA-60044 bone	74°39'36"N 141°59'14"E

S2. Abyland and Adycha sample identity confirmation

The identity of the Adycha faecal sample was confirmed using specifically designed primers, while for Abyland a previously published primer pair was used (Table S2; Barnes et al., 2007). Subsamples (volume ca. 1 ml) were ground in a Retsch CryoMill at -196°C in a dedicated ancient DNA lab. DNA was extracted using silica-based extraction protocol of Rohland and Hofreiter (2007). DNA amplifications were carried out on a Bio-Rad C1000 Touch in 30 µl final volumes. They consisted of 17.8 µl nuclease-free ultrapure water, 6 µl 5X Phire Green reaction buffer, 1.5 µl of each primer, 0.6 µl of dNTPs, 0.6 µl Phire Hot Start II DNA Polymerase and 2 µl DNA sample template. During the PCR a 30 s activation step at 98°C was followed by 40 cycles of 5 sec at 98°C, 5 sec annealing at 55°C and 10 sec at 72°C. The PCR ended with a final extension step at 72°C for 1 min. The products were checked using gel electrophoresis using EtBr staining. The obtained amplicons were Sanger sequenced by BaseClear B.V. (Leiden, The Netherlands) on an ABI3730XL sequencer (Life Technologies). Resulting sequences were matched against reference data in NCBI GenBank using BLAST.

Table S2. Overview of the PCR primers used in this study to identify the identity of the producer of the Abyland and Adycha faeces.

Target taxon	DNA Marker	Primer Name	Primer sequence 5'-3'	Amplicon length (bp)	Reference
M. primigenius (Adycha)	COI	- mam_COI_5771_F - mam_COI_5892_R	TTTTTCACTTCACCTTGCAGGAGTATC TGGACCATACAAATAAGGGTATGTGATA	67	This publication
M. primigenius (Abyland)	mitochondrial control region	- mam_15528F - mam_15656R	TAGACCATACCATGTATAATCG GAGCTTTAATGTGCTATGTAAG	127	Barnes et al. (2007)

Resulting sequences

- Adycha: 5'-CTCTATTTTAAGTGCAATTAATTTTATCACTACCATCATTAACATAAAACCTCCAGCTATGTCTCAA-3' (too short to submit to ENA)
- Abyland: 5'-TGCATCACATTATTTACCCCATGCTTATAAGCAAGTACTGTTTAATCAATGTGTCAAGTCATATTCGTGTAGATTCACAAGTCATGTTTCAGCTCATGGATATTATTCACCTACGATAAACCATAGT-3' (ERA3966948)

S3. Primer selection

Overview of primers used in this study to amplify plants (*trn*L, nrITS1, nrITS2) and fungi (nrITS2 region).

Table S3. Overview of primers used in current study

Target Taxon	DNA Marker	Primer Name	Primer sequence 5'-3'	Annealing T (°)	Amplicon length (bp)	Reference
Plants	ITS1	ITS-p5 (F)	CCTTATCAYTTAGAGGAAGGAG	58	~300-400	Cheng et al. (2016)
		ITS-u2 (R)	GCGTTCAAAGAYTCGATGRTTC			Cheng et al. (2016)
	ITS2	ITS-p3 (F)	YGACTCTCGGCAACGGATA	55	~350-400	Cheng et al. (2016)
		ITS4 (R)	TCCTCCGCTTATTGATATGC			White et al. (1990)
	trnL	*trn*L-g (F)	GGGCAATCCTGAGCCAA	60	~8 – 143	Taberlet et al. (2006)
		*trn*L-h (R)	CCATTGAGTCTCTGCACCTATC			Taberlet et al. (2006)
Fungi	ITS2	fITS7 (F)	GTGARTCATCGAATCTTTG	56	~200-300	Ihrmark et al. (2012)
		ITS4 (R)	TCCTCCGCTTATTGATATGC			White et al. (1990)

S4. Manually removed taxa

Taxon identifications that still remained in the dataset after all filtering steps. These were manually removed from the dataset before further analysis.

***trn*L manually removed**

- likely food contaminants: Musaceae including *Musa* (banana), *Oryza sativa* (rice), *Capsicum* (pepper), *Glycine max* (L.) Merr. (soy), *Zingiber officinale* Roscoe (ginger), *Humulus lupulus* (hops), Laurales, Juglandaceae
- contaminants of unknown origin: Convolvulaceae incl. *Convolvulus*, *Ipomoea* (not in Arctic)

nrITS1 manually removed

- likely food contaminants: *Allium cepa* L. (onion), *Lagenaria siceraria* (Molina) Standl. (calabash)
- non-native species *Celtis tetrandra* Roxb. and *Pteroceltis tatarinowii* Maxim. (native Chinese and South-East Asian tree species)

nrITS2 manually removed:

- likely food contaminants: *Lagenaria siceraria* (Molina) Standl. (calabash)*, Spanicia turkestanica* Iljin (spinach)
- contaminants of unknown origin*: Urtica dioica* L. (Selwyn caribou C)
- non-native species: *Celtis biondii* Pamp. (native South-East Asian tree species), *Chamaecyparis obtusa* (Siebold & Zucc.) Endl. and *Cryptomeria japonica* (Thunb. ex L.f.) D.Don (native to Japan and Taiwan)

S5. *trn*L and nrITS filtering steps

Table S5.1 Number of total reads and unique sequences for plant nrITS remaining after each filtering step. Raw reads for nrITS run = 16,734,333. All paired-end reads were merged using PEAR, resulting in 16,421,333 assembled reads.

		nrITS1		nrITS2	
Filtering steps	**Program/ command**	**Total reads**	**Unique sequences**	**Total reads**	**Unique sequences**
Assignment to samples	Cutadapt	4,888,459		4,307,952	
Removal of sequences with quality <20 and length <150 bp	PRINSEQ	4,854,574		4,305,913	
Dereplication, sorting by size and clustering into OTUs (removing singletons and chimeras)	VSEARCH, unoise3 (USEARCH)		657		1805
Removal of sequences with ≤80% match & <80% cover	R	4,086,417	484	3,790,860	1531
Removal of sequences with maximum abundance in negative controls	R	3,872,930	464	3,710,741	1511
Setting abundance of reads below filtering threshold of 0.3% (nrITS2) or 0.35% (nrITS1 and fungal nrITS2) to 0 for each replicate to account for leaking	R	3,810,820		3,762,751	
Removal of algae, fungi and merging identical identifications	R	2,170,250	79	2,201,842	83
Manual removal of contaminations	R	2,138,759	73	2,177,482	71

Table S5.2 Number of total reads and unique sequences for *trn*L remaining after each filtering step in OBITools and R.

Filtering steps	Program/ command	Total reads	Unique sequences
Raw reads		24,767,590	
Pairwise alignment	*illuminapairedend*, score-min = 40	20,385,514	
Assignment to samples	*ngsfilter*	20,283,841	
Merged identical reads	*obiuniq* & *obiannotate*		497,296
Removal of reads with count <10 & < 10 bp length	*obigrep*	19,655,209	15,780
Identification & removal of PCR/sequencing errors	*obiclean* & R	17,985,094	3,736
Removal of sequences with ≤99% match & <100% cover	R	13,473,872	264
Removal of sequences with maximum abundance in negative controls	R	12,255,382	225
Reduction of reads below filtering threshold of 1.0% of total reads for each replicate to account for leaking	R	11,985,611	
Manual removal of contaminations	R	11,715,436	212

S6. Pollen, DNA and macrofossil results of all samples

Pollen spectra, plant macrofossil data and DNA metabarcoding results of the eleven studied faecal samples. Observations in pollen spectra denoted with a + were made after finishing the counting procedure. Fungal spores are expressed as percentages calculated on the total pollen sum. Abundance categories in macrofossil data are as follows: + = rare/present, ++ = frequent/common and +++ = abundant to dominant. For DNA metabarcoding, any reads below a relative read abundance of 0.1% are shown as + (present).

Table S6.1 Selwyn caribou A (modern) – surface material KfTe-1 Ice Patch

Family/order	**Taxon**	**Pollen (%)**	**Macro***	***trn*L (%)**	**nrITS1 (%)**	**nrITS2 (%)**	**Caribou Diet (Denryter et al., 2017)**
Phanerogams							**A = Avoided** **N = Neutral** **S= Selected** **U = Unknown**
Apiaceae	tribe Oenantheae			0.1			U
	tribe Selineae			+			U
Asteraceae	indet.			+			U
	Artemisia sp.	10.0					N
	Artemisia norvegica subsp. *saxatilis* H.M.Hall & Clem.				+	+	N
	subfamily Asteroideae	5.0		+			U
Betulaceae	*Alnus* sp.	+					
	Betula sp.	16.0	++	89.1	80.9	78.5	S
Boraginaceae	*Mertensia paniculata* (Aiton) G.Don			+		+	N
Campanulaceae	*Campanula* sp.			+			
Caprifoliaceae	*Valeriana* sp.			+			A
Crassulaceae	*Rhodiola integrifolia* Raf.			0.2	+	+	U
Cyperaceae	indet.	1.0					A
	Carex microchaeta Holm			+			A
	Carex podocarpa R.Br.				+		A
Ericaceae	indet.	5.0					U
	Arctostaphylos uva-ursi (L.) Spreng.			+			N
	Arctous alpina (L.) Nied.			+		+	N
	Arctous alpina/rubra			0.4			N
	Arctous rubra (Rehder & E.H.Wilson) Nakai				+		N
	Cassiope tetragona (L.) D.Don			+	+		U
	Empetrum nigrum L.			+	0.1	0.4	S
	Erica sp.			+			U
	Pyrola sp.			+			N
	Pyrola grandiflora Radius			0.2			A
	Pyrola asarifolia Michx.				+	+	A
	Vaccinium sp.					+	U
	Vaccinium uliginosum L.			0.4	0.5	1.2	S
	Vaccinium vitis-idaea L.			+	+	0.1	A
Fabaceae	*Astragalus* sp.			+	+		N
Family indet.		2.0					
Liliaceae	*Gagea serotina* (L.) Ker Gawl.			+			U
Lycopodiaceae	*Lycopodium* sp.	8.0					A
	subfamily Lycopodioideae			+			A

Menyanthaceae	*Menyanthes trifoliata* L.			+		+	U
Onagraceae	*Chamaenerion angustifolium* (L.) Scop.			+	+	+	N
	Epilobium palustre L.					+	N
Ophioglossaceae	*Botrychium* sp.	2.0					A
Orobanchaceae	*Pedicularis capitata* Adams			+			A
	Pedicularis sudetica Willd.			+			A
Pinaceae	indet.	2.0					A
	Abies sp.	5.0					A
	Picea sp.	15.0					A
	Pinus sp.	10.0					A
	Pinus subsect. *Contortae*			+			A
Plantaginaceae	*Veronica* sp.			+			U
	Veronica wormskjoldii Roem. & Schult.			+			U
Poaceae	indet.	+	+				U
	Arctophila fulva (Trin.) Andersson					+	U
	Alopecurus magellanicus Lam.					+	U
	Calamagrostis sp.					+	A
	Deschampsia cespitosa (L.)					+	U
	Festuca altaica Trin.			0.1	+		N
	Poa glauca Vahl				+		N
Polemoniaceae	*Polemonium* sp.	1.0					N
Polygonaceae	*Bistorta vivipara* (L.) Delarbre			+	+		N
	Oxyria digyna (L.) Hill			+			N
Primulaceae	*Primula frigida* (Cham. & Schltdl.) A.R.Mast & Reveal			+			U
Pteridophyta	indet.	4.0					A
Ranunculaceae	indet.	+					
	Anemone sp.			+			U
	Anemonastrum narcissiflorum (L.) Holub			0.1		+	U
	Anemone patens L.					0.1	U
	Anemone richardsonii Hook.			+			U
	Caltha palustris L.					+	A
Rosaceae	indet.	2.0					U
	Comarum palustre L.			0.1			U
	Dryas sp.			0.1			N
	Dryas octopetala L.				0.2	0.3	N
	Geum sp.			0.6		+	N
	Geum aleppicum Jacq.				+		N
	subfamily Rosoidea			+			U
	Rubus arcticus L.					+	A
	Spiraea stevenii (C.K.Schneid.) Rydb.			+			U
Salicaceae	indet.			8.2			S
	Populus sp.	+					S
	Salix sp.	12.0	+		16.4	15.7	S
Saxifragaceae	*Micranthes* sp.			+			A
	Saxifraga (sect. *Mesogyne*)			+			A
Violaceae	*Viola epipsila* var. *repens* (W.Becker) R.J.Little			+			A
Cryptogams							
Bryophyta							
Amblystegiaceae	*Drepanocladus/Sanionia* sp.		+				A
	Sanionia uncinata Loeske			+	+		A
Anastrophyllaceae	*Barbilophozia barbata* (Schmidel ex Schreb.) Loeske					+	A

Aulacomniaceae	*Aulacomnium palustre* (Hedw.) Schwägr.				+	+	A
Brachytheciaceae	*Tomentypnum nitens* Loeske					+	A
Bryaceae	*Bryum* sp.		+	+			A
	Ptychostomum pallescens (Schleich. ex Schwägr.)					+	A
Dicranaceae	indet.			+	+		A
	Dicranum sp.		+		+		A
	Dicranum fuscescens Sm.				1.2	0.2	A
Grimmiaceae	*Bucklandiella* sp.				+		A
	Niphotrichum sp.					+	A
Hylocomiaceae	*Hylocomiastrum pyrenaicum* Fleisch.					+	A
	Hylocomium splendens (Hedw.) Schimp.				+	+	A
	Pleurozium schreberi Mitten		+	+	0.3	0.1	A
Hypnales	indet.			+			A
Mniaceae	indet.					+	A
Polytrichaceae	indet.			+			A
	Polytrichastrum alpinum (Hedw.) G.L. Sm.					0.3	A
	Polytrichum juniperinum Hedw.					0.1	A
	Polytrichum piliferum Hedw.				+	1.4	A
	Polytrichum cf. *strictum* Menzies ex Bridel		++			+	A
	Polytrichum commune var. *commune* Hedw.		++			1.2	A
Pottiaceae	indet.				+		A
Scapaniaceae	*Douinia ovata* (Dicks.) H.Buch					+	A
Sphagnaceae	*Sphagnum* cf. *magellanicum* Brid.		+				A
Takakiaceae	indet.				+		A
Lichen							
Cladoniaceae	*Cladonia* cf. *rangiferina* (L.) Weber ex F.H.Wigg.		+				S

* insufficient material was present for detailed macro analysis

Table S6.2 Selwyn caribou B (±1.5kyr) – core 2, 189-191cm, KfTe-1 Ice Patch

Family/order	**Taxon**	**Pollen (%)**	**Macro**	***trn*L (%)**	**nrITS1 (%)**	**nrITS2 (%)**	**Caribou Diet (Denryter et al., 2017)**
Phanerogams							**A = Avoided** **N = Neutral** **S = Selected** **U = Unknown**
Asteraceae	indet.			+			U
	Antennaria sp.		0.5				A
	Artemisia sp.	40.0	0.2				N
	Artemisia norvegica subsp. *saxatilis* H.M.Hall & Clem.				0.3		N
	subfamily Asteroideae (Tubuliflorae)	10.0					U
Betulaceae	*Betula* sp.		2.3	8.5	18.9	10.9	S
Caryophyllaceae	*Stellaria* sp.		0.3			+	A
Cyperaceae	*Carex* sp.		7.7	0.1			A
	Carex aquatilis Wahlenb.				0.3		A
	Carex subgenus *Euthyceras*		0.3				U
	Carex lachenalii Schkuhr			+			A
	Carex nigra subsp. *juncea* (Fries) Soó				0.4		A
	Eriophorum sp.		2.3				N
Elaeagnaceae	*Shepherdia canadensis* Nutt.	1.0					N
Equisetaceae	*Equisetum* sp.		1.8				N
Ericaceae	*Arctous alpina/rubra*			0.1			N
	Pyrola sp.			+			N
	Pyrola grandiflora Radius			+			A
	Vaccinium sp.					+	U
	Vaccinium uliginosum L.			+			S
	Vaccinium vitis-idaea L.				1.1	1.1	A
Fabaceae	*Astragalus* sp.			+			N
	Hedysarum sp.		0.9				N
Family indet	indet.	20.0					U
Juncaceae	*Juncus* sp.		2.5	0.2			N
	Juncus alpinoarticulatus Chaix			0.1			N
	Juncus effusus L.				2.9		N
	Juncus oxymeris Engelm.				0.4		N
	Luzula sp.			+			A
Juncaginaceae	*Triglochin palustris* L.				1.3		U
Liliaceae	*Gagea serotina* (L.) Ker Gawl.			+			U
Lycopodiaceae	*Lycopodium* sp.		1.1				A
Orobanchaceae	*Pedicularis* sp.			+			A
	Pedicularis sudetica Willd.			0.1		1.0	A
Pinaceae	indet.	5.0					A
	Abies sp.	5.0					A
	Picea sp.	6.0					A
	Pinus sp.	8.0					A
Poaceae	indet.		0.9				U
	Arctagrostis sp.		1.9				U
	Arctagrostis latifolia Griseb.					+	U
	Calamagrostis sp.		0.9				A
	Festuca sp		1.8				N
	Hierochloe sp.		1.4				N
	Poa sp.		9.1				N
	Poa arctica R.Br.					+	N

Plantaginaceae	*Veronica* sp.			+			U
Polygonaceae	*Bistorta vivipara* (L.) Delarbre			+			N
	Rumex sp.		0.9				N
Pteridophyta	indet.	5.0					A
Ranunculaceae	indet.	+					U
	Anemone sp.			+			U
	Anemonastrum narcissiflorum (L.) Holub			0.4	5.1	2.4	U
	Ranunculus trichophyllus Chaix				0.2		A
Rosaceae	*Comarum palustre* L.				+		U
	Dryas sp.		2.5				N
	Geum sp.			6.3		1.7	N
	Geum aleppicum Jacq.				1.0		N
	subfamily Rosoideae			+			U
Salicaceae	*Salix* sp.		9.1	84.1	67.1	78.5	S
	Salix alaxensis (Andersson ex DC.) Coville					0.2	S
Saxifragaceae	*Saxifraga* sp.		0.2				A
Selaginellaceae	*Selaginella* sp.		0.9				U
Taxaceae	*Taxus canadensis* Marshall					1.2	U
Unknown forb			1.4				U
Cryptogams							
Brypophyta							A
Anastrophyllaceae	*Barbilophozia barbata* (Schmidel ex Schreb.) Loeske					0.4	A
Aulacomniaceae	*Aulacomnium* sp.		5.6				A
Dicranaceae	indet.			+			A
	Dicranum-type		15.3				A
	Dicranum fuscescens Sm.				0.5		A
	Dicranum scoparium Hedw.				0.8		A
Hylocomiaceae	*Hylocomium splendens* (Hedw.) Schimp.				+	1.2	A
	Pleurozium schreberi Mitten			+	+	0.1	A
Hypnales	indet.			+			A
Mniaceae	*Pohlia* sp.			+			A
	Pohlia nutans (Hedw.) H. Lindb.					0.5	A
Polytrichaceae	indet.			+			A
	Polytrichum sp.		2.6				A
	Polytrichum piliferum Hedw.					0.8	A
Pottiaceae	indet.				+		A
Sphagnaceae	*Sphagnum* sp.		2.5				U
Lichen							
Cladoniaceae	*Cladonia* sp.		7.2				S
Parmeliaceae	*Alectoria* sp.		4.6				S
	subfamily Parmelioideae (*Cetraria*/*Dactylina* sp.)		7.6				N
Peltigeraceae	*Peltigera* sp.		1.4				A
Stereocaulaceae	*Stereocaulon* sp.		0.9				A
Unknown lichen			0.2				U

Table S6.3 Selwyn caribou C (±2.7kyr) – core 1, 254-256cm, KfTe-1 Ice Patch
N.B. nrITS1 and nrITS2 did not produce any results.

Family/order	**Taxon**	**Pollen (%)**	**Macro**	***trn*L (%)**	**Caribou Diet (Denryter et al., 2017)**
					A = Avoided **N = Neutral** **S= Selected** **U = Unknown**
Amaranthaceae	*Blitum nuttallianum* Schult.			+	U
Apiaceae	subfamily Apioideae			2.4	U
	Cymopterus sessiliflorus (W.L.Theob. & C.C.Tseng) R.L.Hartm.			+	U
Asteraceae	tribe Anthemideae			31.9	U
	subfamily Asteroideae (Tubuliflorae)	10.0			U
	Artemisia sp.	15.0			N
	Artemisia gmelinii Web. ex Stechm.			0.1	N
Betulaceae	*Betula* sp.	3.0	1.1	7.0	S
Boraginaceae	*Mertensia paniculata* (Aiton) G.Don			1.7	N
Caryophyllaceae	*Stellaria* sp.		0.7		A
Cyperaceae	indet.	1.0			A
	Carex sp.		4		A
	Eriophorum sp.		0.4		N
Elaeagnaceae	*Shepherdia canadensis* Nutt.	3.0			N
Ericaceae	indet.	2.0			U
	Cassiope sp.		0.4		A
	Empetrum sp.		1.1		S
Indet.		27.0			U
Juncaceae	*Juncus* sp.		0.5		N
Lycopodiaceae	*Lycopodium* sp.	1.0	0.2		A
Onagraceae	*Chamaenerion angustifolium* (L.) Scop.			2.5	N
Pinaceae	indet.	5.0			A
	Picea sp.	20.0			A
	Abies sp.	2.0			A
Poaceae	indet.	5.0	1.1		U
	Arctagrostis sp.		2.2		U
	Calamagrostis sp.		0.4		A
	Festuca sp.		1.1		N
	Hierochloe sp.		0.4		N
	Poa sp.		2.7		N
Polemoniaceae	*Polemonium* sp.	+			N
Polygonaceae	*Bistorta vivipara* (L.) Delarbre			0.6	N
	Oxyria digyna (L.) Hill			+	N
Pteridophyta	indet.	5.0			
Ranunculaceae	*Anemonastrum narcissiflorum* (L.) Holub			0.4	U
	Ranunculus nivalis L.			3.6	U
	Ranunculus pygmaeus Wahlenb.			2.5	U
Rosaceae	*Dryas* sp.		4.6		N
	subfamily Rosoideae			0.5	U
	Sibbaldia procumbens L.			25.5	N
Salicaceae	*Salix* sp.	+	6	20.4	S
Saxifragaceae	*Micranthes* sp.			0.7	A
	Micranthes nelsoniana (D.Don) Small			0.2	A
	Saxifraga sp.		0.9		A
Selaginellaceae	*Selaginella* sp.		1.1		U

Cryptogams					
Brypophyta					
Aulacomniaceae	*Aulacomnium* sp.		6.7		A
Dicranaceae	*Dicranum*-type		13.3		A
Mniaceae	*Pohlia* sp.			+	A
Polytrichaceae	*Polytrichum* sp.		2.7		A
Sphagnaceae	*Sphagnum* sp.		4		A
Lichen					
Cladoniaceae	*Cladonia* sp.		24.6		S
Parmeliaceae	*Alectoria* sp.		10.2		S
	Subfamily Parmelioideae		5.3		S
Peltigeraceae	*Peltigera* sp.		2.9		A
Stereocaulaceae	*Stereocaulon* sp.		0.5		A

Table S6.4 Oyogos Yar horse (±5.4kyr)

Family/order	Taxon	Pollen (%)	Macro	*trn*L (%)	nrITS1 (%)	nrITS2 (%)
Phanerogams						
Apiaceae	indet.	0.1				
	Cicuta virosa L.				25.5	
Asteraceae	*Artemisia* sp.	+				
	Artemisia scoparia Waldst. & Kit.					+
	subfamily Asteroideae (Tubuliflorae)	0.6				
	Endocellion sibiricum (J.F. Gmel.) J. Toman				0.5	0.1
Betulaceae	*Alnus* sp.	0.9				
	Betula sp.	1.2				
	Betula sect. *Apterocaryon*	0.3				
Cyperaceae	Indet.	3.6	+++			
	Carex aquatilis Wahlenb.			6.1		0.1
	Carex rostrata Stokes				1.7	
	Eriophorum sp.			66.7		
	Eriophorum angustifolium Honck.				14.4	0.8
Ericaceae	*Pyrola* sp.	0.3				
	Vaccinium vitis-idaea L.					+
Indet		0.2				
Menyanthaceae	*Menyanthes trifoliata* L.				0.6	+
Onagraceae	*Epilobium palustre* L.				0.7	
Orobanchaceae	*Pedicularis sudetica* Willd.					+
Papaveraceae	*Papaver* sp. (*Papaver rhoeas*-type)	+				
Pinaceae	indet.	0.1				
	Abies sp.	0.1				
	Pinus subgenus *Pinus*	0.2				
Plantaginaceae	*Plantago* sp.	0.1				
	Hippuris sp.					+
Poaceae	indet.	91.6	+			
	subtribe Agrostidinae			5.6		
	Arctagrostis latifolia Griseb.				2.3	4.2
	Arctophila fulva (Trin.) Andersson					3.4
	Arctophila fulva/ Dupontia fisheri			2.4		
	Calamagrostis sp.					0.7
	Calamagrostis stricta Koeler				5.6	
	Dupontia fisheri R.Br.				44.2	
	Poa arctica R.Br.					1.7
	tribe Poeae			4.5		
Pteridophyta	indet.	0.3				
Ranunculaceae	indet.	0.2				
	Caltha palustris L.				3.1	
Rosaceae	*Comarum palustre* L.				1.4	
	Geum sp.					+
Salicaceae	*Salix* sp.	0.4		14.7		15.7
Cryptogams						
Bryophyta						
Amblystegiaceae	*Campylium* cf. *stellatum* (Hedw.) C.E.O.Jensen		+			
Ditrichaceae	*Ceratodon purpureus* (Hedw.) Brid.					5.3
Family indet.		0.7		+		
Mniaceae	*Plagiomnium* cf. *ellipticum* (Brid.) T.J. Kop.		+			
	Rhizomnium cf. *pseudopunctatum* (Bruch & Schimp.) T.J. Kop.		+			
Polytrichaceae	indet.			+		

	Polytrichastrum alpinum (Hedw.) G.L. Sm.		+			68.1
Sphagnaceae	*Sphagnum* sp.	+	+			

Table S6.5 Yakutian bison (±10.5kyr)

Family/order	**Taxon**	**Pollen (%)**	**Macro**	***trn*L (%)**	**nrITS1 (%)**	**nrITS2 (%)**
Phanerogams						
Adoxaceae	*Sambucus williamsii* Hance			+		
Apiaceae	indet.	8.9	+			
	Cicuta virosa L.				54.9	44.4
	tribe Oenantheae			14.4		
Asteraceae	*Artemisia* sp.	0.1				
	subfamily Asteroideae (Tubuliflorae)	0.1				
	Endocellion sibiricum (J.F. Gmel.) J. Toman				0.8	5.5
Betulaceae	*Alnus* sp.	3.0				
	Betula sp.	1.4				2.1
	Betula sect. *Apterocaryon*	2.3				
	Betula sect. *Betula*	0.8				
Caryophyllaceae	indet.	+				
	Stellaria sp.					+
Cyperaceae	indet.	6.1	++			
	Carex sp.		+			
	Carex aquatilis Wahlenb.			1.4	0.3	0.1
	Carex subgenus *Carex*			13.3		
	Carex chordorrhiza L.f.				0.7	
	Carex nigra subsp. *juncea* (Fries) Soó				+	
	Carex rostrata Stokes				1.7	+
	Carex vesicaria L.				0.2	+
	Eriophorum sp.		+	14.2		
	Eriophorum angustifolium Honck.				16.2	1.2
Dennstaedtiaceae	*Pteridium* sp.	0.2				
Equisetaceae	*Equisetum* sp.	3.0	+	+		
Fabaceae	indet.	1.4				
Liliaceae	indet.	0.2				
Menyanthaceae	*Menyanthes trifoliata* L.		+	2.8	0.5	3.4
Onagraceae	*Epilobium palustre* L.				0.7	1.0
Pinaceae	indet.	0.2				
	Pinus subgenus *Strobus*	0.2				
	Pinus subgenus *Pinus*	0.2				
Plantaginaceae	*Hippuris* sp.					0.6
Poaceae	indet.	71.1	++			
	subtribe Agrostidinae			1.0		
	Arctophila fulva (Trin.) Andersson					0.2
	Dupontia fisheri R.Br.				0.3	
	Calamagrostis sp.					0.1
	Poa arctica R.Br.					+
Pteridophyta	indet.	2.2				
Ranunculaceae	indet.	0.2				
	Anemonastrum narcissiflorum (L.) Holub					+
	Anemone patens L.					0.1
	Caltha palustris L.			2.9	2.5	5.7
Rosaceae	*Alchemilla* sp.			+		
	Comarum palustre L.		+	7.3	2.9	9.3
	subtribe Fragariinae (*Potentilla*-type)	0.6				
	subfamily Rosoideae			+		
Salicaceae	*Salix* sp.	0.5	+	42.6	18.6	26.3
Cryptogams						

Algae						
Zygnemataceae	*Spirogyra* sp.	+				
Bryophyta						
Amblystegiaceae	*Calliergon* cf. *giganteum* (Schimp.) Kindb.		+			
indet.	Type HdV-817 (bryophyte spores)	7.4				
Sphagnaceae	*Sphagnum* sp.	0.4				

Table S6.6 Cape Blossom mammoth (±14.4kyr)

N.B. nrITS1 and nrITS2 did not produce any results.

Family/order	Taxon	Pollen (%)	Macro	*trn*L (%)
Phanerogams				
Amaranthaceae	cf. *Chenopodium* sp.		+	
Apiaceae	indet.	6.0		
	apioid superclade			+
	subfamily Apioideae			0.8
	tribe Oenantheae			0.3
	tribe Selineae			1.1
Asteraceae	indet.			+
	tribe Anthemideae			32.6
	Arnica sp.			1.2
	subtribe Artemisiinae			+
	Artemisia sp.	7.2		
	Artemisia gmelinii Web. ex Stechm.			0.1
	subfamily Asteroideae (Tubuliflorae)	1.9		0.2
Betulaceae	*Betula* sp.	4.5		
Boraginaceae	*Eritrichium* sp.			+
	Mertensia paniculata (Aiton) G.Don			2.1
	Myosotis alpestris F.W.Schmidt			0.6
Brassicaceae	cf. *Draba* sp.		+	
Caryophyllaceae	indet.	0.8		
	tribe Alsineae (*Cerastium/Silene* sp.)		+	
	Minuartia rubella (Wahlenb.) Hiern		+	
Cyperaceae	indet.	4.8	90% (est.)	
	Carex sp.		+++	
	Carex aquatilis Wahlenb.			5.8
	Carex maritima Gunnerus			0.8
	Carex microchaeta Holm			+
	Carex subgenus *Vignea*		+	5.0
	Carex subgenus *Euthyceras*			9.0
Fabaceae	*Astragalus* sp.			+
Juncaceae	*Luzula* sp.		+	
Menyanthaceae	*Menyanthes trifoliata* L.			0.1
Onagraceae	indet.	1.3		
	Chamaenerion angustifolium (L.) Scop.			28.5
Plantaginaceae	*Plantago* sp.	0.7		
	Plantago sect. *Lamprosantha*			0.3
Poaceae	indet.	69.8	5% (est.)	
	tribe Agrostidinae			2.0
	Alopecurus sp.		+	
	Bromus sp.			+
	Bromus pumpellianus Scribn.			1.8
	Elymus sp.		+	
	Festuca kolymensis Drobow			+
	Koeleria asiatica Domin			+
	Poa sp.		+	
	tribe Poeae			1.6
	tribe Triticeae			0.3
Polemoniaceae	*Polemonium* sp.	0.8		
	Polemonium boreale Adams			0.2
Polygonaceae	subfamily Polygonoideae (*Rumex acetosella*-type)	0.2		
	Rumex sp. (*Rumex aquaticus*-type)	0.2		
Ranunculaceae	*Caltha palustris* L.			1.7

Rosaceae	indet.			
	Comarum palustre L.			0.1
	subtribe Fragariinae (*Potentilla*-type)	1.0		
	Potentilla sp.		+	0.2
	Potentilla cf. *hyparctica* Malte		+	
	Potentilla cf. *stipularis* L.		+	
	Sanguisorba officinalis L.	0.5		1.1
Salicaceae	*Salix* sp.	0.3		
Cryptogams				
Bryophyta				
Sphagnaceae	*Sphagnum* sp.	0.2		
Thuidiaceae	*Thuidium abietinum* (Hedw.) Schimp.		+	

Table S6.7 Yukagir mammoth (±22.5kyr)

Family/order	**Taxon**	**Pollen (%)**	**Macro**	***trn*L (%)**	**nrITS1 (%)**	**nrITS2 (%)**
Phanerogams						
Amaranthaceae	indet.	0.1	+			
Apiaceae	indet.	0.3				
	subfamily Apioideae			+		
	tribe Oenantheae			0.1		
Asteraceae	tribe Anthemideae			9.8		
	subtribe Artemisiinae			+		
	Artemisia sp.	16.0				
	Artemisia scoparia Waldst. & Kit.				0.5	2.6
	Artemisia gmelinii Web. ex Stechm.			+		
	subfamily Asteroideae (Tubuliflorae)	0.2	+			
	subfamily Cichorioideae (Liguliflorae)	0.2				
Boraginaceae	*Eritrichium* sp.			0.6		
	Eritrichium sericeum DC.				5.6	8.0
	Myosotis alpestris F.W.Schmidt			16.7	69.0	60.0
Brassicaceae	indet.	0.7				
	Draba sp.		+			
	Parrya nudicaulis (L.) Regel			+		
	Smelowskia sp.			+		
	Smelowskia alba (Pall.) Regel				6.0	11.8
Caryophyllaceae	indet.	4.7				
	Cerastium arvense L.			+	0.1	
	Eremogone sp.			+		
	Eremogone capillaris (Poir.) Fenzl			+		
	Sagina nivalis Fr.		+			
	Silene sp.			+		
Crassulaceae	*Rhodiola rosea* L.			+		
Cyperaceae	indet.	0.1				
	Carex sp.		++	0.1		
	Carex dioica L.		++			
	Carex nardina Fr.		++			
	Carex nigra subsp. *juncea* (Fries) Soó				0.3	
Ericales	indet.	0.1				
Fabaceae	indet.	1.4				
	Astragalus sp.			0.3		
	Astragalus alpinus L.					0.8
	Lotus sp.	0.2				
	Oxytropis sp.			0.1		
	Oxytropis deflexa DC.			+	+	2.0
	Oxytropis splendens Douglas				0.4	
Juncaceae	*Juncus* sp.		+			
Liliaceae	Indet.	+				
Menyanthaceae	*Menyanthes trifoliata* L.			+		
Orchidaceae	*Epipactis* sp.	+				
Orobanchaceae	*Pedicularis* sp.			+		
	Pedicularis sudetica Willd.			+		
Papaveraceae	*Papaver* sp.	0.1		+		
	Papaver sect. *Scapiflora*		+			
Plantaginaceae	*Lagotis* sp.			+		
	Plantago sp.	0.8		0.1		
Plumbaginaceae	tribe Limonieae (*Armeria*-type)	+				
Poaceae	indet.	70.6	+++			
	indet. (cf. *Agrostis* sp.)		+			
	Deschampsia cespitosa (L.) P.Beauv.				0.4	0.9
	Festuca kolymensis Drobow			0.3		
	Festuca ovina L.				0.2	1.0

	Glyceria sp.		+			
	Hordeum sp.		+			
	Pleuropogon sabinei R.Br.			+		
	Poa cf. *arctica* R.Br.		+			
	Poa glauca Vahl				1.1	
	tribe Poeae			0.1		
Polemoniaceae	*Polemonium* sp.	0.2				
	Phlox hoodii Richardson			+		
Polygonaceae	*Persicaria* sp. (*P. maculosa*-type)	0.3				
	subfamily Polygonoideae (*Rumex acetosella*-type)	0.8				
	Rumex sp.		+			
	Rumex acetosella L.		+			
Potamogetonaceae	*Stuckenia* sp.			+		
Primulaceae	cf. *Androsace*	0.2				
	Androsace lehmanniana Spreng.			+		
	Lysimachia sp.		+			
Ranunculaceae	indet.	0.7				
	Anemonastrum narcissiflorum (L.) Holub			+		+
	Caltha palustris L.		+	2.0		
	Ranunculus sp.			+		
	Ranunculus cf. *nivalis* L.		+			
	Ranunculus pedatifidus var. affinis (R.Br.) L.D.Benson			+		
	Ranunculus cf. *pygmaeus* Wahlenb.		+			
Rosaceae	indet.	0.2				
	subtribe Fragariinae (*Potentilla*-type)	0.9				
	Geum sp.			0.2		
	Potentilla sp.		+	1.7	+	
	Potentilla hookeriana Lehm.					0.4
	Potentilla hyparctica Malte		+			
	cf. *Rubus chamaemorus* L.	1.9				
	Sanguisorba officinalis L.	0.1				
Salicaceae	indet.			67.3		
	Salix cf. *arctica* Pall.		+			
	Salix sp.	0.2	+++		15.4	12.4
Saxifragaceae	*Micranthes* sp.			+		
Cryptogams						
Algae						
Zygnemataceae	*Spirogyra* sp.	+				
Hydrodictyaceae	Pediastrum sp.	0.1				
Bryophyta						
Amblystegiaceae	*Drepanocladus* sp.					+
	Drepanocladus aduncus (Hedw.) Warnst.		+	+		
	Drepanocladus sordidus (Müll. Hal.) Hedenäs				+	
Bryaceae	*Bryum* sp.		+	+		
Entodontaceae	*Entodon concinnus* Paris		+			
Hypnales	indet.			+		
Polytrichaceae	*Polytrichastrum alpinum* (Hedw.) G.L. Sm.		+			
Pottiaceae	indet.		+			

Table S6.8 Adycha mammoth (±25.6kyr)

Family/order	Taxon	Pollen (%)	Macro	*trn*L (%)	nrITS1 (%)	nrITS2 (%)
Phanerogams						
Amaranthaceae	indet.	0.8				
Apiaceae	indet.	0.2				
	tribe Apioideae (*Peucedanum*-type)	+				
	Peucedanum sp.			0.2		
	tribe *Selineae*			+		
Asteraceae	tribe *Anthemideae*			4.2		
	Artemisia sp.	18.1				
	Artemisia scoparia Waldst. & Kit.					+
	tribe Cardueae (*Arctium*-type; *Carlina*-type)	0.5				
	subfamily Cichorioideae (Liguliflorae)	0.5				
	tribe Gnaphalieae			+		
	Saussurea sp.			1.0		
Betulaceae	*Betula* sp.	0.7				
Boraginaceae	*Eritrichium* sp.			+		
Brassicaceae	indet.	1.2		+		
Caryophyllaceae	indet.					
	Minuartia sp.	0.1				
	Stellaria sp.				+	
Cyperaceae	indet.	2.9				
	Carex subgenus *Vignea*			0.9		
	Carex maritima Gunnerus			+		
Ericales	indet.	0.1				
	Vaccinium vitis-idaea L.					+
Fabaceae	indet.	0.1				
	Astragalus alpinus L.					+
Juncaceae	*Juncus biglumis* L.			+		
Onagraceae	*Chamaenerion angustifolium* (L.) Scop.			0.4		
Pinaceae	*Pinus* sp.			+		
	Pinus subgenus *Strobus*	0.1				
	Pinus subgenus *Pinus*	1.0				
Poaceae	indet.	72.4	+++			
	Alopecurus magellanicus Lam.				0.2	
	Arctagrostis latifolia Griseb.					+
	Bromus sp.			+		
	Bromus pumpellianus Scribn.			16.2		
	Deschampsia cespitosa (L.) P.Beauv.				0.2	
	Dupontia fisheri R.Br.				0.1	
	Festuca ovina L.					+
	Poa arctica R.Br.					+
	Puccinellia sp.			76.8	38.5	1.0
	Puccinellia tenuiflora/vahliana					99.0
	Puccinellia vahliana Scribn. & Merr.				61.0	
Polypodiophyta	indet.	0.8				
Ranunculaceae	indet.	0.4				
Salicaceae	*Salix* sp.	0.5			+	
Saxifragaceae	*Saxifraga sibirica* L.					+
Cryptogams						
Bryophyta						
Dicranaceae	*Dicranum scoparium* Hedw.				+	
Funariaceae	*Funaria* sp.				+	
Glomeraceae	*Glomus* sp.	5.9				
Hylocomiaceae	*Hylocomium splendens* (Hedw.) Schimp.					+
hypnales	indet.			+		

Algae						
Zygnemataceae	*Zygnema*-type	0.1				

Table S6.9 Yukon horse (±30.9)

N.B. nrITS1 and nrITS2 did not produce any results.

Family/order	**Taxon**	**Pollen (%)**	**Macro**	***trn*L (%)**
Phanerogams				
Amaranthaceae	indet.	2.2		
Amaryllidaceae	*Allium* sp.	0.2		
Apiaceae	indet.	0.9		
Asteraceae	tribe Anthemideae			13.6
	Artemisia sp.	27.4	+	
	Artemisia gmelinii Web. ex Stechm.			+
	subfamily Asteroideae (Tubuliflorae)	1.9		
	subfamily Cichorioideae (Liguliflorae)	0.6		
Betulaceae	*Alnus crispa* (Aiton) Pursh		+	
	Alnus incana (L.) Moench		+	
	Betula sp.		+	
Brassicaceae	indet.	0.2	++	
	Braya sp.			6.0
	Braya rosea Bunge			21.1
Caryophyllaceae	indet.		++	
	Silene sp. (*Silene vulgaris*-type)	0.4		
Cyperaceae	indet.	2.2	++	
Ericaceae	*Pyrola grandiflora* Radius			+
Fabaceae	indet.	0.4		
	Oxytropis sp.			11.7
Gentianaceae	*Gentianella* sp.	0.2		
Juncaceae	*Juncus* sp.			0.1
	Juncus alpinoarticulatus Chaix			+
Orobanchaceae	*Pedicularis sudetica* Willd.			0.1
Papaveraceae	*Papaver* sp.	0.2	++	
Plantaginaceae	*Plantago* sp.	0.6		
Poaceae	indet.	57.1	++	
	Bromus pumpellianus Scribn.			2.9
	tribe Poeae			29.2
	tribe Triticeae			4.9
Polemoniaceae	*Polemonium* sp	0.2		
Polygonaceae	*Persicaria* sp. (*P. maculosa*-type)	3.7		
	subfamily Polygonoideae (*Rumex acetosella*-type)	0.4		
Primulaceae	*Androsace septentrionalis* L.		++	
Ranunculaceae	indet.	0.2		
	Anemonastrum narcissiflorum (L.) Holub			0.1
Rosaceae	subtribe Fragariinae (*Potentilla*-type)	0.9		
	Geum sp.			1.4
	Potentilla sp.		++	8.9
	Sanguisorba officinalis L.	0.2		
Salicaceae	*Salix* sp.		+	

Table S6.10 Abyland mammoth (±32.4kyr)

Family/order	**Taxon**	**Pollen (%)**	**Macro**	***trn*L (%)**	**nrITS1 (%)**	**nrITS2 (%)**
Phanerogams						
Amaranthaceae	indet.	+				
Apiaceae	indet.	+				
	tribe Oenantheae			0.6		
	tribe Selineae			+		
Asteraceae	indet.			+		
	tribe Anthemideae			10.7		
	subtribe Artemisiinae			+		
	Artemisia sp.	26.7				
	Artemisia gmelinii Web. ex Stechm.			+		
	Artemisia scoparia Waldst. & Kit.				0.2	
	Arnica sp.			0.2		
	subfamily Asteroideae (*Aster*-type; *Senecio*-type; Tubuliflorae)	0.3		+		
	tribe Cardueae (*Carduus*-type)	+				
	subfamily Cichorioideae (Liguliflorae)	0.3				
	Saussurea sp.			0.9		
	Tephroseris sp.			0.1		
	Tripleurospermum maritimum (L.) W.D.J.Koch			+		
Boraginaceae	*Eritrichium* sp.			0.1		
	Mertensia paniculata (Aiton) G.Don			+		
	Myosotis alpestris F.W.Schmidt			0.7		
Brassicaceae	indet.	3.8		0.3		
	tribe Thelypodieae			+		
	Sisymbrium linifolium Nutt.				2.2	+
Caryophyllaceae	indet.	1.5				
	Cerastium arvense L.			0.1	+	
	Cerastium maximum L.			+		
	Dianthus sp.	+		+		
	Eremogone capillaris (Poir.) Fenzl			+		
	Silene sp. (*Silene vulgaris*-type)	+				
	tribe Sileneae (*Lychnis*/*Viscaria*-type)	+				
	Silene samojedorum (Sambuk) Oxelman			0.1		
	Stellaria sp.			+		+
	Stellaria borealis Bigelow			+		
Crassulaceae	*Rhodiola integrifolia* Raf.			+		
Cyperaceae	indet.	0.5				
	Carex sp.		+	+		
	Carex nigra subsp. *juncea* (Fries) Soó				7.3	
	Carex duriuscula C.A.Mey.				53.0	0.2
	Carex subgenus *Euthyceras*			+		
	Carex subgenus *Vignea*			10.2		
Fabaceae	indet.	1.0				
	Astragalus sp.			+		
	Oxytropis sp.			+		
	tribe Trifolieae (*Trifolium repens*-type)	+				
Juncaceae	*Juncus* sp.			+		
	Juncus alpinoarticulatus Chaix			+		
	Luzula sp.			+		
Menyanthaceae	*Menyanthes trifoliata* L.			0.1		
Onagraceae	*Chamaenerion angustifolium* (L.) Scop.			1.0		
Orobanchaceae	indet. (*Rhinanthus*-type)	+				
	Pedicularis sp.			+		

	Pedicularis sudetica Willd.			+		
	Pedicularis verticillata L.			+		
Papaveraceae	*Papaver* sp.	0.8		0.2		
Plantaginaceae	*Plantago* sp.	2.1				
	Plantago sect. *Lamprosantha*			2.0		
Poaceae	indet.	61.1	+++			
	subtribe Agrostidinae			0.2		
	Alopecurus magellanicus Lam.				2.9	
	Bromus sp.			+		
	Bromus pumpellianus Scribn.			11.3		
	Deschampsia cespitosa (L.) P.Beauv.					0.3
	Festuca altaica Trin.			+		
	Festuca kolymensis Drobow			+		
	Hordeum sp.			0.2		
	Koeleria asiatica Domin			0.1		
	Poa sp.				2.5	
	tribe Poeae			3.2		
	tribe Triticeae			0.5		
	Puccinellia tenuiflora/vahliana					4.3
Polemoniaceae	*Polemonium* sp.	0.3		+		
Potamogetonaceae	*Stuckenia* sp.			0.3		
Polygonaceae	*Persicaria* sp. (*P. maculosa*-type)	+				
Ranunculaceae	indet.	+				
	Anemonastrum narcissiflorum (L.) Holub			+		+
	Anemone sp.	+		+	0.3	
	Anemone patens L.			49.6	30.9	70.6
	Caltha palustris L.			0.4		
	Thalictrum sp.	+				
Rosaceae	indet.	0.3				
	Comarum palustre L.			0.1		
	subtribe Fragariinae (*Potentilla*-type)	2.1				
	Geum sp.			0.5		
	Potentilla sp.			2.4		
	subfamily Rosoideae			+		
	Sanguisorba officinalis L.	0.3		0.2		
Rubiaceae	tribe Rubieae (*Galium*-type)	+		+		
Salicaceae	*Salix* sp.			3.0		24.6
Saxifragaceae	section Mesogyne			+		
Cryptogams						
Bryophyta						
Pottiaceae	*Barbula unguiculata* Hedw.				0.7	
	Didymodon icmadophilus (Müll.Hal.) K.Saito			+		

Table S6.11 Maly Lyakhovsky mammoth (±32.7kyr)

Family/order	Taxon	Pollen (%)	Macro	*trn*L (%)	nrITS1 (%)	nrITS2 (%)
Phanerogams						
Apiaceae	tribe Oenantheae			0.6		
Asteraceae	*Artemisia* sp.	1.0				
	subtribe Artemisiinae			+		
	subfamily Asteroideae (Tubuliflorae)	0.2				
	subfamily Cichorioideae (Liguliflorae)	+				
	tribe Gnaphalieae			+		
	Saussurea sp.			+		
Brassicaceae	indet.	0.2				
	Arabidopsis lyrata (L.) O'Kane & Al-Shehbaz			0.2		
	Eutrema edwardsii R.Br.			0.1		
Caryophyllaceae	indet.	0.4				
	Stellaria sp.			0.3	2.6	4.9
	Stellaria borealis Bigelow			+		
	Stellaria longifolia Muhl. ex Willd.			+		
Crassulaceae	*Rhodiola rosea* L.			0.1		
Cyperaceae	indet.	0.4				
	Carex sp.			0.1		
	Carex nigra subsp. *juncea* (Fries) Soó				4.2	
	Eriophorum sp.			22.9	0.7	
	Eriophorum angustifolium Honck.				3.3	0.8
Fabaceae	indet.	0.1				
	Oxytropis deflexa DC.					+
Juncaceae	*Juncus biglumis* L.			1.0		
	Juncus oxymeris Engelm.				+	
Menyanthaceae	*Menyanthes trifoliata* L.			0.1	+	+
Orobanchaceae	cf. *Pedicularis* sp.	0.1		0.3		
	Pedicularis sudetica Willd.			0.1		
Poaceae	indet.	96.9	+++			
	tribe Agrostidinae			0.3		
	Arctophila fulva (Trin.) Andersson					7.5
	Arctophila fulva/Dupontia fisheri			21.5		
	Alopecurus magellanicus Lam.				28.7	9.8
	Bromus sp.			+		
	Bromus pumpellianus Scribn.			1.3		
	Dupontia fisheri R.Br.				9.5	5.0
	Deschampsia cespitosa (L.) P.Beauv.				42.2	21.6
	Festuca altaica Trin.			+		
	Hordeum sp.			0.1		
	Pleuropogon sabinei R.Br.			0.6		
	tribe Poeae			40.6		
	tribe Triticeae			+		
	Puccinellia sp.			2.1	3.2	
	Puccinellia vahliana Scribn. & Merr.				0.6	
Papaveraceae	*Papaver* sp.	0.5				
Pinaceae	*Pinus* sp.			+		
Plantaginaceae	*Hippuris* sp.					+
Polemoniaceae	*Polemonium* sp.	+				
Polygonaceae	subfamily Polygonoideae (*Rumex*	0.3				
	Rumex sp.			+		
Ranunculaceae	indet.	+				
	Anemonastrum narcissiflorum (L.) Holub					+
	Anemone patens L.					0.4
	Caltha palustris L.			2.4		7.0
	Comarum palustre L.			0.1		

	Ranunculus pedatifidus var. affinis (R.Br.) L.D.Benson			0.1		
Rosaceae	*Geum* sp.			0.1		
	Potentilla sp.			0.1		
Salicaceae	indet.			3.0		
	Salix sp.				+	
Saxifragaceae	*Micranthes* sp.			0.1		
	Saxifraga sibirica L.					8.1
	Saxifraga sect. *Mesogyne*			1.0		
Cryptogams						
Bryophyta						
Amblystegiaceae	*Campylium stellatum* (cf. var. *stellatum*) (Hedw.) C.E.O.Jensen		+			
	Cratoneuron filicinum (Hedw.) Spruce			+		
	Drepanocladus sp.		+	+		
	Drepanocladus sordidus (Müll. Hal.) Hedenäs				0.2	1.2
	Warnstorfia sarmentosa Hedenäs		+			
Bartramiaceae	*Philonotis cf. arnellii* Husn.		+			
Bryaceae	*Bryum* sp.		+	+		
	Pohlia cf. nutans (Hedw.) H. Lindb.		+			
Dicranaceae	*Dicranum bonjeanii* De Not.			+		
	Dicranoweisia cf. cirrata (Hedw.) Lindb.		+			
Distichiaceae	*Distichium* sp.		+			
Funariaceae	*Funaria* sp.				2.8	
Hypnales	indet.			+		
Mniaceae	*Cinclidium stygium* Sw.		+			
Polytrichaceae	*Polytrichastrum alpinum* (Hedw.) G.L. Sm.		+			33.4
Pottiaceae	*Didymodon icmadophilus* (Müll.Hal.) K.Saito				1.9	
Liverwort						
Ricciaceae	*Riccia* sp.	+				

Multiproxy analysis of permafrost preserved faeces provides an unprecedented insight into the diets and habitats of extinct and extant megafauna

Marcel Polling, Anneke T.M. ter Schure, Bas van Geel, Tom van Bokhoven, Sanne Boessenkool, Glen MacKay, Bram W. Langeveld, María Ariza, Hans van der Plicht, Albert V. Protopopov, Alexei Tikhonov, Hugo de Boer, Barbara Gravendeel

Supporting Information (2/3)

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Table S7. Idenitity and abundance of OTU's *trn* L

id	OTU	Total read c	best_id	best_id	cover	order	family	subfamily	tribe	subtribe	genus/subg.	species	maxid	scientific rank	OTU sequence	length of	type
M01334:3	OTU_0178	5344	1.00	1.00	100	Alismatales	Potamogetonaceae				*Stuckenia*		*Stuckenia*	genus	atccctgttttgagaaaaaagggtttattttctatatttcgaattttctattatatagaaattcaaaaataagaatttaaaaaagg	86	forb
M01334:3	OTU_1518	51	1.00	1.00	100	Apiales	Apiaceae	apioid superclade					apioid superclade	subfamily	atcctattttccaaaaacaaacaaaggcccagaaggcgaaaaaag	45	forb
M01334:3	OTU_0549	117676	1.00	0.91	100	Apiales	Apiaceae	Apioideae	Oenantheae				Oenantheae1	tribe	atcctgttttcaaaaaacaaagaaaggggcagaaggtgaaaaaag	45	forb
M01334:3	OTU_0939	52089	1.00	0.93	100	Apiales	Apiaceae	Apioideae	Oenantheae				Oenantheae2	tribe	atcctgttttcaaaaaacaaacaaaggggcagaaggtgaaaaaag	45	forb
M01334:3	OTU_1356	81	0.98	1.00	100	Apiales	Apiaceae	Apioideae	Selineae		*Cymopterus*	*Cymopterus sessiliflorus*	*Cymopterus sessiliflorus*	species	atcctattttccaaaaacaaacaaaggtccagaaggtgaaaaaag	45	forb
M01334:3	OTU_0374	2278	1.00	1.00	100	Apiales	Apiaceae	Apioideae	Selineae		*Peucedanum*		*Peucedanum*	genus	atcctatttacaaaaacaaacaaaggcccagaaggtgaaaaaag	45	forb
M01334:3	OTU_0950	20590	1.00	1.00	100	Apiales	Apiaceae	Apioideae					Apioideae	subfamily	atcctattttccaaaaacaaacaaaggcccagaaggtgaaaaaag	45	forb
M01334:3	OTU_0554	16971	1.00	1.00	100	Apiales	Apiaceae		Selineae				Selineae	tribe	atcctattttccaaaaacaaacaaaggcccataaggtgaaaaaag	45	forb
M01334:3	OTU_1080	534	1.00	1.00	100	Asterales	Asteraceae	Asteroideae	Anthemideae	Anthemidinae	*Tripleurospermum*	*Tripleurospermum maritimum*	*Tripleurospermum maritimum*	species	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaataaaaaaaaaaaa	55	forb
M01334:3	OTU_0338	2441	0.98	1.00	100	Asterales	Asteraceae	Asteroideae	Anthemideae	Artemisiinae	*Artemisia*	*Artemisia gmelinii*	*Artemisia gmelinii*	species	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaagaaaaaaag	51	forb
M01334:3	OTU_0231	2012	1.00	1.00	100	Asterales	Asteraceae	Asteroideae	Anthemideae	Artemisiinae			Artemisiinae	subtribe	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaagaaaaaaaag	52	forb
M01334:3	OTU_0019	893940	1.00	0.98	100	Asterales	Asteraceae	Asteroideae	Anthemideae				Anthemideae1	tribe	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaagaaaaaaaa	51	forb
M01334:3	OTU_0087	26180	1.00	1.00	100	Asterales	Asteraceae	Asteroideae	Anthemideae				Anthemideae2	tribe	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaagaaaaaaaaa	52	forb
M01334:3	OTU_0179	4772	0.98	1.00	100	Asterales	Asteraceae	Asteroideae	Anthemideae				Anthemideae3	tribe	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaataaaaaaaa	51	forb
M01334:3	OTU_1037	878	0.98	1.00	100	Asterales	Asteraceae	Asteroideae	Anthemideae				Anthemideae4	tribe	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaagaaaaaaaaaa	53	forb
M01334:3	OTU_2119	22	0.96	1.00	100	Asterales	Asteraceae	Asteroideae	Anthemideae				Anthemideae5	tribe	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaagaaaaaaaaaag	54	forb
M01334:3	OTU_2189	20	0.98	1.00	100	Asterales	Asteraceae	Asteroideae	Anthemideae				Anthemideae6	tribe	atcacgttttccgaaaataaacaaaggttcagaaagcgaaaagaaaaaaaaa	52	forb
M01334:3	OTU_0084	634	1.00	1.00	100	Asterales	Asteraceae	Asteroideae	Gnaphalieae				Gnaphalieae	tribe	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaatcaaaag	49	forb
M01334:3	OTU_0375	1907	1.00	1.00	100	Asterales	Asteraceae	Asteroideae	Senecioneae	Tussilagininae	*Tephroseris*		*Tephroseris*	genus	atcacgttttccgaaaacaaacaaaggttcagaaaacgaaaataaaaaag	50	forb
M01334:3	OTU_0984	3278	1.00	1.00	100	Asterales	Asteraceae	Asteroideae (NAC)					Asteroideae NAC	subfamily	atcacgttttccgaaaacaagcaaaggttcagaaagcgaaaatcaaaaag	50	forb
M01334:3	OTU_0030	24771	1.00	1.00	100	Asterales	Asteraceae	Cardueae		Carduinae	*Saussurea*		*Saussurea*	genus	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaatccaaaag	50	forb
M01334:3	OTU_0552	19709	1.00	1.00	100	Asterales	Asteraceae	Madieae		Arnicinae	*Arnica*		*Arnica*	genus	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaataaaaaaaag	52	forb
M01334:3	OTU_0441	535	1.00	1.00	100	Asterales	Asteraceae						Asteraceae1	family	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaagaaaaaag	50	forb
M01334:3	OTU_0574	366	0.98	1.00	100	Asterales	Asteraceae						Asteraceae2	family	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaataaaaaaag	51	forb
M01334:3	OTU_2528	11	0.98	1.00	100	Asterales	Asteraceae						Asteraceae3	family	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaatcaaaaaag	51	forb
M01334:3	OTU_2622	13	0.98	1.00	100	Asterales	Asteraceae						Asteraceae4	family	atcacgttttccgaaaacaaacaaaggttcagaaagcgaaaagaaaaaagg	51	forb
M01334:3	OTU_2293	19	1.00	1.00	100	Asterales	Campanulaceae	Campanuloideae			*Campanula*		*Campanula*	genus	atccggttttctgacaataacaaaaggttcagaaagcgaaaatcaaaaag	50	forb
M01334:3	OTU_0945	33566	1.00	0.98	100	Asterales	Menyanthaceae				*Menyanthes*	*Menyanthes trifoliata*	*Menyanthes trifoliata*	species	atcccgttttccgaaaacaaacaaaggttcagaaagcgaaaataaaaaag	50	forb
M01334:3	OTU_0053	10198	1.00	1.00	100	Boraginales	Boraginaceae	Boraginoideae	Eritrichieae		*Eritrichium*		*Eritrichium*	genus	atccggttttccgaaaacaaaagttgaaaaagaaaaag	38	forb
M01334:3	OTU_0020	233674	1.00	1.00	100	Boraginales	Boraginaceae	Boraginoideae	Eritrichieae		*Myosotis*	*Myosotis alpestris*	*Myosotis alpestris*	species	atccggttttccgaaaacaaaagttgaaaaagaaaaaaggaaggag	46	forb
M01334:3	OTU_0942	37614	1.00	1.00	100	Boraginales	Boraginaceae	Boraginoideae			*Mertensia*	*Mertensia paniculata*	*Mertensia paniculata*	species	atctggttttccgaaaacaaacaaaagttgaaaaagaaaaaag	43	forb
M01334:3	OTU_0061	2088	1.00	1.00	100	Brassicales	Brassicaceae		Camelineae		*Arabidopsis*	*Arabidopsis lyrata*	*Arabidopsis lyrata*	species	atcctggtttacgcgaacaaaccggagtttacaaagcgcgaaaaaagg	48	forb
M01334:3	OTU_1345	78	1.00	1.00	100	Brassicales	Brassicaceae		Chorisporeae		*Parrya*	*Parrya nudicaulis*	*Parrya nudicaulis*	species	atcctggtttacgcgaactaaccagagtttataaagcgagaaaaagg	47	forb
M01334:3	OTU_0228	29598	0.98	1.00	100	Brassicales	Brassicaceae		Euclidieae		*Braya*	*Braya rosea*	*Braya rosea*	species	atcctggtttacgcgaacaaacctgagtttagaaagcgagaaaaaagg	48	forb
M01334:3	OTU_0305	8375	1.00	1.00	100	Brassicales	Brassicaceae		Euclidieae		*Braya*		*Braya*	genus	atcctggtttacgcgaataaacctgagtttagaaagcgagaaaaagg	47	forb
M01334:3	OTU_0092	689	1.00	1.00	100	Brassicales	Brassicaceae		Eutremeae		*Eutrema*	*Eutrema edwardsii*	*Eutrema edwardsii*	species	atcctagtttacgcgaacaaaccagagtttagaaagcgagaaaaaagg	48	forb
M01334:3	OTU_0224	245	1.00	1.00	100	Brassicales	Brassicaceae		Smelowskieae		*Smelowskia*		*Smelowskia*	genus	atcctgggttacgcgaacaaaccggagtttagaaagcgagaaaaaagg	48	forb
M01334:3	OTU_1323	93	0.97	1.00	100	Brassicales	Brassicaceae		Thelypodieae				Thelypodieae	tribe	atcctgggttacgcgaacaaaccagagtttagaaaacgg	39	forb
M01334:3	OTU_0972	5673	1.00	1.00	100	Brassicales	Brassicaceae						Brassicaceae	family	atcctgggttacgcgaacaaaccagagtttagaaagcgg	39	forb
M01334:3	OTU_2052	24	1.00	0.73	100	Bryales	Bryaceae				*Bryum*		*Bryum*	genus	attttattatatattaaaaatga	23	moss
M01334:3	OTU_1669	39	1.00	0.70	100	Bryales	Bryaceae						Bryaceae	family	attttattgtctattaaaaataa	23	moss
M01334:3	OTU_1206	169	1.00	0.53	100	Bryales	Mniaceae				*Pohlia*		*Pohlia*	genus	attttattttatttaa	16	moss
M01334:3	OTU_0986	3273	1.00	0.97	100	Caryophyllales	Amaranthaceae	Chenopodioideae	Anserineae		*Blitum*	Blitum nuttallianum	*Blitum nuttallianum*	species	ctcctttttcaaaagcaagaataaaaaaaaag	33	forb
M01334:3	OTU_0232	1742	1.00	1.00	100	Caryophyllales	Caryophyllaceae		Alsineae		*Cerastium*	*Cerastium arvense*	*Cerastium arvense*	species	ctccttaattgtctcctaaaaaaaacggattcaaaaagaaagaataaaaaag	52	forb
M01334:3	OTU_1069	519	1.00	1.00	100	Caryophyllales	Caryophyllaceae		Alsineae		*Cerastium*	*Cerastium maximum*	*Cerastium maximum*	species	ctccttaattgtctcctaaaaaaaaaacggattcaaaaagcaagaataaaaaaggaaag	59	forb
M01334:3	OTU_1241	132	0.98	1.00	100	Caryophyllales	Caryophyllaceae		Alsineae		*Cerastium*		*Cerastium*	genus	ctccttaattgtctcctaaaaaaaaacggattcaaaaagaaagaataaaaaag	53	forb
M01334:3	OTU_0310	662	1.00	1.00	100	Caryophyllales	Caryophyllaceae		Alsineae		*Stellaria*	*Stellaria borealis*	*Stellaria borealis*	species	ctccttaaatcgtctcctaaaaaaaacggattcaaaaagcaagaataaaaaag	53	forb
M01334:3	OTU_0384	218	1.00	1.00	100	Caryophyllales	Caryophyllaceae		Alsineae		*Stellaria*	*Stellaria longifolia*	*Stellaria longifolia*	species	ctccttaaatcgtctcctaaaaaaaaaacggattcaaaaagcaagaataaaaaag	55	forb
M01334:3	OTU_0121	3903	1.00	1.00	100	Caryophyllales	Caryophyllaceae		Alsineae		*Stellaria*		*Stellaria*	genus	ctccttaaatcgtctcctaaaaaaaaacggattcaaaaagcaagaataaaaaag	54	forb
M01334:3	OTU_1461	60	1.00	1.00	100	Caryophyllales	Caryophyllaceae		Caryophylleae		*Dianthus*		*Dianthus*	genus	ctccttttgtttcctttttcaaaagaaaaaaag	34	forb
M01334:3	OTU_0579	557	0.97	1.00	100	Caryophyllales	Caryophyllaceae		Eremogoneae		*Eremogone*	*Eremogone capillaris*	*Eremogone capillaris*	species	ctcctttatgttttgtttcttttttttttttagcaaag	38	forb
M01334:3	OTU_1333	87	1.00	1.00	100	Caryophyllales	Caryophyllaceae		Eremogoneae		*Eremogone*		*Eremogone*	genus	ctcctttatgttttgtttctttttttttttagcaaag	36	forb
M01334:3	OTU_1050	924	1.00	1.00	100	Caryophyllales	Caryophyllaceae		Sileneae		*Silene*	*Silene samojedorum*	*Silene samojedorum*	species	ctcctttttcaaaagcaaaaaaagtattaagaaagaaaaaaaaag	46	forb
M01334:3	OTU_2444	17	1.00	1.00	100	Caryophyllales	Caryophyllaceae		Sileneae		*Silene*		*Silene*	genus	ctcctttttcaaaagcaaaaaaaagtattaagaaagaaaaaaag	45	forb
M01334:3	OTU_0256	2987	1.00	1.00	100	Caryophyllales	Polygonaceae	Polygonoideae	Persicarieae	Koenigiinae	*Bistorta*	Bistorta vivipara	*Bistorta vivipara*	species	ctcctgctttccaaaaaggaagaataaaaaag	32	forb
M01334:3	OTU_0581	354	1.00	1.00	100	Caryophyllales	Polygonaceae	Polygonoideae	Rumiceae		*Rumex*		*Rumex*	genus	ctcctcctttccaaaaggaagaataaaaaag	31	forb
M01334:3	OTU_1352	86	1.00	1.00	100	Caryophyllales	Polygonaceae				*Oxyria*	*Oxyria digyna*	*Oxyria digyna*	species	ctccttcgtccaaaaggaagaataaaaaag	30	forb
M01334:3	OTU_0630	72	1.00	0.73	100	Dicranales	Dicranaceae				*Dicranum*	*Dicranum bonjeanii*	*Dicranum bonjeanii*	species	attttattttgaaagtaagatataa	25	moss
M01334:3	OTU_1003	1774	1.00	0.58	100	Dicranales	Dicranaceae						Dicranaceae1	family	attttatcttaaaaatga	18	moss
M01334:3	OTU_3451	9	1.00	0.58	100	Dicranales	Dicranaceae						Dicranaceae2	family	attttatcttgaaaatga	18	moss
M01334:3	OTU_1152	215	1.00	0.98	100	Dipsacales	Adoxaceae				*Sambucus*	*Sambucus williamsii*	*Sambucus williamsii*	species	atccggttttccgaaaacaaacaaaggttcagaaagcgaaaataaaaaag	50	shrub/deciduous tree
M01334:3	OTU_1423	66	1.00	1.00	100	Dipsacales	Caprifoliaceae	Valerianoideae			*Valeriana*		*Valeriana*	genus	atccggttttgttttccgaaaacaaacaaaggctaaaatcaaaaaag	48	forb
M01334:3	OTU_1164	206	1.00	0.37	100	Equisetales	Equisetaceae				*Equisetum*		*Equisetum*	genus	attctacctag	11	forb
M01334:3	OTU_1685	39	0.98	1.00	100	Ericales	Ericaceae	Arbutoideae			*Arctostaphylos*	*Arctostaphylos uva-ursi*	*Arctostaphylos uva-ursi*	species	atcttttttttttcgaaaataaagaaagattcagaaagcgaaaaaaaag	50	shrub/deciduous tree
M01334:3	OTU_0573	729	0.98	1.00	100	Ericales	Ericaceae	Arbutoideae			*Arctous*	*Arctous alpina*	*Arctous alpina*	species	atcttttttttttcgaaaataaagaaagattcagaaagcgaaaaaaaag	49	shrub/deciduous tree
M01334:3	OTU_0280	7648	1.00	1.00	100	Ericales	Ericaceae	Arbutoideae			*Arctous*		*Arctous alpina/rubra*	genus	atctttttttttcgaaaataaagaaagattcagaaagcgaaaaaaaag	48	shrub/deciduous tree
M01334:3	OTU_1652	41	1.00	1.00	100	Ericales	Ericaceae	Cassiopoideae			*Cassiope*	*Cassiope tetragona*	*Cassiope tetragona*	species	atcttttttttttttttgctttcaaaatctttgtttgtttgcgaaaaaaag	54	shrub/deciduous tree
M01334:3	OTU_1031	1008	1.00	1.00	100	Ericales	Ericaceae	Ericoideae	Empetreae		*Empetrum*	*Empetrum nigrum*	*Empetrum nigrum*	species	atcctttatttggcaaacaaacaaagattccgaaagctaaaaaag	46	shrub/deciduous tree
M01334:3	OTU_1336	90	0.96	1.00	100	Ericales	Ericaceae	Ericoideae	Ericeae		*Erica*		*Erica*	genus	atcctttttttcgcaaacaaacaaagattccgaaagctaaaaaaag	46	forb
M01334:3	OTU_0983	4104	0.98	1.00	100	Ericales	Ericaceae	Pyroloideae			*Pyrola*	*Pyrola grandiflora*	*Pyrola grandiflora*	species	atcctttttttttttgaaatttttgtttgttttcgaaaaaaagg	43	forb
M01334:3	OTU_1060	609	1.00	1.00	100	Ericales	Ericaceae	Pyroloideae			*Pyrola*		*Pyrola*	genus	atcctttttttttgaaatttttgtttgttttcgaaaaaaagg	42	forb
M01334:3	OTU_0304	7476	1.00	1.00	100	Ericales	Ericaceae	Vaccinioideae	Vaccinieae		*Vaccinium*	*Vaccinium uliginosum*	*Vaccinium uliginosum*	species	atccttctttttggcaaacaaataaagattccgaaagaaataagg	45	shrub/deciduous tree
M01334:3	OTU_1092	411	1.00	1.00	100	Ericales	Ericaceae	Vaccinioideae	Vaccinieae		*Vaccinium*	*Vaccinium vitis-idaea*	*Vaccinium vitis-idaea*	species	atcctttttttttttggcaaacaaataaagattccgaaagaaaaaaaaaagg	50	shrub/deciduous tree
M01334:3	OTU_2360	30	1.00	1.00	100	Ericales	Polemoniaceae	Polemonioideae	Phlocideae		*Phlox*	*Phlox hoodii*	*Phlox hoodii*	species	atccttttttttttttttttcgaaaacaaacaaagattcggaaagtaaaaataagaatcaaaaggaag	68	forb
M01334:3	OTU_1179	222	1.00	1.00	100	Ericales	Polemoniaceae	Polemonioideae	Polemonieae		*Polemonium*	*Polemonium boreale*	*Polemonium boreale*	species	atccttattttcgaaaacaaacaaagattcggaaagcaaaaataagaaaaaaaaaaaggggg	61	forb
M01334:3	OTU_0990	2645	1.00	1.00	100	Ericales	Polemoniaceae	Polemonioideae	Polemonieae		*Polemonium*		*Polemonium*	genus	atccttattttcgaaaacaaacaaagattcggaaagcaaaaataagaaaaaaaaaaggggg	60	forb
M01334:3	OTU_1181	184	1.00	1.00	100	Ericales	Primulaceae	Primuloideae			*Androsace*	*Androsace lehmanniana*	*Androsace lehmanniana*	species	atcctctttttagaaaacaaagattaaaggaaaataaaataaaaaaggggcg	52	forb
M01334:3	OTU_1709	36	1.00	1.00	100	Ericales	Primulaceae	Primuloideae			*Primula*	*Primula frigida*	*Primula frigida*	species	atcctcttttcgaaaacaaaggttaaaggaaaataaaaaaaggggg	47	forb
M01334:3	OTU_0125	3866	1.00	1.00	100	Fabales	Fabaceae	Faboideae	Galegeae		*Astragalus*		*Astragalus*	genus	atcctgctttccgaaaacaaaaaaataaaagttcagaaagttaaaattaaacaaaaaag	59	forb
M01334:3	OTU_1344	85	1.00	1.00	100	Fabales	Fabaceae	Faboideae	Galegeae		*Oxytropis*	*Oxytropis deflexa*	*Oxytropis deflexa*	species	atcctgctttccgaaaacaaaaaaataaaagttcagaaagttaaaattaaacaaataaag	60	forb
M01334:3	OTU_0278	17903	1.00	1.00	100	Fabales	Fabaceae	Faboideae	Galegeae		*Oxytropis*		*Oxytropis*	genus	atcctgctttccgaaaacaaaaaaataaaagttcagaaagttaaaatgaaacaaataaag	60	forb
M01334:3	OTU_0023	1916954	1.00	1.00	100	Fagales	Betulaceae	Betuloideae			*Betula*		*Betula*	genus	atcctgttttccgaaaacaaataaaacaaatttaagggttcataaagtgagaataaaaaag	61	shrub/deciduous tree
M01334:3	OTU_1054	606	1.00	1.00	100	Gentianales	Rubiaceae	Rubioideae	Rubieae		*Galium*		*Galium*	genus	atcctattttccgaaaacaaagtgaaaagggg	32	forb
M01334:3	OTU_0155	502	1.00	0.90	100	Hypnales	Amblystegiaceae				*Cratoneuron*	*Cratoneuron filicinum*	*Cratoneuron filicinum*	species	atcttatttcctttttttgaggataa	27	moss
M01334:3	OTU_0101	1533	1.00	0.83	100	Hypnales	Amblystegiaceae				*Drepanocladus*		*Drepanocladus*	genus	atcttatttcctttggtttgaggataa	27	moss
M01334:3	OTU_1710	37	1.00	0.70	100	Hypnales	Amblystegiaceae				*Sanionia*	*Sanionia uncinata*	*Sanionia uncinata*	species	atcttatttcgttcgagaataa	22	moss
M01334:3	OTU_1117	342	1.00	0.73	100	Hypnales	Hylocomiaceae				*Pleurozium*	*Pleurozium schreberi*	*Pleurozium schreberi*	species	atcttatttcgtttggaaataa	22	moss

M01334:3	OTU_0039	8598	1.00	0.87	100	Hypnales							Hypnales1	order	atcttatttccttttgtttgaggataa	27	moss
M01334:3	OTU_0315	441	1.00	0.73	100	Hypnales							Hypnales2	order	atcttatttcgtttgaagataa	22	moss
M01334:3	OTU_1375	80	1.00	0.73	100	Hypnales							Hypnales3	order	atcttatttcgtttgaaaataa	22	moss
M01334:3	OTU_1381	78	1.00	1.00	100	Lamiales	Orobanchaceae		Pedicularideae		*Pedicularis*	*Pedicularis capitata*	*Pedicularis capitata*	species	atcctcttttttttcaaaacaaaggttcagaaaacgaaaaaag	44	forb
M01334:3	OTU_0069	3890	1.00	1.00	100	Lamiales	Orobanchaceae		Pedicularideae		*Pedicularis*	*Pedicularis sudetica*	*Pedicularis sudetica*	species	atcttcttttttttttcaaaacaaaggtttcgaaaacgaaaaaag	45	forb
M01334:3	OTU_1872	30	1.00	1.00	100	Lamiales	Orobanchaceae		Pedicularideae		*Pedicularis*	*Pedicularis verticillata*	*Pedicularis verticillata*	species	atcttcttttttttttcaaaacaaaggttcagaaaacgaaaaaaag	45	forb
M01334:3	OTU_0049	4332	0.98	1.00	100	Lamiales	Orobanchaceae		Pedicularideae		*Pedicularis*		*Pedicularis*	genus	atcttcttttttttttttcaaaacaaaggtttcgaaaacgaaaaaag	46	forb
M01334:3	OTU_0433	32505	1.00	1.00	100	Lamiales	Plantaginaceae		Plantagineae		*Plantago* subg. *Psyllium*		*Plantago* (sect. *Lamprosantha*)	genus	atcctgtcttctcaaaataaaggttcataaaacgaaaaggg	41	forb
M01334:3	OTU_1256	126	1.00	1.00	100	Lamiales	Plantaginaceae		Veroniceae		*Lagotis*		*Lagotis*	genus	atcccgtcttctaaaaacaaaggttcagaaagcgaaagg	39	forb
M01334:3	OTU_1286	103	1.00	1.00	100	Lamiales	Plantaginaceae		Veroniceae		*Veronica*	*Veronica wormskjoldii*	*Veronica wormskjoldii*	species	atcctgtcttctaaaaacaaaggttcagaaagtgaaaaaaaag	43	forb
M01334:3	OTU_2359	31	1.00	1.00	100	Lamiales	Plantaginaceae		Veroniceae		*Veronica*		*Veronica*	genus	atcctgtcttctaaaaacaaagattcagaaagttaaaaaaaggg	44	forb
M01334:3	OTU_1161	256	1.00	1.00	100	Liliales	Liliaceae	Lilioideae			*Gagea*	*Gagea serotina*	*Gagea serotina*	species	atcttttttttgagaaaaaggtttaattttaattaaagtatagtttttttaatatataaaactacaataaaaaaaaaag	77	forb
M01334:3	OTU_1569	48	1.00	0.65	100	Lycopodiales	Lycopodiaceae	Lycopodioideae					Lycopodioideae	subfamily	atcctgtttagcaaatggcgg	21	forb
M01334:3	OTU_0004	2631745	1.00	1.00	100	Malpighiales	Salicaceae		Saliceae				Saliceae	tribe	atcctattttcgaaaacaaacaaaggttcataaagacagaataagaatacaaaag	56	shrub/deciduous tree
M01334:3	OTU_1549	50	1.00	1.00	100	Malpighiales	Violaceae				*Viola*	*Viola epipsila* var. *repens*	*Viola epipsila* var. *repens*	species	atcctattttttttaaatgaaaaaaagtttatatagacagaataaataaaaaag	53	forb
M01334:3	OTU_0933	431012	1.00	1.00	100	Myrtales	Onagraceae	Onagroideae	Epilobieae		*Chamaenerion*	*Chamaenerion angustifolium*	*Chamaenerion angustifolium*	species	atcctattttacgaaaaccaacaccgggggggttagaaagcgataaaaaaaaaag	54	forb
M01334:3	OTU_1063	527	1.00	1.00	100	Myrtales	Onagraceae	Onagroideae	Epilobieae		*Chamaenerion*	*Chamaenerion latifolium*	*Chamaenerion latifolium*	species	atcctattttacgaaaaccaacaccgggggggttagaaagcgagaaaaaagaag	54	forb
M01334:3	OTU_0135	391	1.00	0.98	100	Pinales	Pinaceae				*Pinus*		*Pinus*	genus	atccggttcatgaagacaatgtttcttctcctaagataggaaggg	45	coniferous tree
M01334:3	OTU_1456	62	0.98	1.00	100	Pinales	Pinaceae				*Pinus* subg. *Pinus*		*Pinus* (subsect. *Contortae*)	genus	atccggttcatgaagacaatgtttcttctcctaatataggaaggg	45	coniferous tree
M01334:3	OTU_0169	56200	1.00	1.00	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex*		*Carex*	genus	atcttctttttgagaaaaagaaatatataaaatatttcttatttcagataagaaataatatttttttcttatctaatattaaa	82	graminoid
M01334:3	OTU_0936	129019	1.00	1.00	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex* (subg. *Carex*)	*Carex aquatilis*	*Carex aquatilis*	species	atcttctttttgagaaaaagaaatatataaaatatttcttatttcagataagaaataatattttttcttaatctaatattaaa	83	graminoid
M01334:3	OTU_1177	199	1.00	1.00	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex* (subg. *Carex*)	*Carex microchaeta*	*Carex microchaeta*	species	atcttctttttgagaaaaagaaatatataaaatatttcttatttaagataagaaataatatttttttcttatctaatattaaa	82	graminoid
M01334:3	OTU_0934	133666	1.00	1.00	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex* (subg. *Carex*)		*Carex* (subg. *Carex*)	genus	atcttctttttgagaaaaagaaatatatatataaaatatttcttatttcagataagaaataatatttttttcttatctaatattaaa	86	graminoid
M01334:3	OTU_0937	127804	1.00	1.00	100	Poales	Cyperaceae	Cyperoideae	Cariceae		Carex (subg. *Euthyceras*)		Carex (subg. *Euthyceras*)	genus	atcttctttttgagaaaaagaaatatataaaatatttcttatttcagataagaaatattttatttcttatctaatattaaa	81	graminoid
M01334:3	OTU_0316	419	1.00	1.00	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex* (subg. *Vignea*)	*Carex lachenalii*	*Carex lachenalii*	species	atcttcttttttagaaaaagaaatatagaaaatatttcttatttcagataagaaagaataatattttttatctaatattaaa	82	graminoid
M01334:3	OTU_0958	10781	1.00	1.00	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex* (subg. *Vignea*)	*Carex maritima*	*Carex maritima*	species	atcttctttttgagaaaaagaaatatataaaatatttcttatttcagataagaaagaaataatatttcttatctaatattaaa	83	graminoid
M01334:3	OTU_0109	204701	1.00	1.00	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex* (subg. *Vignea*)		*Carex* (subg. *Vignea*)	genus	atcttctttttgagaaaaagaaatatataaaatatttcttatttcagataagaaagaataatatttcttatctaatattaaa	82	graminoid
M01334:3	OTU_0026	646430	1.00	1.00	100	Poales	Cyperaceae	Cyperoideae	Scirpeae		*Eriophorum*		*Eriophorum* sp.	genus	atctttcattttgagaaaatgagagatataaaatatctcttatttattataagaaataaaatatttcttatctaatattaaa	82	graminoid
M01334:3	OTU_0027	139081	1.00	1.00	100	Poales	Cyperaceae	Cyperoideae	Scirpeae		*Eriophorum*		*Eriophorum* sp. (scheuchzeri/russeolum)	genus	atctttcattttgaaaaaatgagagatataaaatatctcttatttattataagaaataaaatatttcttatctaatattaaa	82	graminoid
M01334:3	OTU_0259	725	1.00	1.00	100	Poales	Juncaceae				*Juncus*	*Juncus alpinoarticulatus*	*Juncus alpinoarticulatus*	species	atctttattttgagataattggtttttatataaaaacgaaaatcaaaaaa	50	graminoid
M01334:3	OTU_0035	12148	1.00	1.00	100	Poales	Juncaceae				*Juncus*	*Juncus biglumis*	*Juncus biglumis*	species	atctttattttgagataattgatttttatataaaaactaaaatcaaaaaa	50	graminoid
M01334:3	OTU_0103	1870	1.00	0.98	100	Poales	Juncaceae				*Juncus*		*Juncus*	genus	atctttattttgagaaaattggtttttatagaaaaactcaaatcaaaaaa	50	graminoid
M01334:3	OTU_0954	14748	1.00	1.00	100	Poales	Juncaceae				*Luzula*		*Luzula*	genus	atcttaatctggagaaaatgtgtttctctataaaaactagaatcaaaaag	50	graminoid
M01334:3	OTU_0015	396306	1.00	1.00	100	Poales	Poaceae	Pooideae	Bromeae		*Bromus*	*Bromus pumpellianus*	*Bromus pumpellianus*	species	atccgtgttttgagaaaaaaaaaggggggttctcgaactagaatacaaaggaaaag	57	graminoid
M01334:3	OTU_1623	108	1.00	1.00	100	Poales	Poaceae	Pooideae	Bromeae		*Bromus*		*Bromus*	genus	atccgtgttttgagaaaaaaaaagggggttctcgaactagaatacaaaggaaaag	55	graminoid
M01334:3	OTU_0120	4530	1.00	1.00	100	Poales	Poaceae	Pooideae	Hordeeae	Hordeinae	*Hordeum*		*Hordeum*	genus	atccgtgttttgagaagggattctcgaactagaatacaaaggaaaag	47	graminoid
M01334:3	OTU_0045	6917	1.00	1.00	100	Poales	Poaceae	Pooideae	Meliceae		*Pleuropogon*	*Pleuropogon sabinei*	*Pleuropogon sabinei*	species	atccgtgttttgagaaaacaagtggttctcgagctagaatccaaaggaatag	52	graminoid
M01334:3	OTU_0551	76203	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae	Agrostidinae			Agrostidinae1	subtribe	atccgtgttttgagaaaacaaaggggttctcgaatcgaactagaatacaaaggaaaag	58	graminoid
M01334:3	OTU_1317	99	0.98	1.00	100	Poales	Poaceae	Pooideae	Poeae	Agrostidinae			Agrostidinae2	subtribe	atccgtgttttgagaaaacaagggggttctcgaatcgaactagaatacaaaggaaaag	58	graminoid
M01334:3	OTU_1005	1686	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae	Aveninae	*Koeleria*	*Koeleria asiatica*	*Koeleria asiatica*	species	atccgtgttttgagaaaaccaggaggttctctaactagaatacaaaggaaaag	53	graminoid
M01334:3	OTU_0007	936448	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae	Coleanthinae	*Puccinellia*		*Puccinellia*	genus	atccgtgttttgagaaaacctgggggttctcgaactagaatacaaaggaaaag	53	graminoid
M01334:3	OTU_0017	272796	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae	incertae sedis	*Arctophila/Dupontia*		*Arctophila fulva/Dupontia fisheri*	genus	atccgtgttttgagaaaacaagggggttctcgaactagaatacaaaagaaaag	53	graminoid
M01334:3	OTU_0998	1831	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae	Loliinae	*Festuca*	*Festuca altaica*	*Festuca altaica*	species	atctgtgttttgagaaaactaggaggttctcgaactagaatacaaaggaaaag	53	graminoid
M01334:3	OTU_0038	4407	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae	Loliinae	*Festuca*	*Festuca kolymensis*	*Festuca kolymensis*	species	atccgcgttttgagaaaacaaggaggttctcgaactagaatacaaaggaaaag	53	graminoid
M01334:3	OTU_0014	409263	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae				Poeae1	tribe	atccgtgttttgagaaaacaagggggttctcgaactagaatacaaaggaaaag	53	graminoid
M01334:3	OTU_0018	199959	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae				Poeae2	tribe	atccatgttttgagaaaacaagggggttctcgaactagaatgcaaaggaaaag	53	graminoid
M01334:3	OTU_0033	14713	1.00	1.00	100	Poales	Poaceae	Pooideae	Poeae				Poeae3	tribe	atccgtgttttgagaaaaccaggaggttctcgaactagaatacaaaggaaaag	53	graminoid
M01334:3	OTU_0282	804	0.98	1.00	100	Poales	Poaceae	Pooideae	Poeae				Poeae4	tribe	atccgtgttttgagaaaacagggggttctcgaactagaatacaaaggaaaag	53	graminoid
M01334:3	OTU_0260	555	0.98	1.00	100	Poales	Poaceae	Pooideae	Poeae				Poeae5	tribe	atccgtgttttaagaaaacaagggggttctcgaactagaatacaaaggaaaag	53	graminoid
M01334:3	OTU_0587	295	0.98	1.00	100	Poales	Poaceae	Pooideae	Poeae				Poeae6	tribe	atccatgttttgagaaaacaagggggttctcaaactagaatgcaaaggaaaag	53	graminoid
M01334:3	OTU_0059	17831	1.00	1.00	100	Poales	Poaceae	Pooideae	Triticeae				Triticeae1	tribe	atccgtgttttgagaaaacaaggggttctcgaactagaatacaaaggaaaag	52	graminoid
M01334:3	OTU_0320	97	0.98	1.00	100	Poales	Poaceae	Pooideae	Triticeae				Triticeae2	tribe	atccatgttttgagaaaacaaggggttctcgaactagaatacaaaggaaaag	52	graminoid
M01334:3	OTU_1220	153	1.00	0.57	100	Polytrichales	Polytrichaceae						Polytrichaceae	family	atcttattctaaaatga	17	moss
M01334:3	OTU_1189	184	1.00	0.67	100	Pottiales	Pottiaceae				*Didymodon*	*Didymodon icmadophilus*	*Didymodon icmadophilus*	species	attttatattaaaaaaaacaa	21	moss
M01334:3	OTU_0994	2644	1.00	1.00	100	Ranunculales	Papaveraceae	Papaveroideae	Papavereae		*Papaver*		*Papaver*	genus	atcctgttttcagaaaacaaattaagggatttctgaaagcgagaataaaaaaag	54	forb
M01334:3	OTU_0055	7704	1.00	1.00	100	Ranunculales	Ranunculaceae	Ranunculoideae	Anemoneae		*Anemonastrum*	*Anemonastrum narcissiflora*	*Anemonastrum narcissiflorum*	species	atcctttttttgttttcagaaaaaacaaacaaaacaaaagggcttcagaaagcaaaaataaacataaag	68	forb
M01334:3	OTU_0076	695878	1.00	1.00	100	Ranunculales	Ranunculaceae	Ranunculoideae	Anemoneae		*Anemone*	*Anemone patens*	*Anemone patens*	species	atccttatttcagaaaacaaaagagggttcagaaagcaagaataaaaaag	51	forb
M01334:3	OTU_1812	30	1.00	1.00	100	Ranunculales	Ranunculaceae	Ranunculoideae	Anemoneae		*Anemone*	*Anemone richardsonii*	*Anemone richardsonii*	species	atcctttttttgttttcagaaaacaaacaaaagggctttagaaagcaagacaaaag	55	forb
M01334:3	OTU_1006	1629	1.00	1.00	100	Ranunculales	Ranunculaceae	Ranunculoideae	Anemoneae		*Anemone*		*Anemone*	genus	atccttatttcagaaaacaaaagggggttcagaaagcaagaataaaaaag	51	forb
M01334:3	OTU_0025	114615	1.00	1.00	100	Ranunculales	Ranunculaceae	Ranunculoideae	Caltheae		*Caltha*	*Caltha palustris*	*Caltha palustris*	species	atccggttttcagaaaacaaaaaaagggattcagaaagcaagaatcaaaaag	52	forb
M01334:3	OTU_0956	13563	1.00	1.00	100	Ranunculales	Ranunculaceae	Ranunculoideae	Ranunculeae		*Ranunculus*	*Ranunculus nivalis*	*Ranunculus nivalis*	species	atcctgctttcagaaaaaaaaaaaaaagggattcagaaagcgaaaatagg	49	forb
M01334:3	OTU_0066	1536	1.00	1.00	100	Ranunculales	Ranunculaceae	Ranunculoideae	Ranunculeae		*Ranunculus*	*Ranunculus pedatifidus* var. *affinis*	*Ranunculus pedatifidus* var. *affinis*	species	atcctgctttcagaaaacaaaaaaaagggggggattcagaaagcgaaaatagg	52	forb
M01334:3	OTU_0969	9259	1.00	1.00	100	Ranunculales	Ranunculaceae	Ranunculoideae	Ranunculeae		*Ranunculus*	*Ranunculus pygmaeus*	*Ranunculus pygmaeus*	species	atcctgctttcagaaaaaaaaaaaaagggattcagaaagcgaaaatagg	48	forb
M01334:3	OTU_0287	292	0.98	1.00	100	Ranunculales	Ranunculaceae	Ranunculoideae	Ranunculeae		*Ranunculus*		*Ranunculus*	genus	atcctgctttcagaaaacaaaaggggggttcagaaagaaaaaaagg	47	forb
M01334:3	OTU_1224	146	1.00	1.00	100	Rosales	Rosaceae	Amygdaloideae	Spiraeeae		*Spiraea*	*Spiraea stevenii*	*Spiraea stevenii*	species	atcctgttttatgaaaacaagcaagggtttcataaactcataaaccgagaataaaagag	59	shrub/deciduous tree
M01334:3	OTU_1016	1216	1.00	1.00	100	Rosales	Rosaceae	Dryadoideae	Dryadeae		*Dryas*		*Dryas*	genus	atcctgttttatgaaaacaaacaagggtttcagaaagcgcgaataaaaagg	51	shrub/deciduous tree
M01334:3	OTU_0951	18362	1.00	0.98	100	Rosales	Rosaceae	Rosoideae	Agrimonieae	Sanguisorbinae	*Sanguisorba*	*Sanguisorba officinalis*	*Sanguisorba officinalis*	species	atcccgttttatgaaaacaaacaaggatttcacaaagcgagaataaataaag	52	forb
M01334:3	OTU_0021	91811	1.00	1.00	100	Rosales	Rosaceae	Rosoideae	Colurieae		*Geum*		*Geum*	genus	atcccgttttatgaaagaaacaagggtttcagaacgcgagaataaataaag	52	forb
M01334:3	OTU_1319	95	0.98	1.00	100	Rosales	Rosaceae	Rosoideae	Potentilleae	Fragariinae	*Alchemilla*		*Alchemilla*	genus	atcccgttttatgaaaacaaacaagggtttaagaaagcgagaataaataagg	52	forb
M01334:3	OTU_0431	79793	1.00	1.00	100	Rosales	Rosaceae	Rosoideae	Potentilleae	Fragariinae	*Comarum*	*Comarum palustre*	*Comarum palustre*	species	atcccgttttatgaaaacaaacaagggtttcagaaagcgagaataaataagg	52	forb
M01334:3	OTU_0193	95560	1.00	1.00	100	Rosales	Rosaceae	Rosoideae	Potentilleae	Fragariinae	*Sibbaldia*	*Sibbaldia procumbens*	*Sibbaldia procumbens*	species	atcccgttttatgaaaacaaacaaaggtttcagaaagcgagaataaataagg	52	forb
M01334:3	OTU_0037	72319	1.00	1.00	100	Rosales	Rosaceae	Rosoideae	Potentilleae	Potentillinae	*Potentilla*		*Potentilla*	genus	atcccgttttatgaaaacaaacaagggtttcataaagcgagaataaataaag	52	forb
M01334:3	OTU_0999	1929	1.00	0.98	100	Rosales	Rosaceae	Rosoideae					Rosoideae1	subfamily	atcccgttttatgaaaacaaacaagggtttcagaaagcgagaataaataaag	52	forb
M01334:3	OTU_0185	535	1.00	0.96	100	Rosales	Rosaceae	Rosoideae					Rosoideae2	subfamily	atcccgttttatgaaaacaagcaagggtttcagaaagcgagaataaataaag	52	forb
M01334:3	OTU_1349	83	0.98	1.00	100	Rosales	Rosaceae	Rosoideae					Rosoideae3	subfamily	atcccgttttatgaaaacaaacaaaggtttcagaaagcgagaataaataaag	52	forb
M01334:3	OTU_0978	4753	1.00	1.00	100	Saxifragales	Crassulaceae	Sempervivoideae	Umbiliceae		*Rhodiola*	*Rhodiola integrifolia*	*Rhodiola integrifolia*	species	atcttcttttccgaaaccaaacccaaataaaggggaataaaaaag	45	forb
M01334:3	OTU_0062	1773	1.00	1.00	100	Saxifragales	Crassulaceae	Sempervivoideae	Umbiliceae		*Rhodiola*	*Rhodiola rosea*	*Rhodiola rosea*	species	atcttcttttccgaaaccaaacccaaatacaggggttcaaaaagcgcgaataaaaaag	58	forb
M01334:3	OTU_1124	615	1.00	0.83	100	Saxifragales	Saxifragaceae				*Micranthes*	*Micranthes nelsoniana*	*Micranthes nelsoniana*	species	atcctgttttacgaaaaaaaaaaaag	26	forb
M01334:3	OTU_0204	4644	1.00	0.76	100	Saxifragales	Saxifragaceae				*Micranthes*		*Micranthes*	genus	atcctgttttatgaaaaaaaaag	23	forb
M01334:3	OTU_0032	13163	1.00	1.00	100	Saxifragales	Saxifragaceae				*Saxifraga* subg. *Saxifraga*		*Saxifraga* (sect. *Mesogyne*)	genus	atcctgttttaagaaagcaacacacaagggttcagaaaaagcgaaaaaagaatag	55	forb

Table S8. *Trn* L sequence average read counts and relative read abundance per sample

Any OTU with <0.01% relative read abundance is shown as a +

% represents the Relative Read Abundance

				Wolly mammoth										**Horse**				**Bison**		**Caribou**						**Controls**			
				Abyland		**Adycha**		**Cape Blossom**		**Maly Lyakhovsky**		**Yukagir**		**Yukon**		**Oyogas Yar**		**Yakutian**		**Selwyn C**		**Selwyn B**		**Selwyn A**		**Positive**		**Negative**	
id	**OTU**	**Total read**	**maxid**	**Average read**	**%**	**Average read**	**%**	**Average read co**	**%**	**Average read**	**%**	**Average read**	**%**	**Average read**	**%**	**Average read**	**%**	**Average read c**	**%**	**Average read**	**%**	**Average read**	**%**	**Average read c**	**%**	**Reads**	**%**	**Reads**	**%**
M01334:3	OTU_0004	2944014	Saliceae	13647.00	2.92	0	0	0	0	11895.67	2.95	289952.33	67.35	0	0	26959.00	14.69	142549.00	42.62	25495.00	20.26	300691.67	84.09	52521.67	8.22	0	0	0	0
M01334:3	OTU_0007	990799	*Puccinellia*	0	0	303822.67	76.85	0	0	8326.67	2.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0014	429801	Poeae1	10572.00	2.26	0	0	7589.33	1.61	100642.33	24.99	0	0	9397.67	20.13	8219.67	4.48	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0015	413839	*Bromus pumpellianus*	52898.00	11.32	64252.33	16.25	8507.00	1.80	5100.33	1.26	0	0	1332.33	2.85	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0017	287769	*Arctophila fulva/Dupontia fisheri*	0	0	0	0	0	0	86452.67	21.47	0	0	0	0	4479.33	2.44	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0018	213559	Poeae2	4075.00	0.87	0	0	0	0	62578.00	15.54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0019	961592	Anthemideae1	46685.33	9.99	14510.00	3.67	150691.67	31.93	0	0	41067.00	9.54	6134.00	13.14	0	0	0	0	38892.00	30.91	0	0	0	0	0	0	0	0
M01334:3	OTU_0020	250817	*Myosotis alpestris*	3108.67	0.67	0	0	2837.00	0.60	0	0	71945.67	16.71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0021	96354	*Geum*	2459.67	0.53	0	0	0	0	416.00	0.10	699.33	0.16	664.00	1.42	0	0	0	0	0	0	22494.67	6.29	3870.00	0.61	0	0	0	0
M01334:3	OTU_0023	1990846	*Betula*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8757.33	6.96	30434.67	8.51	569348.33	89.13	0	0	0	0
M01334:3	OTU_0025	130531	*Caltha palustris*	2083.33	0.45	0	0	8217.33	1.74	9562.00	2.37	8747.67	2.03	0	0	0	0	9594.67	2.87	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0026	659212	*Eriophorum* sp.	0	0	0	0	0	0	46976.00	11.67	0	0	0	0	121121.67	66.01	47379.00	14.16	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0027	141514	*Eriophorum* sp. (*scheuchzeri/russeo*	0	0	0	0	0	0	45134.67	11.21	0	0	0	0	1225.67	0.67	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0030	26033	*Saussurea*	4156.67	0.89	3916.67	0.99	0	0	183.67	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0032	14392	*Saxifraga* (sect. *Mesogyne*)	220.00	0.05	0	0	0	0	4115.00	1.02	0	0	0	0	0	0	0	0	0	0	0	0	52.67	+	0	0	0	0
M01334:3	OTU_0033	15822	Poeae3	172.33	0.04	0	0	0	0	0	0	513.33	0.12	4218.67	9.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0035	12641	*Juncus biglumis*	0	0	71.33	0.02	0	0	3978.00	0.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0037	75815	*Potentilla*	11178.67	2.39	0	0	1087.33	0.23	298.67	0.07	7393.33	1.72	4134.00	8.85	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0038	5136	*Festuca kolymensis*	36.33	+	0	0	69.67	0.01	0	0	1363.00	0.32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0039	9405	Hypnales1	0	0	48.67	0.01	0	0	2754.33	0.68	63.00	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0045	7228	*Pleuropogon sabinei*	0	0	0	0	0	0	2239.00	0.56	32.33	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0049	4662	*Pedicularis*	158.33	0.03	0	0	0	0	1166.33	0.29	57.33	0.01	0	0	0	0	0	0	0	0	40.67	0.01	0	0	0	0	0	0
M01334:3	OTU_0053	11288	*Eritrichium*	468.00	0.10	144.33	0.04	138.67	0.03	0	0	2648.33	0.62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0055	7954	*Anemonastrum narcissiflorum*	83.67	0.02	0	0	0	0	0	0	47.33	0.01	43.33	0.09	0	0	0	0	500.67	0.40	1352.00	0.38	541.00	0.08	0	0	0	0
M01334:3	OTU_0059	18828	Triticeae1	2197.00	0.47	0	0	1318.33	0.28	157.00	0.04	0	0	2271.33	4.87	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0061	2235	*Arabidopsis lyrata*	0	0	0	0	0	0	675.67	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0062	2015	*Rhodiola rosea*	0	0	0	0	0	0	414.33	0.10	158.00	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0066	1681	*Ranunculus pedatifidus* var. *affinis*	0	0	0	0	0	0	332.33	0.08	164.00	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0069	4105	*Pedicularis sudetica*	57.67	0.01	0	0	0	0	305.67	0.08	94.67	0.02	43.00	0.09	0	0	0	0	0	0	478.33	0.13	310.67	0.04	0	0	0	0
M01334:3	OTU_0076	724601	*Anemone patens*	231959.33	49.63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0084	700	Gnaphalieae	0	0	183.33	0.05	0	0	28.00	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0087	27609	Anthemideae2	3131.00	0.67	641.33	0.16	3038.33	0.64	0	0	863.33	0.20	210.00	0.45	0	0	0	0	842.67	0.67	0	0	0	0	0	0	0	0
M01334:3	OTU_0092	752	*Eutrema edwardsii*	0	0	0	0	0	0	224.67	0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0101	1628	*Drepanocladus*	0	0	0	0	0	0	485.67	0.12	10.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0103	1973	*Juncus*	33.00	+	0	0	0	0	0	0	0	0	42.67	0.09	0	0	0	0	0	0	547.67	0.15	0	0	0	0	0	0
M01334:3	OTU_0109	207829	*Carex* (subg. *Vignea*)	41352.33	8.85	3512.00	0.89	23369.33	4.95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0120	4704	*Hordeum*	1158.33	0.25	0	0	0	0	351.67	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0121	4135	*Stellaria*	32.33	+	0	0	0	0	1268.67	0.32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0125	4403	*Astragalus*	30.33	+	0	0	51.67	0.01	0	0	1126.33	0.26	0	0	0	0	0	0	0	0	58.00	0.02	22.33	+	0	0	0	0
M01334:3	OTU_0135	547	*Pinus*	0	0	3.33	+	0	0	30.33	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0155	534	*Cratoneuron filicinum*	0	0	0	0	0	0	163.67	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0169	59694	*Carex*	6209.67	1.33	0	0	11410.67	2.41	448.67	0.11	385.67	0.09	0	0	0	0	0	0	0	0	275.67	0.08	0	0	0	0	0	0
M01334:3	OTU_0178	5492	*Stuckenia*	1615.00	0.35	0	0	0	0	0	0	166.33	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0179	5091	Anthemideae3	17.33	+	1409.00	0.36	104.00	0.02	0	0	60.33	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0185	574	Rosoideae1	14.67	+	0	0	0	0	0	0	0	0	7.67	0.02	0	0	0	0	0	0	82.33	0.02	69.33	0.01	0	0	0	0
M01334:3	OTU_0193	96865	*Sibbaldia procumbens*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31853.33	25.31	0	0	0	0	0	0	0	0
M01334:3	OTU_0204	4973	*Micranthes*	0	0	0	0	0	0	506.33	0.13	170.33	0.04	0	0	0	0	0	0	858.00	0.68	0	0	13.33	+	0	0	0	0
M01334:3	OTU_0224	257	*Smelowskia*	0	0	0	0	0	0	0	0	72.67	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0228	29862	Euclidieae	0	0	0	0	0	0	0	0	0	0	9866.00	21.13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0231	2732	Artemisiinae	202.67	0.04	0	0	386.00	0.08	11.33	+	43.00	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0232	1873	*Cerastium arvense*	372.33	0.08	0	0	0	0	0	0	201.00	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0256	3131	*Bistorta vivipara*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	740.33	0.58	111.00	0.03	141.67	0.02	0	0	0	0
M01334:3	OTU_0259	747	*Juncus alpinoarticulatus*	15.00	+	0	0	0	0	0	0	0	0	11.33	0.02	0	0	0	0	0	0	215.33	0.06	0	0	0	0	0	0
M01334:3	OTU_0260	595	Poeae4	12.67	+	0	0	0	0	149.00	0.04	0	0	8.00	0.02	6.33	+	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0278	18155	*Oxytropis*	165.00	0.04	0	0	0	0	0	0	364.33	0.08	5438.33	11.65	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0280	7908	*Arctous alpina/rubra*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	228.67	0.06	2320.67	0.36	0	0	0	0
M01334:3	OTU_0282	843	Poeae5	16.00	+	0	0	37.00	+	174.33	0.04	0	0	13.33	0.03	10.00	+	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0287	312	*Ranunculus*	0	0	0	0	0	0	0	0	90.33	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0304	7933	*Vaccinium uliginosum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	99.00	0.03	2393.00	0.37	0	0	0	0
M01334:3	OTU_0305	8464	*Braya*	0	0	0	0	0	0	0	0	0	0	2791.67	5.98	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0310	714	*Stellaria borealis*	17.33	+	0	0	0	0	197.33	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0315	462	Hypnales2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	61.00	0.02	70.00	0.01	0	0	0	0
M01334:3	OTU_0316	429	*Carex lachenalii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	124.33	0.03	0	0	0	0	0	0
M01334:3	OTU_0320	104	Triticeae2	12.00	+	0	0	0	0	0	0	0	0	11.33	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0338	2764	*Artemisia gmelinii*	152.00	0.03	0	0	400.33	0.08	0	0	109.67	0.03	21.67	0.05	0	0	0	0	99.33	0.08	0	0	0	0	0	0	0	0
M01334:3	OTU_0374	2326	*Peucedanum*	0	0	759.33	0.19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0375	2265	*Tephroseris*	628.00	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0384	226	*Stellaria longifolia*	0	0	0	0	0	0	68.67	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_0431	85261	*Comarum palustre*	467.00	0.10	0	0	678.33	0.14	505.33	0.13	0	0	0	0	0	0	24561.00	7.34	0	0	0	0	386.00	0.06	0	0	0	0
M01334:3	OTU_0433	35173	*Plantago* (sect. *Lamprosantha*)	8958.67	1.92	0	0	1338.67	0.28	0	0	537.67	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

M01334:3:	OTU_0441	730	Asteraceae1	19.33	+	0	0	128.33	0.03	0	0	0	0	0	0	0	0	0	0	5.67	+	4.33	+	10.67	+	0	0	0	0
M01334:3:	OTU_0549	124653	Oenantheae1	2345.33	0.50	0	0	779.33	0.17	1702.33	0.42	601.00	0.14	0	0	0	0	33363.67	9.97	0	0	0	0	433.67	0.07	0	0	0	0
M01334:3:	OTU_0551	78091	Agrostidinae1	1108.67	0.24	0	0	9227.33	1.95	1247.33	0.31	0	0	0	0	10321.67	5.63	3496.00	1.05	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0552	21192	*Arnica*	1082.33	0.23	0	0	5487.33	1.16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0554	18062	Selineae	341.00	0.07	166.00	0.04	5051.67	1.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	98.33	0.02	0	0	0	0
M01334:3:	OTU_0573	755	*Arctous alpina*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.33	+	226.33	0.03	0	0	0	0
M01334:3:	OTU_0574	542	Asteraceae2	26.00	+	0	0	93.67	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0579	601	*Eremogone capillaris*	59.67	+	0	0	0	0	0	0	119.00	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0581	366	*Rumex*	0	0	0	0	0	0	107.67	0.03	6.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0587	315	Poeae6	0	0	0	0	0	0	95.67	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0630	76	*Dicranum bonjeanii*	0	0	0	0	0	0	23.33	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0727	116	*Bromus*	7.33	+	15.33	+	7.00	+	2.33	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0933	447614	*Chamaenerion angustifolium*	4757.67	1.02	1565.00	0.40	134262.67	28.45	0	0	0	0	0	0	0	0	0	0	3085.33	2.45	0	0	0	0	0	0	0	0
M01334:3:	OTU_0934	138234	*Carex* (subg. *Carex*)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44555.33	13.32	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0936	133044	*Carex aquatilis*	0	0	0	0	27167.33	5.76	0	0	0	0	0	0	11107.00	6.05	4732.00	1.41	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0937	130842	Carex (subg. *Euthyceras*)	5.00	+	0	0	42592.67	9.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0939	56769	Oenantheae2	637.33	0.14	0	0	809.33	0.17	747.33	0.19	0	0	0	0	0	0	14854.33	4.44	0	0	0	0	314.67	0.05	0	0	0	0
M01334:3:	OTU_0942	40548	*Mertensia paniculata*	168.67	0.04	0	0	9967.33	2.11	0	0	0	0	0	0	0	0	0	0	2106.00	1.67	0	0	296.00	0.05	0	0	0	0
M01334:3:	OTU_0945	37100	*Menyanthes trifoliata*	480.00	0.10	0	0	700.33	0.15	374.33	0.09	162.33	0.04	0	0	0	0	9279.67	2.77	0	0	0	0	191.33	0.03	0	0	0	0
M01334:3:	OTU_0950	21745	Apioideae	0	0	0	0	3700.33	0.78	0	0	142.67	0.03	0	0	0	0	0	0	3020.33	2.40	0	0	0	0	0	0	0	0
M01334:3:	OTU_0951	19572	*Sanguisorba officinalis*	833.33	0.18	0	0	5283.67	1.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0954	15398	*Luzula*	314.00	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	67.67	0.02	0	0	0	0	0	0
M01334:3:	OTU_0956	13688	*Ranunculus nivalis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4521.00	3.59	0	0	0	0	0	0	0	0
M01334:3:	OTU_0958	10897	*Carex maritima*	0	0	37.67	0.01	3556.00	0.75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0969	9341	*Ranunculus pygmaeus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3086.33	2.45	0	0	0	0	0	0	0	0
M01334:3:	OTU_0972	5944	Brassicaceae	1582.67	0.34	308.33	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0978	5055	*Rhodiola integrifolia*	27.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1454.33	0.23	0	0	0	0
M01334:3:	OTU_0983	4174	*Pyrola grandiflora*	0	0	0	0	0	0	0	0	0	0	26.67	0.06	0	0	0	0	0	0	97.00	0.03	1241.67	0.19	0	0	0	0
M01334:3:	OTU_0984	3328	Asteroideae NAC	125.33	0.03	0	0	925.33	0.20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26.00	+	0	0	0	0
M01334:3:	OTU_0986	3399	*Blitum nuttallianum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1091.00	0.87	0	0	0	0	0	0	0	0
M01334:3:	OTU_0990	2703	*Polemonium*	48.33	0.01	0	0	833.33	0.18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0994	2756	*Papaver*	761.00	0.16	0	0	0	0	0	0	109.33	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_0998	2075	*Festuca altaica*	15.33	+	0	0	0	0	80.00	0.02	0	0	0	0	0	0	0	0	0	0	0	0	503.33	0.08	0	0	0	0
M01334:3:	OTU_0999	2026	Rosoideae2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.00	+	576.67	0.46	0	0	53.33	+	0	0	0	0
M01334:3:	OTU_1003	1833	Dicranaceae1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18.33	+	33.00	+	0	0	0	0
M01334:3:	OTU_1005	1753	*Koeleria asiatica*	531.33	0.11	0	0	15.00	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1006	1733	*Anemone*	360.67	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	53.33	0.01	110.33	0.02	0	0	0	0
M01334:3:	OTU_1016	1271	*Dryas*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	382.67	0.06	0	0	0	0
M01334:3:	OTU_1031	1046	*Empetrum nigrum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	313.67	0.05	0	0	0	0
M01334:3:	OTU_1037	934	Anthemideae4	128.33	0.03	0	0	87.00	0.02	0	0	26.67	+	0	0	0	0	0	0	19.33	0.02	0	0	0	0	0	0	0	0
M01334:3:	OTU_1050	962	*Silene samojedorum*	305.33	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1054	628	*Galium*	191.00	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1060	617	*Pyrola*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.67	+	194.33	0.03	0	0	0	0
M01334:3:	OTU_1063	554	*Chamaenerion latifolium*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	156.67	0.02	0	0	0	0
M01334:3:	OTU_1069	544	*Cerastium maximum*	165.33	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1080	547	*Tripleurospermum maritimum*	171.33	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1092	422	*Vaccinium vitis-idaea*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.67	+	135.33	0.02	0	0	0	0
M01334:3:	OTU_1117	352	*Pleurozium schreberi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38.33	0.01	71.33	0.01	0	0	0	0
M01334:3:	OTU_1124	618	*Micranthes nelsoniana*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	203.00	0.16	0	0	0	0	0	0	0	0
M01334:3:	OTU_1152	258	*Sambucus williamsii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	51.33	0.02	0	0	0	0	11.00	+	0	0	0	0
M01334:3:	OTU_1161	260	*Gagea serotina*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39.00	0.01	46.33	+	0	0	0	0
M01334:3:	OTU_1164	227	*Equisetum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	54.33	0.02	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1177	204	*Carex microchaeta*	0	0	0	0	10.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	47.33	+	0	0	0	0
M01334:3:	OTU_1179	229	*Polemonium boreale*	0	0	0	0	69.00	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1181	199	*Androsace lehmanniana*	0	0	0	0	0	0	0	0	57.67	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1189	189	*Didymodon icmadophilus*	46.00	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1206	172	*Pohlia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17.33	0.01	7.00	+	0	0	0	0	0	0
M01334:3:	OTU_1220	158	Polytrichaceae	0	0	0	0	0	0	0	0	0	0	0	0	1.67	+	0	0	0	0	2.00	+	10.00	+	0	0	0	0
M01334:3:	OTU_1224	155	*Spiraea stevenii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	46.67	+	0	0	0	0
M01334:3:	OTU_1241	143	*Cerastium*	0	0	0	0	0	0	0	0	33.00	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1256	131	*Lagotis*	0	0	0	0	0	0	0	0	38.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1286	114	*Veronica wormskjoldii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32.33	+	0	0	0	0
M01334:3:	OTU_1317	100	Agrostidinae2	0	0	0	0	0	0	0	0	0	0	0	0	10.67	+	5.00	+	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1319	100	*Alchemilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27.00	+	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1323	97	Thelypodieae	29.00	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1333	92	*Eremogone*	0	0	0	0	0	0	0	0	21.33	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1336	91	*Erica*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29.67	+	0	0	0	0
M01334:3:	OTU_1344	89	*Oxytropis deflexa*	0	0	0	0	0	0	0	0	23.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1345	89	*Parrya nudicaulis*	0	0	0	0	0	0	0	0	22.00	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1349	88	Rosoideae3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26.33	0.02	0	0	0	0	0	0	0	0
M01334:3:	OTU_1352	86	*Oxyria digyna*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18.67	0.01	0	0	10.00	+	0	0	0	0
M01334:3:	OTU_1356	85	*Cymopterus sessiliflorus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15.67	0.01	0	0	0	0	0	0	0	0
M01334:3:	OTU_1375	80	Hypnales3	0	0	0	0	0	0	0	0	0	0	0	0	25.67	0.01	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3:	OTU_1381	78	*Pedicularis capitata*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25.00	+	0	0	0	0
M01334:3:	OTU_1423	69	*Valeriana*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20.67	+	0	0	0	0
M01334:3:	OTU_1456	63	*Pinus* (subsect. *Contortae*)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19.00	+	0	0	0	0
M01334:3:	OTU_1461	62	*Dianthus*	19.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

M01334:3	OTU_1518	53	apioid superclade	0	0	0	0	10.67	+	0	0	0	0	0	0	0	0	0	0	0.00	+	0	0	0	0	0	0	0	0
M01334:3	OTU_1549	50	*Viola epipsila* var. *repens*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15.33	+	0	0	0	0
M01334:3	OTU_1569	48	Lycopodioideae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16.00	+	0	0	0	0
M01334:3	OTU_1652	41	*Cassiope tetragona*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.67	+	0	0	0	0
M01334:3	OTU_1669	40	Bryaceae	0	0	0	0	0	0	0	0	11.33	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_1685	39	*Arctostaphylos uva-ursi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.67	+	0	0	0	0
M01334:3	OTU_1709	37	*Primula frigida*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.33	+	0	0	0	0
M01334:3	OTU_1710	37	*Sanionia uncinata*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.00	+	0	0	0	0
M01334:3	OTU_1812	32	*Anemone richardsonii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.00	+	0	0	0	0
M01334:3	OTU_1872	30	*Pedicularis verticillata*	10.00	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_2052	24	*Bryum*	0	0	0	0	0	0	7.00	+	0	0	0	0	0	0	0	0	0	0	0	0	1.00	+	0	0	0	0
M01334:3	OTU_2119	23	Anthemideae5	4.67	+	0	0	2.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_2189	21	Anthemideae6	0	0	0	0	2.00	+	0	0	2.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_2293	19	*Campanula*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.00	+	0	0	0	0
M01334:3	OTU_2359	31	*Veronica*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.00	+	4.33	+	0	0	0	0
M01334:3	OTU_2360	30	*Phlox hoodii*	0	0	0	0	0	0	0	0	7.66	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_2444	17	*Silene*	0	0	0	0	0	0	0	0	4.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_2528	16	Asteraceae3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_2622	15	Asteraceae4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M01334:3	OTU_3451	10	Dicranaceae2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.67	+	2.33	+	0	0	0	0

Table S9. Idenitity and abundance of OTU's nrITS1

OTU	Total read c	best_id_I	cover	order	family	subfamily	tribe	subtribe	genus/subgenus	species	maxid	scientific ra	OTU sequence (truncated)	length of	type
Otu042	7678	100	89	Alismatales	Juncaginaceae				*Triglochin*	*Triglochin palustris*	*Triglochin palustris*	species	AAGTCGTAACAAGGTTTCCGTA	387	forb
Otu008	142153	100	100	Apiales	Apiaceae	Apioideae	Oenantheae		*Cicuta*	*Cicuta virosa*	*Cicuta virosa*	species	AAGTCGTAACAAGGTTTCCGTA	345	forb
Otu020	52109	100	100	Asparagales	Orchidaceae	Coelogyne				*Coelogyne fimbriata*	*Coelogyne fimbriata*	species	AAGTCGTAACAAGGTTTCCGTA	370	positive control
Otu085	1540	100	100	Asterales	Asteraceae	Asteroideae	Anthemideae	Artemisiinae	*Artemisia*	*Artemisia norvegica* subsp. *saxatilis*	*Artemisia norvegica subsp. saxatilis*	species	AAGTCGTAACAAGGTTTCCGTA	391	forb
Otu114	929	100	100	Asterales	Asteraceae	Asteroideae	Anthemideae	Artemisiinae	*Artemisia*	*Artemisia scoparia*	*Artemisia scoparia*	species	AAGTCGTAACAAGGTTTCCGTA	390	forb
Otu084	2127	98.98	100	Asterales	Asteraceae	Asteroideae	Senecioneae	Tussilagininae	*Endocellion*	*Endocellion sibiricum*	*Endocellion sibiricum*	species	AAGTCGTAACAAGGTTTCCGTA	392	forb
Otu090	1243	99.19	93	Asterales	Menyanthaceae				*Menyanthes*	*Menyanthes trifoliata*	*Menyanthes trifoliata*	species	AAGTCGTAACAAGGTTTCCGTA	396	forb
Otu049	9753	99.73	98	Boraginales	Boraginaceae	Boraginoideae	Eritrichieae		*Eritrichium*	*Eritrichium sericeum*	*Eritrichium sericeum*	species	AAGTCGTAACAAGGTTTCCGTA	373	forb
Otu017	120776	99.18	100	Boraginales	Boraginaceae	Boraginoideae	Eritrichieae		*Myosotis*	*Myosotis alpestris*	*Myosotis alpestris*	species	AAGTCGTAACAAGGTTTCCGTA	366	forb
Otu131	408	99.49	100	Brassicales	Brassicaceae		Sisymbrieae		*Sisymbrium*	*Sisymbrium linifolium*	*Sisymbrium linifolium*	species	AAGTCGTAACAAGGTTTCCGTA	387	forb
Otu053	10501	100	86	Brassicales	Brassicaceae		Smelowskieae		*Smelowskia*	*Smelowskia alba*	*Smelowskia alba*	species	AAGTCGTAACAAGGTTTCCGTA	385	forb
Otu110	1122	95.58	88	Brassicales	Brassicaceae		Smelowskieae		*Smelowskia*		*Smelowskia*	genus	AAGTCGTAACAAGGTTTCCGTA	385	forb
Otu105	780	97.34	99	Caryophyllales	Caryophyllaceae		Alsineae		*Cerastium*	*Cerastium arvense*	*Cerastium arvense*	species	AAGTCGTAACAAGGTTTCCGTA	381	forb
Otu044	5978	98.68	100	Caryophyllales	Caryophyllaceae		Alsineae		*Stellaria*		*Stellaria*	genus	AAGTCGTAACAAGGTTTCCGTA	380	forb
Otu255	28	99.40	100	Caryophyllales	Polygonaceae	Polygonoideae	Persicarieae	Koenigiinae	*Bistorta*	*Bistorta vivipara*	*Bistorta vivipara*	species	AAGTCGTAACAAGGTTTCCGTA	331	forb
Otu045	7176	100	89	Dicranales	Dicranaceae				*Dicranum*	*Dicranum fuscescens*	*Dicranum fuscescens*	species	AAGTCGTAACAAGGTTTCCGTA	419	moss
Otu058	4475	100	100	Dicranales	Dicranaceae				*Dicranum*	*Dicranum scoparium*	*Dicranum scoparium*	species	AAGTCGTAACAAGGTTTCCGTA	400	moss
Otu207	77	100	89	Dicranales	Dicranaceae				*Dicranum*		*Dicranum*	genus	AAGTCGTAACAAGGTTTCCGTA	417	moss
Otu273	27	100	88	Ericales	Ericaceae	Arbutoideae			*Arctous*	*Arctous rubra*	*Arctous rubra*	species	AAGTCGTAACAAGGTTTCCGTA	372	shrub/deciduous tree
Otu261	25	100	85	Ericales	Ericaceae	Cassiopoideae			*Cassiope*	*Cassiope tetragona*	*Cassiope tetragona*	species	AAGTCGTAACAAGGTTTCCGTA	384	shrub/deciduous tree
Otu149	400	99.50	100	Ericales	Ericaceae	Ericoideae	Empetreae		*Empetrum*	*Empetrum nigrum*	*Empetrum nigrum*	species	AAGTCGTAACAAGGTTTCCGTA	396	shrub/deciduous tree
Otu219	80	99.74	100	Ericales	Ericaceae	Pyroloideae			*Pyrola*	*Pyrola asarifolia*	*Pyrola asarifolia*	species	AAGTCGTAACAAGGTTTCCGTA	380	shrub/deciduous tree
Otu098	1824	98.47	100	Ericales	Ericaceae	Vaccinioideae	Vaccinieae		*Vaccinium*	*Vaccinium uliginosum*	*Vaccinium uliginosum*	species	AAGTCGTAACAAGGTTTCCGTA	392	shrub/deciduous tree
Otu061	6695	99.74	100	Ericales	Ericaceae	Vaccinioideae	Vaccinieae		*Vaccinium*	*Vaccinium vitis-idaea*	*Vaccinium vitis-idaea*	species	AAGTCGTAACAAGGTTTCCGTA	388	shrub/deciduous tree
Otu125	709	99.73	100	Fabales	Fabaceae	Faboideae	Galegeae		*Oxytropis*	*Oxytropis splendens*	*Oxytropis splendens*	species	AAGTCGTAACAAGGTTTCCGTA	363	forb
Otu282	15	98.63	100	Fabales	Fabaceae	Faboideae			*Astragalus*		*Astragalus*	genus	AAGTCGTAACAAGGTTTCCGTA	364	forb
Otu005	383914	99.72	100	Fagales	Betulaceae	Betuloideae			*Betula*		*Betula*	genus	AAGTCGTAACAAGGTTTCCGTA	352	shrub/deciduous tree
Otu043	6469	95.19	100	Funariales	Funariaceae				*Funaria*		*Funaria*	genus	AAGTCGTAACAAGGTTTCCGTA	398	moss
Otu303	13	98.44	86	Grimmiales	Grimmiaceae				*Bucklandiella*		*Bucklandiella*	genus	AAGTCGTAACAAGGTTTCCGTA	295	moss
Otu157	362	99.75	100	Hypnales	Amblystegiaceae				*Drepanocladus*	*Drepanocladus sordidus*	*Drepanocladus sordidus*	species	AAGTCGTAACAAGGTTTCCGTA	398	moss
Otu246	20	99.73	96	Hypnales	Amblystegiaceae				*Sanionia*	*Sanionia uncinata*	*Sanionia uncinata*	species	AAGTCGTAACAAGGTTTCCGTA	389	moss
Otu215	47	98.19	97	Hypnales	Hylocomiaceae				*Hylocomium*	*Hylocomium splendens*	*Hylocomium splendens*	species	AAGTCGTAACAAGGTTTCCGTA	399	moss
Otu106	992	100	94	Hypnales	Hylocomiaceae				*Pleurozium*	*Pleurozium schreberi*	*Pleurozium schreberi*	species	AAGTCGTAACAAGGTTTCCGTA	386	moss
Otu002	518851	100	100	Malpighiales	Salicaceae		Saliceae		*Salix*		*Salix*	genus	AAGTCGTAACAAGGTTTCCGTA	357	shrub/deciduous tree
Otu096	1764	98.67	100	Myrtales	Onagraceae	Onagroideae	Epilobieae		*Epilobium*	*Epilobium palustre*	*Epilobium palustre*	species	AAGTCGTAACAAGGTTTCCGTA	377	forb
Otu233	18	99.70	89	Myrtales	Onagraceae	Onagroideae	Epilobieae		*Chamaenerion*	*Chamaenerion angustifolium*	*Chamaenerion angustifolium*	species	AAGTCGTAACAAGGTTTCCGTA	379	forb
Otu161	150	100	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex* subg. *Carex*	*Carex aquatilis*	*Carex aquatilis*	species	AAGTCGTAACAAGGTTTCCGTA	357	graminoid
Otu038	13727	100	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex* subg. *Carex*	*Carex nigra* subsp. *juncea*	*Carex nigra subsp. juncea*	species	AAGTCGTAACAAGGTTTCCGTA	355	graminoid
Otu315	15	99.44	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex* subg. *Carex*	*Carex podocarpa*	*Carex podocarpa*	species	AAGTCGTAACAAGGTTTCCGTA	356	graminoid
Otu123	4400	100	99	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex* subg. *Carex*	*Carex rostrata*	*Carex rostrata*	species	AAGTCGTAACAAGGTTTCCGTA	358	graminoid
Otu254	615	99.44	99	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex* subg. *Carex*	*Carex vesicaria*	*Carex vesicaria*	species	AAGTCGTAACAAGGTTTCCGTA	357	graminoid
Otu080	1702	99.16	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex* subg. *Vignea*	*Carex chordorrhiza*	*Carex chordorrhiza*	species	AAGTCGTAACAAGGTTTCCGTA	357	graminoid
Otu077	9807	100	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex* subg. *Vignea*	*Carex duriuscula*	*Carex duriuscula*	species	AAGTCGTAACAAGGTTTCCGTA	357	graminoid
Otu034	49655	98.93	100	Poales	Cyperaceae	Cyperoideae	Scirpeae		*Eriophorum*	*Eriophorum angustifolium*	*Eriophorum angustifolium*	species	AAGTCGTAACAAGGTTTCCGTA	375	graminoid
Otu087	1611	96.60	99	Poales	Cyperaceae	Cyperoideae	Scirpeae		*Eriophorum*		*Eriophorum*	genus	AAGTCGTAACAAGGTTTCCGTA	384	graminoid
Otu026	16788	99.12	100	Poales	Juncaceae				*Juncus*	*Juncus effusus*	*Juncus effusus*	species	AAGTCGTAACAAGGTTTCCGTA	338	graminoid
Otu072	2072	98.51	100	Poales	Juncaceae				*Juncus*	*Juncus oxymeris*	*Juncus oxymeris*	species	AAGTCGTAACAAGGTTTCCGTA	336	graminoid
Otu209	48	100	100	Poales	Poaceae	Pooideae	Poeae	Agrostidinae	*Arctagrostis*	*Arctagrostis latifolia*	*Arctagrostis latifolia*	species	AAGTCGTAACAAGGTTTCCGTA	354	graminoid
Otu183	116	99.72	100	Poales	Poaceae	Pooideae	Poeae	Agrostidinae	*Calamagrostis*	*Calamagrostis stricta*	*Calamagrostis stricta*	species	AAGTCGTAACAAGGTTTCCGTA	353	graminoid
Otu016	66916	100	100	Poales	Poaceae	Pooideae	Poeae	Alopecurinae	*Alopecurus*	*Alopecurus magellanicus*	*Alopecurus magellanicus*	species	AAGTCGTAACAAGGTTTCCGTA	354	graminoid
Otu015	98214	99.72	100	Poales	Poaceae	Pooideae	Poeae	Aristaveninae	*Deschampsia*	*Deschampsia cespitosa*	*Deschampsia cespitosa*	species	AAGTCGTAACAAGGTTTCCGTA	353	graminoid
Otu004	330714	98.87	100	Poales	Poaceae	Pooideae	Poeae	Coleanthinae	*Puccinellia*	*Puccinellia vahliana*	*Puccinellia vahliana*	species	AAGTCGTAACAAGGTTTCCGTA	353	graminoid
Otu056	215237	100	100	Poales	Poaceae	Pooideae	Poeae	Coleanthinae	*Puccinellia*		*Puccinellia*	genus	AAGTCGTAACAAGGTTTCCGTA	349	graminoid
Otu156	23623	99.43	100	Poales	Poaceae	Pooideae	Poeae	incertae sedis	*Dupontia*	*Dupontia fisheri*	*Dupontia fisheri*	species	AAGTCATAACAAGGTTTCCGTA	353	graminoid
Otu221	101	98.31	100	Poales	Poaceae	Pooideae	Poeae	Loliinae	*Festuca*	*Festuca altaica*	*Festuca altaica*	species	AAGTCGTAACAAGGTTTCCGTA	355	graminoid
Otu144	414	99.72	100	Poales	Poaceae	Pooideae	Poeae	Loliinae	*Festuca*	*Festuca ovina*	*Festuca ovina*	species	AAGTCGTAACAAGGTTTCCGTA	353	graminoid
Otu097	1917	100	100	Poales	Poaceae	Pooideae	Poeae	Poinae	*Poa*	*Poa glauca*	*Poa glauca*	species	AAGTCGTAACAAGGTTTCCGTA	353	graminoid
Otu122	471	96.05	100	Poales	Poaceae	Pooideae	Poeae	Poinae	*Poa*		*Poa*	genus	AAGTCGTAACAAGGTTTCCGTA	353	graminoid
Otu343	12	97.69	96	Polytrichales	Polytrichaceae				*Polytrichum*	*Polytrichum piliferum*	*Polytrichum piliferum*	species	GGACTTCTGCCGGGAGGATCCC	136	moss
Otu165	132	98.79	91	Pottiales	Pottiaceae				*Barbula*	*Barbula unguiculata*	*Barbula unguiculata*	species	AAGTCGTAACAAGGTTTCCGTA	365	moss
Otu060	4367	96.19	92	Pottiales	Pottiaceae				*Didymodon*	*Didymodon icmadophilus*	*Didymodon icmadophilus*	species	AAGTCGTAACAAGGTTTCCGTA	426	moss
Otu172	158	84.53	95	Pottiales	Pottiaceae						Pottiaceae	family	AAGTCGTAACAAGGTTTCCGTA	380	moss
Otu024	29336	99.66	100	Ranunculales	Ranunculaceae	Ranunculoideae	Anemoneae		*Anemonastrum*	*Anemonastrum narcissiflora*	*Anemonastrum narcissiflora*	species	AAGTCGTAACAAGGTTTCCGTA	295	forb
Otu063	5717	98.79	100	Ranunculales	Ranunculaceae	Ranunculoideae	Anemoneae		*Anemone*	*Anemone patens*	*Anemone patens*	species	AAGTCGTAACAAGGTTTCCGTA	331	forb
Otu286	57	98.68	100	Ranunculales	Ranunculaceae	Ranunculoideae	Anemoneae		*Anemone*		*Anemone*	genus	AAGTCGTAACAAGGTTTCCGTA	380	forb
Otu068	6587	99.71	92	Ranunculales	Ranunculaceae	Ranunculoideae	Caltheae		*Caltha*	*Caltha palustris*	*Caltha palustris*	species	AAGTCGTAACAAGGTTTCCGTA	378	forb
Otu100	949	99.73	99	Ranunculales	Ranunculaceae	Ranunculoideae	Ranunculeae		*Ranunculus*	*Ranunculus trichophyllus*	*Ranunculus trichophyllus*	species	AAGTCGTAACAAGGTTTCCGTA	375	forb
Otu247	28	99.42	100	Rhizogoniales	Aulacomniaceae				*Aulacomnium*	*Aulacomnium palustre*	*Aulacomnium palustre*	species	AAGTCGTAACAAGGTTTCCGTA	343	moss
Otu147	776	99.69	87	Rosales	Rosaceae	Dryadoideae	Dryadeae		*Dryas*	*Dryas octopetala*	*Dryas octopetala*	species	AAGTCGTAACAAGGTTTCCGTA	364	forb
Otu047	5607	97.55	100	Rosales	Rosaceae	Rosoideae	Colurieae		*Geum*	*Geum aleppicum*	*Geum aleppicum*	species	AAGTCGTAACAAGGTTTCCGTA	365	forb
Otu066	7675	99.48	100	Rosales	Rosaceae	Rosoideae	Potentilleae	Fragariinae	*Comarum*	*Comarum palustre*	*Comarum palustre*	species	AAGTCGTAACAAGGTTTCCGTA	384	forb

Otu267	16	97.42	100	Rosales	Rosaceae	Rosoideae	Potentilleae	Potentillinae	*Potentilla*		*Potentilla*	genus	AAGTCGTAACAAGGTTTCCGTA(	388	forb
Otu272	12	100	100	Saxifragales	Crassulaceae	Sempervivoideae	Umbiliceae		*Rhodiola*	*Rhodiola integrifolia*	*Rhodiola integrifolia*	species	AAGTCGTAACAAGGTTTCCGTA(	362	forb
Otu206	48	98.47	100	Takakiales	Takakiaceae						Takakiaceae	family	AAGTCGTAACAAGGTTTCCGTA(	392	moss

Table S10. nrITS1 sequence average read counts and relative read abundance per sample

Any OTU with <0.01% relative read abundance is shown as a +

% represents the Relative Read Abundance

No nrITS results were obtained for Cape Blossom mammoth, Yukon horse and Selwyn caribou C

			Wolly mammoth								Horse		Bison		Caribou				Controls			
			Abyland		Adycha		Maly Lyakhovsky		Yukagir		Oyogas Yar		Yakutian		Selwyn B		Selwyn A		Positive		Negative	
OTU	**Total read**	**maxid**	**Average re**	**%**	**Average rea**	**%**	**Average re**	**%**	**Average re**	**%**	**Average re**	**%**	**Average re**	**%**	**Average read**	**%**	**Average read c**	**%**	**Reads**	**%**	**Reads**	**%**
Otu002	518851	*Salix*	0	0	7.00	+	3.00	+	9002.00	15.44	0	0	15967.00	18.56	129319.00	66.89	18650.67	16.50	0	0	0	0
Otu004	330714	*Puccinellia vahliana*	0	0	109800.00	61.00	438.00	0.58	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu005	383914	*Betula*	0	0	0	0	0	0	0	0	0	0	0	0	36572.67	18.92	91398.67	80.92	0	0	0	0
Otu008	142153	*Cicuta virosa*	0	0	0	0	0	0	0	0	175.67	25.5	47208.67	54.87	0	0	0	0	0	0	0	0
Otu015	98214	*Deschampsia cespitosa*	0	0	443.00	0.25	32082.67	42.23	212.33	0.36	0	0	0	0	0	0	0	0	0	0	0	0
Otu016	66916	*Alopecurus magellanicus*	178.33	2.89	312.67	0.17	21814.33	28.71	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu017	120776	*Myosotis alpestris*	0	0	0	0	0	0	40258.67	69.03	0	0	0	0	0	0	0	0	0	0	0	0
Otu020	52109	*Coelogyne fimbriata*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	52109	100	0	0
Otu024	29336	*Anemonastrum narcissiflora*	0	0	0	0	0	0	0	0	0	0	0	0	9778.67	5.06	0	0	0	0	0	0
Otu026	16788	*Juncus effusus*	0	0	0	0	0	0	0	0	0	0	0	0	5596.00	2.89	0	0	0	0	0	0
Otu034	49655	*Eriophorum angustifolium*	0	0	0	0	2514.00	3.31	0	0	99.00	14.35	13938.67	16.20	0	0	0	0	0	0	0	0
Otu038	13727	*Carex nigra subsp. juncea*	452.00	7.32	0	0	3220.67	4.24	154.67	0.27	0	0	62.33	0.07	686.00	0.35	0	0	0	0	0	0
Otu042	7678	*Triglochin palustris*	0	0	0	0	0	0	0	0	0	0	0	0	2545.33	1.32	0	0	0	0	0	0
Otu043	6469	*Funaria*	0	0	9.00	0.01	2147.33	2.83	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu044	5978	*Stellaria*	0	0	7.33	+	1985.33	2.61	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu045	7176	*Dicranum fuscescens*	0	0	0	0	0	0	0	0	0	0	0	0	1030.33	0.53	1361.67	1.21	0	0	0	0
Otu047	5607	*Geum aleppicum*	0	0	0	0	0	0	0	0	0	0	0	0	1861.67	0.96	7.33	+	0	0	0	0
Otu049	9753	*Eritrichium sericeum*	0	0	0	0	0	0	3251.00	5.57	0	0	0	0	0	0	0	0	0	0	0	0
Otu053	10501	*Smelowskia alba*	0	0	0	0	0	0	3500.33	6.00	0	0	0	0	0	0	0	0	0	0	0	0
Otu056	215237	*Puccinellia*	0	0	69299.00	38.50	2446.67	3.22	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu058	4475	*Dicranum scoparium*	0	0	6.33	+	0	0	0	0	0	0	0	0	1485.33	0.77	0	0	0	0	0	0
Otu060	4367	*Didymodon icmadophilus*	0	0	0	0	1455.67	1.92	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu061	6695	*Vaccinium vitis-idaea*	0	0	0	0	0	0	0	0	0	0	0	0	2204.00	1.14	27.67	0.02	0	0	0	0
Otu063	5717	*Anemone patens*	1905.67	30.88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu066	7675	*Comarum palustre*	0	0	0	0	0	0	0	0	9.67	1.4	2477.33	2.88	71.33	0.04	0	0	0	0	0	0
Otu068	6587	*Caltha palustris*	0	0	0	0	0	0	0	0	21.33	3.1	2174.33	2.53	0	0	0	0	0	0	0	0
Otu072	2072	*Juncus oxymeris*	0	0	0	0	3.67	+	0	0	0	0	0	0	687.00	0.36	0	0	0	0	0	0
Otu077	9807	*Carex duriuscula*	3269.00	52.98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu080	1702	*Carex chordorrhiza*	0	0	0	0	0	0	0	0	0	0	565.00	0.66	0	0	0	0	0	0	0	0
Otu084	2127	*Endocellion sibiricum*	0	0	0	0	0	0	0	0	3.33	0.5	705.67	0.82	0	0	0	0	0	0	0	0
Otu085	1540	*Artemisia norvegica subsp. saxatilis*	0	0	0	0	0	0	0	0	0	0	0	0	498.33	0.26	10.00	+	0	0	0	0
Otu087	1611	*Eriophorum*	0	0	0	0	537.00	0.71	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu090	1243	*Menyanthes trifoliata*	0	0	0	0	2.00	+	0	0	4.33	0.6	408.00	0.47	0	0	0	0	0	0	0	0
Otu096	1764	*Epilobium palustre*	0	0	0	0	0	0	0	0	5.00	0.7	583.00	0.68	0	0	0	0	0	0	0	0
Otu097	1917	*Poa glauca*	0	0	0	0	0	0	634.00	1.09	0	0	0	0	0	0	5.00	+	0	0	0	0
Otu098	1824	*Vaccinium uliginosum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	608.00	0.54	0	0	0	0
Otu100	949	*Ranunculus trichophyllus*	0	0	0	0	0	0	0	0	0	0	0	0	316.33	0.16	0	0	0	0	0	0
Otu105	780	*Cerastium arvense*	2.00	+	0	0	0	0	258.67	0.44	0	0	0	0	0	0	0	0	0	0	0	0
Otu106	992	*Pleurozium schreberi*	0	0	0	0	0	0	0	0	0	0	0	0	24.00	0.01	305.33	0.27	0	0	0	0
Otu110	1122	*Smelowskia*	0	0	0	0	0	0	372.33	0.64	0	0	0	0	0	0	0	0	0	0	0	0
Otu114	929	*Artemisia scoparia*	13.33	0.22	0	0	0	0	296.33	0.51	0	0	0	0	0	0	0	0	0	0	0	0
Otu122	471	*Poa*	154.67	2.51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu123	4400	*Carex rostrata*	0	0	0	0	0	0	0	0	11.67	1.7	1455.00	1.69	0	0	0	0	0	0	0	0
Otu125	709	*Oxytropis splendens*	0	0	0	0	0	0	233.00	0.40	0	0	0	0	0	0	0	0	0	0	0	0

Otu131	408	*Sisymbrium linifolium*	135.33	2.19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu144	414	*Festuca ovina*	0	0	0	0	0	0	136.00	0.23	0	0	0	0	0	0	0	0	0	0	0	0
Otu147	776	*Dryas octopetala*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	258.67	0.23	0	0	0	0
Otu149	400	*Empetrum nigrum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	133.33	0.12	0	0	0	0
Otu156	23623	*Dupontia fisheri*	0	0	105.33	0.06	7218.00	9.50	0	0	305.00	44.2	246.00	0.29	0	0	0	0	0	0	0	0
Otu157	362	*Drepanocladus sordidus*	0	0	0	0	116.00	0.15	4.00	+	0	0	0	0	0	0	0	0	0	0	0	0
Otu161	150	*Carex aquatilis*	0	0	0	0	0	0	0	0	0	0	50.00	0.06	600.00	0.31	0	0	0	0	0	0
Otu165	132	*Barbula unguiculata*	43.00	0.70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu172	158	Pottiaceae	0	0	0	0	0	0	0	0	0	0	0	0	48.67	0.03	4.00	+	0	0	0	0
Otu183	116	*Calamagrostis stricta*	0	0	0	0	0	0	0	0	38.67	5.6	0	0	0	0	0	0	0	0	0	0
Otu206	48	Takakiaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16.00	0.01	0	0	0	0
Otu207	77	*Dicranum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25.67	0.02	0	0	0	0
Otu209	48	*Arctagrostis latifolia*	0	0	0	0	0	0	0	0	16.00	2.3	0	0	0	0	0	0	0	0	0	0
Otu215	47	*Hylocomium splendens*	0	0	0	0	0	0	0	0	0	0	0	0	13.33	+	2.00	+	0	0	0	0
Otu219	80	*Pyrola asarifolia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26.67	0.02	0	0	0	0
Otu221	101	*Festuca altaica*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33.67	0.03	0	0	0	0
Otu233	18	*Chamaenerion angustifolium*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.00	+	0	0	0	0
Otu246	20	*Sanionia uncinata*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.67	+	0	0	0	0
Otu247	28	*Aulacomnium palustre*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.33	+	0	0	0	0
Otu254	615	*Carex vesicaria*	0	0	0	0	0	0	0	0	0	0	202.00	0.23	0	0	0	0	0	0	0	0
Otu255	28	*Bistorta vivipara*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.33	+	0	0	0	0
Otu261	25	*Cassiope tetragona*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.33	+	0	0	0	0
Otu267	16	*Potentilla*	0	0	0	0	0	0	5.33	+	0	0	0	0	0	0	0	0	0	0	0	0
Otu272	12	*Rhodiola integrifolia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.00	+	0	0	0	0
Otu273	27	*Arctous rubra*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.00	+	0	0	0	0
Otu282	15	*Astragalus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.00	+	0	0	0	0
Otu286	57	*Anemone*	19.00	0.31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu303	13	*Bucklandiella*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.33	+	0	0	0	0
Otu315	15	*Carex podocarpa*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.00	+	0	0	0	0
Otu343	12	*Polytrichum piliferum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.00	+	0	0	0	0

Table S11. Idenitity and abundance of OTU's nrITS2

OTU	Total read	best_id_N(	cover	order	family	subfamily	tribe	subtribe	genus/subgenus	species	maxid	scientific ra	OTU sequence (truncated)	length	type
Otu005	166638	100	100	Apiales	Apiaceae	Apioideae	Oenantheae		*Cicuta*	*Cicuta virosa*	*Cicuta virosa*	species	TCCCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	406	forb
Otu020	109350	100	100	Asparagales	Orchidaceae				*Coelogyne*	*Coelogyne fimbriata*	*Coelogyne fimbriata*	species	TCTCGGCTCTCGCATCGATGAAGAGCGCAGCGAAAT	429	positive control
Otu180	185	99.50	100	Asterales	Asteraceae	Asteroideae	Anthemideae	Artemisiinae	*Artemisia*	*Artemisia norvegica*	*Artemisia norvegica subsp. saxatilis*	species	TCTCGGCTCATGCATCGATGAAGAACGTAGCAAAAT	400	forb
Otu070	7099	99.50	100	Asterales	Asteraceae	Asteroideae	Anthemideae	Artemisiinae	*Artemisia*	*Artemisia scoparia*	*Artemisia scoparia*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	401	forb
Otu026	20691	99.75	100	Asterales	Asteraceae	Asteroideae	Senecioneae	Tussilagininae	*Endocellion*	*Endocellion sibiricum*	*Endocellion sibiricum*	species	TCTCGGCTCACGCATCGATGAAGAACGTAGCAAAAT	400	forb
Otu051	13113	99.01	100	Asterales	Menyanthaceae				*Menyanthes*	*Menyanthes trifoliata*	*Menyanthes trifoliata*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	402	forb
Otu030	21115	100	100	Boraginales	Boraginaceae	Boraginoideae	Eritrichieae		*Eritrichium*	*Eritrichium sericeum*	*Eritrichium sericeum*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	400	forb
Otu008	157471	100	100	Boraginales	Boraginaceae	Boraginoideae	Eritrichieae		*Myosotis*	*Myosotis alpestris*	*Myosotis alpestris*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	403	forb
Otu160	111	99.75	100	Boraginales	Boraginaceae	Boraginoideae			*Mertensia*	*Mertensia paniculata*	*Mertensia paniculata*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	399	forb
Otu251	5	97.27	100	Brassicales	Brassicaceae		Sisymbrieae		*Sisymbrium*	*Sisymbrium linifolium*	*Sisymbrium linifolium*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	366	forb
Otu017	30925	98.35	98	Brassicales	Brassicaceae		Smelowskieae		*Smelowskia*	*Smelowskia alba*	*Smelowskia alba*	species	TCTTGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	369	forb
Otu227	15	99.78	100	Bryales	Aulacomniaceae				*Aulacomnium*	*Aulacomnium palustre*	*Aulacomnium palustre*	species	TCTTGGCTCCTGCAACGATGAAGAACGCAGCGAAAT	459	moss
Otu224	31	98.54	100	Bryales	Bryaceae				*Ptychostomum*	*Ptychostomum pallescens*	*Ptychostomum pallescens*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	477	moss
Otu100	1627	99.78	100	Bryales	Mniaceae				*Pohlia*	*Pohlia nutans*	*Pohlia nutans*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	444	moss
Otu210	78	88.64	100	Bryales	Mniaceae						Mniaceae	family	TCTTGGCTCCCGTATCGATGAAGAACGCAGCGAAAT	384	moss
Otu075	4854	98.25	100	Caryophyllales	Caryophyllaceae		Alsineae		*Stellaria*		*Stellaria*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	401	forb
Otu143	1084	100	100	Dicranales	Dicranaceae				*Dicranum*	*Dicranum fuscescens*	*Dicranum fuscescens*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	506	moss
Otu054	8506	100	99	Dicranales	Ditrichaceae				*Ceratodon*	*Ceratodon purpureus*	*Ceratodon purpureus*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	477	moss
Otu225	39	98.53	100	Ericales	Ericaceae	Arbutoideae			*Arctous*	*Arctous rubra*	*Arctous alpina*	species	TCTCGGCTCTTGCATCGATGAAGAACGTAGCGAAAT	402	shrub/deciduous tree
Otu107	1960	100	100	Ericales	Ericaceae	Ericoideae	Empetreae		*Empetrum*	*Empetrum nigrum*	*Empetrum nigrum*	species	TCTCGGCTCTTGCATCGATGAAGAACGTAGCGAAAT	405	shrub/deciduous tree
Otu173	121	100	100	Ericales	Ericaceae	Pyroloideae			*Pyrola*	*Pyrola asarifolia*	*Pyrola asarifolia*	species	TCTCGGCTCTTGCATCGATGAAGAACGTAGCGAAAT	412	shrub/deciduous tree
Otu071	5734	98.03	100	Ericales	Ericaceae	Vaccinioideae	Vaccinieae		*Vaccinium*	*Vaccinium uliginosum*	*Vaccinium uliginosum*	species	TCTCGGCTCTTGCATCGATGAAGAACGTAGCGAAAT	407	shrub/deciduous tree
Otu234	3953	99.75	100	Ericales	Ericaceae	Vaccinioideae	Vaccinieae		*Vaccinium*	*Vaccinium vitis-idaea*	*Vaccinium vitis-idaea*	species	TCTCGGCTCTTGCATCGATGAAGAACGTAGCGAAAT	407	shrub/deciduous tree
Otu242	48	98.77	100	Ericales	Ericaceae	Vaccinioideae	Vaccinieae		*Vaccinium*		*Vaccinium*	genus	TCTCGGCTCTTGCATCGATGAAGAACGTAGCGAAAT	407	shrub/deciduous tree
Otu090	1993	100	100	Fabales	Fabaceae	Faboideae	Galegeae		*Astragalus*	*Astragalus alpinus*	*Astragalus alpinus*	species	TCTAGGCTCTTGCATCGATGAAGAACGTAGCGAAAT	391	forb
Otu052	5231	100	100	Fabales	Fabaceae	Faboideae	Galegeae		*Oxytropis*	*Oxytropis deflexa*	*Oxytropis deflexa*	species	TCTAGGCTCTTGCATCGATGAAGAACGTAGCGAAAT	392	forb
Otu001	412353	100	100	Fagales	Betulaceae	Betuloideae			*Betula*		*Betula*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	402	shrub/deciduous tree
Otu246	14	96.87	93	Grimmiales	Grimmiaceae				*Niphotrichum*		*Niphotrichum*	genus	TCTTGGCTCCTGCAACGATGAAGAACGCAGCGAAAT	481	moss
Otu121	1340	100	100	Hypnales	Amblystegiaceae				*Drepanocladus*	*Drepanocladus sordidus*	*Drepanocladus sordidus*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	442	moss
Otu244	13	99.06	99	Hypnales	Brachytheciaceae				*Tomentypnum*	*Tomentypnum nitens*	*Tomentypnum nitens*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	427	moss
Otu228	13	100	99	Hypnales	Hylocomiaceae				*Hylocomiastrum*	*Hylocomiastrum pyrenaicum*	*Hylocomiastrum pyrenaicum*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	439	moss
Otu079	3809	100	98	Hypnales	Hylocomiaceae				*Hylocomium*	*Hylocomium splendens*	*Hylocomium splendens*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	424	moss
Otu130	510	100	100	Hypnales	Hylocomiaceae				*Pleurozium*	*Pleurozium schreberi*	*Pleurozium schreberi*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	426	moss
Otu125	1272	97.14	100	Jungermanniales	Anastrophyllaceae				*Barbilophozia*	*Barbilophozia barbata*	*Barbilophozia barbata*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	453	moss
Otu262	16	100	99	Jungermanniales	Scapaniaceae				*Douinia*	*Douinia ovata*	*Douinia ovata*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	439	moss
Otu069	3140	99.76	100	Lamiales	Orobanchaceae		Pedicularideae		*Pedicularis*	*Pedicularis sudetica*	*Pedicularis sudetica*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	408	forb
Otu088	2342	95.40	100	Lamiales	Plantaginaceae		Callitricheae		*Hippuris*		*Hippuris*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	388	forb
Otu122	699	97.95	100	Malpighiales	Salicaceae		Saliceae		*Salix*	*Salix alaxensis*	*Salix alaxensis*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	391	shrub/deciduous tree
Otu002	512554	99.744	100	Malpighiales	Salicaceae		Saliceae		*Salix*		*Salix*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	391	shrub/deciduous tree
Otu178	169	97.51	92	Myrtales	Onagraceae	Onagroideae	Epilobieae		*Chamaenerion*	*Chamaenerion angustifolium*	*Chamaenerion angustifolium*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	393	forb
Otu089	3682	99.74	100	Myrtales	Onagraceae	Onagroideae	Epilobieae		*Epilobium*	*Epilobium palustre*	*Epilobium palustre*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	391	forb
Otu084	3684	99.50	100	Pinales	Taxaceae				*Taxus*	*Taxus canadensis*	*Taxus canadensis*	species	TCTCGGCTCTCGCCACGATGAAGAATGTAGCGAAAT	403	coniferous tree
Otu148	476	99.26	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex subg. Carex*	*Carex aquatilis*	*Carex aquatilis*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	407	graminoid
Otu187	87	99.75	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex subg. Carex*	*Carex rostrata*	*Carex rostrata*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	407	graminoid
Otu261	10	99.26	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex subg. Carex*	*Carex vesicaria*	*Carex vesicaria*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	407	graminoid
Otu146	247	99.50	100	Poales	Cyperaceae	Cyperoideae	Cariceae		*Carex subg. Vignea*	*Carex duriuscula*	*Carex duriuscula*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	402	graminoid
Otu073	6714	99.31	100	Poales	Cyperaceae	Cyperoideae	Scirpeae		*Eriophorum*	*Eriophorum angustifolium*	*Eriophorum angustifolium*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	434	graminoid
Otu068	6830	98.97	100	Poales	Poaceae	Pooideae	Poeae	Agrostidinae	*Arctagrostis*	*Arctagrostis latifolia*	*Arctagrostis latifolia*	species	TCTCGGCTCTCGCATCGATGAAGAATGTAGCGAAAT	390	graminoid
Otu140	1352	99.75	100	Poales	Poaceae	Pooideae	Poeae	Agrostidinae	*Calamagrostis*		*Calamagrostis*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	392	graminoid
Otu050	9804	98.98	100	Poales	Poaceae	Pooideae	Poeae	Alopecurinae	*Alopecurus*	*Alopecurus magellanicus*	*Alopecurus magellanicus*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	393	graminoid
Otu042	24297	99.24	100	Poales	Poaceae	Pooideae	Poeae	Aristaveninae	*Deschampsia*	*Deschampsia cespitosa*	*Deschampsia cespitosa*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	395	graminoid
Otu003	348562	99.24	100	Poales	Poaceae	Pooideae	Poeae	Coleanthinae	*Puccinellia*		*Puccinellia (tenuiflora/vahliana)*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	393	graminoid
Otu062	3332	99.49	100	Poales	Poaceae	Pooideae	Poeae	Coleanthinae	*Puccinellia*		*Puccinellia*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	393	graminoid
Otu040	13996	99.24	100	Poales	Poaceae	Pooideae	Poeae	incertae sedis	*Arctophila*	*Arctophila fulva*	*Arctophila fulva*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	394	graminoid
Otu055	4934	100	100	Poales	Poaceae	Pooideae	Poeae	incertae sedis	*Dupontia*	*Dupontia fisheri*	*Dupontia fisheri*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	394	graminoid
Otu092	2582	100	100	Poales	Poaceae	Pooideae	Poeae	Loliinae	*Festuca*	*Festuca ovina*	*Festuca ovina*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	394	graminoid
Otu074	2755	99.49	100	Poales	Poaceae	Pooideae	Poeae	Poinae	*Poa*	*Poa arctica*	*Poa arctica*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	391	graminoid
Otu010	144687	100	100	Polytrichales	Polytrichaceae				*Polytrichastrum*	*Polytrichastrum alpinum*	*Polytrichastrum alpinum*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	418	moss
Otu104	5535	99.78	94	Polytrichales	Polytrichaceae				*Polytrichum*	*Polytrichum commune*	*Polytrichum commune*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	493	moss
Otu162	429	100	100	Polytrichales	Polytrichaceae				*Polytrichum*	*Polytrichum juniperinum*	*Polytrichum juniperinum*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	442	moss
Otu064	8899	100	93	Polytrichales	Polytrichaceae				*Polytrichum*	*Polytrichum piliferum*	*Polytrichum piliferum*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	473	moss
Otu263	88	100	100	Polytrichales	Polytrichaceae				*Polytrichum*	*Polytrichum strictum*	*Polytrichum strictum*	species	TCTTGGCTCTTGCAACGATGAAGAACGCAGCGAAAT	438	moss
Otu080	7613	99.48	100	Ranunculales	Ranunculaceae	Ranunculoideae	Anemoneae		*Anemonastrum*	*Anemonastrum narcissiflora*	*Anemonastrum narcissiflorum*	species	TCTCGGCTCTTGCATCGATGAAGAACGTAGCGAAAT	385	forb
Otu011	106430	98.73	100	Ranunculales	Ranunculaceae	Ranunculoideae	Anemoneae		*Anemone*	*Anemone patens*	*Anemone patens*	species	TCTCGGCTCTTGCATCGATGAAGAACGTAGCGAAAT	395	forb
Otu024	28383	98.62	92	Ranunculales	Ranunculaceae	Ranunculoideae	Caltheae		*Caltha*	*Caltha palustris*	*Caltha palustris*	species	TCTCGGCTCTTGCATCGATGAAGAACGTAGCGAAAT	396	forb
Otu145	1325	98.63	92	Rosales	Rosaceae	Dryadoideae	Dryadeae		*Dryas*	*Dryas octopetala*	*Dryas octopetala*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	396	forb
Otu057	5448	96.37	100	Rosales	Rosaceae	Rosoideae	Colurieae		*Geum*		*Geum*	genus	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	386	forb
Otu032	34930	99.48	100	Rosales	Rosaceae	Rosoideae	Potentilleae	Fragariinae	*Comarum*	*Comarum palustre*	*Comarum palustre*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	386	forb
Otu103	1183	98.71	100	Rosales	Rosaceae	Rosoideae	Potentilleae	Potentillinae	*Potentilla*	*Potentilla hookeriana*	*Potentilla hookeriana*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	386	forb
Otu182	99	100	96	Rosales	Rosaceae	Rosoideae	Rubeae		*Rubus*	*Rubus arcticus*	*Rubus arcticus*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAT	386	shrub/deciduous tree
Otu192	152	99.24	100	Saxifragales	Crassulaceae	Sempervivoideae	Umbiliceae		*Rhodiola*	*Rhodiola integrifolia*	*Rhodiola integrifolia*	species	TCTCGGCTCTCGCATCGATGAAGAACGTAGCAAAAT	392	forb
Otu037	8066	99.27	100	Saxifragales	Saxifragaceae				*Saxifraga* subg. *Saxifraga*	*Saxifraga sibirica*	*Saxifraga sibirica*	species	TCTCGGCTCTTACATCGATGAAGAACGTAGCAAAAT	413	forb

Table S12. nrITS2 sequence average read counts and relative read abundance per sample

Any OTU with <0.01% relative read abundance is shown as a +

% represents the Relative Read Abundance

No nrITS results were obtained for Cape Blossom mammoth, Yukon horse and Selwyn caribou C

			Wolly mammoth								Horse		Bison		Caribou				Controls			
			Abyland		Adycha		Maly Lyakhovsky		Yukagir		Oyogas Yar		Yakutian		Selwyn B		Selwyn A		Positive		Negative	
OTU	**Total read**	**maxid**	**Average re**	**%**	**Average reac**	**%**	**Average rea**	**%**	**Average re**	**%**	**Average re**	**%**	**Average re**	**%**	**Average re**	**%**	**Average reac**	**%**	**Reads**	**%**	**Reads**	**%**
Otu001	412353	*Betula*	0	0	0	0	0	0	0	0	0	0	2589.33	2.06	11308.33	10.88	123552.33	78.51	0	0	11.00	5.42
Otu002	512554	*Salix*	12237.33	24.65	0	0	0	0	10854.00	12.41	8458.33	15.66	32951.33	26.31	81608.00	78.54	24741.33	15.71	0	0	40.00	19.70
Otu003	348562	*Puccinellia (tenuiflora/vahliana)*	2140.33	4.31	114047.00	98.95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	122.00	60.10
Otu005	166638	*Cicuta virosa*	0	0	0	0	0	0	0	0	0	0	55546.00	44.36	0	0	0	0	0	0	0	0
Otu008	157471	*Myosotis alpestris*	0	0	0	0	0	0	52490.33	60.00	0	0	0	0	0	0	0	0	0	0	30.00	14.78
Otu010	144687	*Polytrichastrum alpinum*	0	0	0	0	10992.33	33.43	0	0	36765.67	68.09	0	0	0	0	471.00	0.30	0	0	0	0
Otu011	106430	*Anemone patens*	35041.67	70.58	0	0	144.67	0.44	0	0	0	0	138.33	0.11	0	0	152.00	0.10	0	0	0	0
Otu017	30925	*Smelowskia alba*	0	0	0	0	0	0	10308.33	11.78	0	0	0	0	0	0	0	0	0	0	0	0
Otu020	109350	*Coelogyne fimbriata*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	109350	100	0	0
Otu024	28383	*Caltha palustris*	0	0	0	0	2293.33	6.98	0	0	0	0	7124.00	5.69	0	0	43.67	0.03	0	0	0	0
Otu026	20691	*Endocellion sibiricum*	0	0	0	0	0	0	0	0	38.33	0.07	6858.67	5.48	0	0	0	0	0	0	0	0
Otu030	21115	*Eritrichium sericeum*	0	0	0	0	0	0	7038.33	8.05	0	0	0	0	0	0	0	0	0	0	0	0
Otu032	34930	*Comarum palustre*	0	0	0	0	0	0	0	0	0	0	11643.33	9.30	0	0	0	0	0	0	0	0
Otu037	8066	*Saxifraga sibirica*	0	0	11.33	+	2677.33	8.14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu040	13996	*Arctophila fulva*	0	0	0	0	2480.00	7.54	0	0	1839.33	3.41	305.33	0.24	0	0	40.67	0.03	0	0	0	0
Otu042	24297	*Deschampsia cespitosa*	127.33	0.26	0	0	7087.00	21.56	821.33	0.94	0	0	0	0	0	0	63.33	0.04	0	0	0	0
Otu050	9804	*Alopecurus magellanicus*	0	0	0	0	3228.33	9.82	0	0	0	0	0	0	0	0	39.67	0.03	0	0	0	0
Otu051	13113	*Menyanthes trifoliata*	0	0	0	0	19.67	0.06	0	0	20.00	0.04	4309.33	3.44	0	0	22.00	0.01	0	0	0	0
Otu052	5231	*Oxytropis deflexa*	0	0	0	0	13.00	0.04	1730.67	1.98	0	0	0	0	0	0	0	0	0	0	0	0
Otu054	8506	*Ceratodon purpureus*	0	0	0	0	0	0	0	0	2835.33	5.25	0	0	0	0	0	0	0	0	0	0
Otu055	4934	*Dupontia fisheri*	0	0	0	0	1644.67	5.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu057	5448	*Geum*	0	0	0	0	0	0	0	0	7.00	0.01	0	0	1762.67	1.70	46.33	0.03	0	0	0	0
Otu062	3332	*Puccinellia*	0	0	1110.67	0.96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu064	8899	*Polytrichum piliferum*	0	0	0	0	0	0	0	0	0	0	0	0	839.00	0.81	2127.33	1.35	0	0	0	0
Otu068	6830	*Arctagrostis latifolia*	0	0	16.67	0.01	0	0	0	0	2252.00	4.17	0	0	8.00	+	0	0	0	0	0	0
Otu069	3140	*Pedicularis sudetica*	0	0	0	0	0	0	0	0	8.33	0.02	0	0	1038.33	1.00	0	0	0	0	0	0
Otu070	7099	*Artemisia scoparia*	0	0	42.33	0.04	0	0	2315.67	2.65	8.33	0.02	0	0	0	0	0	0	0	0	0	0
Otu071	5734	*Vaccinium uliginosum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1911.33	1.21	0	0	0	0
Otu073	6714	*Eriophorum angustifolium*	0	0	0	0	268.67	0.82	0	0	441.00	0.82	1528.33	1.22	0	0	0	0	0	0	0	0
Otu074	2755	*Poa arctica*	0	0	9.33	+	0	0	0	0	900.00	1.67	4.00	+	5.00	+	0	0	0	0	0	0
Otu075	4854	*Stellaria*	6.33	0.01	0	0	1597.67	4.86	0	0	0	0	7.67	+	6.33	+	0	0	0	0	0	0
Otu079	3809	*Hylocomium splendens*	0	0	8.00	+	0	0	0	0	0	0	0	0	1222.00	1.18	39.67	0.03	0	0	0	0
Otu080	7613	*Anemonastrum narcissiflorum*	10.00	0.02	0	0	15.67	0.05	9.67	+	0	0	13.00	+	2441.00	2.35	48.33	0.03	0	0	0	0
Otu084	3684	*Taxus canadensis*	0	0	0	0	0	0	0	0	0	0	0	0	1222.67	1.18	0	0	0	0	0	0
Otu088	2342	*Hippuris*	0	0	0	0	5.67	0.02	0	0	7.00	0.01	764.33	0.61	0	0	0	0	0	0	0	0
Otu089	3682	*Epilobium palustre*	0	0	0	0	0	0	0	0	0	0	1220.67	0.97	0	0	6.67	+	0	0	0	0
Otu090	1993	*Astragalus alpinus*	0	0	4.00	+	0	0	656.33	0.75	0	0	0	0	0	0	0	0	0	0	0	0
Otu092	2582	*Festuca ovina*	0	0	7.00	+	0	0	844.33	0.97	0	0	0	0	0	0	0	0	0	0	0	0
Otu100	1627	*Pohlia nutans*	0	0	0	0	0	0	0	0	0	0	0	0	540.00	0.52	0	0	0	0	0	0
Otu103	1183	*Potentilla hookeriana*	0	0	0	0	0	0	384.67	0.44	0	0	0	0	0	0	0	0	0	0	0	0
Otu104	5535	*Polytrichum commune*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1845.00	1.17	0	0	0	0
Otu107	1960	*Empetrum nigrum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	653.33	0.42	0	0	0	0
Otu121	1340	*Drepanocladus sordidus*	0	0	0	0	410.00	1.25	30.67	0.04	0	0	0	0	0	0	0	0	0	0	0	0

Otu122	699	*Salix alaxensis*	0	0	0	0	0	0	0	0	0	0	0	0	230.67	0.22	0	0	0	0	0	0
Otu125	1272	*Barbilophozia barbata*	0	0	0	0	0	0	0	0	0	0	0	0	414.00	0.40	7.67	+	0	0	0	0
Otu130	510	*Pleurozium schreberi*	0	0	0	0	0	0	0	0	0	0	0	0	78.33	0.08	91.67	0.06	0	0	0	0
Otu140	1352	*Calamagrostis*	0	0	0	0	0	0	0	0	358.67	0.66	85.00	0.07	0	0	5.00	+	0	0	0	0
Otu143	1084	*Dicranum fuscescens*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	361.33	0.23	0	0	0	0
Otu145	1325	*Dryas octopetala*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	439.33	0.28	0	0	0	0
Otu146	247	*Carex duriuscula*	81.33	0.16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu148	476	*Carex aquatilis*	0	0	0	0	0	0	0	0	51.67	0.10	106.00	0.08	0	0	0	0	0	0	0	0
Otu160	111	*Mertensia paniculata*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37.00	0.02	0	0	0	0
Otu162	429	*Polytrichum juniperinum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	142.33	0.09	0	0	0	0
Otu173	121	*Pyrola asarifolia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40.33	0.03	0	0	0	0
Otu178	169	*Chamaenerion angustifolium*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	55.00	0.03	0	0	0	0
Otu180	185	*Artemisia norvegica* subsp. *saxatilis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60.67	0.04	0	0	0	0
Otu182	99	*Rubus arcticus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33.00	0.02	0	0	0	0
Otu187	87	*Carex rostrata*	0	0	0	0	0	0	0	0	0	0	28.33	0.02	0	0	0	0	0	0	0	0
Otu192	152	*Rhodiola integrifolia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50.00	0.03	0	0	0	0
Otu210	78	*Mniaceae*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26.00	0.02	0	0	0	0
Otu224	31	*Ptychostomum pallescens*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.33	+	0	0	0	0
Otu225	39	*Arctous alpina*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.33	+	0	0	0	0
Otu227	15	*Aulacomnium palustre*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.00	+	0	0	0	0
Otu228	13	*Hylocomiastrum pyrenaicum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.33	+	0	0	0	0
Otu234	3953	*Vaccinium vitis-idaea*	0	0	4.67	+	0	0	0	0	8.00	0.01	0	0	1179.67	1.14	125.33	0.08	0	0	0	0
Otu242	48	*Vaccinium*	0	0	0	0	0	0	0	0	0	0	0	0	7.00	+	8.67	+	0	0	0	0
Otu244	13	*Tomentypnum nitens*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.33	+	0	0	0	0
Otu246	14	*Niphotrichum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.67	+	0	0	0	0
Otu251	5	*Sisymbrium linifolium*	1.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu261	10	*Carex vesicaria*	0	0	0	0	0	0	0	0	0	0	3.33	+	0	0	0	0	0	0	0	0
Otu262	16	*Douinia ovata*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.00	+	0	0	0	0
Otu263	88	*Polytrichum strictum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31.00	0.02	0	0	0	0

Table S13. Idenitity and abundance of OTU's fungal nrITS2

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OTU	Total read c	best_id_UN	cover	phylum	class	order	family	genus	species	maxid	rank	OTU sequence (truncated)	length	funguild_ta	funguild_tr	funguild_g	funguild_g	potential_food?
Otu010	90857	100	100	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Cladosporium*		*Cladosporium*	genus	AACGCACATTGCGCCCCCTGGT	243	Mycosphae	Pathotroph	Plant Path	Microfungus	
Otu037	21827	90.265	97	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Mycosphaerella*		*Mycosphaerella*	genus	AACGCACATTGCGCCCCCTGGT	237	Mycosphae	Pathotroph	Plant Path	Microfungus	
Otu049	16571	94.397	100	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Sphaerulina*		*Sphaerulina*	genus	AACGCACATTGCGCCCTCTGGT	237	Sphaerulin	Pathotroph	Plant Path	Microfungus	
Otu154	9134	99.565	100	Ascomycota	Dothideomycetes	Dothideales	Aureobasidiaceae	*Aureobasidium*	*Aureobasidium pullulans*	*Aureobasidium pullulans*	species	AACGCACATTGTGCCCCTTGGT	230	Aureobasic	Pathotroph	Animal Pat	Facultative Yeast	
Otu096	3130	100	100	Ascomycota	Dothideomycetes	Pleosporales	Coniothyriaceae	*Coniothyrium*		*Coniothyrium*	genus	AACGCACATTGCGCCCCTTGGT	248	Coniothyri	Pathotroph	Plant Path	-	
Otu360	28	100	100	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	*Calophoma*	*Calophoma sandfjordenica*	*Calophoma sandfjordenica*	species	AACGCACATTGCGCCCCTTGGT	230	Calophoma	Pathotroph	Plant Path	Microfungus	
Otu035	22353	100	100	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	*Didymella*	*Didymella microchlamydospora*	*Didymella microchlamydospora*	species	AACGCACATTGCGCCCCTTGGT	249	Didymella	Pathotroph	Animal Pat	Microfungus	
Otu271	68	100	100	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	*Phoma*	*Phoma herbarum*	*Phoma herbarum*	species	AACGCACATTGCGCCCCTTGGT	252	Phoma	Pathotroph	Plant Path	Microfungus	
Otu220	158	99.565	100	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	*Paraconiothyrium*	*Paraconiothyrium sporulosum*	*Paraconiothyrium sporulosum*	species	AACGCACATTGCGCCCCTTGGT	247	Paraconiot	Saprotroph	Undefined	-	
Otu377	11	100	100	Ascomycota	Dothideomycetes	Pleosporales	Leptosphaeriaceae	*Plenodomus*	*Plenodomus biglobosus*	*Plenodomus biglobosus*	species	AACGCACATTGCGCCCCTTGGT	249	Plenodomu	Saprotroph	Undefined	-	
Otu095	3062	100	100	Ascomycota	Dothideomycetes	Pleosporales	Massarinaceae	*Stagonospora*	*Stagonospora trichophoricola*	*Stagonospora trichophoricola*	species	AACGCACATTGCGCCCCTCGGT	247	Stagonosp	Pathotroph	Plant Path	Microfungus	
Otu173	442	100	100	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Neosetophoma*	*Neosetophoma rosarum*	*Neosetophoma rosarum*	species	AACGCACATTGCGCCCCTTGGT	249	Neosetoph	Saprotroph	Undefined	-	
Otu126	1223	100	100	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Paraphoma*	*Paraphoma fimeti*	*Paraphoma fimeti*	species	AACGCACATTGCGCCCCTTGGT	247	Paraphoma	Pathotroph	Plant Path	Microfungus	
Otu253	92	100	100	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Phaeosphaeriopsis*		*Phaeosphaeriopsis*	genus	AACGCACATTGCGCCCCTT	251	Phaeospha	Saprotroph	Undefined	Microfungus	
Otu246	97	100	100	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae			Phaeosphaeriaceae	family	AACGCACATTGCGCCCCTTGGT	245	Phaeospha	Pathotroph	Fungal Para	Microfungus	
Otu098	3933	96.537	100	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Preussia*	*Preussia flanaganii*	*Preussia flanaganii*	species	AACGCACATTGCGCCCTAT	248	Preussia	Saprotroph	Dung Sapr	-	
Otu383	28	99.13	100	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Preussia*	*Preussia longisporopsis*	*Preussia longisporopsis*	species	AACGCACATTGCGCCCTTTGGT	246	Preussia	Saprotroph	Dung Sapr	-	
Otu392	17	99.565	100	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Preussia*	*Preussia minipascua*	*Preussia minipascua*	species	AACGCACATTGCGCCCTTTGGT	241	Preussia	Saprotroph	Dung Sapr	-	
Otu117	2997	99.13	100	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Preussia*	*Preussia tetramera*	*Preussia tetramera*	species	AACGCACATTGCGCCCTTTGGT	244	Preussia	Saprotroph	Dung Sapr	-	
Otu015	136505	100	100	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Preussia*		*Preussia*	genus	AACGCACATTGCGCCCTTTGGT	249	Preussia	Saprotroph	Dung Sapr	-	
Otu135	8913	100	100	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Sporormiella*	*Sporormiella intermedia*	*Sporormiella intermedia*	species	AACGCACATTGCGCCCTTT	244	Sporormiel	Saprotroph	Dung Sapr	Microfungus	
Otu382	18	100	100	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Sporormiella*	*Sporormiella leporina*	*Sporormiella leporina*	species	AACGCACATTGCGCCCTTTGGT	244	Sporormiel	Saprotroph	Dung Sapr	Microfungus	
Otu269	111	100	100	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Sporormiella*	*Sporormiella vexans*	*Sporormiella vexans*	species	AACGCACATTGCGCCCTTT	244	Sporormiel	Saprotroph	Dung Sapr	Microfungus	
Otu311	42	97.391	100	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Sporormiella*		*Sporormiella*	genus	AACGCACATTGCGCCCTTTGGT	263	Sporormiel	Saprotroph	Dung Sapr	Microfungus	
Otu033	27109	96.522	100	Ascomycota	Dothideomycetes					Dothideomycetes1	class	AACGCACATTGCGCCCCCTGGT	247	-	-	-	-	
Otu065	8096	96.104	100	Ascomycota	Dothideomycetes					Dothideomycetes2	class	AACGCACATTGCGCCCTCTGGT	271	-	-	-	-	
Otu288	94	98.261	100	Ascomycota	Dothideomycetes					Dothideomycetes3	class	AACGCACATTGCGCCCTTTGGT	271	-	-	-	-	
Otu297	101	90	100	Ascomycota	Dothideomycetes					Dothideomycetes4	class	AACGCACATTGCGCCCTCTGGT	270	-	-	-	-	
Otu287	98	98.696	100	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Capronia*		*Capronia*	genus	AACGCATATTGCGCCCTTTGGT	252	Capronia	Symbiotrop	Endophyte	Facultative Yeast	
Otu036	22519	100	100	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	*Cladophialophora minutissima*	*Cladophialophora minutissima*	species	AACGCACATTGCGCCCTTTAGT	255	Cladophial	Saprotroph	Moss Sapr	Microfungus	
Otu359	23	99.13	100	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*		*Cladophialophora*	genus	AACGCACATTGCGCCCTTTGGT	230	Cladophial	Saprotroph	Undefined	Microfungus	
Otu069	6029	98.69	99	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae			Herpotrichiellaceae	family	AACGCACATTGCGCCCTTTGGT	291	Herpotrich	Pathotroph	Animal Pat	Facultative Yeast-Microfungus	
Otu007	250160	99.565	100	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus*	*Aspergillus versicolor*	*Aspergillus versicolor*	species	AACGCACATTGCGCCCCCTGGC	259	-	-	-	-	
Otu022	106644	100	100	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium aethiopicum*	*Penicillium aethiopicum*	species	AACGCACATTGCGCCCCCTGGT	258	Penicillium	Pathotroph	Animal Pat	-	
Otu396	4068	97.009	100	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium melinii*	*Penicillium melinii*	species	AACGCACATTGCGCCCCCTGGT	260	Penicillium	Pathotroph	Animal Pat	-	
Otu222	3619	97.823	100	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium paradoxum*	*Penicillium paradoxum*	species	AACGCACATTGCGCCCCCTGGC	230	Penicillium	Pathotroph	Animal Pat	-	
Otu001	890607	97.826	100	Ascomycota	Eurotiomycetes	Onygenales	Arachnomycetaceae	*Arachnomyces*		*Arachnomyces*	genus	AACGCACATTGCGCCCCCTGGT	255	Arachnomy	Saprotroph	Dung Sapr	-	
Otu005	279349	100	100	Ascomycota	Eurotiomycetes	Onygenales	*incertae sedis*	*Chrysosporium*	*Chrysosporium merdarium*	*Chrysosporium merdarium*	species	AACGCACATTGCGCCCCCTGGT	240	Chrysospor	Saprotroph	Undefined	-	
Otu009	120543	100	100	Ascomycota	Eurotiomycetes	Onygenales	*incertae sedis*	*Chrysosporium*	*Chrysosporium pseudomerdarium*	*Chrysosporium pseudomerdarium*	species	AACGCACATTGCGCCCCCTGGT	240	Chrysospor	Saprotroph	Undefined	-	
Otu145	846	100	100	Ascomycota	Eurotiomycetes	Onygenales		*Chrysosporium*	*Chrysosporium synchronum*	*Chrysosporium synchronum*	species	AACGCACATTGCGCCCGCCAGT	248	Botryotrich	Saprotroph	Wood Sapr	-	
Otu331	42	94.783	100	Ascomycota	Eurotiomycetes	Onygenales				Onygenales	order	AACGCACATTGCGCCCCCTGGT	259	-	-	-	-	
Otu312	44	96.522	100	Ascomycota	Eurotiomycetes	Verrucariales				Verrucariales1	order	AACGCATATTGCGCCCTTTG	247	Verrucarial	Pathotroph	Lichen Para	Thallus	
Otu320	53	100	100	Ascomycota	Eurotiomycetes	Verrucariales				Verrucariales2	order	AACGCATATTGCGCCCTTTG	248	Verrucarial	Pathotroph	Lichen Para	Thallus	
Otu347	54	100	100	Ascomycota	Eurotiomycetes	Verrucariales				Verrucariales3	order	AACGCATATTGCGCCCTTTG	246	Verrucarial	Pathotroph	Lichen Para	Thallus	
Otu355	17	100	100	Ascomycota	Eurotiomycetes	Verrucariales				Verrucariales4	order	AACGCATATTGCGCCCTTTG	246	Verrucarial	Pathotroph	Lichen Para	Thallus	
Otu386	23	100	100	Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	*Cladonia*	*Cladonia cornuta*	*Cladonia cornuta*	species	AACGCACATTGCGCCCCTCGGT	255	Cladonia	Symbiotrop	Lichenized	Thallus	yes
Otu217	421	100	100	Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	*Cladonia*	*Cladonia mitis*	*Cladonia mitis*	species	AACGCACATTGCGCCCCTCGGT	256	Cladonia	Symbiotrop	Lichenized	Thallus	yes
Otu243	380	100	100	Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	*Cladonia*	*Cladonia rangiferina*	*Cladonia rangiferina*	species	AACGCACATTGCGCCCCTTGGT	256	Cladonia	Symbiotrop	Lichenized	Thallus	yes
Otu239	309	100	100	Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	*Cladonia*	*Cladonia stellaris*	*Cladonia stellaris*	species	AACGCACATTGCGCCCCTCGGT	263	Cladonia	Symbiotrop	Lichenized	Thallus	yes
Otu299	124	100	100	Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	*Cladonia*	*Cladonia submitis*	*Cladonia submitis*	species	AACGCACATTGCGCCCCTCGGT	256	Cladonia	Symbiotrop	Lichenized	Thallus	yes
Otu445	14	100	100	Ascomycota	Lecanoromycetes	Lecanorales	Parmeliaceae	*Bryocaulon*	*Bryocaulon divergens*	*Bryocaulon divergens*	species	AACGCACATTGCGCCCCTCGGT	230	Bryocaulon	Symbiotrop	Lichenized	Thallus	yes
Otu335	39	99.565	100	Ascomycota	Lecanoromycetes	Lecanorales	Stereocaulaceae	*Stereocaulon*	*Stereocaulon saxatile*	*Stereocaulon saxatile*	species	AACGCACATTGCGCCCCTCGGA	246	Stereocaul	Symbiotrop	Lichenized	Thallus	yes
Otu064	9123	100	100	Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	*Patinella*	*Patinella hyalophaea*	*Patinella hyalophaea*	species	AACGCACATTGCGCCCTCTGGT	240	Patinella	Saprotroph	Undefined	-	
Otu254	99	100	100	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Collophora*		*Collophora*	genus	AACGCACATTGCGCCCTCTGGT	242	Collophora	Pathotroph	Plant Path	Microfungus	
Otu209	186	100	100	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Tetracladium*		*Tetracladium*	genus	AACGCACATTGCGCCCCTTGGT	238	Tetracladiu	Saprotroph	Undefined	-	
Otu315	69	100	100	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae			Hyaloscyphaceae	family	AACGCACATTGCGCCCTCTGGT	242	Hyaloscyph	Saprotroph	Plant Sapr	Microfungus	
Otu008	253365	100	100	Ascomycota	Leotiomycetes	Helotiales	*incertae sedis*	*Cadophora*	*Cadophora luteo-olivacea*	*Cadophora luteo-olivacea*	genus	AACGCACATTGCGCCCTCTGGT	241	Tricladium	Symbiotrop	Endophyte	Microfungus	
Otu047	19764	95.671	100	Ascomycota	Leotiomycetes	Helotiales	Lachnaceae	*Lachnellula*		*Lachnellula*	genus	AACGCACATTGCGCCCCTTGGT	239	Lachnellula	Saprotroph	Undefined	Helotioid	
Otu452	13	100	100	Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	*Oidiodendron*	*Oidiodendron cereale*	*Oidiodendron cereale*	species	AACGCACATTGCGCCCTGTGGT	234	Oidiodendr	Pathotroph	Ericoid My	Dark Septate Endophyte	
Otu053	15522	100	100	Ascomycota	Leotiomycetes	Helotiales	Ploettnerulaceae	*Cadophora*		*Cadophora*	genus	AACGCACATTGCGCCCTTT	243	Cadophora	Symbiotrop	Endophyte	Microfungus	
Otu072	5338	92.241	100	Ascomycota	Leotiomycetes	Helotiales				Helotiales1	order	AACGCACATTGCGCCCTCTGGT	242	-	-	-	-	
Otu283	62	100	100	Ascomycota	Leotiomycetes	Helotiales				Helotiales2	order	AACGCACATTGCGCCCTCTGGT	242	Alatospora	Saprotroph	Undefined	-	
Otu352	17	100	100	Ascomycota	Leotiomycetes	Helotiales				Helotiales3	order	AACGCACATTGCGCCCCCTGGT	241	Botrytis	Pathotroph	Plant Path	Facultative Yeast-Microfungus	
Otu002	520344	100	100	Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	*Pseudeurotium*	*Pseudeurotium hygrophilum*	*Pseudeurotium hygrophilum*	species	AACGCACATTGCGCCCCCTGGT	241	Pseudeuro	Saprotroph	Soil saprot	Microfungus	
Otu192	383	100	100	Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	*Pseudeurotium*		*Pseudeurotium*	genus	AACGCACATTGCGCCCCCTGGT	241	Pseudeuro	Saprotroph	Undefined	Microfungus	
Otu079	8935	100	100	Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	*Pseudogymnoascus*	*Pseudogymnoascus roseus*	*Pseudogymnoascus roseus*	species	AACGCACATTGCGCCCCCTGGT	239	Pseudogym	Saprotroph	Soil Saprot	-	
Otu140	1432	99.565	100	Ascomycota	Leotiomycetes	Thelebolales	Thelebolaceae	*Antarctomyces*	*Antarctomyces psychrotrophicus*	*Antarctomyces psychrotrophicus*	species	AACGCACATTGCGCCCTCTGGT	241	Antarctom	Saprotroph	Undefined	Yeast	
Otu282	144	99.565	100	Ascomycota	Leotiomycetes	Thelebolales	Thelebolaceae	*Cleistothelebolus*	*Cleistothelebolus nipigonensis*	*Cleistothelebolus nipigonensis*	species	AACGCACATTGCGCCCTCTGGT	242	Cleistothel	Saprotroph	Dung Sapr	Microfungus	
Otu006	818163	100	100	Ascomycota	Leotiomycetes	Thelebolales	Thelebolaceae	*Thelebolus*	*Thelebolus globosus*	*Thelebolus globosus*	species	AACGCACATTGCGCCCTCTGGT	242	Thelebolus	Saprotroph	Dung Sapr	Microfungus	
Otu153	1115	100	100	Ascomycota	Leotiomycetes	Thelebolales	Thelebolaceae	*Thelebolus*		*Thelebolus*	genus	AACGCACATTGCGCCCTCTGGT	242	Thelebolus	Saprotroph	Dung Sapr	Microfungus	
Otu393	10	97.826	100	Ascomycota	Lichinomycetes	Lichinales	Lichinaceae	*Phylliscum*	*Phylliscum demangeonii*	*Phylliscum demangeonii*	species	AACGCACATTGCGCCCTTTGGT	230	Phylliscum	Symbiotrop	Lichenized	Thallus	yes
Otu026	41022	98.696	100	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Arthrobotrys*	*Arthrobotrys superba*	*Arthrobotrys superba*	species	AACGCACATTGCGCCCATTGGT	267	Arthrobotr	Saprotroph	Wood Sapr	Microfungus	
Otu028	27752	99.565	100	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Dactylella*		*Dactylella*	genus	AACGCACATTGCGCCCATAGGT	273	Dactylella	Saprotroph	Undefined	-	
Otu180	455	100	100	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Dactylellina*	*Dactylellina cionopaga*	*Dactylellina cionopaga*	species	AACGCACATTGCGCCCATCGGT	260	Dactylellina	Saprotroph	Undefined	-	
Otu144	792	99.087	100	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Orbilia*	*Orbilia rectispora*	*Orbilia rectispora*	species	AACGCACATTGCGCCTATTGGT	266	Orbilia	Saprotroph	Wood Sapr	Helotioid	

Otu067	7372	99.565	100	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Orbilia*		*Orbilia*	genus	AACGCACATTGCGCCTATTGGT	266	Orbilia	Saprotroph	Wood Sapr	Helotioid	
Otu343	24	99.565	100	Ascomycota	Pezizomycetes	Pertusariales	Microcaliciaceae	*Microcalicium*	*Microcalicium ahlneri*	*Microcalicium ahlneri*	species	AACGCACATTGCGCCCTTTGGT	248	Microcalici	Pathotroph	Lichen Para	Microfungus	
Otu091	4910	100	100	Ascomycota	Pezizomycetes	Pezizales	Ascobolaceae	*Ascobolus*	*Ascobolus equinus*	*Ascobolus equinus*	species	AACGCACATTGCGCCCTTTGGT	248	Ascobolus	Saprotroph	Dung Sapro	-	
Otu003	357130	99.565	100	Ascomycota	Pezizomycetes	Pezizales	Ascobolaceae			*Ascobolaceae*	family	AACGCACATTGCGCCCACTGGT	247	Ascobolace	Saprotroph	Undefined	-	
Otu242	163	99.13	100	Ascomycota	Pezizomycetes	Pezizales	Pezizaceae	*Peziza*	*Peziza ampliata*	*Peziza ampliata*	species	AACGCACATTGCGCCTTATGGT	268	Peziza	Saprotroph	Wood Sapr	Pezizoid	
Otu373	23	100	100	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	*Byssonectria*	*Byssonectria deformis*	*Byssonectria deformis*	species	AACGCACATTGCGCCTCCTGGT	252	Byssonectr	Saprotroph	Undefined	Gasteroid-I	yes
Otu341	27	100	100	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	*Cheilymenia*	*Cheilymenia stercorea*	*Cheilymenia stercorea*	species	AACGCACATTGCGCCTCCTGGT	254	Cheilymeni	Saprotroph	Undefined	Pezizoid	
Otu188	336	99.07	93	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	*Cheilymenia*		*Cheilymenia*	genus	AACGCACATTGCGCCTCCTGGT	254	Cheilymeni	Saprotroph	Dung Sapro	Pezizoid	
Otu221	216	99.13	100	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae			Pyronemataceae	family	AACGCACATTGCGCCTTCTGGT	253	Pyronemat	Saprotroph	Dung Sapro	Gasteroid-Pezizoid	
Otu004	343946	100	100	Ascomycota	Saccharomycetes	Saccharomycetales	*incertae sedis*	*Candida*	*Candida zeylanoides*	*Candida zeylanoides*	species	AACGCACATTGCGCCCTATGGT	281	Candida ze	Pathotroph	Animal Pat	Yeast	
Otu099	2638	99.565	100	Ascomycota	Sordariomycetes	Amphisphaeriales	Amphisphaeriaceae	*Microdochium*		*Microdochium*	genus	AACGCACATTGCGCCCATTAGT	262	Microdoch	Pathotroph	Endophyte	Dark Septate Endophyte	
Otu012	78660	100	100	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	*Coniochaeta*	*Coniochaeta hoffmannii*	*Coniochaeta hoffmannii*	species	AACGCACATTGCGCCCGGCAGT	250	Coniochaet	Pathotroph	Animal Pat	Microfungus	
Otu354	32	100	100	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae			*Coniochaetaceae*	family	AACGCACATTGCGCCCGGCAGT	249	Coniochaet	Pathotroph	Animal Pat	Microfungus	
Otu286	67	100	100	Ascomycota	Sordariomycetes	Coniochaetales				*Coniochaetales*	order	AACGCACATTGCGCCCGCTAGT	248	-	-	-	-	
Otu071	6150	100	100	Ascomycota	Sordariomycetes	Hypocreales	*incertae sedis*	*Fusariella*	*Fusariella hughesii*	*Fusariella hughesii*	species	AACGCACATTGCGCCCGCCAGT	268	Hypocreale	Saprotroph	Undefined	Microfungus	
Otu011	80413	100	100	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Cosmospora*	*Cosmospora viridescens*	*Cosmospora viridescens*	species	AACGCACATTGCGCCCGCC	253	Cosmospor	Pathotroph	Fungal Para	-	
Otu101	2341	100	98	Ascomycota	Sordariomycetes	Microascales	Microascaceae	*Pithoascus*	*Pithoascus ater*	*Pithoascus ater*	species	AACGCACATTGCGCCCAGCAG	265	Pithoascus	Saprotroph	Undefined	-	
Otu040	32284	100	100	Ascomycota	Sordariomycetes	Microascales				Microascales	order	AACGCATATTGCGCTCGAGGCT	297	-	-	-	-	
Otu237	391	99.565	100	Ascomycota	Sordariomycetes	Sordariales	*incertae sedis*	*Ramophialophora*	*Ramophialophora humicola*	*Ramophialophora humicola*	species	AACGCACATTGCGCCCGCTAGT	245	Ramophial	Saprotroph	Soil Saprot	Microfungus	
Otu166	1466	99.569	100	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Apodus*	*Apodus deciduus*	*Apodus deciduus*	species	AACGCACATTGCGCCCGCTAGT	247	Apodus	Saprotroph	Undefined	Saprotroph	
Otu110	3915	98.261	100	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Podospora*	*Podospora pleiospora*	*Podospora pleiospora*	species	AACGCACATTGCGCCCGCCAG	256	Podospora	Saprotroph	Dung Sapro	Microfungus	
Otu107	17593	99.565	100	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Podospora*		*Podospora*	genus	AACGCACATTGCGCCCGCTAGT	247	Podospora	Saprotroph	Dung Sapro	Microfungus	
Otu046	66149	99.567	100	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Schizothecium*	*Schizothecium carpinicola*	*Schizothecium carpinicola*	species	AACGCACATTGCGCCCGCTAGT	245	Schizotheci	Saprotroph	Dung Sapro	-	
Otu063	14676	98.696	100	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Schizothecium*		*Schizothecium*	genus	AACGCACATTGCGCCCGCCAGT	244	Schizotheci	Saprotroph	Dung Sapro	-	
Otu301	78	97.391	100	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae			Lasiosphaeriaceae	family	AACGCACATTGCGCCCGCTAGT	244	Lasiosphae	Saprotroph	Undefined	Microfungus	
Otu319	48	100	100	Ascomycota	Sordariomycetes	Sordariales	Sordariaceae	*Sordaria*	*Sordaria fimicola*	*Sordaria fimicola*	species	AACGCACATTGCGCTCGCCAGT	243	Sordaria fir	Saprotroph	Dung Sapro	-	
Otu085	8454	98.26	100	Ascomycota	Sordariomycetes	Sordariales				Sordariales1	order	AACGCACATTGCGCCCGCCAGT	245	-	-	-	-	
Otu285	63	100	100	Ascomycota	Sordariomycetes	Sordariales				Sordariales2	order	AACGCACATTGCGCCCGCCAGT	245	-	-	-	-	
Otu025	33058	100	100	Ascomycota	Taphrinomycetes	Taphrinales	Protomycetaceae	*Protomyces*	*Protomyces inouyei*	*Protomyces inouyei*	species	AACGCACATTGCGCCCTCTGGT	261	Protomyce	Pathotroph	Plant Pathogen		
Otu233	140	99.565	100	Ascomycota	Taphrinomycetes	Taphrinales	Taphrinaceae	*Taphrina*	*Taphrina carpini*	*Taphrina carpini*	species	AACGCACATTGCGCCCTCT	293	Taphrina	Pathotroph	Plant Patho	Microfungus	
Otu212	167	100	100	Basidiomycota	Agaricomycetes	Agaricales	Bolbitiaceae	*Conocybe*	*Conocybe lenticulospora*	*Conocybe lenticulospora*	species	AACGCACCTTGCGCTCCTTGGT	298	Conocybe	Saprotroph	Dung Sapro	Agaricoid	yes
Otu274	60	100	100	Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Entoloma*		*Entoloma*	species	AACGCACCTTGCGCTCCTTGGT	298	Entoloma	Pathotroph	Ectomycor	Agaricoid	yes
Otu172	748	98.701	100	Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	*Inocybe*		*Inocybe*	genus	AACGCATCTTGCGCTTCTTGGT	300	Inocybe	Symbiotrop	Ectomycor	Agaricoid	yes
Otu195	295	100	100	Basidiomycota	Agaricomycetes	Agaricales	Lycoperdaceae	*Bovista*	*Bovista plumbea*	*Bovista plumbea*	species	AACGCACCTTGCGCTCCTT	305	Bovista	Saprotroph	Soil Saprot	Gasteroid	yes
Otu103	2094	99.565	100	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Coprinopsis*	*Coprinopsis kubickae*	*Coprinopsis kubickae*	species	AACGCACCTTGCGCTCCTTGGT	308	Coprinopsi	Saprotroph	Leaf Saprot	Agaricoid	yes
Otu197	293	100	100	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Psathyrella*	*Psathyrella ammophila*	*Psathyrella ammophila*	species	AACGCACCTTGCGCTCCTTGGT	296	Psathyrella	Saprotroph	Wood Sapr	Agaricoid	yes
Otu013	139431	100	100	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	*Sebacina*		*Sebacina*	genus	AACGCACCTTGCACCCTTTGGT	295	Sebacina	Symbiotrop	Ectomycor	-	
Otu058	28457	100	100	Basidiomycota	Cystobasidiomycetes	Cystobasidiales	Cystobasidiaceae	*Cystobasidium*	*Cystobasidium minuta*	*Cystobasidium minuta*	species	AACGCACCTTGCACTCTTTGGT	297	Cystobasid	Pathotroph	Fungal Para	Facultative Yeast	
Otu177	843	100	100	Basidiomycota	Cystobasidiomycetes	Cystobasidiales	Cystobasidiaceae	*Cystobasidium*	*Cystobasidium pinicola*	*Cystobasidium pinicola*	species	AACGCACCTTGCACTCTTTGGT	296	Cystobasid	Pathotroph	Fungal Para	Facultative Yeast	
Otu357	25	100	100	Basidiomycota	Cystobasidiomycetes	Cystobasidiales	Cystobasidiaceae	*Cystobasidium*	*Cystobasidium psychroaquaticum*	*Cystobasidium psychroaquaticum*	species	AACGCACCTTGCACTCTTTGGT	294	Cystobasid	Pathotroph	Fungal Para	Facultative Yeast	
Otu029	28397	98.696	100	Basidiomycota	Cystobasidiomycetes		Symmetrosporaceae	*Symmetrospora*	*Symmetrospora gracilis*	*Symmetrospora gracilis*	species	AACGCACCTTGCACTCTTTGGT	303	-	-	-	-	
Otu016	154914	100	100	Basidiomycota	Malasseziomycetes	Malasseziales				Malasseziales	order	AACGCACCTTGCGCTCTATGGC	369	-	-	-	-	
Otu061	8921	100	100	Basidiomycota	Microbotryomycetes	Leucosporidiales	Leucosporidiaceae	*Leucosporidium*	*Leucosporidium creatinivorum*	*Leucosporidium creatinivorum*	species	AACGCACCTTGCGCTCTCTGGT	306	Leucospori	Saprotroph	Soil Saprot	Yeast	
Otu181	1468	99.565	100	Basidiomycota	Microbotryomycetes	Leucosporidiales	Leucosporidiaceae	*Leucosporidium*	*Leucosporidium fragarium*	*Leucosporidium fragarium*	species	AACGCACCTTGCGCTCCGTGGT	306	Leucospori	Saprotroph	Soil Saprot	Yeast	
Otu054	12873	93.913	100	Basidiomycota	Microbotryomycetes	Leucosporidiales				Leucosporidiales	order	AACGCACCTTGCGCTCCCTGGT	307	-	-	-	-	
Otu024	36256	89.565	100	Basidiomycota	Microbotryomycetes					Microbotryomycetes	class	AACGCACCTTGCGCTCCCTGGT	315	-	-	-	-	
Otu062	10013	100	100	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Cystofilobasidiaceae	*Cystofilobasidium*	*Cystofilobasidium infirmominiatun*	*Cystofilobasidium infirmominiatum*	species	AACGCATCTTGCGCTCTTTGGT	328	Cystofiloba	Saprotroph	Leaf Saprot	Yeast	
Otu115	1775	99.565	100	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Cystofilobasidiaceae	*Cystofilobasidium*	*Cystofilobasidium macerans*	*Cystofilobasidium macerans*	species	AACGCATCTTGCGCTCTTTGGT	328	Cystofiloba	Saprotroph	Leaf Saprot	Yeast	
Otu048	36809	100	100	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	*Mrakia*	*Mrakia blollopis*	*Mrakia blollopis*	species	AACGCACCTTGCGCTCCTTGGT	327	Mrakia	Saprotroph	Soil Saprot	Yeast	
Otu223	7823	97.391	100	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	*Mrakia*	*Mrakia frigida*	*Mrakia frigida*	species	AACGCACCTTGCGCTCCTTGGT	230	Mrakia	Saprotroph	Soil Saprot	Yeast	
Otu023	92173	98.696	100	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	*Mrakia*	*Mrakia niccombsii*	*Mrakia aquatica*	species	AACGCACCTTGCGCTCCTTGGT	327	Mrakia	Saprotroph	Soil Saprot	Yeast	
Otu105	3024	100	100	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	*Mrakia*		*Mrakia*	genus	AACGCACCTTGCGCTCCTTGGT	327	Mrakia	Saprotroph	Soil Saprot	Yeast	
Otu059	11886	100	100	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	*Tausonia*	*Tausonia pullulans*	*Tausonia pullulans*	species	AACGCACCTTGCGCTCCTTGGT	314	-	-	-	-	
Otu147	1170	99.13	100	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Filobasidium*	*Filobasidium oeirense*	*Filobasidium oeirense*	species	AACGCACCTTGCGCTCCTTGGT	336	Filobasidiu	Saprotroph	Undefined	Facultative Yeast	
Otu043	22000	100	100	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Goffeauzyma*	*Goffeauzyma gilvescens*	*Goffeauzyma gilvescens*	species	AACGCACCTTGCACTCTTTGGT	337	Cryptococc	Pathotroph	Animal Pat	Yeast	
Otu039	30434	100	100	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Naganishia*	*Naganishia adeliensis*	*Naganishia adeliensis*	species	AACGCACCTTGCGCTCCTTGGT	314	Cryptococc	Pathotroph	Animal Pat	Yeast	
Otu466	5245	99.13	100	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Naganishia*	*Naganishia friedmannii*	*Naganishia friedmannii*	species	AACGCACCTTGCGCTCCTTGGT	314	Cryptococc	Pathotroph	Animal Pat	Yeast	
Otu161	24004	98.659	100	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Naganishia*	*Naganishia liquefaciens*	*Naganishia liquefaciens*	species	AACGCACCTTGCGCTCCCTGGT	314	Cryptococc	Pathotroph	Animal Pat	Yeast	
Otu068	10945	99.13	100	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Naganishia*	*Naganishia randhawae*	*Naganishia randhawae*	species	AACGCACCTTGCGCTCCTTGGT	316	Cryptococc	Pathotroph	Animal Pat	Yeast	
Otu155	1289	99.565	100	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	*Solicoccozyma*	*Solicoccozyma terricola*	*Solicoccozyma terricola*	species	AACGCACCTTGCGCTCTTTGGT	329	-	-	-	-	
Otu127	1151	100	100	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	*Vishniacozyma*	*Vishniacozyma dimennae*	*Vishniacozyma dimennae*	species	AACGCACCTTGCGCCCTCT	247	-	-	-	-	
Otu118	1920	100	100	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	*Vishniacozyma*	*Vishniacozyma tephrensis*	*Vishniacozyma tephrensis*	species	AACGCACCTTGCGCCCTTTGGT	235	-	-	-	-	
Otu374	954	98.261	100	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	*Cryptococcus*		*Cryptococcus*	genus	AACGCACCTTGCGCTCCCTGGT	230	Cryptococc	Pathotroph	Animal Pat	Yeast	
Otu252	111	100	99	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	*Tremella*	*Tremella diploschistina*	*Tremella diploschistina*	species	AACGCACCTTGCGCCCTCT	235	Tremella	Pathotroph	Fungal Parasite-Lichen Parasite		
Otu405	26	100	100	Basidiomycota	Wallemiomycetes	Wallemiales	Wallemiaceae	*Wallemia*	*Wallemia sebi*	*Wallemia sebi*	species	AACGCAAATGGCACTCTATGGT	230	Wallemia	Saprotroph	Undefined	Saprotroph	
Otu225	138	95.299	100	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*		*Mortierella*	genus	AACGCATATTGCGCTCTTTGGT	344	Mortierella	Saprotroph	Endophyte	Microfungus	
Otu100	2840	100	100	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*Mucor hiemalis*	*Mucor hiemalis*	species	AACGCAACTTGCGCTCAATGGT	272	Mucor	Saprotroph	Undefined	-	
Otu124	2080	95.595	99	Neocallimastigomycota	Neocallimastigomycetes	Neocallimastigales	Neocallimastigaceae	*Piromyces*		*Piromyces*	genus	AACGCATATTGCACTTTTTTAGT	327	Piromyces	Symbiotrop	Animal Enc	-	

Table S14. fungal nrITS2 sequence average read counts and relative read abundance per sample

Any OTU with <0.01% relative read abundance is shown as a +

% represents the Relative Read Abundance

			Wolly mammoth										Horse				Bison		Caribou						Controls	
			Abyland		Adycha		Cape Blossom		Maly Lyakhovsky		Yukagir		Yukon		Oyogas Yar		Yakutian		Selwyn C		Selwyn B		Selwyn A		Negative	
OTU	Total read maxid		Average read c	%	Average read	%	Average read	%	Average reac	%	Average read	%	Average read	%	Average reac	%	Average read	%	Average re:	%	Average read coi	%	Average read	%	Reads	%
Otu001	890607	*Arachnomyces*	0	0	284467.33	89.33	1069.67	0.59	11324.00	26.52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu002	520344	*Pseudeurotium hygrophilum*	8077.33	4.06	0	0	0	0	0	0	0	0	0	0	112018.67	48.76	27415.33	36.62	0	0	6249.67	4.08	0	0	0	0
Otu003	357130	Ascobolaceae	113036.00	56.86	0	0	484.33	0.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu004	343946	*Candida zeylanoides*	0	0	0	0	1155.33	0.64	0	0	0	0	0	0	109434.67	47.64	1127.33	1.51	0	0	0	0	0	0	0	0
Otu005	279349	*Chrysosporium merdarium*	443.33	0.22	0	0	0	0	0	0	81177.00	60.51	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu006	818163	*Thelebolus globosus*	0	0	15046.00	4.72	84164.33	46.60	9736.33	22.80	0	0	1039.00	1.15	0	0	0	0	159.67	0.20	43148.67	28.20	99994.00	67.80	0	0
Otu007	250160	*Aspergillus versicolor*	0	0	0	0	0	0	0	0	0	0	83386.67	91.98	0	0	0	0	0	0	0	0	0	0	0	0
Otu008	253365	*Cadophora luteo-olivacea*	0	0	0	0	280.67	0.16	0	0	5.00	+	0	0	0	0	0	0	1794.00	2.22	82001.00	53.59	0	0	0	0
Otu009	120543	*Chrysosporium pseudomerdarium*	0	0	0	0	615.33	0.34	0	0	28429.00	21.19	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu010	90857	*Cladosporium*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12861.33	15.95	0	0	0	0	0	0
Otu011	80413	*Cosmospora viridescens*	0	0	0	0	0	0	0	0	24504.00	18.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu012	78660	*Coniochaeta hoffmannii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26220.00	35.02	0	0	0	0	0	0	0	0
Otu013	139431	*Sebacina*	46065.67	23.17	0	0	190.00	0.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu015	136505	*Preussia*	0	0	45.00	0.01	0	0	1330.00	3.11	0	0	465.00	0.51	0	0	0	0	0	0	0	0	35567.33	24.11	0	0
Otu016	154914	Malasseziales	0	0	0	0	0	0	6046.33	14.16	0	0	0	0	0	0	0	0	4981.67	6.18	0	0	0	0	0	0
Otu022	106644	*Penicillium aethiopicum*	0	0	0	0	0	0	0	0	0	0	2481.67	2.74	0	0	0	0	153.00	0.19	0	0	0	0	0	0
Otu023	92173	*Mrakia aquatica*	0	0	0	0	30724.33	17.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu024	36256	Microbotryomycetes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12085.33	14.98	0	0	0	0	0	0
Otu025	33058	*Protomyces inouyei*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11019.33	13.66	0	0	0	0	0	0
Otu026	41022	*Arthrobotrys superba*	0	0	13119.00	4.12	56.00	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu028	27752	*Dactylella*	9157.33	4.61	0	0	49.00	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu029	28397	*Symmetrospora gracilis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9431.33	11.69	0	0	0	0	0	0
Otu033	27109	Dothideomycetes1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9036.33	11.20	0	0	0	0	0	0
Otu035	22353	*Didymella microchlamydospora*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7451.00	9.24	0	0	0	0	0	0
Otu036	22519	*Cladophialophora minutissima*	0	0	0	0	0	0	0	0	0	0	0	0	33.00	+	7366.00	9.84	0	0	100.67	0.06	0	0	0	0
Otu037	21827	*Mycosphaerella*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7275.67	9.02	0	0	0	0	0	0
Otu039	30434	*Naganishia adeliensis*	0	0	0	0	10144.67	5.62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu040	32284	Microascales	534.00	0.27	0	0	0	0	9682.33	22.68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu043	22000	*Goffeauzyma gilvescens*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7299.67	4.77	0	0	0	0
Otu046	66149	*Schizothecium carpinicola*	17663.33	8.88	0	0	4244.33	2.35	0	0	0	0	0	0	0	0	140.00	0.19	0	0	0	0	0	0	0	0
Otu047	19764	*Lachnellula calyciformis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6545.67	4.28	0	0	0	0
Otu048	36809	*Mrakia blollopis*	121.00	0.06	0	0	400.67	0.22	0	0	0	0	0	0	2436.67	1.06	0	0	0	0	0	0	0	0	0	0
Otu049	16571	*Sphaerulina hyperici*	0	0	0	0	0	0	0	0	0	0	0	0	28.00	+	5495.67	7.34	0	0	0	0	0	0	0	0
Otu053	15522	*Cadophora*	0	0	0	0	0	0	0	0	0	0	0	0	5174.00	2.25	0	0	0	0	0	0	0	0	0	0
Otu054	12873	Leucosporidiales	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4291.00	5.32	0	0	0	0	0	0
Otu058	28457	*Cystobasidium minuta*	125.33	0.06	0	0	0	0	0	0	0	0	36.33	0.04	0	0	0	0	0	0	3594.33	2.35	4231.33	2.87	0	0
Otu059	11886	*Tausonia pullulans*	0	0	0	0	3962.00	2.19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu061	8921	*Leucosporidium creatinivorum*	0	0	0	0	2973.67	1.65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu062	10013	*Cystofilobasidium infirmominiatum*	0	0	0	0	3337.67	1.85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu063	14676	*Schizothecium*	0	0	0	0	4892.00	2.71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu064	9123	*Patinella hyalophaea*	0	0	0	0	0	0	0	0	0	0	12.00	0.01	0	0	0	0	0	0	3005.67	1.96	0	0	0	0
Otu065	8096	Dothideomycetes2	0	0	0	0	0	0	0	0	0	0	36.67	0.04	0	0	0	0	0	0	0	0	2662.00	1.80	0	0
Otu067	7372	*Orbilia*	0	0	2439.00	0.77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu068	10945	*Naganishia randhawae*	0	0	0	0	3648.33	2.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu069	6029	Herpotrichiellaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2009.67	2.68	0	0	0	0	0	0	0	0
Otu071	6150	*Fusariella hughesii*	2028.67	1.02	0	0	12.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu072	5338	Helotiales1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1779.33	2.38	0	0	0	0	0	0	0	0
Otu079	8935	*Pseudogymnoascus roseus*	0	0	1703.67	0.54	0	0	1244.67	2.92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu085	8454	Sordariales1	0	0	0	0	2818.00	1.56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu091	4910	*Ascobolus equinus*	0	0	0	0	0	0	0	0	0	0	26.00	0.02	0	0	0	0	0	0	44.67	0.03	1561.00	1.06	0	0
Otu095	3062	*Stagonospora trichophoricola*	0	0	0	0	0	0	0	0	0	0	0	0	5.33	+	1015.33	1.36	0	0	0	0	0	0	0	0
Otu096	3130	*Coniothyrium*	0	0	5.33	+	0	0	1034.00	2.42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu098	3933	*Preussia flanaganii*	0	0	20.33	+	1290.00	0.71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu099	2638	*Microdochium*	0	0	869.00	0.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu100	2840	*Mucor hiemalis*	0	0	0	0	0	0	350.00	0.82	0	0	596.67	0.66	0	0	0	0	0	0	0	0	0	0	0	0
Otu101	2341	*Pithoascus ater*	0	0	206.67	0.06	0	0	573.67	1.34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu103	2094	*Coprinopsis kubickae*	690.33	0.35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu105	3024	*Mrakia*	0	0	0	0	955.33	0.53	0	0	0	0	0	0	0	0	0	0	0	0	52.67	0.03	0	0	0	0
Otu107	13678	*Podospora*	0	0	0	0	4549.00	2.52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu110	3915	*Podospora pleiospora*	0	0	0	0	1299.67	0.72	0	0	5.33	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu115	1775	*Cystofilobasidium macerans*	0	0	0	0	586.00	0.32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu117	2997	*Preussia tetramera*	0	0	4.00	+	990.00	0.55	0	0	0	0	5.00	+	0	0	0	0	0	0	0	0	0	0	0	0
Otu118	1920	*Vishniacozyma tephrensis*	0	0	0	0	640.00	0.35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu126	1223	*Paraphoma fimeti*	0	0	0	0	0	0	0	0	0	0	0	0	407.67	0.18	0	0	0	0	0	0	0	0	0	0
Otu127	1151	*Vishniacozyma dimennae*	0	0	0	0	0	0	380.00	0.89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu135	8913	*Sporormiella intermedia*	0	0	0	0	0	0	7.33	+	0	0	16.67	0.01	0	0	0	0	0	0	0	0	2906.00	1.97	0	0
Otu140	1432	*Antarctomyces psychrotrophicus*	5.33	+	18.67	+	0	0	303.67	0.71	0	0	0	0	0	0	0	0	0	0	89.00	0.05	56.00	0.04	0	0
Otu144	792	*Orbilia rectispora*	258.33	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu145	846	*Chrysosporium synchronum*	0	0	279.33	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu147	1170	*Filobasidium oeirense*	0	0	0	0	390.00	0.22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu153	1115	*Thelebolus*	0	0	0	0	357.67	0.20	0	0	0	0	0	0	0	0	0	0	0	0	4.33	+	0	0	0	0
Otu154	9134	*Aureobasidium pullulans*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2286.67	3.05	0	0	0	0	0	0	0	0

Otu155	1289	*Solicoccozyma terricola*	0	0	0	0	427.33	0.24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu161	24004	*Naganishia liquefaciens*	0	0	0	0	8001.33	4.43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu166	1466	*Apodus deciduus*	0	0	0	0	486.67	0.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu172	748	*Inocybe*	0	0	0	0	215.67	0.12	0	0	0	0	0	0	0	0	0	0	0	0	32.33	0.02	0	0	0	0
Otu173	442	*Neosetophoma rosarum*	143.33	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu177	843	*Cystobasidium pinicola*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	141.00	0.09	120.33	0.08	0	0
Otu180	455	*Dactylellina cionopaga*	147.00	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu181	1468	*Leucosporidium fragarium*	0	0	0	0	489.33	0.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu188	336	*Cheilymenia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	110.00	0.14	0	0	0	0	0	0
Otu192	383	*Pseudeurotium*	0	0	0	0	0	0	0	0	0	0	0	0	125.00	+	0	0	0	0	0	0	0	0	0	0
Otu194	2080	*Piromyces*	32.00	+	120.00	0.03	48.00	0.03	443.33	1.04	23.67	0.02	0	0	0	0	0	0	0	0	4.00	+	0	0	0	0
Otu195	295	*Bovista plumbea*	97.00	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu197	293	*Psathyrella ammophila*	0	0	0	0	0	0	97.67	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu209	186	*Tetracladium*	57.67	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu212	167	*Conocybe lenticulospora*	0	0	0	0	0	0	54.67	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu217	421	*Cladonia mitis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	137.67	0.09	0	0	0	0
Otu220	158	*Paraconiothyrium sporulosum*	0	0	0	0	0	0	52.33	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu221	216	Pyronemataceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17.33	0.01	52.00	0.04	0	0
Otu222	3619	*Penicillium paradoxum*	0	0	0	0	0	0	0	0	0	0	1206.33	1.33	0	0	0	0	0	0	0	0	0	0	0	0
Otu223	7823	*Mrakia frigida*	0	0	0	0	2607.67	1.44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu225	138	*Mortierella*	0	0	0	0	0	0	0	0	0	0	0	0	45.33	+	0	0	0	0	0	0	0	0	0	0
Otu233	140	*Taphrina carpini*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44.33	0.03	0	0	0	0
Otu237	391	*Ramophialophora humicola*	32.33	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	91.67	0.06	0	0
Otu239	309	*Cladonia stellaris*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	66.00	0.04	35.67	0.02	0	0
Otu242	163	*Peziza ampliata*	45.67	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu243	459	*Cladonia rangiferina*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	142.33	0.09	4.00	+	0	0
Otu246	97	Phaeosphaeriaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30.33	0.02	0	0
Otu252	111	*Tremella diploschistina*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36.00	0.02	0	0	0	0
Otu253	92	*Phaeosphaeriopsis*	0	0	27.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu254	99	*Collophora*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31.67	0.02	0	0	0	0
Otu269	111	*Sporormiella vexans*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34.00	0.02	0	0
Otu271	68	*Phoma herbarum*	0	0	0	0	0	0	0	0	0	0	0	0	22.00	+	0	0	0	0	0	0	0	0	0	0
Otu274	60	*Entoloma*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19.33	0.01	0	0	0	0
Otu282	144	*Cleistothelebolus nipigonensis*	0	0	28.33	+	0	0	16.33	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu283	62	Helotiales2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19.33	0.01	0	0	0	0
Otu285	63	Sordariales2	20.67	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu286	67	Coniochaetales	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20.33	0.01	0	0
Otu287	98	*Capronia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.00	+	0	0	25.67	0.02	0	0	0	0
Otu288	94	Dothideomycetes3	17.67	+	6.33	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.00	+	0	0	0	0
Otu297	101	Dothideomycetes4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32.00	0.02	0	0
Otu299	124	*Cladonia submitis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37.33	0.02	0	0	0	0
Otu301	78	Lasiosphaeriaceae	0	0	14.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.33	+	0	0	0	0
Otu311	42	*Sporormiella*	0	0	12.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu312	44	Verrucariales1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14.33	+	0	0	0	0
Otu315	69	Hyaloscyphaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21.00	0.01	0	0
Otu319	48	*Sordaria fimicola*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15.67	0.01	0	0
Otu320	53	Verrucariales2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16.33	0.01	0	0	0	0
Otu331	42	Onygenales	0	0	0	0	0	0	11.00	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu335	39	*Stereocaulon saxatile*	4.67	+	0	0	0	0	0	0	4.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu341	27	*Cheilymenia stercorea*	0	0	0	0	0	0	9.00	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu343	24	*Microcalicium ahlneri*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.67	+	0	0	0	0
Otu347	54	Verrucariales3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16.67	0.01	0	0	0	0
Otu352	17	Helotiales3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.00	+	0	0	0	0	0	0	0	0
Otu354	32	Coniochaetaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.67	+	0	0
Otu355	17	Verrucariales4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.33	+	0	0
Otu357	25	*Cystobasidium psychroaquaticum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.33	+	0	0
Otu359	23	*Cladophialophora*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.67	+	0	0
Otu360	28	*Calophoma sandfjordenica*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.00	+	0	0	0	0
Otu373	23	*Byssonectria deformis*	4.67	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu374	954	*Cryptococcus*	0	0	0	0	318.00	0.18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu377	11	*Plenodomus biglobosus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.67	+	0	0	0	0
Otu382	18	*Sporormiella leporina*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.67	+	0	0
Otu383	28	*Preussia longisporopsis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.33	+	0	0
Otu386	23	*Cladonia cornuta*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.00	+	0	0	0	0
Otu392	17	*Preussia minipascua*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.67	+	0	0
Otu393	10	*Phylliscum demangeonii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.33	+	0	0	0	0
Otu396	4068	*Penicillium melinii*	0	0	0	0	0	0	0	0	0	0	1350.00	1.49	0	0	0	0	0	0	0	0	6.00	+	0	0
Otu405	26	*Wallemia sebi*	5.33	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu445	14	*Bryocaulon divergens*	0	0	4.00	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu452	13	*Oidiodendron cereale*	0	0	4.00	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Otu466	5245	*Naganishia friedmannii*	0	0	0	0	1719.00	0.95	0	0	0	0	0	0	0	0	0	0	0	0	29.33	0.02	0	0	0	0

Multiproxy analysis of permafrost preserved faeces provides an unprecedented insight into the diets and habitats of extinct and extant megafauna

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Supporting Information (3/3)

Contents

S15. Taxonomic resolution nrITS primers

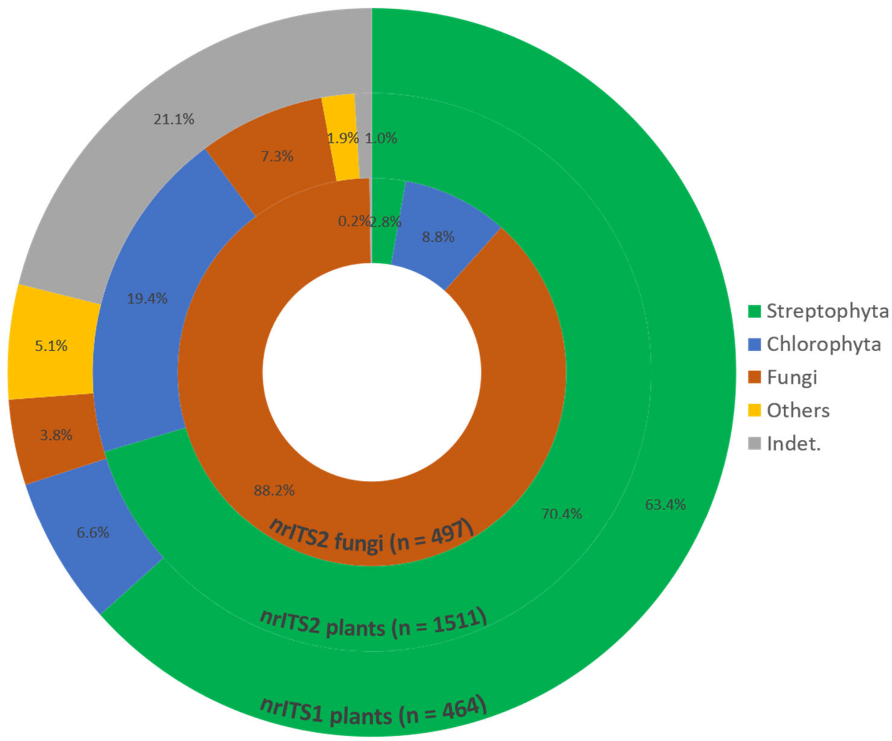

Figure S15. Taxonomic results of the three nrITS markers for all samples. Numbers represent the percentage of OTUs that were assigned to the different clades. The group Others contains Bacteria, Eukaryota and Alveolata. N = number of OTUs found.

S16. Sample read and OTU numbers

f_nrITS2 = fungal nrITS2

Sample	Age (kyr)	Average read counts				no. of OTUs			
		*trn*L	nrITS1	nrITS2	f_nrITS2	*trn*L	nrITS1	nrITS2	f_nrITS2
Selwyn A	0	6.4E+05	1.1E+05	1.6E+05	1.5E+05	56	28	40	26
Selwyn B	±1.5	3.6E+05	1.9E+05	1.0E+05	1.5E+05	30	18	17	37
Selwyn C	±2.7	1.3E+05	0	0	8.1E+04	23	0	0	13
Oyogas Yar	±5.4	1.8E+05	6.9E+02	5.4E+04	2.3E+05	12	11	16	11
Yakutian bison	±10.5	3.3E+05	8.6E+04	1.3E+05	7.5E+04	15	14	19	12
Cape Blossom	±14.4	4.7E+05	0	0	1.8E+05	44	0	0	38
Yukagir	±22.5	4.3E+05	5.8E+04	8.7E+04	1.3E+05	47	14	12	7
Adycha	±25.6	4.0E+05	1.8E+05	1.2E+05	3.2E+05	18	9	10	21
Yukon horse	±30.9	4.7E+04	0	0	9.1E+04	21	0	0	13
Abyland	±32.4	4.7E+05	6.2E+03	5.0E+04	2.0E+05	74	10	8	25
Maly Lyakhovsky	±32.7	4.0E+05	7.6E+04	3.3E+04	4.3E+04	47	15	15	19

S17. Species habitat types

Table S17. Habitat types of all species and some genera for which clear habitat preference were identified. The habitat types used are steppe, dry disturbed sites, meadow (dry), meadow (saline), mountainous/rocks, tundra (arctic/alpine), snow patches, gravelly slopes, woods (dry), woods (wet), meadow (wet), wetland (along lakes, ponds, streams, rivers) and wetland (marsh, bog, fen, swamp).

Family	Taxon	DNA	Macro	Pollen	Selwyn caribou A	Selwyn caribou B	Selwyn caribou C	Oyogas Yar horse	Yakutian Bison	Cape Blossom mammoth	Yukagir mammoth	Adycha mammoth	Yukon horse	Abyland mammoth	Maly Lyakhovsky	Habitat type
Adoxaceae	*Sambucus williamsii*								X							Gravelly slopes
Amaranthaceae	*Blitum nuttallianum*						X									Dry disturbed site
Amblystegiaceae	*Calliergon cf. giganteum*								X							Wetland (marsh, bog, fen, swamp)
Amblystegiaceae	*Campylium stellatum*							X							X	Wetland (marsh, bog, fen, swamp)
Amblystegiaceae	*Cratoneuron filicinum*														X	Wetland (marsh, bog, fen, swamp)
Amblystegiaceae	*Drepanocladus aduncus*										X					Wetland (marsh, bog, fen, swamp)
Amblystegiaceae	*Drepanocladus sordidus*														X	Wetland (marsh, bog, fen, swamp)
Amblystegiaceae	*Sanionia uncinata*				X											Woods (wet)
Anastrophyllaceae	*Barbilophozia barbata*				X	X										Mountainous/rocks
Apiaceae	*Cicuta virosa*							X	X							Wetland (marsh, bog, fen, swamp)
Apiaceae	*Cymopterus sessiliflorus*						X									Gravelly slopes
Apiaceae	*Thalictrum*													X		Meadow (wet)
Asteraceae	*Artemisia*				X	X	X	X	X	X	X	X	X	X	X	Meadow (dry)
Asteraceae	*Artemisia gmelinii*						X			X	X		X	X		Steppe
Asteraceae	*Artemisia norvegica*				X	X										Tundra (arctic/alpine)
Asteraceae	*Artemisia scoparia*							X			X	X		X		Steppe
Asteraceae	*Endocellion sibiricum*							X	X							Wetland (along lakes, ponds, streams,
Asteraceae	*Tripleurospermum*													X		Meadow (saline)
Aulacomniaceae	*Aulacomnium palustre*				X											Wetland (marsh, bog, fen, swamp)
Bartramiaceae	*Philonotis cf. arnellii*														X	Mountainous/rocks
Betulaceae	*Alnus crispa*												X			Wetland (along lakes, ponds, streams,
Betulaceae	*Alnus incana*												X			Wetland (along lakes, ponds, streams,
Boraginaceae	*Eritrichium*									X	X	X		X		Gravelly slopes
Boraginaceae	*Eritrichium sericeum*										X					Steppe
Boraginaceae	*Mertensia paniculata*				X		X			X				X		Woods (wet)
Boraginaceae	*Myosotis alpestris*									X	X			X		Meadow (dry)
Brachytheciaceae	*Tomentypnum nitens*				X											Wetland (marsh, bog, fen, swamp)
Brassicaceae	*Arabidopsis lyrata*														X	Tundra (arctic/alpine)
Brassicaceae	*Braya rosea*												X			Gravelly slopes
Brassicaceae	*Eutrema edwardsii*														X	Gravelly slopes
Brassicaceae	*Parrya nudicaulis*										X					Tundra (arctic/alpine)
Brassicaceae	*Sisymbrium linifolium*													X		Gravelly slopes
Brassicaceae	*Smelowskia alba*										X					Gravelly slopes
Bryaceae	*Ptychostomum pallescens*				X											Wetland (along lakes, ponds, streams,
Calliergonaceae	*Warnstorfia sarmentosa*														X	Wetland (marsh, bog, fen, swamp)
Caryophyllaceae	*Cerastium arvense*										X			X		Meadow (dry)
Caryophyllaceae	*Cerastium maximum*													X		Meadow (dry)
Caryophyllaceae	*Eremogone capillaris*										X			X		Meadow (dry)
Caryophyllaceae	*Minuartia rubella*									X						Gravelly slopes
Caryophyllaceae	*Sagina nivalis*										X					Gravelly slopes
Caryophyllaceae	*Silene samojedorum*													X		Steppe
Caryophyllaceae	*Stellaria*					X	X					X		X	X	Meadow (wet)
Caryophyllaceae	*Stellaria borealis*													X	X	Meadow (wet)

Family	Taxon	DNA	Macro	Pollen	Selwyn caribou A	Selwyn caribou B	Selwyn caribou C	Oyogas Yar horse	Yakutian Bison	Cape Blossom mammoth	Yukagir mammoth	Adycha mammoth	Yukon horse	Abyland mammoth	Maly Lyakhovsky	Habitat type
Caryophyllaceae	*Stellaria longifolia*														X	Meadow (wet)
Crassulaceae	*Rhodiola integrifolia*				X									X		Mountainous/rocks
Crassulaceae	*Rhodiola rosea*										X				X	Mountainous/rocks
Cyperaceae	*Carex aquatilis*							X	X	X						Wetland (marsh, bog, fen, swamp)
Cyperaceae	*Carex chordorrhiza*								X							Wetland (marsh, bog, fen, swamp)
Cyperaceae	*Carex dioica*										X					Wetland (marsh, bog, fen, swamp)
Cyperaceae	*Carex duriuscula*													X		Steppe
Cyperaceae	*Carex lachenalii*					X										Tundra (arctic/alpine)
Cyperaceae	*Carex maritima*									X		X				Meadow (saline)
Cyperaceae	*Carex microchaeta*				X					X						Wetland (marsh, bog, fen, swamp)
Cyperaceae	*Carex nardina*										X					Tundra (arctic/alpine)
Cyperaceae	*Carex nigra subsp. juncea*					X			X		X			X	X	Meadow (wet)
Cyperaceae	*Carex podocarpa*				X											Tundra (arctic/alpine)
Cyperaceae	*Carex rostrata*							X	X							Wetland (marsh, bog, fen, swamp)
Cyperaceae	*Carex vesicaria*								X							Wetland (marsh, bog, fen, swamp)
Cyperaceae	*Eriophorum*					X	X	X	X						X	Wetland (marsh, bog, fen, swamp)
Cyperaceae	*Eriophorum angustifolium*							X	X						X	Wetland (marsh, bog, fen, swamp)
Dicranaceae	*Dicranum bonjeanii*														X	Wetland (marsh, bog, fen, swamp)
Dicranaceae	*Dicranum fuscescens*				X	X										Woods (wet)
Ditrichaceae	*Ceratodon purpureus*															n/a (various)
Elaeagnaceae	*Shepherdia canadensis*					X	X									Woods (dry)
Entodontaceae	*Entodon concinnus*										X					Meadow (dry)
Ericaceae	*Arctostaphylos uva-ursi*				X											Woods (dry)
Ericaceae	*Arctous alpina*				X											Tundra (arctic/alpine)
Ericaceae	*Arctous alpina/rubra*				X	X										Tundra (arctic/alpine)
Ericaceae	*Arctous rubra*				X											Tundra (arctic/alpine)
Ericaceae	*Cassiope tetragona*				X											Tundra (arctic/alpine)
Ericaceae	*Empetrum nigrum*				X											Tundra (arctic/alpine)
Ericaceae	*Pyrola grandifolia*				X	X							X			Tundra (arctic/alpine)
Ericaceae	*Vaccinium uliginosum*				X	X										Tundra (arctic/alpine)
Ericaceae	*Vaccinium vitis-idaea*				X	X		X				X				Tundra (arctic/alpine)
Fabaceae	*Astragalus alpinus*										X	X				Tundra (arctic/alpine)
Fabaceae	*Oxytropis deflexa*										X				X	Meadow (dry)
Fabaceae	*Oxytropis splendens*										X					Meadow (dry)
Funariaceae	*Funaria* sp.											X			X	Dry disturbed sites
Grimmiaceae	*Niphotrichum*				X											Mountainous/rocks
Hylocomiaceae	*Hylocomiastrum pyrenaicum*				X											Wetland (along lakes, ponds, streams,
Hylocomiaceae	*Hylocomium splendens*				X	X						X				Woods (wet)
Hylocomiaceae	*Pleurozium schreberi*				X	X										Woods (dry)
Juncaceae	*Juncus*					X	X				X		X	X		Wetland (along lakes, ponds, streams,
Juncaceae	*Juncus alpinoarticulatus*					X							X	X		Meadow (wet)
Juncaceae	*Juncus biglumis*											X			X	Wetland (along lakes, ponds, streams,
Juncaceae	*Juncus effusus*					X										Meadow (wet)
Juncaceae	*Juncus oxymeris*					X										Wetland (along lakes, ponds, streams,
Juncaginaceae	*Triglochin palustris*					X										Wetland (marsh, bog, fen, swamp)
Liliaceae	*Gagea serotina*				X	X										Mountainous/rocks
Menyanthaceae	*Menyanthes trifoliata*				X			X	X	X	X			X	X	Wetland (marsh, bog, fen, swamp)
Mniaceae	*Cinclidium stygium*														X	Wetland (marsh, bog, fen, swamp)
Mniaceae	*Plagiomnium cf. ellipticum*							X								Wetland (marsh, bog, fen, swamp)
Mniaceae	*Rhizomnium cf.*							X								Wetland (marsh, bog, fen, swamp)
Onagraceae	*Chamaenerion angustifolium*				X		X			X		X		X		Meadow (wet)

Family	Taxon	DNA	Macro	Pollen	Selwyn caribou A	Selwyn caribou B	Selwyn caribou C	Oyogas Yar horse	Yakutian Bison	Cape Blossom mammoth	Yukagir mammoth	Adycha mammoth	Yukon horse	Abyland mammoth	Maly Lyakhovsky	Habitat type
Onagraceae	*Chamaenerion latifolium*	■														Wetland (along lakes, ponds, streams,
Onagraceae	*Epilobium palustre*	■			X			X	X							Wetland (along lakes, ponds, streams,
Orobanchaceae	*Pedicularis capitata*	■			X											Meadow (wet)
Orobanchaceae	*Pedicularis sudetica*	■			X	X		X			X		X	X	X	Meadow (wet)
Orobanchaceae	*Pedicularis verticillata*	■												X		Meadow (wet)
Plantaginaceae	*Hippuris*	■						X	X							Wetland (marsh, bog, fen, swamp)
Plantaginaceae	*Plantago media/canescens*	■								X	X			X		Meadow (wet)
Plantaginaceae	*Veronica wormskjoldii*	■			X											Woods (wet)
Plumbaginaceae	*Armeria-type*			■							X					Meadow (saline)
Poaceae	*Alopecurus magellanicus*	■			X							X		X	X	Meadow (wet)
Poaceae	*Arctagrostis latifolia*	■				X		X				X				Wetland (along lakes, ponds, streams,
Poaceae	*Arctophila fulva*	■			X			X	X						X	Wetland (along lakes, ponds, streams,
Poaceae	*Arctophila fulva/Dupontia*	■						X							X	Wetland (along lakes, ponds, streams,
Poaceae	*Bromus pumpellianus*	■								X		X	X	X	X	Meadow (dry)
Poaceae	*Calamagrostis stricta*	■						X								Wetland (marsh, bog, fen, swamp)
Poaceae	*Deschampsia cespitosa*	■			X						X	X		X	X	Meadow (wet)
Poaceae	*Dupontia fisheri*	■						X	X			X			X	Wetland (along lakes, ponds, streams,
Poaceae	*Festuca altaica*	■			X									X	X	Gravelly slopes
Poaceae	*Festuca kolymensis*	■								X	X			X		Steppe
Poaceae	*Festuca ovina*	■									X					Steppe
Poaceae	*Koeleria asiatica*	■								X				X		Meadow (dry)
Poaceae	*Pleuropogon sabinei*	■									X				X	Wetland (marsh, bog, fen, swamp)
Poaceae	*Poa arctica*	■				X		X				X				Meadow (wet)
Poaceae	*Poa glauca*	■			X						X					Meadow (dry)
Poaceae	*Puccinellia*	■										X			X	Meadow (saline)
Poaceae	*Puccinellia tenuiflora / vahliana*	■										X		X		Meadow (saline)
Poaceae	*Puccinellia vahliana*	■										X			X	Meadow (saline)
Polemoniaceae	*Phlox hoodii*			■							X					Steppe
Polemoniaceae	*Polemonium boreale*			■						X						Gravelly slopes
Polygonaceae	*Bistorta vivipara*	■			X	X	X									Tundra (arctic/alpine)
Polygonaceae	*Oxyria digyna*	■			X		X									Snow patches
Polygonaceae	*Rumex acetosella*		■								X					Dry disturbed sites
Polygonaceae	*Rumex aquaticus-type*			■						X						Wetland (along lakes, ponds, streams,
Polytrichaceae	*Polytrichastrum alpinum*	■	■		X			X			X				X	Woods (dry)
Polytrichaceae	*Polytrichum cf. strictum*	■	■		X											Wetland (marsh, bog, fen, swamp)
Polytrichaceae	*Polytrichum commune*	■	■		X											Woods (wet)
Polytrichaceae	*Polytrichum juniperinum*	■			X											Woods (dry)
Polytrichaceae	*Polytrichum piliferum*	■			X	X										Tundra (arctic/alpine)
Potamogetonaceae	*Stuckenia*	■									X			X		Wetland (marsh, bog, fen, swamp)
Pottiaceae	*Barbula unguiculata*	■												X		n/a (various)
Pottiaceae	*Didymodon icmadophilus*	■												X	X	n/a (various)
Primulaceae	*Androsace lehmanniana*	■									X					Mountainous/rocks
Primulaceae	*Androsace septentrionalis*		■										X			Meadow (dry)
Primulaceae	*Primula frigida*	■			X											Meadow (wet)
Ranunculaceae	*Anemonastrum narcissiflora*	■			X	X	X				X		X	X	X	Tundra (arctic/alpine)
Ranunculaceae	*Anemone patens*	■			X				X					X	X	Meadow (dry)
Ranunculaceae	*Anemone richardsonii*	■			X											Tundra (arctic/alpine)
Ranunculaceae	*Caltha palustris*	■	■		X			X	X	X	X			X	X	Wetland (marsh, bog, fen, swamp)
Ranunculaceae	*Ranunculus nivalis*	■					X									Snow patches
Ranunculaceae	*Ranunculus pedatifidus var.*	■									X				X	Meadow (dry)

Family	**Taxon**	**DNA**	**Macro**	**Pollen**	**Selwyn caribou A**	**Selwyn caribou B**	**Selwyn caribou C**	**Oyogas Yar horse**	**Yakutian Bison**	**Cape Blossom mammoth**	**Yukagir mammoth**	**Adycha mammoth**	**Yukon horse**	**Abyland mammoth**	**Maly Lyakhovsky**	**Habitat type**
Ranunculaceae	*Ranunculus pygmaeus*						X									Snow patches
Ranunculaceae	*Ranunculus trichophyllus*					X										Wetland (along lakes, ponds, streams,
Rosaceae	*Comarum palustre*				X	X		X	X	X				X	X	Wetland (marsh, bog, fen, swamp)
Rosaceae	*Dryas*				X	X	X									Tundra (arctic/alpine)
Rosaceae	*Dryas octopetala*				X											Tundra (arctic/alpine)
Rosaceae	*Geum aleppicum*				X	X										Wetland (along lakes, ponds, streams,
Rosaceae	*Potentilla hookeriana*										X					Mountainous/rocks
Rosaceae	*Potentilla hyparctica*										X					Gravelly slopes
Rosaceae	*Rubus arcticus*				X											Tundra (arctic/alpine)
Rosaceae	*Sanguisorba officinalis*									X	X		X	X		Meadow (wet)
Rosaceae	*Sibbaldia procumbens*						X									Snow patches
Rosaceae	*Spiraea stevenii*				X											Meadow (wet)
Salicaceae	*Salix alaxensis*					X										Woods (wet)
Saxifragaceae	*Micranthes*				X		X				X				X	Tundra (arctic/alpine)
Saxifragaceae	*Micranthes nelsoniana*						X									Tundra (arctic/alpine)
Saxifragaceae	*Saxifraga sibirica*											X			X	Gravelly slopes
Scapaniaceae	*Douinia ovata*				X											Mountainous/rocks
Sphagnaceae	*Sphagnum*					X	X	X	X	X						Wetland (marsh, bog, fen, swamp)
Sphagnaceae	*Sphagnum cf. magellanicum*				X											Wetland (marsh, bog, fen, swamp)
Taxaceae	*Taxus canadensis*					X										Woods (wet)
Thuidiaceae	*Thuidium abietinum*									X						Meadow (dry)
Violaceae	*Viola epipsila var. repens*				X											Wetland (marsh, bog, fen, swamp)

S18. Lichen phycobionts

Identified using plant nrITS2 (only showing Selwyn Caribou samples, as no phycobionts were identified in any of the other samples, or using nrITS1)

OTU	#Identity percentage	#Coverage	maxid	Selwyn A	Selwyn B	Selwyn C
Otu792	99,202	100	*Asterochloris*	1	0	0
Otu227	100,000	100	*Asterochloris (pseudo)irregularis*	1	0	0
Otu428	100,000	100	*Asterochloris phycobiontica*	1	0	0
Otu089	99,505	100	*Coccomyxa solorinae*	1	1	1
Otu499	100,000	100	*Coccomyxa* sp. gbA3	1	0	0
Otu896	96,552	99	*Coccomyxa* sp. NEM-1	1	0	0
Otu907	98,473	100	*Coccomyxa subellipsoidea*	1	0	0
Otu355	99,229	96	*Elliptochloris bilobata*	1	1	0
Otu203	92,647	100	*Elliptochloris* sp.	0	1	0
Otu349	91,803	95	*Symbiochloris* sp.	1	0	0
Otu493	99,496	100	*Trebouxia impressa*	1	0	0
Otu362	100,000	100	*Trebouxia* sp.	1	0	0
Otu599	99,501	100	*Trebouxia vagua*	1	0	0

S19. Caribou diet selection

Table S19. Comparison of known caribou dietary preferences (Denryter et al., 2017) as detected using the different proxies.

*for the modern caribou, insufficient material was available for a detailed analysis of plant macroremains.

Sample	Caribou Diet preference	Pollen (%)	Macro (%)	*trn*L (%)	ITS1 (%)	ITS2 (%)
Selwyn caribou A	- Selected	28.0	++*	97.7	98.1	95.9
	- Neutral	11.0	+	1.3	0.3	0.4
	- Avoided	47.0		0.3	1.6	3.4
	- Unknown	14.0		0.6	0.0	0.3
Selwyn caribou B	- Selected	0.0	22.4	92.6	86.0	89.6
	- Neutral	41.0	46.0	6.6	4.5	1.7
	- Avoided	29.0	21.0	0.3	3.0	5.1
	- Unknown	30.0	10.6	0.4	6.4	3.5
Selwyn caribou C	- Selected	4.0	25.8	11.4		
	- Neutral	5.0	30.5	44.4	n/a	n/a
	- Avoided	27.0	20.7	1.4		
	- Unknown	64.0	23.0	42.7		

S20 Sample metadata

*trn*L (run ERR5880341)

nrITS (run ERR5881895)

Sample	Rep	*trn*L tag combination	nrITS tag combination	nrITS2 tag combination	Fungal nrITS2 tag combination
Abyland	1	TGCAGATCCAAC:CCTATGTGATGG	TGCAGATCCAAC:CCTATGTGATGG	TGCAGATCCAAC:CCTATGTGATGG	TGCAGATCCAAC:CCTATGTGATGG
Abyland	2	TGCAGATCCAAC:CTCCCATACCAC	TGCAGATCCAAC:CTCCCATACCAC	TGCAGATCCAAC:CTCCCATACCAC	TGCAGATCCAAC:CTCCCATACCAC
Abyland	3	TGCAGATCCAAC:CACCCTTAAAGT	TGCAGATCCAAC:CACCCTTAAAGT	TGCAGATCCAAC:CACCCTTAAAGT	TGCAGATCCAAC:CACCCTTAAAGT
Adycha	1	TGCAGATCCAAC:AGAAACGCAACA	TGCAGATCCAAC:AGAAACGCAACA	TGCAGATCCAAC:AGAAACGCAACA	TGCAGATCCAAC:AGAAACGCAACA
Adycha	2	CCATCACATAGG:CCGTAGTTTAGG	CCATCACATAGG:CCGTAGTTTAGG	CCATCACATAGG:CCGTAGTTTAGG	CCATCACATAGG:CCGTAGTTTAGG
Adycha	3	CCATCACATAGG:GTTGGATCTGCA	CCATCACATAGG:GTTGGATCTGCA	CCATCACATAGG:GTTGGATCTGCA	CCATCACATAGG:GTTGGATCTGCA
Bison	1	TGTTGCGTTTCT:CTCCCATACCAC	TGTTGCGTTTCT:CTCCCATACCAC	TGTTGCGTTTCT:CTCCCATACCAC	TGTTGCGTTTCT:CTCCCATACCAC
Bison	2	TGTTGCGTTTCT:CACCCTTAAAGT	TGTTGCGTTTCT:CACCCTTAAAGT	TGTTGCGTTTCT:CACCCTTAAAGT	TGTTGCGTTTCT:CACCCTTAAAGT
Bison	3	TGTTGCGTTTCT:AGGATGTTGCTC	TGTTGCGTTTCT:AGGATGTTGCTC	TGTTGCGTTTCT:AGGATGTTGCTC	TGTTGCGTTTCT:AGGATGTTGCTC
Selwyn A	1	GTGGTATGGGAG:CACCCTTAAAGT	GTGGTATGGGAG:CACCCTTAAAGT	GTGGTATGGGAG:CACCCTTAAAGT	GTGGTATGGGAG:CACCCTTAAAGT
Selwyn A	2	GTGGTATGGGAG:AGGATGTTGCTC	GTGGTATGGGAG:AGGATGTTGCTC	GTGGTATGGGAG:AGGATGTTGCTC	GTGGTATGGGAG:AGGATGTTGCTC
Selwyn A	3	GTGGTATGGGAG:AGAAACGCAACA	GTGGTATGGGAG:AGAAACGCAACA	GTGGTATGGGAG:AGAAACGCAACA	GTGGTATGGGAG:AGAAACGCAACA
Selwyn B	1	ACTTTAAGGGTG:GTTGGATCTGCA	ACTTTAAGGGTG:GTTGGATCTGCA	ACTTTAAGGGTG:GTTGGATCTGCA	ACTTTAAGGGTG:GTTGGATCTGCA
Selwyn B	2	ACTTTAAGGGTG:CCTATGTGATGG	ACTTTAAGGGTG:CCTATGTGATGG	ACTTTAAGGGTG:CCTATGTGATGG	ACTTTAAGGGTG:CCTATGTGATGG
Selwyn B	3	ACTTTAAGGGTG:CTCCCATACCAC	ACTTTAAGGGTG:CTCCCATACCAC	ACTTTAAGGGTG:CTCCCATACCAC	ACTTTAAGGGTG:CTCCCATACCAC
Selwyn C	1	ACTTTAAGGGTG:AGGATGTTGCTC	ACTTTAAGGGTG:AGGATGTTGCTC	ACTTTAAGGGTG:AGGATGTTGCTC	ACTTTAAGGGTG:AGGATGTTGCTC
Selwyn C	2	GAGCAACATCCT:CCGTAGTTTAGG	GAGCAACATCCT:CCGTAGTTTAGG	GAGCAACATCCT:CCGTAGTTTAGG	GAGCAACATCCT:CCGTAGTTTAGG
Selwyn C	3	GAGCAACATCCT:GTTGGATCTGCA	GAGCAACATCCT:GTTGGATCTGCA	GAGCAACATCCT:GTTGGATCTGCA	GAGCAACATCCT:GTTGGATCTGCA
Cape Blossom	1	CCTAAACTACGG:AGGATGTTGCTC	CCTAAACTACGG:AGGATGTTGCTC	CCTAAACTACGG:AGGATGTTGCTC	CCTAAACTACGG:AGGATGTTGCTC
Cape Blossom	2	CCTAAACTACGG:AGAAACGCAACA	CCTAAACTACGG:AGAAACGCAACA	CCTAAACTACGG:AGAAACGCAACA	CCTAAACTACGG:AGAAACGCAACA
Cape Blossom	3	TGCAGATCCAAC:CCGTAGTTTAGG	TGCAGATCCAAC:CCGTAGTTTAGG	TGCAGATCCAAC:CCGTAGTTTAGG	TGCAGATCCAAC:CCGTAGTTTAGG
Yukon horse	1	GTGGTATGGGAG:CCGTAGTTTAGG	GTGGTATGGGAG:CCGTAGTTTAGG	GTGGTATGGGAG:CCGTAGTTTAGG	GTGGTATGGGAG:CCGTAGTTTAGG
Yukon horse	2	GTGGTATGGGAG:GTTGGATCTGCA	GTGGTATGGGAG:GTTGGATCTGCA	GTGGTATGGGAG:GTTGGATCTGCA	GTGGTATGGGAG:GTTGGATCTGCA
Yukon horse	3	GTGGTATGGGAG:CCTATGTGATGG	GTGGTATGGGAG:CCTATGTGATGG	GTGGTATGGGAG:CCTATGTGATGG	GTGGTATGGGAG:CCTATGTGATGG
Maly Lyakh.	1	CCATCACATAGG:CTCCCATACCAC	CCATCACATAGG:CTCCCATACCAC	CCATCACATAGG:CTCCCATACCAC	CCATCACATAGG:CTCCCATACCAC
Maly Lyakh.	2	CCATCACATAGG:CACCCTTAAAGT	CCATCACATAGG:CACCCTTAAAGT	CCATCACATAGG:CACCCTTAAAGT	CCATCACATAGG:CACCCTTAAAGT
Maly Lyakh.	3	CCATCACATAGG:AGGATGTTGCTC	CCATCACATAGG:AGGATGTTGCTC	CCATCACATAGG:AGGATGTTGCTC	CCATCACATAGG:AGGATGTTGCTC
Oyogas Yar	1	TGTTGCGTTTCT:CCGTAGTTTAGG	TGTTGCGTTTCT:CCGTAGTTTAGG	TGTTGCGTTTCT:CCGTAGTTTAGG	TGTTGCGTTTCT:CCGTAGTTTAGG
Oyogas Yar	2	TGTTGCGTTTCT:GTTGGATCTGCA	TGTTGCGTTTCT:GTTGGATCTGCA	TGTTGCGTTTCT:GTTGGATCTGCA	TGTTGCGTTTCT:GTTGGATCTGCA
Oyogas Yar	3	TGTTGCGTTTCT:CCTATGTGATGG	TGTTGCGTTTCT:CCTATGTGATGG	TGTTGCGTTTCT:CCTATGTGATGG	TGTTGCGTTTCT:CCTATGTGATGG
Yukagir	1	CCTAAACTACGG:GTTGGATCTGCA	CCTAAACTACGG:GTTGGATCTGCA	CCTAAACTACGG:GTTGGATCTGCA	CCTAAACTACGG:GTTGGATCTGCA
Yukagir	2	CCTAAACTACGG:CCTATGTGATGG	CCTAAACTACGG:CCTATGTGATGG	CCTAAACTACGG:CCTATGTGATGG	CCTAAACTACGG:CCTATGTGATGG
Yukagir	3	CCTAAACTACGG:CTCCCATACCAC	CCTAAACTACGG:CTCCCATACCAC	CCTAAACTACGG:CTCCCATACCAC	CCTAAACTACGG:CTCCCATACCAC
Pos. Control		ATGTCCGACCAA:CCGTAGTTTAGG	ATGTCCGACCAA:CCGTAGTTTAGG	ATGTCCGACCAA:CCGTAGTTTAGG	ATGTCCGACCAA:CCGTAGTTTAGG
Neg. Control		ATGTCCGACCAA:GTTGGATCTGCA	ATGTCCGACCAA:GTTGGATCTGCA	ATGTCCGACCAA:GTTGGATCTGCA	ATGTCCGACCAA:GTTGGATCTGCA
ExBl	1	TGCAGATCCAAC:GTTGGATCTGCA	TGCAGATCCAAC:GTTGGATCTGCA	TGCAGATCCAAC:GTTGGATCTGCA	CCATCACATAGG:AGAAACGCAACA
ExBl	2	TGCAGATCCAAC:AGGATGTTGCTC	TGCAGATCCAAC:AGGATGTTGCTC	TGCAGATCCAAC:AGGATGTTGCTC	CCTAAACTACGG:CACCCTTAAAGT
ExBl	3	GTGGTATGGGAG:CTCCCATACCAC	GTGGTATGGGAG:CTCCCATACCAC	GTGGTATGGGAG:CTCCCATACCAC	ACTTTAAGGGTG:CCGTAGTTTAGG
ExBl	4	ACTTTAAGGGTG:CCGTAGTTTAGG	CCATCACATAGG:CCTATGTGATGG	CCATCACATAGG:CCTATGTGATGG	CCATCACATAGG:CCTATGTGATGG
ExBl	5	TGCAGATCCAAC:AGGATGTTGCTC	TGCAGATCCAAC:AGGATGTTGCTC	TGCAGATCCAAC:AGGATGTTGCTC	TGCAGATCCAAC:AGGATGTTGCTC

Supplementary Reference

Denryter, K.A., Cook, R.C., Cook, J.G., Parker, K.L., 2017. Straight from the caribou's (*Rangifer tarandus*) mouth: detailed observations of tame caribou reveal new insights into summer–autumn diets. Canadian Journal of Zoology 95, 81-94.