

Original article

Islands in a sea of stone: Distinct microbial soil communities in *Tilia* tree pits compared to paved soilsSjoerd Gremmen^{a,b,*,1} , Panji Cahya Mawarda^{b,c,2}, Tisja Meijers^b, Jorinde Nuytinck^{b,d,3}, Michael Stech^{b,e,4}^a Evolutionary and Population Biology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, the Netherlands^b Understanding Evolution, Naturalis Biodiversity Center, Darwinweg 2, Leiden 2333 CR, the Netherlands^c Research Center for Applied Microbiology, National Research and Innovation Agency Republic of Indonesia (BRIN), KST Samaun Sadikun, Bandung, Indonesia^d Department of Biology, Ghent University, Ghent 9000, Belgium^e Leiden University, Leiden 2300 RA, the Netherlands

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ABSTRACT

With ongoing population growth and large-scale urbanization, urban ecosystems are now the predominant habitats for humans. Yet, the biodiversity and dynamics of these ecosystems have been studied only sparsely, especially considering soil microbes. Soils in cities are disturbed and largely covered by (semi-)impervious covering, except in the highly fragmented urban greenspaces. Street trees are one of the smallest and often most confined greenspaces and can serve as a model system to better understand the urban soil microbiome. We used DNA metabarcoding to characterize bacterial and fungal communities in five urban typologies: 1) open soils around street trees (tree pits, *Tilia* spp.), soils underneath 2) sidewalks and 3) roadways, 4) with and 5) without these trees in five cities in the Netherlands. Bacterial alpha diversity was consistent in all typologies, while fungal richness was 91% higher with street trees nearby and was 304% higher in open tree pits, when compared to typologies without trees. Bacterial and fungal community structure were influenced by soil covering as well as presence of trees consistently throughout the cities. Trait annotation indicates that tree pits harbor more bacteria with putative nitrogen-cycling potential and nearly all putative fungal traits are overrepresented in the presence of trees, implying more functional redundancy. Inferred community assembly differed between typologies: bacterial communities were shaped by homogeneous selection which correlates with sensitivity to edaphic factors, whereas fungal assembly was dominated by dispersal limitation in covered areas, but not in the tree pits. Taken together, street trees have a distinct influence on soil microbial communities, even beneath paving.

1. Introduction

Rapid urbanization has tripled the land area covered by urban landscapes over the past half century, creating highly fragmented ecosystems dominated by impervious surfaces and small patches of urban green (Ehrlich et al., 2024; Grimm et al., 2008; UN DESA, 2018). Street trees are one of the smallest, but most ubiquitous forms of urban green. Their positive above-ground impact on the urban ecosystem, including humans, is well-documented (Mullaney et al., 2015; Savo et al., 2025).

However, the soil microbiome associated with these trees has barely been studied, even though soil bacterial and fungal diversity are crucial for ecosystem functioning (Wagg et al., 2019). Singular street trees are often planted in small patches of open soil (tree pits) separated from each other by (semi-)impervious surfaces like roads or sidewalks. Therefore, they provide an excellent model to test how extreme fragmentation, soil sealing and microbial interactions with a single tree shape microbial communities.

Previous work in this system has largely focused on mycorrhizal

* Corresponding author at: Evolutionary and Population Biology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, the Netherlands.

E-mail address: Sjoerd.gremmen@naturalis.nl (S. Gremmen).

¹ <https://orcid.org/0000-0002-0107-1623>

² <https://orcid.org/0000-0003-3473-0581>

³ <https://orcid.org/0000-0001-9250-377X>

⁴ <https://orcid.org/0000-0001-9804-0120>

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colonization (e.g. Alzetta et al., 2012; Geel et al., 2019; Grassi et al., 2023; Martinová et al., 2016; Timonen and Kauppinen, 2008; Tonn and Ibáñez, 2017; Verbeek et al., 2025), showing slightly decreased diversity of arbuscular mycorrhizal and ectomycorrhizal fungi when compared to rural and natural areas, and significant differences in colonization rates between cities (Geel et al., 2019). Soil available carbon and nitrogen were identified as key drivers for mycorrhizal diversity (Alzetta et al., 2012). Furthermore, soil sealing by roads did not prevent AM colonization of the roots of Dutch elm (*Ulmus × hollandica* Mill.) trees in Amsterdam (Verbeek et al., 2025), and ash trees (*Fraxinus ornus* L.) in Italy (Grassi et al., 2023), suggesting that the influence of street trees on soil biota extends beyond the physical confines of the tree pit.

The few existing studies on broader bacterial and non-mycorrhizal fungal communities indicate that the presence of pavement overrides the effect of nearby urban green on bacterial communities (Hu et al., 2021, 2018), and that the quality of paving (pervious or impervious) had no effect (Yu et al., 2019). This could be explained by the lower soil respiration, moisture, carbon and nitrogen content and higher pH found in the paved soils (Pereira et al., 2021; Piotrowska-Długosz and Charzyński, 2015). Dispersal limitation has been inferred to be another dominant factor in fungal, but not bacterial, community structure in tree pit soils in the city of Leiden (Mawarda et al., 2025).

This study extends beyond the current knowledge by comparing fungal and bacterial diversity, community composition, trait annotation and assembly analysis between multiple urban typologies within a multi-city design, all in one framework. To achieve this, we used DNA metabarcoding of fungal (ITS2) and bacterial (16S-V4) markers to explore the microbial diversity in tree pits and underneath semi-impervious streets and sidewalks in five cities situated in the western and southern Netherlands. We compared streets with or without lime trees (*Tilia* spp.) and analysed microbial community composition, functional ecology and assembly processes in five different typologies (tree pits, sidewalks and roadways with or without trees), to infer how tree pits influence microbial alpha diversity, community composition and functional diversity compared to their paved surroundings (sidewalks and roadways).

We hypothesize, firstly, that both soil paving and tree presence act as strong environmental filters, resulting in significant differences in bacterial and fungal alpha diversity (ASV-richness), community structure, and putative functional diversity as a whole between soil typologies. Secondly, we hypothesize that soil paving and tree presence have opposite effects on microbial community assembly processes, resulting in community assembly in paved typologies without trees being

dominated by dispersal limitation, in tree pits by selection and in paved areas with trees with a mix of both (undominated by either, part of the community being limited in dispersal by paving, another part being influenced by selection due to tree presence). Thirdly, we hypothesize that the chemical properties of the soil function as an additional filter, influencing both the bacterial and fungal community structure and ASV-richness.

By addressing these hypotheses, we aim to advance our understanding of how urban planning, soil chemistry, and dispersal limitation interact to shape microbial communities in highly modified ecosystems, and provide general insights into microbial ecology under conditions of fragmentation and global urbanization.

2. Material and methods

2.1. Study sites, soil sampling

The central-western part of the Netherlands is a highly urbanized conglomeration of several cities and smaller towns (Randstad). Soil was sampled in four cities in the Randstad (Amsterdam, Leiden, The Hague, Dordrecht) as well as in Tilburg (Fig. 1A). Four to eight sites were selected per city, each consisting of two adjacent streets, of which one street was flanked by *Tilia* trees and the other one lacked planted vegetation. All streets were more or less small streets in residential areas with comparable paving design (roadways with bricks and sidewalks with 30 × 30 cm tiles or rarely bricks). Soil under pavement was generally sand (sidewalks) or more gravelly substrate (roadways). No further factors such as travel intensity, cleaning regimes etc. were considered, given that the number of street pairs fulfilling the above-mentioned characteristics across all five cities was limited. *Tilia* was chosen as it is one of the most commonly planted genera in Europe at least from the 16th century (Pigott, 2012). In streets with trees, three sets of soil samples were taken in three separate tree pits (Fig. 1B/C) (0–1.5 m from the tree) as well as 2–4 m from the same trees underneath the sidewalk (Fig. 1B/C) and the roadway (Fig. 1B/C). In streets without trees, three sets of soil samples were taken underneath the sidewalk (Fig. 1B/C) and roadway (Fig. 1B/C). One brick or tile was removed carefully to sample underneath roadways and sidewalks (paved typologies). Sampling was performed using PVC tubes (12.5 cm long, ø32 mm) inserted to a depth of 10–12.5 cm. A total of 465 samples were taken in 31 street pairs spread over 5 cities in June/July of 2023. All samples were handled with sterile gloves, instruments were cleaned with bleach and demi-water between collections, and soils were stored

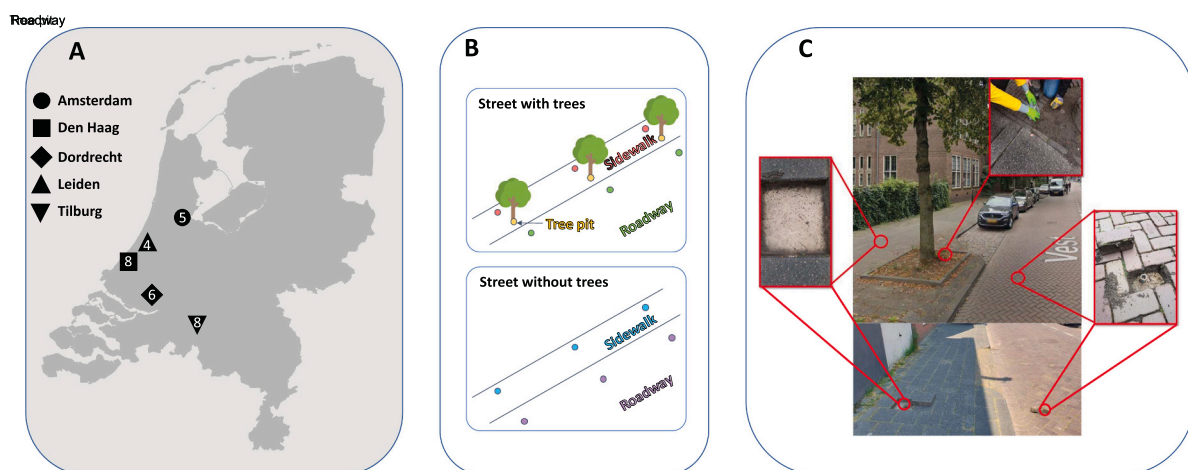


Fig. 1. Overview of sampling sites and framework. A) Map of the Netherlands indicating the number of street pairs selected in each sampled city. Cities are indicated by different symbols. B) Sampling setup in one pair of streets with trees (top) and without trees (bottom). Three replicates of each typology (see C) were sampled per street pair. C) Examples of soil sampling sites for each typology: In tree pits, underneath roadways and sidewalks close to trees and underneath roadways and sidewalks in streets without trees.

at -20°C until DNA extraction.

2.2. DNA extraction, PCR, library preparation and Illumina sequencing

DNA was extracted from 0.25 g of manually homogenized soil using the MagAttract PowerSoil Pro DNA kit (Qiagen) and Powerbead Pro plates on a Kingfisher Flex Purification System (Thermo Fisher Scientific). If roots were present in a sample, they were removed before extraction. In each extraction plate, one extraction blank was included. DNA concentrations were measured using a Lunatic UV/VIS spectrophotometer (Unchained Labs). PCR amplifications were performed on each sample, including the extraction blank, to amplify the 16S V4-V5 region (primers 515 F and 806 R (Apprill et al., 2015; Parada et al., 2016)) for prokaryotes and ITS2 for fungi (forward primer mix ITS3tagmix1–5, reverse primer ITS4ngs (Tedersoo et al., 2015)) and to attach IDT10 labels (Integrated DNA Technologies). PCR-products were cleaned with the NucleoMag NGS Clean-up and Size Select beads (Macherey-Nagel). DNA concentrations were measured on a Fragment Analyzer (Agilent) with the Qualitative DNA Kit DNF–910. Samples were pooled equimolar using the QIAgility pipetting robot (Qiagen). One mixed extraction-blank/PCR-negative per 96-well plate was included in the library prep and end pool. The pool was sent to Baseclear BV for NovaSeq 6000 250 bp paired-end sequencing (Illumina). Raw sequence reads were demultiplexed by the company.

2.3. Processing of raw reads

All data analysis was done in Rstudio (R Core Team, 2024). The demultiplexed sequences were processed to amplicon sequence variants (ASVs) using DADA2 (Callahan et al., 2016). Primers were removed using 'Dada2::Filterandtrim' for 16S, and 'cutadapt' (Martin, 2011) for ITS2. Forward and reverse sequences were cut at 200 bp (16S) or 220 and 210 bp (ITS forward and reverse), respectively based on estimated quality scores ($Q > 30$). Chimeras were removed using 'Dada2::removeBimeraDenovo'. Taxonomy was added with 'Dada2::assignTaxonomy' using the Silva v138.2 (Quast et al., 2013; Yilmaz et al., 2014) and UNITE (all eukaryotes; release: 25–02–2025) (Abarenkov et al., 2025; Kõljalg et al., 2020) databases for 16S and ITS2, respectively.

Two 'phyloseq' elements were created, for bacterial and fungal data separately (McMurdie and Holmes, 2013), and putative contaminants were removed using 'decontam::IsContaminant' (Davis et al., 2018). ASVs that were not classified as bacteria or fungi were excluded from all downstream analysis.

2.4. Analysis of bacterial and fungal richness and community composition

For alpha diversity calculations, both phyloseq elements were rarefied with 'phyloseq::rarefy_even_depth' to correct for differences in sequencing depth between samples (16S: 44,521; ITS2 11,731). Beforehand, 31 samples of the ITS2 dataset with $< 10,000$ sequences were removed based on rarefaction curves (Supplemental Fig. 1), leaving 435 samples (93 tree pit; 91 sidewalk with trees; 92 street with trees; 74 sidewalk without trees; 85 street without trees) for subsequent analysis. No sample of the 16S dataset fell below the threshold of 10,000 ASVs and therefore all samples were analysed.

ASV richness was calculated using 'phyloseq::estimate_richness'. Normality and homogeneity of alpha diversity were calculated using the Shapiro-Wilk test and Bartlett test (Bartlett and Fowler, 1937; Shapiro and Wilk, 1965). For fungi in tree pit samples, richness was both homogenous and normally distributed ($p > 0.05$ for both Shapiro-Wilk and Bartlett) and differences in alpha diversity between cities were tested using Anova with post-hoc Tukey and pairwise Wilcoxon signed-rank tests (Chambers et al., 1992; Hollander et al., 1973; Tukey, 1949). For all other data (full datasets and selections of cities and/or typologies) Kruskal-Wallis with post-hoc Dunn tests of multiple comparisons ('rstatix::dunn_test' (Dunn, 1964)) were performed. Alpha diversity was

visualized in boxplots using 'ggplot2::ggplot' (Wickham, 2009).

To assess the impact of rarefying on patterns in ASV richness, a sensitivity check was performed with unrarefied data. To visualize the relative taxonomic diversity per typology of the unrarefied dataset, the ASV-tables were transformed to presence/absence and ASV counts per typology were aggregated per taxon. Differences per taxon between typologies were compared using Kruskal-Wallis tests (Benjamini-Hochberg FDR correction), and further analyzed using Dunn's pairwise post hoc test (FDR correction) if significant ($p < 0.05$).

To assess differences in community composition between typologies, a Centered log-ratio (CLR) transformation was performed on both the 16S and ITS unrarefied ASV-tables ('Phyloseq::transform_sample_counts') (McMurdie and Holmes, 2013). Aitchison distances were calculated ('phyloseq::ordinate') and Principal Component Analysis (PCA) plots were made using 'ggplot2::ggplot'. The significance of the effect of city and typology on beta diversity was calculated with a PERMANOVA using 'vegan::adonis2 (strata = street pair)' (Oksanen et al., 2001) followed by pairwise PERMANOVAs with 'RVAideMemoire::pair.perm.manova' (Herve, 2025). Permutational multivariate analysis of dispersal (PERMDISP) was performed to test for homogeneity of dispersions ('vegan::betadisper') (Anderson, 2006). A sensitivity check was performed using Bray-Curtis dissimilarity on the rarefied datasets.

2.5. Analysis of putative functional diversity

The 'phyloseq' elements were transformed to a presence-absence ASV table and imported to a 'microeco' environment using 'file2meco::phyloseq2meco' (Liu et al., 2022, 2021). Putative functional traits were assigned using 'microeco::trans_func' with Faprotax and FungalTraits as reference databases (Louca et al., 2016; Pölme et al., 2020). The relative abundance of ASVs corresponding to putative traits was calculated per typology using 'microeco::cal_spec_func_perc' and visualized with 'microeco::plot_spe_func_perc'. Differences in functional diversity between typologies were checked for significance with Kruskal-Wallis and post-hoc Dunn's tests. P-values were adjusted for multiple comparisons using Benjamini-Hochberg correction with 'stats::p.adjust' (Benjamini and Hochberg, 1995).

2.6. Inferred community assembly processes

To infer the bacterial and fungal community assembly process, we calculated the β -nearest taxon index (β NTI) and Raup-Crick (RCBray) metrics on the rarefied datasets. The combination of phylogenetic turnover (β NTI) and taxonomic dissimilarity (RCBray) allows us to quantify different processes (homogeneous/heterogeneous selection, homogenizing dispersal, dispersal limitation) that may contribute to the microbial community assembly, as per a pre-established null modeling approach (Stegen et al., 2013, 2015). The fungal and bacterial β NTI was calculated for each typology in each city to quantify phylogenetic turnover. Two reference phylogenetic trees were constructed. The bacterial tree was constructed from an alignment of 16S sequences using Fasttree ver.2.1.11 (Approximately-Maximum-Likelihood) (Price et al., 2010). For ITS, a tree was constructed based on the assigned taxonomy of all ASVs in our data with 'taxonomy_to_tree.pl' (Tedersoo et al., 2018) (default settings). For both trees, we included only ASVs that were present in more than two reads in at least four percent of the samples. Data was filtered with 'picante::match.phylo.data' (Kembel et al., 2010). The phylogenetic trees were used to calculate the mean nearest taxon distances (MNTD) with 'picante::comdistnt' (Kembel et al., 2010), which were standardized using null-models generated with 999 randomization of the ASV-tables using the independent swap method (Gotelli, 2000). The standardized MNTD was multiplied by -1 to yield the β NTI and tested for significance with a Kruskal-Wallis rank sum test, followed by a Dunn post hoc test. Comparisons with β NTI values between -2 and 2 were classified as stochastic selection and tested for

dispersal limitation using modified Raup-Crick index based on Bray-Curtis dissimilarity with 'ICAMP::RC.cm' (Ning et al., 2020). Community assembly processes were assigned following established thresholds (Mawarda et al., 2025; Stegen et al., 2015, 2013): $\beta\text{NTI} > 2$: heterogeneous selection (selection by the environment for heterogeneous communities), $\beta\text{NTI} < -2$: homogeneous selection (selection by the environment for homogeneous communities), $\beta\text{NTI} < |2|$ and $\text{RCBray} > 0.95$: dispersal limitation (communities are very dissimilar but without a clear taxonomic pattern), $\beta\text{NTI} < |2|$ and $\text{RCBray} < -0.95$: homogenizing dispersal (communities are very similar without a clear taxonomic pattern), and intermediate values as "undominated" (the assembly process is mixed or weakly resolved). Process frequencies were summarized per habitat typology and visualized using ggplot2.

To evaluate if community similarity declined with increasing geographic distance, a spatial-distance decay analysis was performed using the Bray-Curtis dissimilarity of the rarefied datasets and distances between sample points calculated using the Haversine formula ('Geosphere::distm') (Hijmans et al., 2026). To test whether spatial distance explained variation in community composition, we performed a partial Mantel test ('Vegan::mantel.partial'; permutations = 9999) (Borcard and Legendre, 2012) between the geographic distances and the Bray-Curtis matrices for each typology independently.

2.7. Analysis of edaphic properties

A selection of soil samples from the same street and typology were pooled equally. Bulk samples ($n = 95$) were sent to Eurofins Nederland BV for Near-Infrared Spectroscopy (NIRS) analysis to measure soil reserve of nitrogen, sulphur, phosphate, potassium, calcium, magnesium, sodium, organic carbon, inorganic carbon, organic matter, cation exchange capacity (CEC), pH, and soil structure. All edaphic properties above the detection limit, and bacterial as well as fungal richness per sample were standardized to their z-score, which corresponds to the number of standard deviations of the value above/below the mean. To avoid pseudo-replication, samples in both the bacterial and fungal ASV-tables were merged per bulk sample ('Phyloseq::merge.samples'). Samples not included in the NIRS analysis (not part of a pool) were excluded from further analysis. Linear correlations between all factors were determined using the Pearson coefficient (r) (Pearson, 1895). When properties were highly correlated ($r > |0.6|$), only one was used for further analysis (Supplemental Table 3). After removal of collinear properties based on Pearson, eight soil properties as well as bacterial and fungal ASV richness remained for analysis: C/N ratio (collinear with C/organic matter ratio), nitrogen supply (collinear with CEC, C/S-ratio, total calcium, soil density), total phosphate, sulfur supply, pH (collinear with C/S ratio, soil density), crusting index, calcium saturation (collinear with CEC saturation), and percentage of silt (collinear with soil density and percentage of sand) (Supplemental Table 3). We constructed general linear models (GLMs) with the remaining properties for both ITS and 16S. The variance inflation factor (VIF) was calculated for the properties in the GLM to test for multicollinearity with 'olsrr::ols_vif_tol' (Heballi, 2024). No VIF value exceeded 3.0 indicating low multi-collinearity (Supplemental Table 3). Two final GLMs were used:

Fungal.Richness $\sim$ Bacterial.Richness + C/N-ratio + nitrogen supply + total phosphate + sulfur supply + pH + crusting index + calcium saturation + Silt;

Bacterial.Richness $\sim$ Fungal.Richness + C/N-ratio + nitrogen supply + total phosphate + sulfur supply + pH + crusting index + calcium saturation + Silt.

The results were visualized using 'sjPlot::tab_model' (Lüdecke, 2024).

We assessed correlations between the soil microbial communities and environmental parameters by normalizing both ASV-tables with a Hellinger transformation ('vegan::decostand'), calculating the Bray distance ('vegan::vegdist') and applying a Mantel test ('vegan::mantel')

for each factor. We performed forward selection with the 'adespatial::forward.sel' (Dray et al., 2025) to identify soil variables that explained community variation; p-values were adjusted (Holm transformation) to account for multiple testing (Holm, 1979). Redundancy analysis (RDA) was visualized to show the relationships between soil properties and microbial community composition, displaying only the environmental variables retained after forward selection.

3. Results

3.1. Patterns of microbial richness and community composition across urban typologies

Bacterial communities consistently harboured higher numbers of ASVs than fungal communities across all five typologies (tree pits, sidewalks and roadways with or without trees; Fig. 2) and cities (Supplemental Fig. 2). While bacterial ASV richness showed insensitivity to urban typology (Fig. 2A), fungal ASV richness varied significantly with both tree presence and presence/absence of soil paving (Kruskal-Wallis, $p = 2.2e-16$) (Fig. 2B). Soils in streets with trees consistently contained a higher fungal richness than those in streets without trees. Richness peaked in the open soil of tree pits (median = 441 ASVs; Wilcoxon, $p \leq 2.8e-13$), declined to intermediate levels beneath sidewalks and streets adjacent to trees (medians = 223 and 230 ASVs respectively; Wilcoxon, $p \leq 5.7e-7$), and reached its lowest values under paved surfaces without trees (medians = 128 and 109 ASVs respectively; Wilcoxon $p \leq 5.7e-7$).

While the same bacterial and fungal phyla dominate all five typologies (Fig. 3; Supplementary Table 1), twelve bacterial and ten fungal phyla showed clear patterns between typologies, namely 1) an overrepresentation in all typologies with trees (Verrucomicrobia and Myxococcota A; Wilcoxon: $p = 0.04-1.67e-15$); 2) an overrepresentation in tree pits compared to all paved typologies (Ascomycota and Mucoromycota; Dunn $p = 2e-4-4.19e-11$); 3) a decreasing richness from tree pits to paved typologies with trees to paved typologies without trees (Actinobacteria, Bacteroidia, Fibrobacterota; Wilcoxon $p = 0.05-3.67e-24$; Chytridiomycota and Sanchytriomycota; Dunn $p < 0.05$); and 4) a richness gradient opposite to 3) (CSP1.3, Methylobacteriales, KSB1, Nitrospira A, and Desulfobacterota B, D and G; Wilcoxon $p < 0.05$; Mortierellomycota; Dunn $p = 0.00-2.90e-7$).

There were no significant differences at lower taxonomic levels for bacteria, although 97 bacterial ASVs were found in > 25% of the covered typologies, but not in tree pits. No other combination of typologies showed such a pattern for either bacteria or fungi. Thirty-five fungal classes, 86 orders, 180 families and 287 genera were proportionally differentially present between the typologies (Kruskal-Wallis $p < 0.05$; Supplementary Table 2).

Pairwise PERMANOVA based on Aitchison distances suggest that both bacterial and fungal communities differed significantly across typologies (PERMANOVA, 999 permutations, bacteria: $R^2 = 0.064$, $p < 0.001$, $F = 8.0$; fungi: $R^2 = 0.040$, $p < 0.001$, $F = 5.5935$; Fig. 4). Pairwise PERMANOVA indicated significant differences between most typologies for both bacterial and fungal communities ($p < 0.05$), with the exception of fungal communities underneath the sidewalk and the street. However, the fungal PERMANOVA results need to be interpreted carefully, since there is significant heterogeneity of dispersion among typologies (PERMDISP: $F=30.2$, $p < 0.001$). Multivariate dispersion did not differ among typologies for bacterial communities (PERMDISP: $p > 0.05$).

The pairwise comparison of typologies shows additional patterns in community similarity. For bacteria, communities separated into three distinct clusters: tree pits, paved soils with trees, and paved soils without trees (Fig. 4A). Paving exerted the strongest influence, with the largest dissimilarity observed between tree pits and sidewalks without trees (pairwise PERMANOVA, $R^2 = 0.08$, $F = 16.8$, $p < 0.001$). Nevertheless, bacterial communities in tree pits were somewhat closer to paved soils

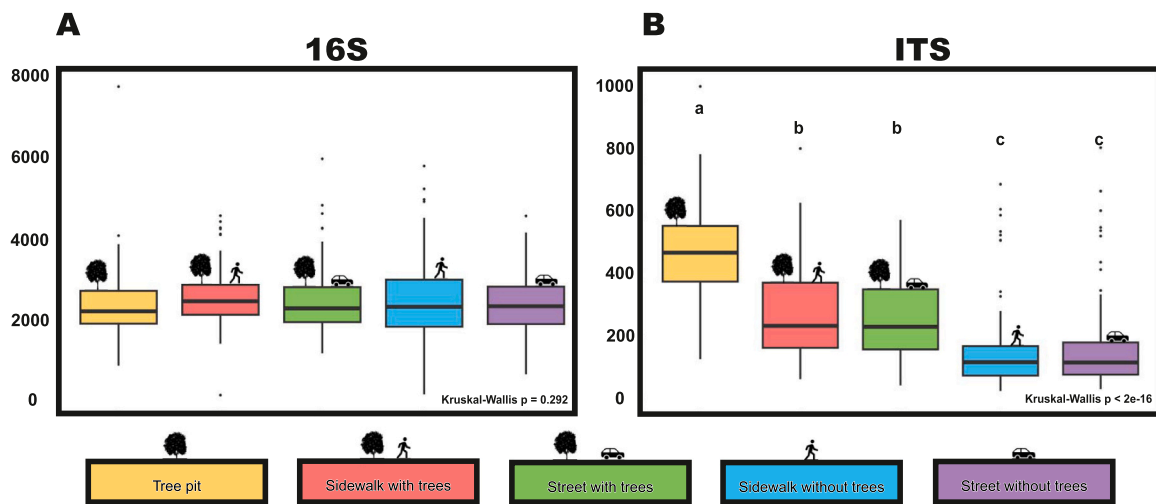


Fig. 2. ASV richness per sample in each typology (tree pit, sidewalk with trees, street with trees, sidewalk without trees, and street without trees). P-values of Kruskal-Wallis test are shown in each graph. A) bacterial ASVs from 16S sequences ($n = 93$ for each typology; rarefied to 44,521 sequences per sample); B) fungal ASVs from ITS sequences. Letters (a–c) above the boxplots indicate statistical groups based on a pairwise Wilcoxon signed-rank tests, different letter means $p < 0.05$ (Tree pit $n = 93$; Sidewalk with trees $n = 91$; Street with trees $n = 92$; Sidewalk without trees $n = 74$; Street without trees $n = 85$, rarefied to 11,731 sequences per sample).

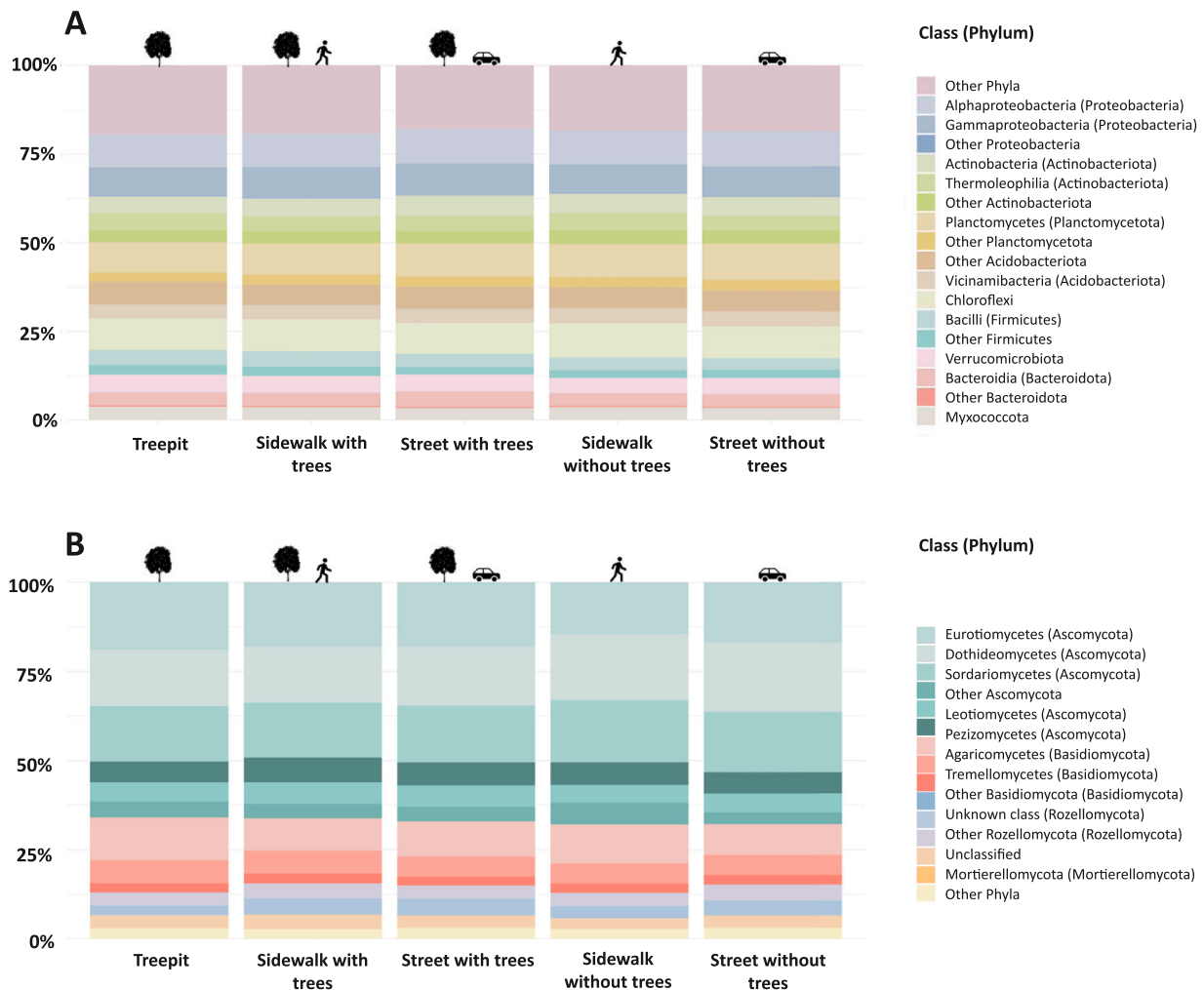


Fig. 3. Relative inferred taxonomic diversity at class and phylum level per typology, using presence/absence of ASVs per sample for each typology (tree pit, sidewalk with trees, street with trees, sidewalk without trees, and street without trees). A) Bacteria inferred from 16S sequences ($n = 93$ for each typology) B) Fungi, inferred from ITS2 sequences (Tree pit $n = 93$; Sidewalk with trees $n = 91$; Street with trees $n = 92$; Sidewalk without trees $n = 74$; Street without trees $n = 85$). Taxa with an abundance of $< 3\%$ are aggregated to “other phyla” for both bacteria and fungi separately.

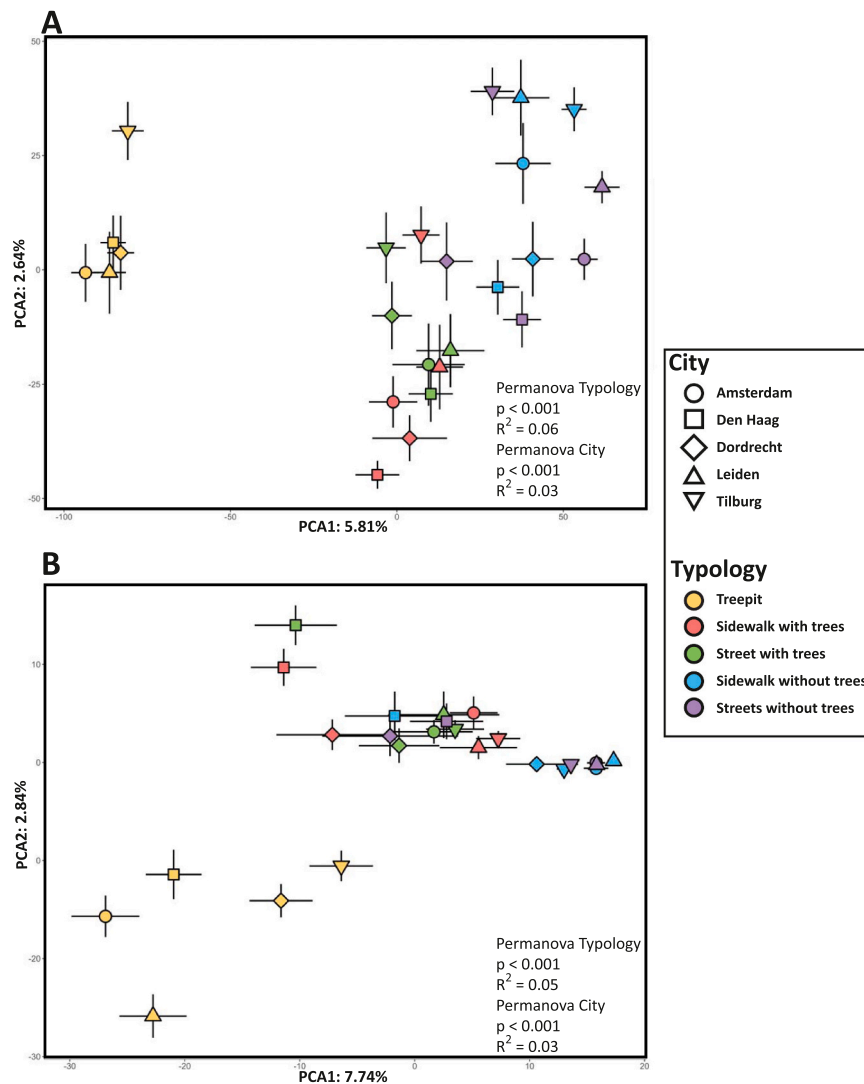


Fig. 4. Principal component analysis (PCA) of Aitchison distances on Centered Log-Ratio (CLR) transformed, unrarefied ASV tables for A) bacteria based on 16S sequences ($n = 93$ for each typology) and B) fungi based on ITS2 sequences ($n = 93$ for each typology). Each point represents the mean location of all samples from the corresponding typology and city (color and shape respectively, as indicated in legend), the standard deviations shown with bars. P-values and R^2 values are the result of PERMANOVAs.

with trees ($R^2 = 0.6$, $F = 11.7$, $p < 0.001$) than to paved soils without trees ($R^2 = 0.8$, $F \approx 16$, $p < 0.001$), suggesting that trees partially mitigate the homogenizing effect of pavement. Fungal communities showed a less pronounced clustering pattern (Fig. 4B), but still differed significantly among all typologies (pairwise PERMANOVA, $R^2 = 0.03$, $F = 5.6$, $p < 0.001$), except the paved typologies without trees.

We found that geographic location also contributed to community differences, as both bacterial and fungal assemblages varied among cities (Fig. 4). These effects were less pronounced (PERMANOVA: bacteria: $R^2 = 0.05$, $F = 3.6$, $p < 0.001$; fungi: $R^2 = 0.03$, $F = 3.3$, $p < 0.001$) compared to the stronger structuring by typology, but nevertheless significant. These results hold in a pairwise PERMANOVA, where all bacterial and fungal communities slightly differ between all cities (Pairwise PERMANOVA $p < 0.05$). However, within-group variability differed between cities for bacterial as well as fungal communities (PERMDISP: $p < 0.05$), which could (partly) explain the differences.

3.2. Patterns of inferred functional diversity across urban typologies

Putative functional traits could be assigned to 21% of bacterial and 53% of the fungal ASVs, respectively. Fifty-six out of the 75 bacterial

traits (Fig. 5A) and 86 out of the 134 fungal traits (Fig. 5B) showed significant differences between typologies (Kruskal-Wallis $p < 0.05$).

The primary bacterial energy source in each typology was aerobic chemoheterotrophy, although it was significantly more abundant in tree pits (32%) than in the other typologies (17–20%) (Wilcoxon signed-rank test with BH correction: $p < 0.001$). Notably, 11 out of the 13 traits that are part of nitrogen cycling were slightly but consistently overrepresented in tree pits as well (Wilcoxon signed-rank test with BH correction < 0.05). The other two N-cycle traits were not differentially abundant from the other typologies and were either ubiquitously present (nitrite reduction) or very rare (nitrous oxide denitrification, aerobic nitrite oxidation). For many of the N-cycle traits, streets and sidewalks with trees showed an intermediate abundance between tree pits and paved typologies without trees (nitrogen fixation, ureolysis nitrification, nitrate denitrification).

Ectomycorrhizal fungi were found in all typologies, but made up a bigger percentage of the communities in sidewalks with trees and tree pits (resp. 18% and 14% of assigned ASVs). Underneath streets with trees, the amount of ectomycorrhizal fungi was higher than in paved typologies without trees (7.0%, 0.40%, 0.50% respectively; $p < 0.0001$). Similarly, root endophytes were more abundant when

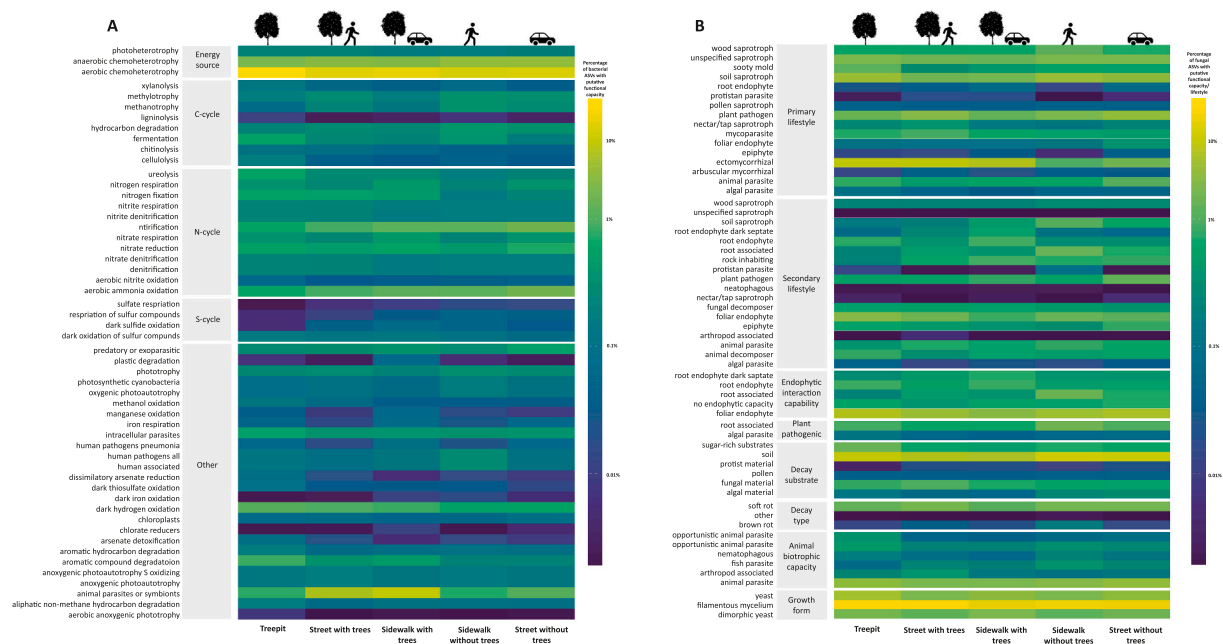


Fig. 5. Heatmaps of putative functional characteristics of A) bacterial communities classified using FAPROTAX (Louca et al., 2016) and B) fungal communities classified using FungalTraits (Pöhlme et al., 2020). Heatmaps show the average percentage of ASVs attributed to each trait in each typology (tree pit, sidewalk with trees, street with trees, sidewalk without trees, and street without trees), using presence-absence data of ASVs. Only categories that differ significantly between typologies (Kruskal-Wallis $p < 0.05$) are shown.

trees were present, more so in the tree pits than in the paved areas. Algal parasites, root pathogens, and pollen saprotrophs were exclusively found in tree pits, all with a relatively low abundance of $< 1\%$.

3.3. Patterns of inferred microbial community assembly processes across urban typologies

For bacteria, the community composition in samples from the same typologies were phylogenetically more similar than expected by chance

(mean $\beta NTI < -2$; Kruskal-Wallis, $p < 2.2 \times 10^{-16}$). Therefore, homogeneous selection is the dominant inferred assembly process in all typologies (Fig. 6A). This effect was strongest and most prominent in tree pits (mean $\beta NTI = -6.5$; 92%), suggesting that the conditions around trees intensify environmental filtering compared to paved soils (mean $\beta NTI = -3.5$; 83–90%). Dispersal limitation putatively contributed modestly across all typologies, slightly more so under paved conditions (8–12%) than in tree pits (6%), whereas homogenizing dispersal was inferred to be negligible ($< 1\%$). Only a minor fraction of bacterial

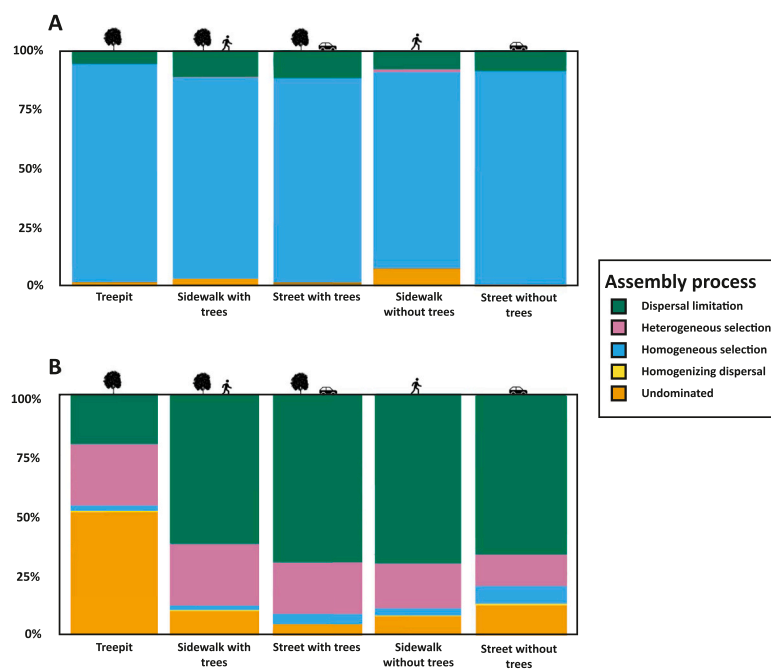


Fig. 6. Relative occurrence (in %) of community assembly processes based on β -nearest taxon index (βNTI) and Bray-Curtis based Raup-Crick values (RCBray) for A) bacteria (rarefied to 44,521 sequences per sample) and B) fungi (rarefied to 11,718 sequences per sample). $\beta NTI > 2$ = heterogeneous selection, $\beta NTI < -2$ = homogeneous selection, RCBray > 0.95 = dispersal limitation, RCBray < -0.95 = homogenizing dispersal, $|\beta NTI| < 2$ & $|RCBray| < 0.95$ = Undominated.

variation was attributable to heterogeneous selection or undominated by any of the processes. Overall, bacterial communities appear primarily structured by consistent environmental filters, with trees amplifying this effect. Consistent with this interpretation, bacterial communities showed weak (but significant) distance-decay relationships within each typology (Pearson: $r = 0.15\text{--}0.23$; Mantel: all $p = 0.0001$), indicating only weak spatial structuring (Supplemental Fig. 3 A).

In contrast, fungal communities were inferred to be shaped mainly by dispersal limitation in paved typologies ($|\beta\text{NTI}| < 2$; $\text{RCBray} > 0.95$; Kruskal-Wallis, $p < 2.2 \times 10^{-16}$, 62–70%), but not in tree pits, where no single process dominated ($|\beta\text{NTI}| < 2$; $|\text{RCBray}| \leq 0.95$; 51%) (Fig. 6B). Unlike bacteria, fungi showed a notable contribution of heterogeneous selection: 13–26% of communities were less phylogenetically similar than expected ($\beta\text{NTI} > 2$), reflecting divergent responses to local conditions. This signal was relatively stronger in the presence (21–26%) than in absence (13–19%) of trees, consistent with the host-driven structuring of fungal functional groups. Homogeneous selection and homogenizing dispersal putatively played only minor roles in fungal community assembly. Fungal communities also exhibited significant distance-decay patterns (Pearson: $r = 0.06\text{--}0.16$; Mantel: all $p < 0.0001$) across all typologies independently (Supplemental Fig. 3B).

3.4. Edaphic properties and their influence on microbial communities

All bulk soil samples consisted mostly of sand (grain size $> 50\text{ }\mu\text{m}$; 56–98%), complemented with silt (2–50 μm ; 1–37%) and clay ($< 2\text{ }\mu\text{m}$;

1–33%), with no significant differences between typologies (ANOVA, $p = 0.88$). Other edaphic factors differed between typologies (ANOVA $p < 0.05$), mostly between tree pits and all paved typologies (Tukey $p < 0.05$). For instance, soil in tree pits contained more calcium, magnesium, nitrogen, organic carbon, and soil organic matter (SOM), had a lower pH and density and showed increased microbial activity and cation exchange capability (CEC) (Tukey $p < 0.05$). Bacterial richness was associated with only one of the measured variables, i.e. it showed a negative correlation with silt percentage (ANOVA: $p = 0.028$) (Fig. 7A). The GLM explained only 10.1% of the variance ($R^2 = 0.101$). In contrast, fungal richness was lower at an increased nitrogen content, total phosphate, a higher C/N ratio, and a lower fraction of silt (ANOVA: $p = 0.029$, $p = 0.005$, $p = 0.002$, and $p = 0.038$ respectively) (Fig. 7C). Of these parameters, the silt percentage is the only one not significantly different between typologies. The model explained 47.8% of the variance in fungal richness ($R^2 = 0.478$). Fungal and bacterial ASV richness did not influence each other.

The redundancy analysis (RDA) explained 18.9% of the total variance of the bacterial dataset (ANOVA: $p = 0.001$). The community composition showed significant changes with varying pH, calcium saturation, total phosphate, nitrogen supply potential, C/N ratio, and silt percentage (Fig. 7B). Especially high nitrogen and C/N ratio coincided with samples from typologies without trees. This indicates that the bacterial communities are tightly shaped by environmental gradients and environmental gradients might be shaped by presence of trees and paving. The explained inertia by the fungal RDA was lower with only

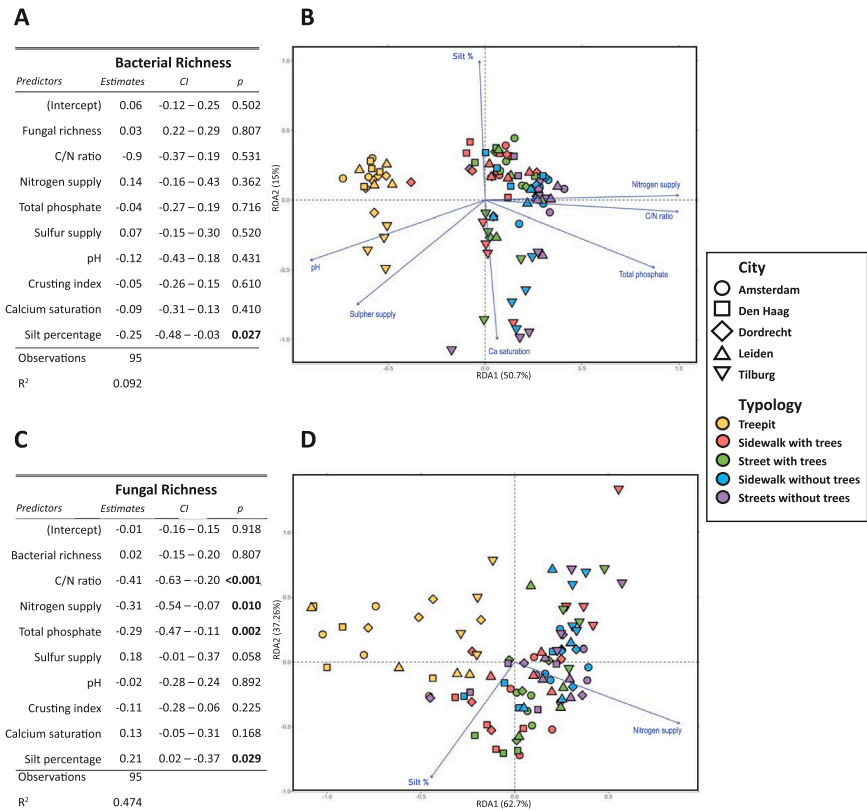


Fig. 7. Correlations of edaphic properties and bacterial and fungal alpha (A/C) and beta diversity (B/D). Edaphic properties were successfully obtained with Near infrared spectroscopy (NIRS) on 95 bulk soil samples; $n = 20$ for tree pit, streets with trees and streets without trees ($n = 17$ for sidewalk without trees; $n = 18$ for streets without trees). A) Results of the generalized linear model (GLM) using edaphic factors and fungal ASV-richness to predict bacterial ASV-richness (rarefied to 44,521). For each predictor, the regression estimates, 95% confidence intervals (CI) and p-values are shown; B) Redundancy analysis (RDA) on community structure of bacteria based on Hellinger transformed ASV-table of merged bacterial data (explains 18.9% of total variation). The merged samples correspond with the bulk soil samples. Edaphic factors that were significant predictors for community composition (Holm adjusted p-value < 0.05) are indicated with blue arrows. C) Results of the generalized linear model (GLM) using edaphic factors and bacterial ASV-richness to predict fungal ASV-richness (rarefied to 11,718). For each predictor, the regression estimates, 95% confidence intervals (CI) and p-values are shown; and D) RDA showing community structure of fungi based on Hellinger transformed ASV-table of merged fungal data (explains 3.6% of total variation). The merged samples correspond with the bulk soil samples. Edaphic factors that were significant predictors for community composition (Holm adjusted p-value < 0.05) are indicated with blue arrows.

3.6% (ANOVA: $p = 0.001$). Fungal community structure was, however, significantly correlated with nitrogen supply potential and silt percentage of soils (Fig. 7C). While both microbial groups are influenced by typology, the bacterial communities showed a greater sensitivity to measured environmental factors than the fungal communities.

4. Discussion

Due to rapid urbanization, understanding the biodiversity and dynamics of ecosystems in the urban landscape is essential (Grimm et al., 2008). Soil microbes play a crucial role in maintaining ecosystem health and flexibility (Nugent and Allison, 2022). In this study, we characterized the urban soil bacterial and fungal biomes using DNA metabarcoding in five common city typologies: tree pits, roadways and sidewalks in streets with trees, as well as roadways and sidewalks in streets without trees, within a multi-city design. With this data we inferred the putative ecological functionality and assembly processes of microbial communities in the different typologies.

Our results are partly in line with the hypotheses. Regarding our first hypothesis, bacterial richness was similar in all typologies, while fungal richness was highest in tree pits, but also elevated under paving in the presence of trees. However, community structure in each typology is significantly different for both bacteria and fungi, supporting that both soil paving and tree presence act as environmental filters. Key ecological functions were putatively present in all typologies, with higher inferred functional redundancy potential in tree pits, notably mainly for the fungal community. Our second hypothesis is partly supported for the fungal community, where differences in community structure underneath paving could indeed be explained mainly by dispersal limitation while being largely undominated in tree pits. In contrast, bacterial communities were inferred to be shaped mainly by deterministic (homogeneous) selection in all typologies, indicating that dispersal is not a dominant limitation for bacteria, even underneath paving. In line with our third hypothesis, the bacterial community is sensitive to multiple edaphic factors, which may contribute more strongly to community composition than in fungi, where these factors mainly contribute to differences in alpha diversity.

The bacterial alpha diversity is similar across all typologies, whereas the mean fungal richness increased with 91% under paving in streets with trees, and with 304% in the tree pits, compared to under the paving in streets without trees. The difference in the effect of degree of urbanization on bacterial and fungal richness is consistent with previous studies (Chen et al., 2024; Grierson et al., 2023), although results vary (Scholier et al., 2023; Whitehead et al., 2022; Yao et al., 2023). The increase in fungal richness in presence of trees might be explained by an addition of fungal niche diversity. The rhizosphere, for instance, harbours a wide variety of fungi (Pattnaik and Busi, 2019). On top of that, the tree pit is the only typology in which dispersal limitation is not the primary community assembly process as inferred from our data (see below), meaning that more fungi should be able to disperse to this typology. It is somewhat counterintuitive that presence of trees, absence of paving, and increase of fungal richness do not influence bacterial alpha diversity, if only because rhizospheres (and mycospheres) also form niches for many specific bacteria (Boer et al., 2005). Bacteria, however, live on a different scale than most fungi and bacterial diversity differs in samples taken even a few centimetres apart. Furthermore, their niche formation is highly dependent on micro-scale variations of determinants (Baquero et al., 2021; Vos et al., 2013). On this microscale, the total heterogeneity in each individual sample might not differ significantly, leading to a stable bacterial alpha diversity in all typologies.

The nature of these microniches, however, does differ between the different typologies. This can be seen in the bacterial community composition that clusters significantly on each typology in our principal component analysis based on Aitchison distance. The same clustering is found for the fungal community composition, although this could be partly due to heterogeneity of dispersion among typologies and the low

R^2 values indicate that a large portion of variation remains unexplained. Additionally, bacterial and fungal community compositions underneath sidewalks and roadways with trees are more similar to each other than to the same typologies without trees. Between these typologies, none of the measured edaphic factors differed significantly, while the community assembly processes are largely the same. This suggests that the mere presence of a tree and/or a nearby small patch of open soil (tree pit) is a defining factor in microbial community composition underneath streets and sidewalks. This effect is bigger for bacteria than for fungi, which is in line with the dominant community assembly processes inferred for both groups.

For bacteria, homogeneous selection is the key inferred driver for community assembly in all typologies. In addition, dispersal limitation increases with higher urbanization (under the pavement versus in the tree pits). The latter finding is in line with the bacterial community assembly within *Tilia* tree pits across an urbanization gradient in a previous study (Mawarda et al., 2025). However, while the community assembly was largely undominated, i.e. not found to be dominantly shaped by any of the processes (Mawarda et al., 2025), our results clearly indicate that the bacterial communities are shaped by environmental filters more so than by stochastic factors like drift or dispersal, similar to findings in urban parks in Beijing (Chen et al., 2024). This appears consistent with the classical hypothesis ‘Everything is everywhere, but the environment selects’ (Baas Becking, 1934). Indeed, even though we lost statistical power in pooling the soil samples, we still observe the community structure (beta diversity) of bacteria to be sensitive to edaphic factors. It should be noted that a very small distance-decay pattern indicates that other processes might contribute on a larger spatial scale, which is widely accepted (Feng et al., 2019). The importance of the interplay between soil parameters and bacterial community structure is well established (Fierer, 2017; Philippot et al., 2024). We have identified and quantified some of these factors, namely: pH, calcium supply, total phosphate, total nitrogen, C/N-ratio, crusting and silt percentage, although correlation to other edaphic factors makes it difficult to determine direct causality. These variables are interconnected and are known, important factors. For instance, pH is regarded as the best predictor for microbial community composition (Fierer, 2017), and bacterial diversity peaks with a relatively high phosphate and low total nitrogen and carbon content (Delgado-Baquerizo et al., 2016). Crusting and silt percentage are both texture parameters, and soil texture is known to influence microbial diversity directly and indirectly by influencing, for instance, soil moisture (Xia et al., 2020), as is true for soil capacity to supply and retain cations (Ca). Interestingly, variation of these edaphic factors do not lead to variation in bacterial alpha diversity in this study.

In contrast, the fungal community assembly is mainly inferred to be driven by dispersal limitation in covered typologies, but largely undominated by dispersal or selection in tree pits. The significant (but small) distance-decay correlation confirms that dispersal is indeed important. Thus, fungal communities are able to disperse to tree pits, but less to covered typologies with or without trees. Fungal spores can disperse over long distances even on a continental scale (Favet et al., 2013; Golan and Pringle, 2017), but it seems ground covering is an obstacle that is difficult to overcome. As with bacteria, increasing urbanization leads to stronger dispersal limitation, although the contribution of dispersal limitation in our study is lower than seen previously (Mawarda et al., 2025). It should be noted that the regional species pool in our analysis only includes taxa from urban samples, while (Mawarda et al., 2025) included suburban samples as well. This could lead to an underestimation of the role of dispersal limitation in tree pits in our study, because the taxa that are limited in dispersal are simply not all in our null-model. In all typologies with trees, heterogeneous selection contributes more to community assembly than in typologies without trees, suggesting that trees have a diversifying effect on fungal communities and increase phylogenetic diversity in their sphere of influence. Another increase of fungal community diversity is correlated with an increased silt

percentage, indicating that varying soil structure could enhance fungal diversity. However, the causal relationship might be *vice versa* since (mycorrhizal) fungi are known to influence soil structure significantly (Rillig and Mummey, 2006). No other edaphic property is significantly correlated with fungal community structure. However, increases of total nitrogen, total phosphate, and the C/N ratio all have a negative effect on fungal alpha diversity. Decreased diversity with an increase of phosphorus has previously been shown for arbuscular mycorrhizal fungi (Ma et al., 2023), as well as all fungi in tropical forests (Ma et al., 2022), while the negative effects on fungal alpha diversity of too much nitrogen have been established for a long time (Cairney and Meharg, 1999; Lilleskov et al., 2002).

Despite the differences in bacterial and fungal communities between the typologies, the overall taxonomic composition of these communities was similar between all typologies and to previously studied urban soils (e.g. Hu et al., 2021; Huot et al., 2017; Labouyrie et al., 2023; Nugent and Allison, 2022), supporting the observation that soil diversity in cities around the world is highly homogenized (Delgado-Baquerizo et al., 2021). Differences on lower taxonomic levels, as well as the full functional potential of the communities, are difficult to quantify. The majority of the microbial genetic diversity cannot be identified due to limitations of available DNA barcodes in databases, even for relatively well-studied groups like ectomycorrhizal fungi (van Galen et al., 2025), leaving room for the presence of (yet unknown) microorganisms adapted specifically to these extremely urbanized soils. In terms of identifiable putative functions, bacterial ASVs (known or hypothesized to be) attributing to nitrogen-cycling are more often present in tree pits, coinciding with a higher level of nitrogen in this typology, as are almost all inferred fungal traits. The increased coexistence of microbes sharing the same putative functional potential, indicates that there is more functional redundancy in tree pits, which is associated with more stable and resilient ecosystems (Biggs et al., 2020; Ramond et al., 2025).

Patterns in richness, community structure, and inferred community assembly and putative community functional potential were similar in all cities, suggesting that urban planning has a bigger effect on microbial communities than location. This corresponds with previous findings that land use determines diversity and abundance of microbes in types of urban green in cities (Christel et al., 2023).

In conclusion, *Tilia* tree pits support higher fungal richness, distinct microbial community composition, and higher putative functional potential than surrounding paved soils. The effect of trees, in particular on the fungal alpha diversity, is most pronounced in the tree pits, but extends under the adjacent paved surfaces. This increased diversity and putative functional redundancy might lead to higher ecosystem stability and resilience towards disturbance (Biggs et al., 2020). Our findings suggest that despite high fragmentation and disturbance, urban soils can support microbial communities with consistent composition across cities, highlighting the strong influence of urban typology, and therefore urban planning, on community structure. In these highly urbanized areas, *Tilia* tree pits function as islands of biodiversity. Furthermore, our results show that even the largely unidentified ASV data provide meaningful ecological signals to assess microbial communities in urban soils. As with all metabarcoding studies however, there are limitations to our research. For instance, data was rarefied for some analyses (e.g. alpha diversity), which corrects for sequencing bias but also introduces other biases (Schloss, 2023). Furthermore, microbial putative functions and community assembly processes were not directly measured, but were based on annotations and inferred from community similarity/taxonomic diversity within typologies, respectively.

Future studies could further explore the dynamics of tree pits as semi-isolated habitats to better understand microbial dispersal, plant-microbiome interactions, and the functional ecology of urban soils under anthropogenic influence. Investigating additional tree species, other soil types and urban green structures, comparing cities in other climates, performing metagenomic analyses, or revisiting our data as reference databases expand by taxonomic studies, culturing and DNA

barcoding, could all provide valuable opportunities to validate and extend our findings. Such efforts would also help link microbial composition to functional potential more directly.

This study contributes to our fundamental understanding of soil microbial communities in urban environments and how they are formed, by addressing understudied urban habitats. Furthermore, it helps us understand the ecology of urban green in our increasingly urbanized world, showing that planting a single *Tilia* tree already considerably increases soil microbial diversity, a result that can be directly translated to urban greening policy. We advocate to assess urban ecosystems not only by macro-organisms, but to dig deeper and consider soil microbial communities as well. Doing so will help pave the way for building healthier, greener and more sustainable cities.

CRediT authorship contribution statement

Jorinde Nuytinck: Writing – review & editing, Supervision, Investigation, Conceptualization. **Michael Stech:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Sjoerd Gremmen:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Panji Cahya Mawarda:** Writing – review & editing, Software, Methodology, Formal analysis. **Tisja Meijers:** Project administration, Methodology, Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ufug.2026.129603.

Data Availability

The raw sequences generated for the current study are available in the SRA repository: <https://www.ncbi.nlm.nih.gov/sra/PRJNA1353108>; All metadata and sample information can be found on Figshare: <https://figshare.com/s/f7b6024e6232756c3273>; R-scripts can be publicly available on Github: <https://github.com/sjogre/citydeal>.

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