

Standard Paper

Urban heat island effect as a driver for Specific Thallus Mass (STM) in lichens

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Abstract

Lichens, renowned for their resilience in extreme environments, serve as valuable bio-indicators of environmental conditions. Despite this recognition, environmental influences on lichen ecophysiology are not well understood in urban environments. In this study, we explore the use of functional traits in analyzing the impact of the urban heat island (UHI) on epiphytic chlorolichens. Lichen material was collected from 12 sites across an UHI gradient in Amsterdam and Leiden, the Netherlands. For each lichen specimen, the specific thallus mass (STM) and water-holding capacity (WHC) were calculated. The relationship between the UHI and STM/WHC was assessed using linear mixed models and ANOVA. Our study provides functional trait values (STM and WHC) for 18 species for which no prior data were available. Furthermore, our findings reveal a significant correlation between the UHI and the STM, which suggests STM as a potential indicator for the UHI.

Keywords: epiphytes; heat gradient; macrolichens; The Netherlands; trait-based community ecology; water-holding capacity

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Introduction

Due to climate change, the likelihood of extremely hot summers in Europe has increased by a factor of ten (Christidis *et al.* 2014). This becomes especially acute in urban areas where more thermal energy is stored during the day, so cooling during the night is slower relative to the surrounding rural areas (Oke 1982, 1995). This phenomenon is described as the urban heat island (UHI). As the global average air temperature is expected to rise by up to 4 °C by 2100 (IPCC 2023), this temperature difference between urban and rural areas is expected to become more pronounced under future climate scenarios (see e.g. Silva *et al.* (2022) for Lisbon, Portugal). As a result, species living in anthropogenic ecosystems will face extreme temperatures more frequently. This affects not only the species themselves, but also the species communities of which they are a part. Therefore, an ecological understanding of these communities in response to urban environmental change is vital for biodiversity conservation and ecosystem service maintenance in cities (Buchholz & Egerer 2020).

Trait-based community ecology originates from plant community ecology and has typically been used in that discipline to survey environmental changes, responses to climate change and the provision of ecosystem services (Asner *et al.* 2016; Dawson *et al.* 2021; Watkins *et al.* 2021). Here, we consider a trait as ‘a well-defined, measurable property of organisms, usually measured at the individual

level and used comparatively across species’, following McGill *et al.* (2006). Identifying traits that exhibit fitness differences across environmental gradients could lead to insights into the environmental controls of those traits (Weiher *et al.* 1998; Ellis *et al.* 2021). The independence from taxonomic assemblages and geographical locations in trait-based community ecology is considered one of the reasons for its success (Shipley *et al.* 2016). Although still heavily plant-focused, the method is increasingly being applied to other species groups such as fungi (Aguilar-Trigueros *et al.* 2015; Dawson *et al.* 2019) and invertebrates (Bertelsmeier 2017; Moretti *et al.* 2017).

Lichens are increasingly being studied in trait-based community ecology. They exhibit a symbiotic partnership between a certain fungus and a green alga and/or cyanobacterium. With *c.* 20 000 species (Hawksworth & Lücking 2017), they form a substantial part of biodiversity, being distributed from the polar regions to the tropics. Lacking true roots, lichens depend on atmospheric sources to acquire nutrients (Nieboer *et al.* 1978). Additionally, they are very responsive to changes in ambient moisture and temperature regimes because of their poikilohydric and anhydrobiotic properties (Green *et al.* 2011). These enable lichens to survive long periods in a desiccated state. Moreover, lichen secondary metabolites allow for a high tolerance against stress (e.g. UV-radiation, rapid environmental change) (de Vera *et al.* 2004). This lifestyle has allowed them to survive in a wide range of environments and stressful conditions, such as urban environments (Lorenz *et al.* 2022; Phinney *et al.* 2022). Their physiological properties have enabled a widespread and historical use as biological indicators of (changes in) environmental conditions (Skye 1979) and have made them a promising study subject in trait-based community ecology.

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For vascular plants, continuous traits that function as a proxy for the response of species to environmental factors (Garnier *et al.* 2015), such as specific leaf area (SLA), leaf dry matter content and seed mass, are already well established. In contrast, most studies on lichens have focused on categorical traits such as growth form (crustose, foliose, fruticose), photobiont type and reproductive type (Ellis *et al.* 2021), or ecological indicator values (Dengler *et al.* 2023). Despite some continuous traits being already known for lichens, they can be difficult to measure or are applicable only on a subset of the lichen community (typically foliose and fruticose species) (Stanton *et al.* 2023). This lack of easy-to-measure and widely applicable continuous functional traits hampers our understanding of community assemblies. Nevertheless, several studies have already analyzed such traits in relation to environmental and other variables in natural ecosystems (Giordani *et al.* 2012; Koch *et al.* 2013; Nelson *et al.* 2015; Boch *et al.* 2021; Phinney *et al.* 2022).

With respect to urban environments and measuring the UHI effect, Gauslaa (2014) introduced two promising water-related variables for epiphytic macrolichens (i.e. foliose and fruticose lichens): specific thallus mass (STM) and water-holding capacity (WHC). The STM and WHC are correlated (see Materials and Methods). Additionally, Gauslaa & Coxson (2011) state that STM is the driver for WHC in foliose lichens and directly translates to hydration sources such as dew or rain (Gauslaa 2014). This is corroborated by the 1:1 relationship between both variables in foliose chlorolichens (Gauslaa & Coxson 2011), that is, lichens with a green alga as their main photobiont (Ahmadjian 1989; Lange & Wagenitz 2004). This 1:1 relationship for foliose chlorolichens indicates a more opportunistic water economy in contrast to the 2:1 relationship in cyanolichens, lichens with cyanobacteria as their main photobiont (Ahmadjian 1989). This is due to a lower mass per thallus area allowing a more rapid use of water sources from humid air and dew. Lichens respond more strongly to variables relating to humidity than those relating to temperature (e.g. Jørgensen 1996), therefore dew acts as an important water source and driver for C-assimilation (Lange *et al.* 1970, 1986; Lange 2003; Gauslaa 2014). Since evaporation due to higher overall temperature is high and humidity is generally lower in city centres (Liu *et al.* 2009), dew and other humidity-related water sources are less available for epiphytic lichens. Therefore, water in the form of rain becomes more important as a water source, as illustrated by Beysens *et al.* (2017) for Paris, where the amount of water from rain greatly exceeded that from humidity. Nonetheless, temperature may also play a role. For example, Meyer *et al.* (2023) found that an increase of 2 °C in experimental warming led to a significant loss of STM and WHC.

The interplay between temperature- and water-related variables in an urban environment is insufficiently understood, reinforced by the lack of data for certain abiotic factors in urban environments such as dew and humidity (Richards 2004). Nonetheless, some generalizations can be inferred regarding the humidity, the supposed absence of dew, and the temperature in urban areas. A general deficit in urban humidity during the daytime can be connected to 1) a reduced evapotranspiration due to fewer areas with open soil, less vegetation and fewer water bodies; 2) more concealed surfaces and run-off; 3) lower wind speeds. This latter factor may also increase humidity by increasing the formation of dew in optimal wind conditions (Richards 2005; Gauslaa 2014). At night, humidity may be higher than in rural environments, creating an urban moisture island (UMI), because of a lack of vertical mixing of vapour fluxes (Wang *et al.* 2021). Higher temperatures pertaining to the UHI inhibit dew formation (Richards 2004) and can even promote evapotranspiration (Zipper *et al.* 2017), further increasing

humidity levels (Wang *et al.* 2021). This increase in humidity is a significant factor in the formation of the UHI as latent heat (the thermal energy needed to trigger a phase change without altering the substance's temperature), together with the accompanied moisture formation, is a greater contributor than sensible heat (the temperature of the ambient air) to the increase in surface air heat-content (Wang *et al.* 2021).

The aims of this study were: 1) to determine the relationship between the variation in STM and WHC and the UHI as an explanatory variable; 2) to provide new STM and WHC values for lichens from which this information has not yet been collected. To gain a better understanding of the response of lichens to urban environments, we measured the STM and WHC for 18 epiphytic macrolichens across a gradient of UHI in the cities of Amsterdam and Leiden in the Netherlands. As an increased STM and WHC could buffer the increase in temperature and decrease in humidity in city centres (Harlan *et al.* 2006; Hass *et al.* 2016), we hypothesize that within species, STM and WHC follow a significant positive linear relationship with increasing UHI intensity. Our hypothesis is based on the findings of Gauslaa & Coxson (2011) that STM has a positive linear relationship with solar exposure.

Materials and Methods

Sampling area

Lichen thallus samples were collected in the cities of Amsterdam and Leiden in December 2022 and January 2023, respectively. Samples were taken along a gradient of increasing urban heat island effect (UHI; average temperature difference with baseline situation, the Dutch countryside), whereby the UHI was divided into four categories (Table 1, Fig. 1). Twelve sampling sites (seven in Amsterdam and five in Leiden) were selected across the four UHI categories (Table 1), based on the occurrence of two tree genera with a neutral bark-pH (*Acer* and *Ulmus*) and average trunk diameter (c. 30–100 cm). UHI values were derived from the UHI map of the RIVM (Rijksinstituut voor Volksgezondheid en Milieu 2020; www.atlasleefomgeving.nl). Phorophytes were extracted from the tree datasets provided by the municipalities of Amsterdam (Gemeente Amsterdam 2023) and Leiden.

Lichen sampling

In each site, epiphytic lichens were sampled at breast height (c. 1.5 m). We aimed to sample at least three individuals for each focal lichen species (Table 2; 18 most frequent macrolichens according to data from Amsterdam; H. Timans & S. van Zon, unpublished data) in each UHI category. Samples were collected and stored in sorting boxes with adequate ventilation until processing in the laboratory. Lichens were identified *in situ*, following van Herk *et al.* (2022).

Functional traits

We employed the following protocol for measuring STM and WHC. The collected lichen thalli were acclimated in the laboratory under stable climatic conditions for one day, before they were immersed in distilled water overnight. One large or multiple small marginal lobes or branches of c. 1 cm² were cut from the fully saturated specimens using a scalpel and cleaned under a dissecting microscope, removing remains of tree bark if present. Excess water was removed using blotting tissue, similar to Phinney *et al.* (2018). The clean thallus

Table 1. Sample site information: city (A: Amsterdam, L: Leiden); site number (cf. Fig. 1); site name; coordinates following the Dutch national triangulation (RD) format; urban heat island (UHI) category (UHI cat; 1: ≤ 1.0 °C UHI temperature difference; 2: 1.0–1.5 °C; 3: 1.5–2.0 °C; 4: ≥ 2.0 °C); actual UHI temperatures (UHI); phorophyte tree species (A: *Acer* sp.; U: *Ulmus* sp.).

City	Site no.	Site name	Coordinates (RD)	UHI cat	UHI (°C)	Tree sp.
A	1	Sloterplas	116102:487173	1	0.50	A
A	2	Rembrandtpark	118122:486956	2	1.24	U
A	3	Vondelpark (North)	119371:485678	2	1.46	A
A	4	Vondelpark (East)	120072:485823	3	1.57	A
A	5	Frederiksplein	121823:486019	3	1.67	A
A	6	Valkenburgerstraat	122193:486910	4	2.06	A
A	7	Prinsengracht	120727:487680	4	2.08	U
L	8	Annie van Hattemstraat	90508:462874	1	0.94	A
L	9	Sara de Bronovoplein	90774:462733	2	1.07	A
L	10	Emmy Andriesselaats	91163:462514	2	1.26	A
L	11	Zeemanslaan	94026:462769	3	1.62	A
L	12	Ir. Driessensstraat	94213:463830	4	2.04	A

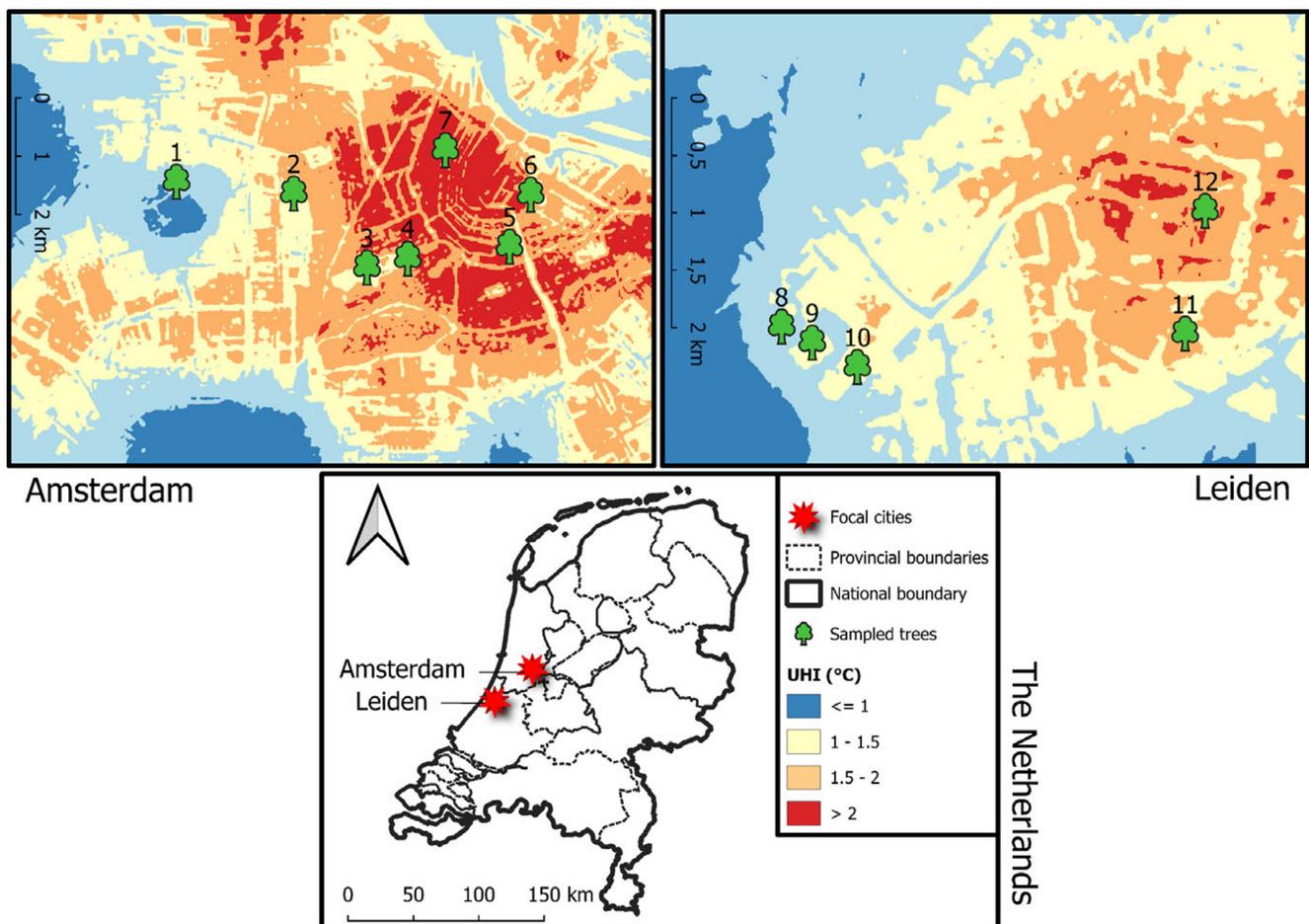


Figure 1. Sampling sites in Amsterdam and Leiden, The Netherlands. Illustrated here is the urban heat island (UHI) map of the RIVM (Rijksinstituut voor Volksgezondheid en Milieu 2020; www.atlasleefomgeving.nl), showing four UHI categories. Numbers of the sampled sites follow 'Site no.' in Table 1.

fragments were photographed at a consistent distance using a Nikon D300S camera, together with a ruler. The thallus samples were flattened using a coverslip and a pair of tweezers to avoid overlap of thallus

material. The planar photosynthetic area (PhA) was calculated using Fiji v.1.51h99 (Schindelin *et al.* 2012). The wet mass (Mw) was weighed in Eppendorf tubes, which were weighed themselves beforehand using

Table 2. Target lichen species with information on their growth form, photobiont and the number of samples collected (*n*). Species in bold were excluded from the species-specific linear regressions because they lack data points in one or more UHI category. Photobiont types follow Sanders & Masumoto (2021).

#	Species	Growth form	Photobiont	<i>n</i>
1	<i>Candelaria concolor</i> (Dicks.) Stein	Foliose	Chlorococcoid	24
2	<i>Flavoparmelia caperata</i> (L.) Hale	Foliose	Trebouxioid	18
3	<i>Flavoparmelia soredians</i> (Nyl.) Hale	Foliose	Trebouxioid	24
4	<i>Hyperphyscia adglutinata</i> (Flörke) H. Mayrhofer & Poelt	Foliose	Trebouxioid	24
5	<i>Hypotrachyna revoluta</i> (Flörke) Hale	Foliose	Trebouxioid	12
6	<i>Melanelixia subaurifera</i> (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	Foliose	Trebouxioid	9
7	<i>Parmelia sulcata</i> Taylor	Foliose	Trebouxioid	21
8	<i>Parmotrema perlatum</i> (Huds.) M. Choisy	Foliose	Trebouxioid	15
9	<i>Phaeophyscia orbicularis</i> (Neck.) Moberg	Foliose	Trebouxioid	33
10	<i>Physcia adscendens</i> H. Olivier	Foliose	<i>Trebouxia</i>	24
11	<i>Physcia tenella</i> (Scop.) DC.	Foliose	<i>Trebouxia</i>	12
12	<i>Physconia grisea</i> (Lam.) Poelt	Foliose	Trebouxioid	33
13	<i>Punctelia borreeri</i> (Sm.) Krog	Foliose	Trebouxioid	18
14	<i>Punctelia jeckeri</i> (Roum.) Kalb	Foliose	Trebouxioid	18
15	<i>Punctelia subrudecta</i> (Nyl.) Krog	Foliose	Trebouxioid	21
16	<i>Ramalina farinacea</i> (L.) Ach.	Fruticose	Trebouxioid	3
17	<i>Ramalina fastigiata</i> (Pers.) Ach.	Fruticose	Trebouxioid	9
18	<i>Xanthoria parietina</i> (L.) Th. Fr.	Foliose	Trebouxioid	33

a Sartorius BP211S analytical balance. The open Eppendorf tubes, containing the lichen material, were then placed in an oven and dried at 30 °C for 24 h and weighed again to obtain their dry mass (Md). Measurements (PhA and weight) were taken immediately after hydration/desiccation. WHC and STM were subsequently calculated as $WHC = (Mw - Md)/PhA$ and $STM = Md/PhA$.

Data analysis

Arithmetic means and standard errors (SEs) of STM and WHC were calculated for each species in general, for each UHI category and for each combination of species and location. Species' average STM and WHC, including the SEs, were plotted against each other.

All statistical analyses were performed in R v. 4.2.2 (R Core Team 2023). To assess the effect of the UHI (predictor variable; numerical values) on STM and WHC (response variables), we used a linear mixed model in the R package *lme4* (Bates *et al.* 2015). Normality was tested visually with a quantile-quantile plot. Normality was achieved after log-transformation of the response variables 'STM' and 'WHC'. Homoskedasticity was tested visually using the 'simulateResiduals' function in the R package *DHARMA* (Hartig 2022). We included species as a random effect in the model to account for species-specific physiological differences. A random slope of UHI with species was included since the intensity of these physiological responses to the UHI may differ between species. We did not include location as a random effect since location provides little more additional information than UHI. Thus, the final model resulted in:

$$\log(STM \text{ or } WHC) \sim UHI + (UHI | Species)$$

Estimates and 95% confidence intervals (CIs) of the regression slopes were calculated. Furthermore, species-specific linear regressions were run for species with data points in every UHI category.

Additionally, a type III analysis of variance (ANOVA) with the R package *car* (Fox & Weisberg 2019) was performed at the interspecific level to evaluate the influence of the UHI category (UHI cat) on STM and WHC.

Results

STM and WHC per lichen species

The raw data regarding STM and WHC values can be found in [Supplementary Material File S1](#) (available online). For STM, values ranged from 1.087 mg cm⁻² to 27.619 mg cm⁻², with a mean of 7.925 mg cm⁻² (± 0.154 SE). For the WHC, they ranged from 0.719 mg H₂O cm⁻² to 46.465 mg H₂O cm⁻², with a mean of 9.661 mg H₂O cm⁻² (± 0.317 SE).

The species' STM and WHC averages are plotted against each other in [Fig. 2](#), together with their standard errors. Mean species-level STM and WHC values varied considerably between species, with the highest mean value being three and four times larger than the lowest mean STM and WHC value, respectively ([Table 3](#)). *Physcia tenella* and *Physconia grisea* had the highest mean STM (10.972 \pm 0.673 mg cm⁻² and 10.249 \pm 0.358 mg cm⁻², respectively; [Table 3](#)). *Candelaria concolor* and *Physcia tenella* had the highest mean WHC (19.352 \pm 1.607 mg H₂O cm⁻² and 15.514 \pm 1.466 mg H₂O cm⁻², respectively; [Table 3](#)). The lowest mean STM and WHC of the sampled species was observed in *Melanelixia subaurifera* (3.984 \pm 0.733 mg cm⁻² and 4.576 \pm 0.978 mg H₂O cm⁻²; [Table 3](#)).

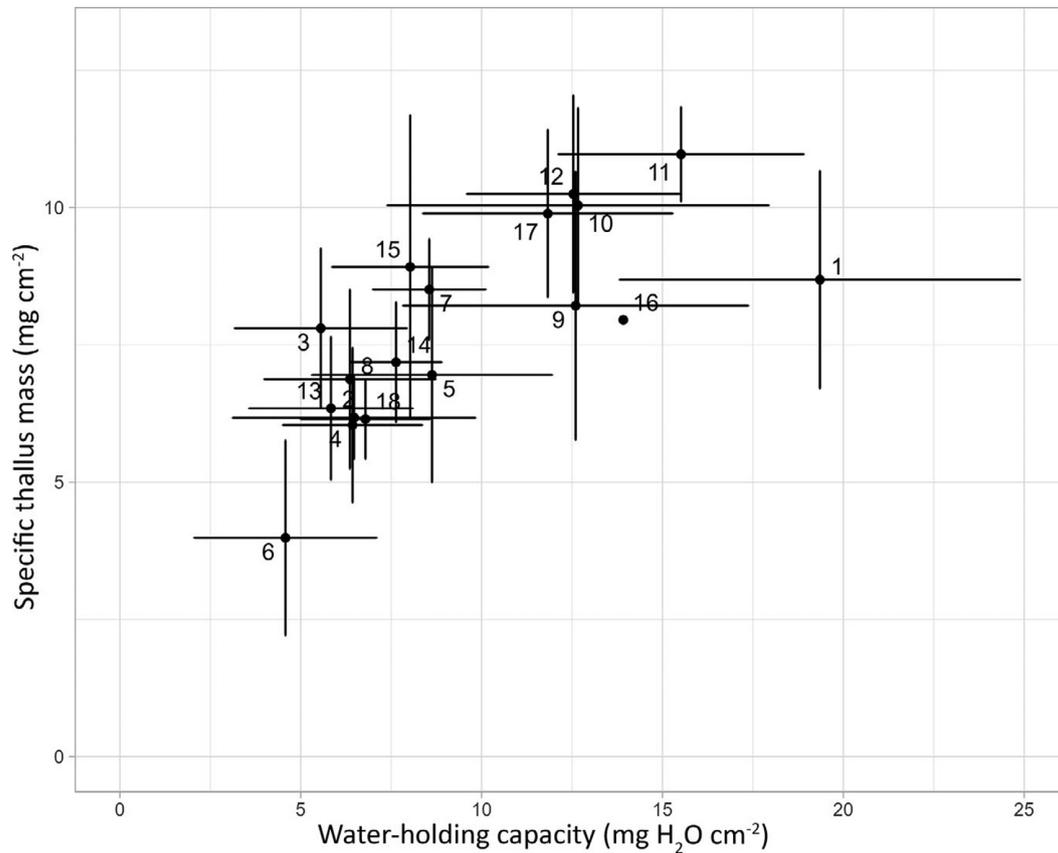


Figure 2. Mean water-holding capacity (WHC; $\text{mg H}_2\text{O cm}^{-2}$) and specific thallus mass (STM; mg cm^{-2}), including standard errors, for each measured lichen species. Numbers follow the species numbers (“#”) in Table 2.

Table 3. Arithmetic means and standard errors (SE) of the specific thallus mass (STM; mg cm^{-2}) and water-holding capacity (WHC; $\text{mg H}_2\text{O cm}^{-2}$) for each species and for all species sampled within a certain Urban Heat Island (UHI) category. Species in bold were excluded from the species-specific linear regressions because they lack data points in one or more UHI category. n = sample size. Urban Heat Island category (UHI cat): 1 = ≤ 1.0 °C UHI temperature difference; 2 = 1.0–1.5 °C; 3 = 1.5–2.0 °C; 4 = ≥ 2.0 °C.

Species	n	STM		WHC	
		Mean	SE	Mean	SE
<i>Candelaria concolor</i>	24	8.687	0.536	19.352	1.607
<i>Flavoparmelia caperata</i>	18	6.174	0.293	6.473	0.863
<i>F. soredians</i>	24	7.803	0.386	5.553	0.586
<i>Hyperphyscia adglutinata</i>	24	6.039	0.365	6.431	0.598
<i>Hypotrachyna revoluta</i>	12	6.954	0.673	8.629	1.230
<i>Melanelixia subaurifera</i>	9	3.984	0.733	4.576	0.978
<i>Parmelia sulcata</i>	21	8.507	0.363	8.553	0.695
<i>Parmotrema perlatum</i>	15	6.875	0.598	6.358	0.714
<i>Phaeophyscia orbicularis</i>	33	8.214	0.608	12.600	1.176
<i>Physcia adscendens</i>	24	10.042	0.498	12.667	1.174
<i>P. tenella</i>	12	10.972	0.673	15.514	1.466
<i>Physconia grisea</i>	33	10.249	0.358	12.534	0.784
<i>Punctelia borrieri</i>	18	6.344	0.340	5.834	0.711
<i>P. jeckeri</i>	18	7.184	0.321	7.634	0.640
<i>P. subrudecta</i>	21	8.921	1.026	8.026	0.683
<i>Ramalina farinacea</i>	3	7.953	1.107	13.917	2.448

(Continued)

Table 3. (Continued)

Species	n	STM		WHC	
		Mean	SE	Mean	SE
<i>R. fastigiata</i>	9	9.891	1.176	11.830	2.071
<i>Xanthoria parietina</i>	33	6.149	0.208	6.788	0.529
UHI cat					
1	75	7.656	0.297	9.266	0.661
2	114	7.252	0.226	9.157	0.464
3	90	8.146	0.285	10.039	0.614
4	72	8.992	0.432	10.399	0.890

Table 4. Results of the regression with a linear mixed model for specific thallus mass (STM; mg cm⁻²) and water-holding capacity (WHC; mg H₂O cm⁻²). CI_[2.5;97.5] = 95% confidence intervals. P-value in bold is significant with α = 0.05. R²M = marginal R² (proportion of variance explained by the fixed effects only, relative to the overall variance; Nakagawa & Schielzeth 2013). R²C = conditional R² (variance explained by the full model (fixed and random effects); Nakagawa & Schielzeth 2013).

Response variable	Effects	Estimate ± SE	CI _[2.5;97.5]	df	P-value	R ² M	R ² C
STM	Intercept	0.006 ± 0.0003	[0.005; 0.007]	15.5	<2.00 × 10 ⁻¹⁶	0.02	0.46
	UHI	1.115 ± 0.041	[1.031; 1.201]	13.7	0.011		
WHC	Intercept	0.007 ± 0.005	[0.004; 0.010]	15.7	2.58 × 10 ⁻¹⁴	0.01	0.48
	UHI	1.146 ± 1.038	[0.942; 1.403]	14.8	0.188		

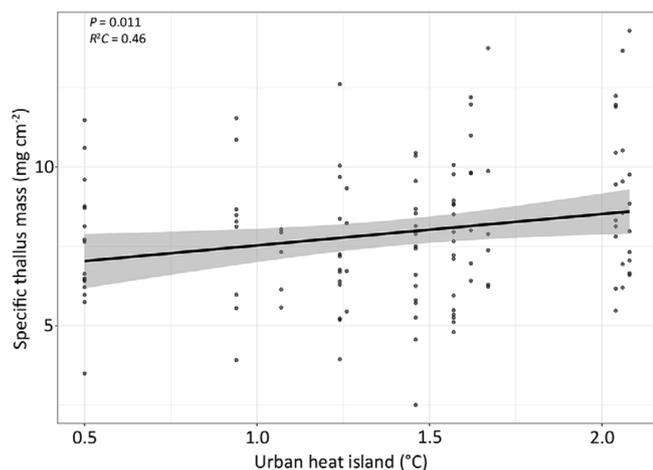


Figure 3. Overall regression line (black) with standard error (grey) of the mean specific thallus mass (STM; mg cm⁻²) across the urban heat island (UHI; °C) effect for every species–location combination.

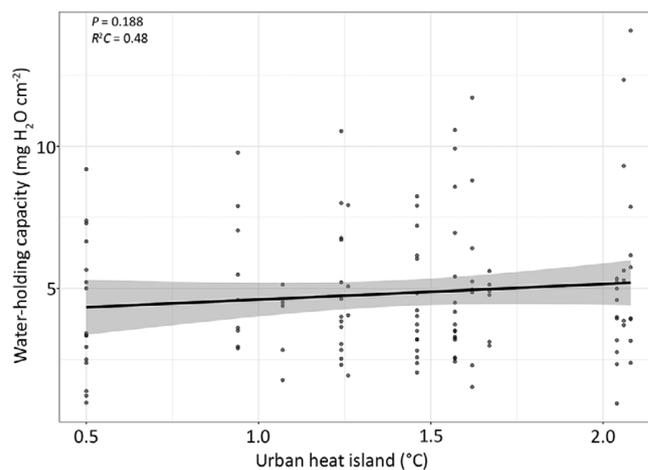


Figure 4. Regression line (black) with standard error (grey) of the mean water-holding capacity (WHC; mg H₂O cm⁻²) across the urban heat island (UHI; °C) effect for every species–location combination.

The lowest mean STM and WHC for the UHI categories was attributed to category 2, followed by 1, 3, and lastly 4 with the highest means (Table 3). The comparison of every species–location combination can be found in Supplementary Material File S2 (available online).

Linear mixed model (LMM) and analysis of variance (ANOVA)

Linear mixed model results (LMM; in log-scale), including confidence intervals, are given in Table 4. The STM was significantly correlated with UHI (P = 0.0111), but WHC was not. The model estimates in log-scale are given as ‘STM = 0.006 + 1.115 × UHI’ and ‘WHC = 0.007 + 1.146 × UHI’.

The models are visualized in Figs 3 and 4. Considering the high variability attributed to UHI, the conditional R² (R²C, variance explained by the full model (fixed and random effects); Nakagawa & Schielzeth 2013) is considered high by the authors of this paper; particularly when considering the low marginal R² (R²M, proportion of variance explained by the fixed effects only, relative to the overall variance; Nakagawa & Schielzeth 2013). A posteriori, we calculated that 35% of the variance was explained by the random factor ‘Species’.

Species-specific responses of STM and WHC to the UHI are visualized in Figs 5 and 6, respectively. The STM of *Phaeophyscia orbicularis* and *Punctelia subrudecta* as well as the WHC of

Candelaria concolor and *Punctelia jeckeri* showed a significantly positive linear relationship with the UHI, whereas the respective relationships for all other species were not significant. The influence of the UHI category, using a type III analysis of variance (ANOVA), significantly affected STM ($F(3) = 6.0191, P < 0.001$), while WHC was not affected ($F(3) = 0.8755, P = 0.454$).

Discussion

In this study, we measured STM and WHC values for 18 species of lichens for which this information had so far been lacking. Thereby, we contribute to the knowledge, and promote the use of, trait-based community ecology in lichenology, a promising addition to the field.

A significant positive relationship between STM and UHI was found, both in a regression analysis (Fig. 3, Table 4) and an ANOVA of the entire dataset, as well as in two of the 12 studied species which were sampled in every UHI category (*Phaeophyscia orbicularis* and *Punctelia subrudecta*; Fig. 5). For these species, we conclude that specific thallus weight or thickness is greater in dense urban areas. The relationship between WHC and UHI did not prove to be significant (Fig. 4, Table 4), except for the species *Candelaria concolor* and *Punctelia jeckeri* (Fig. 6). The following section will discuss these findings in light of the species' niches, potential confounder variables and the broader scientific findings regarding trait variation in other groups of primary producers (plants and bryophytes).

Our findings regarding the magnitude of the STM and WHC values are in line with previous studies such as Gauslaa & Coxson (2011), Phinney *et al.* (2018) and Wan & Ellis (2020). Wan & Ellis (2020) found the STM values to be between *c.* 0.5 mg cm⁻² and *c.* 16.5 mg cm⁻² and the WHC values to be between *c.* 0.5 mg H₂O cm⁻² and *c.* 44.5 mg H₂O cm⁻². Phinney *et al.* (2018) identified STM values ranging from 7.3 ± 0.5 mg cm⁻² to 22.9 ± 2.3 mg cm⁻² and WHC values from 6.5 ± 0.4 mg H₂O cm⁻² to 33.3 ± 4.3 mg H₂O cm⁻². Compared to the data in Phinney *et al.* (2018) and Wan & Ellis (2020), our species would fall in the same category of species from 'closed old boreal spruce forest canopy'; however, most of the species from this category consist of fruticose species, while our study included only two of this growth form. Similarly, the mean STM and WHC in our study lie close to the mean STM and WHC for chlorolichens in old forests (8.0 ± 0.1 mg cm⁻² and 10.8 ± 0.2 mg H₂O cm⁻², respectively) (Gauslaa & Coxson 2011). Additionally, mean STM and WHC values and their respective standard errors (Fig. 2) show a similar distribution to that in Wan & Ellis (2020).

Our findings suggest a greater importance of interspecific variability (Messier *et al.* 2010) since only two of the 12 investigated species of lichens showed a significant relationship between STM or WHC and the UHI (Figs 5 & 6). This is corroborated by the generally significant positive relationship across all species (Fig. 3, Table 4) and the low marginal R^2 (Table 4), which demonstrates that most of the unexplained variation is found between species. In contrast to vascular plants, where it is generally accepted that

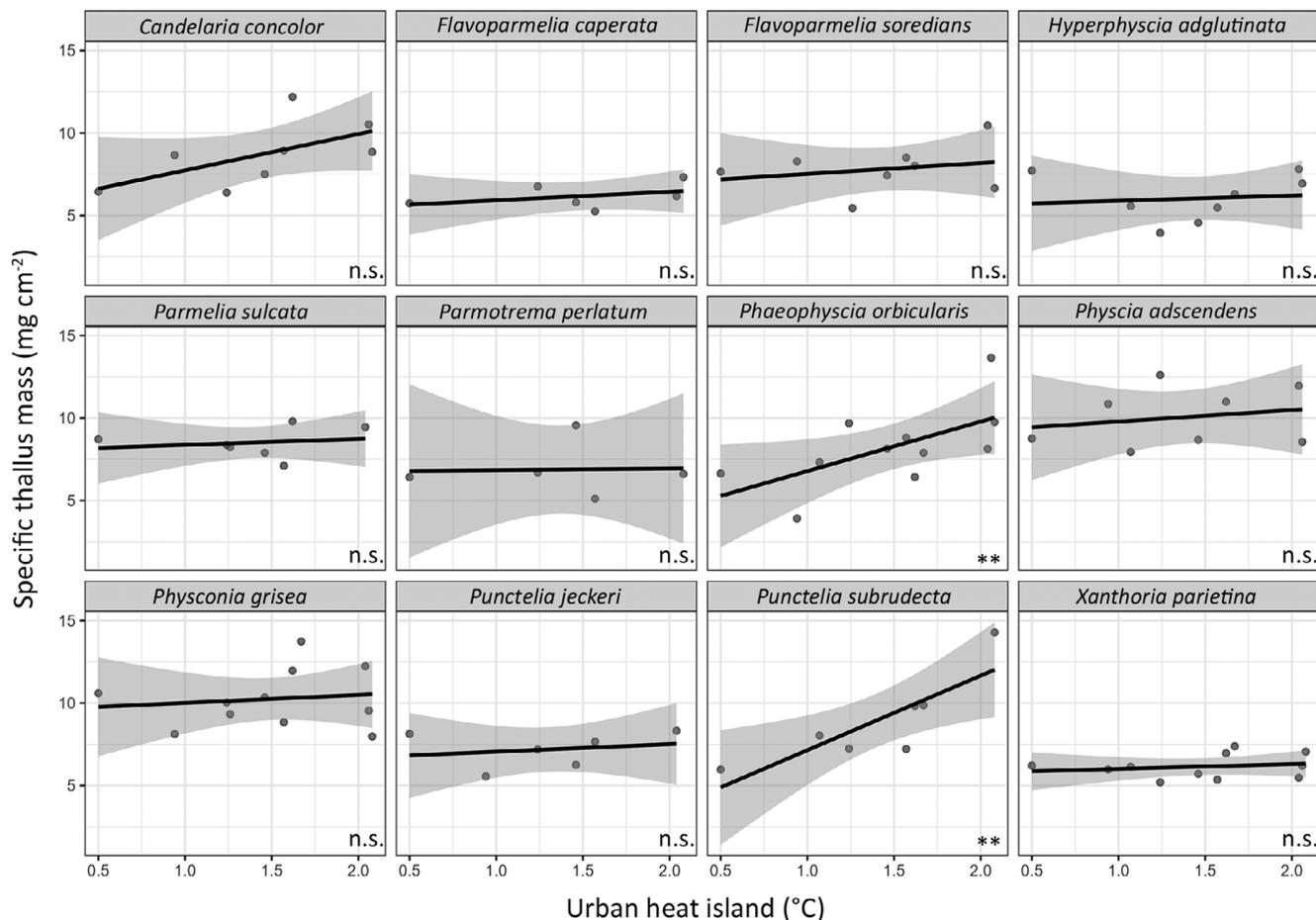


Figure 5. Species-specific regression lines with standard error (grey) of the mean specific thallus mass (STM; mg cm⁻²) across the urban heat island (UHI; °C) effect for every species–location combination (only species included which have data for each urban heat island category). n.s. = non-significant; ** = $P < 0.05$.

interspecific variation is the most important source of variation, few studies have explored this topic in lichens and bryophytes. Asplund & Wardle (2014) have investigated this for lichens and Roos *et al.* (2019) and van Zuijlen *et al.* (2022) for bryophytes, lichens and vascular plants. The research by Asplund & Wardle (2014) and van Zuijlen *et al.* (2022) found that intraspecific variation was substantially more important than interspecific variation in lichens. Conversely, Roos *et al.* (2019) found that interspecific variation was the driving force for bryophytes, lichens and vascular plants, as did van Zuijlen *et al.* (2022) for bryophytes. Thus, we may conclude that lichens can show a substantial amount of intra- and interspecific variation, and groups of primary producers may respond differently, depending on the environmental gradient in question.

The significant positive STM–UHI relationship may explain the stress response to an increasingly hot, dry and stressful environment in lichens. The two species that exhibited a significant positive response in STM to the UHI (*Phaeophyscia orbicularis* and *Punctelia subrudecta*; Fig. 5) have already been found to be indicators for the UHI (*P. orbicularis*; T. Claerhout *et al.*, unpublished data) or climate change (*Punctelia subrudecta*; Stapper & John 2015), respectively. These species are increasing in abundance over time (van Herk *et al.* 2002; Gauslaa 2024) and across the UHI gradient (T. Claerhout *et al.*, unpublished data). Since these species have niches extending into highly urbanized areas, it may follow that they are better adapted to the urban environment than the other

investigated species. The question remains whether these trends lie within the range of the species’ physiological variability or are the result of novel evolutionary adaptations. Based on our findings, lichens develop thicker lobes under stressful conditions. This is similar to an increase in leaf mass per area (LMA) during environmental stress in *Sphagnum* species (Rice *et al.* 2008), with altitude as a temperature response in alpine plants (Bresson *et al.* 2011; Scheepens *et al.* 2010), with increased urbanization in *Plantago lanceolata* (Kardel *et al.* 2010) and with increased solar exposure in the cyanolichen *Pseudocyphellaria dissimilis* (Nyl.) D. J. Galloway & P. James (Snelgar & Green 1981). Moreover, Woudstra *et al.* (2023) found that *Taraxacum officinale* exhibited a significant increase in growth after germination from seeds collected from individuals growing in a higher UHI environment at 20 °C and 26 °C. This experiment showed for the first time a genetic adaptation to the UHI. In Wright *et al.* (2004), high LMA values have been interpreted as adaptations to dry conditions, based on a positive correlation with mean annual temperature, irradiance and vapour pressure deficit, and a negative correlation with mean annual rainfall. In urban environments, other confounding variables could also be at work. The UHI is mainly an effect related to temperature and humidity but it is also partly caused or amplified by urban factors such as anthropogenic emissions, street canyons, urban roughness and urban interconnectedness (Ulpiani 2021). However, since we sampled species which are generally nitrophytic, on phorophytes with a neutral bark-pH, across a gradient

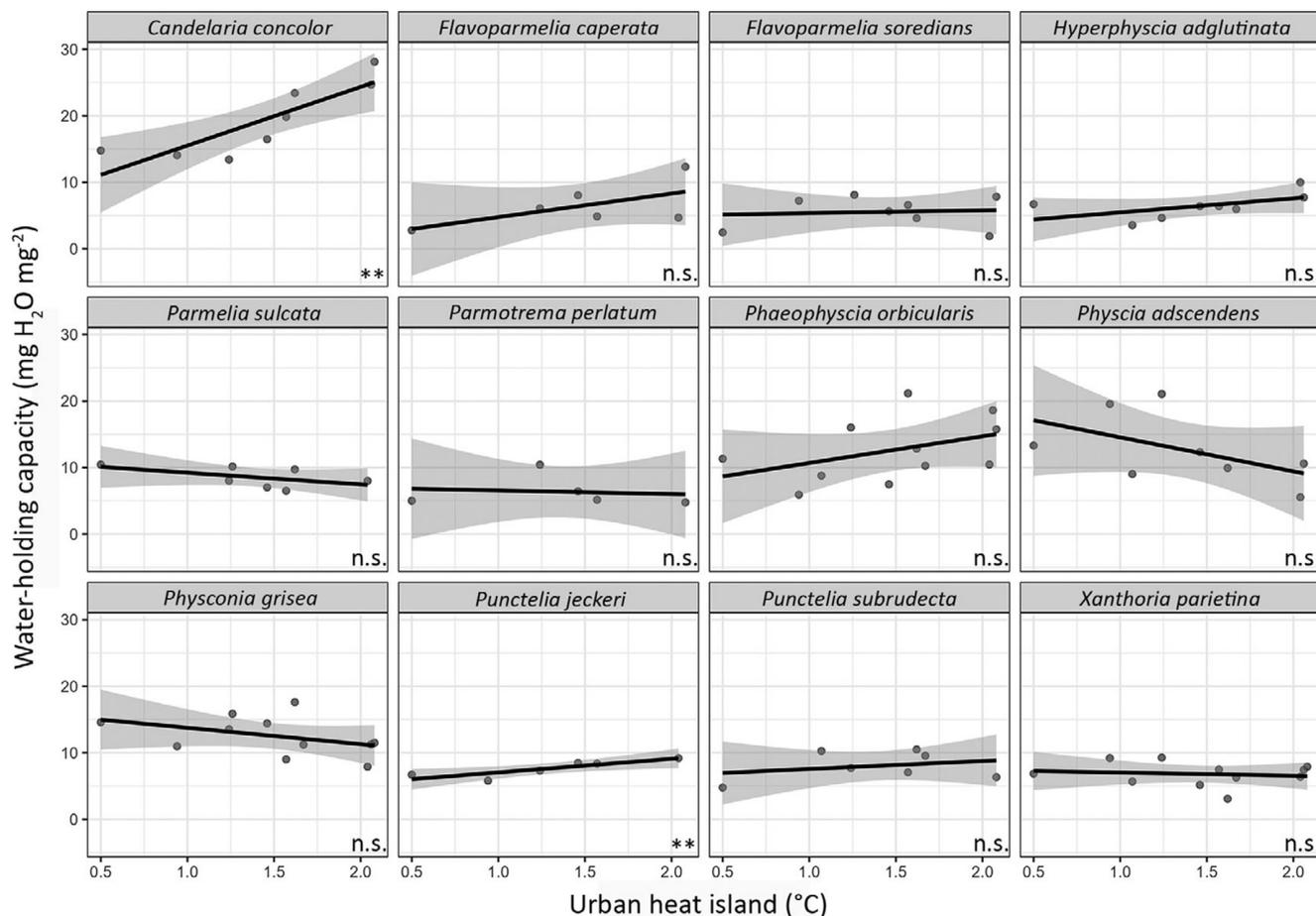


Figure 6. Species-specific regression lines with standard error (grey) of the mean water-holding capacity (WHC; mg H₂O cm⁻²) across the urban heat island (UHI; °C) effect for every species–location combination (only species included which have data for each urban heat island category). n.s. = non-significant; ** = P < 0.05.

specifically chosen for the UHI, we expect these influences to be minimal.

Despite the correlation between STM and WHC (Fig. 2), no significant positive correlation was found between WHC and UHI (Table 4). We suspect this to be a result of the degree of investment in dry matter and the usage of water sources. Chlorolichens are generally rather thin and produce their optimum photosynthetic performance in humid, not water-soaked, conditions (Asplund *et al.* 2012; Phinney *et al.* 2018). Consequently, they have a higher resource allocation to biomass (i.e. the thallus), resulting in a higher STM (Gauslaa & Coxson 2011). Despite this, Gauslaa & Coxson (2011) propose that STM drives WHC as more dry matter is necessary to increase the capacity to store water. WHC is not raised equally when resources are allocated to different thallus parts. Which thallus parts are allocated more resources to increase STM without increasing the WHC remains a topic for further research.

Conclusions

This study demonstrates that the urban heat island (UHI) effect has a significant positive relationship with the specific thallus mass (STM) of foliose lichens in an urban environment. This pattern was not found for the water-holding capacity (WHC). Our findings suggest a greater importance of interspecific variability since only two of the 12 investigated species of lichen showed a significant relationship of STM or WHC across the UHI. Species-specific differences could not be explained by indicator values.

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Competing Interests. The authors declare none.

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