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Using Lichen Diversity to track the effects of climate change: can we do this at a global scale?

"Documento Definitivo"

Doutoramento em Biologia
Especialidade de Ecologia

Bernardo Reis Rocha

Tese orientada por:
Professora Doutora Paula Matos
Professor Doutor Pedro Pinho
Professor Doutor Paolo Giordani

Documento especialmente elaborado para a obtenção do grau de doutor

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Nota Prévia

A presente tese apresenta resultados de trabalhos já publicados ou em preparação para publicação (capítulos 2 a 4), de acordo com o previsto no nº2 do artigo 25º do Regulamento de Estudos Pós-Graduados da Universidade de Lisboa, publicado em Diário da República, série II, nº57 de 23 de Março de 2015. Para mais se informa que, tendo os trabalhos sido realizados em colaboração, o candidato esclarece que participou integralmente na conceção dos trabalhos, obtenção dos dados, análise e discussão dos resultados e redação dos manuscritos.

Lisboa, Março de 2026

Bernardo Reis Rocha

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[Porque inglês ainda não é o teu forte, beijinho avó, o teu neto vai ser doutor.]

Abstract

As climate change continues to jeopardize ecosystems worldwide, it is more critical than ever to build biodiversity-based ecological indicators capable of tracking these impacts at the global scale. Despite excelling in this task, lichen diversity is still limited in its global application due to several overarching knowledge gaps. The aim of this work was to bridge three gaps that currently limit the integration of lichen diversity into climate change research and policy applications: i) disentangle climate from other environmental pressures, ii) application across extreme environments, and iii) space-for-time validation. First, under complex environmental gradients in European cities, lichen taxonomic and trait-based metrics were modelled with non-linear machine learning statistical methods. This enabled us to disentangle the effects of climate from other environmental drivers and importantly, to detect thresholds for effects. Second lichen diversity was sampled in the maritime Antarctic, a region characterized by extreme environmental conditions. We found that lichen diversity could be used to track climate effects in the region, and importantly, that accounting for the mediating effects of biotic interactions with other vegetation groups, plants and bryophytes, was critical. Finally, we used a long-term lichen diversity monitoring dataset of 17 years to validate spatial models' predictions over time. Models' predictive accuracy supports the validity of the space-for-time assumption, but with important caveats, mainly associated with the trait considered and an ecological time lag in lichen response to climate. Based on these results, we strongly towards the inclusion of lichens in international monitoring frameworks. Ultimately, and from an ecological indicator perspective, this work contributed to the reinforcement of lichen diversity as robust ecological indicators of environmental change, and we hope it encourages further efforts towards its global application.

Keywords

Ecological indicators; Global application; Climate change; Lichen diversity; Monitoring frameworks

Resumo alargado

À medida que as alterações climáticas continuam a impactar a biodiversidade e a colocar em risco ecossistemas um pouco por todo o mundo, é cada vez mais importante desenvolver indicadores ecológicos baseados na biodiversidade que sejam capazes de monitorizar estes impactos à escala global. Os líquenes são cada vez mais reconhecidos como poderosos indicadores ecológicos das alterações ambientais devido à sua sensibilidade a múltiplos fatores ambientais, à sua natureza ubíqua e à sua longevidade. No entanto, apesar da sua ampla utilização, o potencial da diversidade de líquenes como indicador ecológico global das alterações climáticas permanece subdesenvolvido, nomeadamente na sua aplicação global. Esta tese visa por isso desenvolver esse potencial, abordando três lacunas que atualmente limitam a integração do indicador tanto na investigação científica como nas estratégias de adaptação e conservação relacionadas com as alterações climáticas: i) distinguir os efeitos do clima de outros fatores ambientais, ii) aplicação em ambientes extremos, e iii) validação da metodologia espaço-tempo.

Para responder a estas lacunas, a tese é composta por cinco capítulos principais, dois dos quais subdivididos em dois subcapítulos cada. O Capítulo 1 começa com uma introdução geral que enquadra o problema da deteção e atribuição de respostas ecológicas às alterações climáticas no contexto mais amplo das alterações globais. Segue-se uma revisão atualizada do conhecimento existente sobre a diversidade de líquenes como indicador ecológico das alterações climáticas. Por fim, contextualiza as principais lacunas de conhecimento que impedem a utilização global da diversidade de líquenes como indicador ecológico dos efeitos das alterações climáticas, definindo assim os desafios científicos que os capítulos seguintes procuram abordar. O Capítulo 2, dividido em dois subcapítulos, aborda o desafio de distinguir os efeitos das alterações climáticas dos de outros fatores de mudança global. Os sistemas naturais e humanos são raramente moldados apenas pelo clima. A poluição atmosférica, os usos do solo e outros fatores ambientais de grande escala interagem com o clima de formas que podem ofuscar o seu sinal. Uma dificuldade central reside, portanto, em determinar quais as alterações observadas na diversidade de líquenes que são predominantemente impulsionadas pelo clima. O subcapítulo 2.1 examina quanto da variância em vários índices tradicionais de diversidade pode ser atribuída ao clima e à poluição atmosférica em larga escala em múltiplas cidades europeias, utilizando uma abordagem de modelação linear convencional e, portanto, de aplicação simples, mas incapaz de captar relações não lineares. O subcapítulo 2.2 baseia-se no mesmo gradiente ambiental, mas aplica um enquadramento metodológico distinto para

ultrapassar as limitações da modelação linear. Uma abordagem de aprendizagem automática não linear, baseada em *Random Forests*, foi utilizada para captar relações complexas entre a diversidade de líquenes e o clima, a poluição atmosférica e a pressão antrópica, em múltiplas escalas espaciais de ação. A expectativa do potencial desta abordagem para distinguir os efeitos do clima e detetar limiares nas respostas ecológicas foi confirmada, permitindo separar os efeitos do clima de outros fatores ambientais e, também importante, detetar limiares de resposta. O Capítulo 3, igualmente dividido em dois subcapítulos, aborda o desafio da aplicação do indicador ecológico em ambientes extremos. O indicador foi originalmente desenvolvido para líquenes epifíticos, enquanto em ecossistemas extremos, como a Antártida, os líquenes são predominantemente terrícolos ou saxícolos devido à inexistência de árvores. Nestes ecossistemas, o substrato e outras variáveis abióticas e bióticas à microescala podem ofuscar o sinal climático, levantando dúvidas sobre a aplicabilidade do indicador. Além disso, a aplicação deste método em ambientes extremos, onde as campanhas de amostragem são complexas e dispendiosas, requer também a expansão da abordagem baseada em características funcionais para além dos líquenes apenas, focando assim nos três principais grupos de vegetação existentes - líquenes, briófitos e plantas vasculares. Estes grupos podem ser detetados por sensoriamento remoto, permitindo uma monitorização em larga escala e repetível dos efeitos das alterações climáticas, ao mesmo tempo que se tem em conta as suas distintas sensibilidades ecológicas. O subcapítulo 3.1 começa por testar a resposta destes três grupos ao clima na Antártida marítima, de modo a avaliar o potencial desta abordagem baseada em traços funcionais como indicador ecológico dos efeitos das alterações climáticas em regiões extremas. O subcapítulo 3.2 expande esta análise quantificando explicitamente o papel dos fatores abióticos locais e das interações bióticas na modelação das respostas da vegetação antártica ao clima, testando simultaneamente esta abordagem como ferramenta para prever as distribuições espaciais atuais e futuras da vegetação antártica. que a diversidade de líquenes foi amostrada na Antártica marítima, uma região caracterizada por condições ambientais extremas. No global, verificámos que a diversidade de líquenes pode ser utilizada para monitorizar os efeitos do clima nesta região e, de forma crucial, que a inclusão das interações bióticas mediadas por outros grupos de vegetação, briófitos e plantas, é fundamental. O Capítulo 4 aborda o desafio de validar o método espaço-tempo, testando se os modelos construídos a partir das relações entre diversidade de líquenes e clima no espaço conseguem prever com precisão as mudanças temporais observadas. Trabalhos preliminares sugerem que o espaço pode ser um bom preditor do tempo, mas discrepâncias entre as variáveis climáticas espaciais e temporais, bem como a falta de séries temporais longas, limitaram a robustez da validação. Para isso, utilizámos um

conjunto de dados de monitorização de diversidade de líquenes com 17 anos para validar, em três momentos distintos - cinco anos antes da avaliação espacial, e cinco e dez anos depois, as previsões de modelos espaciais. A precisão preditiva dos modelos apoia a validade do pressuposto do espaço-tempo, mas com importantes ressalvas, principalmente associadas à característica funcional considerada e a um desfasamento temporal na resposta dos líquenes ao clima. Por fim, o capítulo 5 apresenta uma discussão geral, integrando os resultados obtidos nos capítulos anteriores no contexto mais amplo da literatura existente, em particular no que diz respeito à aplicação global da diversidade de líquenes como indicador ecológico das alterações climáticas. Por fim, o capítulo destaca as principais oportunidades, desafios e limitações que poderão surgir no futuro, especialmente no que se refere à sua potencial integração em programas internacionais de monitorização da biodiversidade no contexto das políticas de adaptação e mitigação das alterações climáticas.

Em resumo, estes resultados constituem um forte contributo para a inclusão dos líquenes em programas internacionais de monitorização. Em última análise, e numa perspetiva de indicadores ecológicos, este trabalho contribuiu para reforçar a robustez da diversidade de líquenes enquanto indicador das alterações ambientais, e esperamos que incentive continuados esforços para a sua aplicação global.

Palavras-chave

Indicadores ecológicos; Aplicação global; Alterações climáticas; Diversidade de líquenes

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Chapter 1

1. GENERAL INTRODUCTION, AIM AND RATIONALE

1. General Introduction

1.1 The Anthropocene – a threat to global biodiversity

In an era dominated by human-induced global environmental change – the Anthropocene – it is ever clearer that climate change is progressing towards being one of the most serious threats to biodiversity (Habibullahet al., 2022; Pereira et al., 2024; Thomas et al., 2004). Fuelled by an increase in greenhouse gases concentrations (Lamb et al., 2021; Olivier, 2022), climate change effects are felt at the global scale and threatens to disrupt ecological and evolutionary processes alike (Parmesan, 2006; Vázquez et al., 2017), the basis of fundamental ecosystem functions and services to humankind (Oliver et al., 2015; Pires et al., 2018). As these effects range from local biodiversity losses (Franco et al., 2006; Holzmann et al., 2023) to regional environmental catastrophes (Nicholson, 2014; van Woesik et al., 2022; Varga et al., 2022), scientific community, politicians, and general society alike, becomes progressively aware of its consequences to nature and humankind. Thus, a growing prioritization of climate change impacts assessment has occurred in the last decades.

Although local assessments of climate change impacts have been done in recent years (Vanneste et al., 2017; Zhu et al., 2024), to reproduce this at a global scale remains challenging, as it requires a comprehensive understanding and predictive power on how climate change impacts biodiversity at the ecosystem level, across biomes. With that intent, several regional to international conventions, monitoring networks and frameworks have and are being developed to assess climate and biodiversity change across space and time (Fellous, 2008; Pereira et al., 2013; Tsioumani, 2020). However, as 2020 marked the end of the United Nations *Decade on Biodiversity*, the Strategic Plan for Biodiversity and its ambitious Aichi targets were only partially met (Buchanan et al., 2020; Butchart et al., 2010). Thus, downward biodiversity trends are still alarming, and urgent action is required (Liu et al., 2025; Pereira et al., 2024). New targets have been set for 2050 under the Kunming-Montreal Global Biodiversity Framework (GBF) to track progress towards protecting and restoring ecosystems and the sustainable use of biodiversity, with indicators ranging from ecological to socio-economic (UNEP-WCMC, 2025). Within these first, the development of robust (i.e. generalizable, reliable and consistent) biodiversity-based ecological indicators is seen as a priority to globally monitor, diagnose and predict climate change impacts on biodiversity and ecosystems, and to evaluate the effectiveness of already applied mitigation and adaptation actions (CBD, 2022).

1.2 Ecological indicators of climate change

Ecological indicators are based on measurable and biologically meaningful metrics which have the capacity to convey vital information on ecosystem health in a simple and cost-effective way, without the need to quantify ecosystems in their entirety (Niemi & McDonald, 2004). Within these, and as biodiversity is tightly linked to ecosystem functions and services (Zhang et al., 2022), biodiversity-based ecological indicators are well suited to assess climate change effects at the ecosystem level (Branquinho et al., 2019). Developing robust, globally applicable and predictable biodiversity-based ecological indicators should be a priority to achieve the GBF goals and target. Choosing the most appropriate indicator and metrics is nevertheless challenging (Balmford et al., 2005; Lindenmayer & Likens, 2011), and there is a set of criteria that ecological indicators should follow. In summary, and based on existing literature (Dale & Beyeler, 2001; Niemi & McDonald, 2004), an ecological indicator of climate change must be highly sensitive and integrate both spatial and temporal changes in climate conditions, exhibiting early responses to even subtle changes in temperature or water availability. For its global application, it must also exhibit a broad geographical range and respond consistently to climate across different ecosystems, with specific biodiversity-based metrics exhibiting strong and predictable explanatory relationships with specific climate variables. From a methodological perspective, the ecological indicator must be globally applicable, comparable, and reproducible, through simple and cost-effective standardized sampling protocols and reporting systems, thus producing easily interpretable results, like defined environmental thresholds, which can be shared among decision-makers and general public alike.

1.3 A path towards a global application

Lichens meet most of the requirements presented above, reason why lichen diversity has long been used as an ecological indicator to track several environmental drivers' effects (e.g. air pollution, land-use), and more recently of climate (Aptroot et al., 2021; Stanton et al., 2023). They are rootless non-vascular organisms, which originate from a symbiotic relationship between a fungus and a photobiont, an algae and/or cyanobacteria (Honegger, 2009; Lücking & Spribille, 2024). Due to the lack of roots and cuticle, lichen physiology reflects surrounding climate conditions, thus having a high sensitivity and quick response to changes in air temperature and water availability, as under dry conditions they dehydrate and become dormant but quickly reactivate under wetter conditions (Boruah et al., 2024; Gauslaa, 2014).

As climate effects are felt globally, it is important that lichen diversity can be applied as ecological indicators of climate at that same scale, and for that standard sampling methods are necessary. Such standard sampling methods have long been used in Europe and the United States of America for epiphytic lichen diversity (Asta et al., 2002; USDA, 2011) and, though quite different, they can be used jointly to assess global trends (Matos et al., 2017), with standard methods existing also for terricolous and saxicolous lichens that can be applied in treeless extreme environments (Concostrina-Zubiri et al., 2014; Matos et al., 2024). The high cost-effectiveness of these sampling methods has allowed its application in a high number of sites (Pinho et al., 2008), increasing spatial coverage (Stanton et al., 2023). Over the years this has led to important contributions for policy making, helping map areas for conservation (Czarnota et al., 2023; Lendemer & Allen, 2014), characterize spatially air quality (Rocha et al., 2019a; Rocha et al, 2019b), and define critical levels and loads for several pollutants after which potentially irreversible impacts to the ecosystem can occur (Aguillaume et al., 2017; Geiser et al., 2010; Pinho et al., 2012).

The development of well-defined metrics to indicate climate effects has also been crucial. While in the past taxonomic metrics (e.g. species richness, abundance) were widely employed due to their easier interpretability and linear response to high intensity drivers such as pollution (Pescott et al., 2015), a set of easily measure trait-based metrics (e.g. growth form, main photobiont type, size) have since been determined as particularly responsive to climate (Matos et al., 2015) and thus suggested as candidates to track climate effects at the global scale (Ellis et al., 2021; Koch et al., 2019; Phinney et al., 2021). Despite the lack of an international lichen-trait database (Branquinho et al., 2019; Ellis et al., 2021), this set of traits more responsive to climate are easily identifiable and hold the highest potential for global application, as they are not directly linked to species identity. Though lichen diversity is undoubtedly a promising tool to track climate change at the global scale, there are still several overarching knowledge gaps that hinder its global application and its formal adoption into regional to international monitoring frameworks.

1.4 Disentangling climate change effects from those of other global change drivers

Due to their sensitivity to atmospheric changes, lichens excel as ecological indicators of several environmental drivers' effects (Boggess et al., 2024; Matos et al., 2015; Robert et al., 2020). However, in most real-world conditions, these environmental drivers act simultaneously, with different drivers having different spatial scales and intensities (Coffey &

Fahrig, 2012; Lättman et al., 2014; Massad et al., 2019). This may hinder our capacity to interpret lichens response to climate, particularly while having to account for the effects of all other drivers. Thus, from a global ecological indicator perspective, it is fundamental to determine whether we can use lichen diversity to disentangle (i.e. see the independent effects) climate change effects, even in areas where other high intensity global change drivers (i.e. air pollution, land-use) occur concurrently.

Cities are the optimal location to investigate this due to the extreme anthropic pressure present. The industrial revolution has changed cities (Atack et al., 2022; Azzulin et al., 2022; De Vries, 2013), which saw air pollution and land-use changes become major drivers of urban ecosystems transformation (Zhang et al., 2022). As regulatory measures lacked for much of the XIXth and XXth centuries (Fowler et al., 2020; Hanzl, 2010), these drivers' intensity rose to impact urban ecosystems to the point of biodiversity loss and ecosystem services and functions degradation (Elmqvist et al., 2013). However, as society increasingly recognizes that ecosystem services are critical to support urban wellbeing, an increasing number of policies have been put in place to achieve more resilient and sustainable cities (Carter, 2011; Klopp & Petretta, 2017; Spiliotopoulou & Roseland, 2020). As a result, these drivers have changed their relative importance in time and space and so, others, like climate change, are gradually gaining importance as main drivers of urban biodiversity change (Simkin et al., 2022), making data interpretation more complex. So far, lichen diversity has successfully been used in cities as ecological indicators of local scale climatic conditions (i.e. urban heat island effect), but only in a context in which other environmental drivers were comparatively less intense (Koch et al., 2019; Munzi et al., 2014). Thus, it is still unknown if lichen diversity can be used as an indicator of climate change in areas where climate may not be the most intense driver.

Sampling lichen diversity over multiple cities and along regional to continental gradients of climate and then using a straightforward modelling approach to disentangle its effects from those of air pollution and other environmental drivers could allow us to bridge this knowledge gap. However, conventional modelling approaches can underperform in the presence of non-linear biodiversity-climate relationships associated with threshold-like responses (Spake et al., 2022). If these conventional modelling approaches fail, alternative statistical modelling approaches (Schratz et al., 2019) such as non-parametric methods like machine learning, could enable the identification of these thresholds and the separation of climate change effects from other drivers. Confirming the ecological indicator capacity to track the individual effects of climate in cities is fundamental as it expands their application to other mixed anthropic gradients. Besides, and from an environmental policy perspective and management planning, successfully disentangling and determining thresholds for individual urban drivers is a critical step towards the development of better supported policies to mitigate key environmental challenges in cities. Nevertheless, this has not yet been done.

1.5 Application across extreme environments

Lichens' ubiquitous nature is a major advantage when considering their potential application as ecological indicators of climate change at the global scale. However, their potential as ecological indicator of climate change effects was developed focusing on epiphytic lichens, as the vast majority of studies addressing lichen diversity response to climate have been conducted in forested ecosystems (Di Nuzzo et al., 2021; Geiser & Neitlich, 2007; Root et al., 2014; Hurtado et al., 2020; Matos et al., 2015). In extreme environments however, where soil- or rock-dwelling lichens are commonly one of the dominant life-forms (Honegger, 2009; Lutzoni & Miadlikowska, 2009), the indicator applicability may be hindered by the presence of potential confounding factors (e.g. soil characteristics). From the perspective of a global ecological indicator, it is fundamental to determine whether lichen diversity can be used also across extreme environments to track climate effects.

Antarctica would be a good place to investigate this, as it is characterized by some of the harshest climate conditions on Earth. The maritime Antarctic in particular, has been stage for one of the fastest warming trends recorded during the XXth century, while also registering higher frequency of prolonged warm events (Feron et al. 2021). The extreme climate conditions shaped Antarctica terrestrial biodiversity, where the vegetation is dominated by lichens, with bryophytes, and two vascular plants, occurring in a fragile equilibrium with the surrounding environment (Colesie et al., 2023; Robinson et al., 2003). Recent monitoring efforts have detected changes in the abundance of these three main vegetation groups (Amaral et al., 2025; Cannone et al., 2022; Roland et al., 2024), suggesting that they could potentially be used as ecological indicators of climate in the region. However, it is still unknown whether these vegetation groups diversity can be applied here as ecological indicators of climate effects.

Nevertheless, testing lichen diversity potential as an indicator of climate change in these extreme environments must also account for other factors. In a region where logistical constraints (i.e. remoteness and difficulty to access) limit traditional sampling, the adoption of cost-effective approaches like remote sensing to monitor biodiversity change over space and time gains paramount importance. In these extreme environments, it becomes particularly relevant to test whether imagery-identifiable, trait-based diversity metrics, such as the cover and distribution of the three main vegetation groups (lichens, bryophytes, and vascular plants) can be effectively used (Matos et al., 2024). This would enable consistent and repeatable monitoring efforts across large and otherwise inaccessible areas, reduce dependency on costly and time-consuming field campaigns, and provide the temporal continuity necessary to detect biodiversity responses to climate change (Koerich et al., 2023). Moreover, because these vegetation groups represent different functional strategies and ecological sensitivities, they could be used together to disentangle climate change effects from other environmental drivers, thereby strengthening the robustness and scalability of biodiversity assessments. In addition to this, previous works have highlighted the importance of microscale abiotic and critically, the role of biotic interactions, i.e. biotic factors, to have a more comprehensive understanding of the direct and indirect effects of climate in these functional vegetation groups (Lee et al., 2019; Matos et al., 2024). Thus, to disentangle the effects of climate, it is crucial to also account for the relative contribution of microscale abiotic and biotic factors, as these can either amplify or buffer vegetation responses, and thereby shape the observed functional diversity patterns. Yet it is still unclear to which extent the three vegetation functional groups respond to macroclimate gradients per se, and what role microscale abiotic and biotic processes play in shaping their distribution within such gradients.

Understanding the applicability of lichen diversity as ecological indicators of climate change in Antarctica is thus fundamental from a global application perspective, as it further expands their application to other extreme environments where major changes are also expected, namely the Arctic tundra and alpine ecosystems (IPCC, 2021). Besides, from an environmental policy perspective, developing key metrics of vegetation diversity that can act as early warning ecological indicators of climate effects in this region is a critical step to better understand climate change impacts across maritime Antarctic terrestrial ecosystems, anticipate future impacts based on climate projections and guide better supported conservation actions.

1.6. Space-for-time validation

A globally applicable ecological indicator of climate change should ideally be developed using long-term datasets, as these provide direct evidence on how lichen diversity integrates changing climate conditions over time (van Herk et al., 2002). However, such datasets remain scarce, particularly for lichens, limiting our ability to predict past and future responses of lichen diversity to climate.

The space-for-time substitution method, an approach anchored in the key assumption that biodiversity-climate relationships in both space and time are equivalent (Pickett, 1989) can be useful to bypass this limitation. This method has been pivotal to develop the ecological indicator based on observing lichen diversity change under climate gradients across space, inherently assuming that those signals can be transferred through time (Lovell et al., 2023). Nevertheless, there are known inherent limitations to this approach, namely the assumption of stationarity environments, inferences about causal relationships and lack of temporal validation (Lovell et al., 2023). For that reason, using climate change space-for-time substitution studies across ecological fields has produced mixed results, with several in support (Blois et al., 2013; Matos et al., 2016) and other opposing (Angert, 2024; Kreyling, 2025).

It would be important to use the few existing long-term lichen datasets to test the temporal predictive ability of spatial climate models based on lichen diversity, i.e., to validate the space-for-time substitution approach. An almost 20-year monitoring effort conducted in the Iberian Peninsula, one of the longest in Europe targeting lichen diversity, would be an optimal dataset to validate the space-for-time assumption in the context of climate change. Findings from a preliminary work with a shortened version of this dataset suggested that space is a good predictor of time, as several lichen trait-based metrics that responded to relative air humidity variables in space also shifted over time in response to changing conditions in this climate variable (Matos et al., 2016). However, in that work the climate variables used to model spatial patterns of relative air humidity were not the same as those available for the temporal analysis, which may have limited the strength of the validation. Therefore, it is necessary to further explore this validation with longer temporal datasets, broader sets of climate predictors, and a more accurate match between spatial and temporal variables to fully assess the predictive performance of the space-for-time approach in explaining lichen diversity response to climate. Such validation is essential to ensure that lichen diversity–based ecological indicators developed under this framework are both scientifically robust and reliable for projecting biodiversity responses under future climate change scenarios.

1.7 Thesis aim and rationale

Lichens are increasingly recognized as powerful ecological indicators of environmental change due to their sensitivity to multiple environmental drivers, their ubiquitous nature, and their long lifespan. Yet, despite their widespread use, the potential of lichen diversity as a global ecological indicator of climate change remains underdeveloped. This thesis aims to advance that potential by addressing three major knowledge gaps that currently limit the integration of lichen diversity into climate change research and policy applications. To address these gaps, the thesis is comprised of five main chapters, two of them divided into two sub-chapters each.

Chapter 1 establishes the foundation of the thesis. It begins with a general introduction that frames the problem of detecting and attributing ecological responses to climate change within the broader context of global change. It then provides a state-of-the-art review of current knowledge on lichen diversity as ecological indicators of climate change. Finally, it contextualizes the overarching knowledge gaps that prevent the global use of lichen diversity as an ecological indicator of climate change effects, thereby defining the scientific challenges that the subsequent chapters aim to address.

Chapter 2, divided into two sub-chapters, addresses the challenge of disentangling climate change effects from those of other global change drivers. Natural and human systems are rarely shaped by climate alone. Air pollution, land-use and other major environmental drivers interact with climate in ways that can obscure the climate signal. A key difficulty thus lies in determining which observed shifts in lichen diversity are driven primarily by climate. **Subchapter 2.1** examines how much of the variance in several traditional diversity metrics can be attributed to broad scale climate and air pollution across multiple European cities using a conventional linear modelling approach, which is easily applicable but cannot account for nonlinear relationships. **Subchapter 2.2** builds on the same environmental gradient but applies a different methodological framework to overcome the limitations of linear modelling. A non-linear machine learning approach, based on Random Forest, was employed to capture complex relationships between lichen diversity and climate, air pollution and anthropic pressure, at multiple spatial scales of action. This sub-chapter aims to test this approach potential to disentangle the effects of climate and detect thresholds in ecological responses.

Chapter 3, divided also into two sub-chapters, addresses the challenge of the ecological indicator application across extreme environments. The indicator was originally developed for epiphytic lichens, whereas in extreme ecosystems such as Antarctica lichens are primarily soil- or rock-dwelling due to the inexistence of trees. In such ecosystems, the substrate and other microscale abiotic and biotic variables may obscure climatic signals, raising questions about the indicator's applicability. In addition, applying this method in extreme environments where sampling assessment is difficult and costly requires also expanding the trait-based approach for the three main vegetation groups, lichens, bryophytes, and vascular plants. These groups can be detected by remote sensing, enabling broad scale and repeatable monitoring of climate change effects while accounting for their distinct ecological sensitivities. **Subchapter 3.1** begins by testing these three groups response to climate in the maritime Antarctic, to test this trait-based potential as an ecological indicator of climate change effects in extreme regions. **Subchapter 3.2** expands this analysis by explicitly quantifying the role of local abiotic drivers and biotic interactions in shaping Antarctic vegetation responses to climate, simultaneously testing this approach as a tool predict current and future spatial distributions of Antarctic vegetation.

Chapter 4 addresses the challenge of validating the space-for-time method by testing whether spatial models of lichen diversity and climate relationships can accurately predict observed temporal changes. Preliminary work suggested that space can be a good predictor of time, but mismatches between the spatial and temporal climate variables, and the lack of longer datasets limited the strength of validation. In this chapter, spatial models were applied to predict lichen trait-based metrics at three time points, five years before the spatial assessment and five and ten years after, and tested against independent temporal observations, including both the direction and magnitude of change.

Chapter 5 displays a general discussion, integrating the results obtained throughout the previous chapters within the broader body of existing literature, in particular with regard to the global application of lichen diversity as ecological indicator of climate change. Finally, the chapter highlights the main opportunities, challenges, and limitations that may arise in the future, particularly in relation to a potential integration in international biodiversity monitoring frameworks in the context of climate change adaptation and mitigation policies.

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Chapter 2

2. DISENTANGLING CLIMATE CHANGE EFFECTS FROM THOSE OF OTHER GLOBAL CHANGE DRIVERS

Subchapter 2.1

2.1 Modelling the response of urban lichens to broad scale changes in air pollution and climate

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Abstract

To create more resilient cities, it is important that we understand the effects of the global change drivers in cities. Biodiversity-based ecological indicators (EIs) can be used for this, as biodiversity is the basis of ecosystem structure, composition, and function. In previous studies, lichens have been used as EIs to monitor the effects of global change drivers in an urban context, but only in single-city studies. Thus, we currently do not understand how lichens are affected by drivers that work on a broader scale. Therefore, our aim was to quantify the variance in lichen biodiversity-based metrics (taxonomic and trait-based) that can be explained by environmental drivers working on a broad spatial scale, in an urban context where local drivers are superimposed. To this end, we performed an unprecedented effort to sample epiphytic lichens in 219 green spaces across a continental gradient from Portugal to Estonia. Twenty-six broad scale drivers were retrieved, including air pollution and bio-climatic variables, and their dimensionality reduced by means of a principal component analysis (PCA). Thirty-eight lichen metrics were then modelled against the scores of the first two axes of each PCA, and their variance partitioned into pollution and climate components. For the first time, we determined that 15% of the metric variance was explained by broad scale drivers, with broad scale air pollution showing more importance than climate across the majority of metrics. Taxonomic metrics were better explained by air pollution, as expected, while climate did not surpass air pollution in any of the trait-based metric groups. Consequently, 85% of the metric variance was shown to occur at the local scale. This suggests that further work is necessary to decipher the effects of climate change. Furthermore, although drivers working within cities are prevailing, both spatial scales must be considered simultaneously if we are to use lichens as EIs in cities at continental to global scales.

Keywords

Urban macroecology; Spatial scales; Atmospheric pollution; Ecological indicator; Biodiversity-based metrics; Trait-based diversity.

2.1.1. Introduction

Cities are becoming more densely populated, with 80% of the European population estimated to live in them by 2050 (UN, 2019). Thus, the government-led transition to more sustainable and resilient cities must be hastened (Cartalis, 2014). Together with increased anthropic pressure, a city's sustainability and resilience is threatened by both local drivers (i.e. having a spatial scale of action at the city level), such as the heat island effect (Tam, Gough, & Mohsin, 2015) or the gradual conversion of land from non-urban to urban uses (Romero, Ihl, Rivera, Zalazar, & Azocar, 1999), and broad scale drivers (e.g. climate change, which has a broad spatial range of action) (Dawson et al., 2017). However, we currently do not understand the effects of drivers working on a broad spatial scale in cities. Therefore, the cities' responses to these drivers must be compared at the continental level and not only within cities. Although this is more commonly done by examining physicochemical parameters (e.g. temperature, precipitation, and pollutant concentration), these parameters are unable to quantify the effects of drivers in ecosystems.

Epiphytic lichens have long been used as ecological indicators (EIs) of the effects of global change drivers on ecosystems (Aptroot, Stapper, Košuthová, & van Herk, 2021; Asta et al., 2002; Brunialti, Frati, & Loppi, 2012). Consequently, lichen biodiversity-based metrics (taxonomic and trait-based) can be associated with particular environmental drivers (Branquinho, Matos, & Pinho, 2015). Taxonomic-based diversity metrics (e.g. species richness or total species abundance) respond to high-intensity drivers, such as air pollution (Hauck, Bruyn, & Leuschner, 2013; Lättman et al., 2014), whereas less intense drivers cause shifts in species composition rather than species loss (Ellis & Coppins, 2006). Thus, taxonomic metrics should be complemented by trait-based diversity metrics as these are better at detecting compositional shifts in the communities, are potentially more universal as they are not linked to species identity, and are better indicators of ecosystem function in response to global change drivers (Van Der Plas, 2019).

EIs should quantify the effects of broad scale drivers, even when local scale drivers are present. However, this task is particularly challenging as i) the effects of local scale drivers can overshadow broad scale effects (Branquinho, Serrano, Nunes, Pinho, & Matos, 2019); and ii) the same environmental driver (e.g. temperature) works at both spatial scales (e.g. climate change and the urban heat island effect) (Jenerette et al., 2007). Lichens have been used as the EI of drivers working at the local scale in cities, which include urbanisation (Lättman et al., 2014), air quality (McCarthy, Craig, & Brand, 2009), and the urban heat island effect (Munzi et al., 2014). In studies at the continental level, they have also been used to track broad scale drivers, such as pollution (Mccune, Dey, Peck, Heiman, & Will-Wolf, 1997) and climate (Phinney, Gauslaa, Palmqvist, & Esseen, 2021), but only in natural and semi-natural ecosystems. Thus, it is still not known whether lichen biodiversity-based metrics can monitor the effects of broad scale drivers on urban ecosystems. This could potentially be tested by looking into epiphytic lichen communities across multiple cities along a continental gradient of climate and air pollution for example, but this has not yet been tested.

The aim of this study was to quantify the variance in lichen biodiversity-based metrics (taxonomic and trait-based) that is explained by environmental drivers working on a broad spatial scale, in an urban context where local scale drivers are also present and can potentially overshadow their effects. To do so, we sampled epiphytic lichens, to an extent that has never before been done in cities. We also calculated several taxonomic and trait-based metrics, and tested them as the EI of broad scale air pollution and climate. This was done across a large spatial gradient in Europe (in seven cities from Lisbon to Tartu), representing a continental gradient of climate and pollution. Based on the existing knowledge, we expect part of the metric variance to be related to local scale drivers. Nevertheless, we believe that by exploring such a large continental gradient, lichen metrics will also reflect the influence of broad scale urban drivers. Additionally, broad scale air pollution intensity is expected to surpass that of climate, as urban pollutants present a more deleterious effect on lichens than climate (Evju & Bruteig, 2013; Molina & Molina, 2004). For this reason, we expect taxonomic metrics to better reflect the effects of high-intensity drivers, such as broad scale air pollution, whereas trait-based metrics are expected to be more responsive to less intense drivers, such as climate.

2.1.2 Materials and Methods

2.1.2.1 Sampling sites

Lichen communities were sampled across seven European cities in 2018, under the BIOVEINS project (BiodivERsA32015014). Almada and Lisbon (Portugal), Paris (France), Zurich (Switzerland), Antwerp (Belgium), Poznan (Poland), and Tartu (Estonia) were chosen to represent a large continental gradient of air pollution, climate, and local city characteristics (Fig. S2.1.1). Cities present distinct spatial extensions, namely at local administrative unit level 2 (LAU-2 Level) (http://ec.europa.eu/eurostat/web/nuts/local-administrative-units). Thus, for consistency in the spatial extent, only one section was used for very large cities (e.g. Paris). In all the cities, the sampling sites represented a gradient of urban density. Sampling sites were selected within the “Green Urban Area” class of the pan-European land-cover classification from the European Urban Atlas (EEA, 2018), to confirm the land-use typology and intensity. Stratification was performed using a patch size and connectivity index to randomly select up to 36 patches per city (219 sites in total). See the supplementary material for further details.

2.1.2.2 Field sampling

Epiphytic lichen diversity was sampled following the European standard method (Asta et al., 2002; Cristofolini et al., 2014) (Fig. S2.1.2). The four trees closest to the centroid were sampled while following the method selection criteria (see Supporting information for more details). A sampling grid (50 cm × 10 cm, divided into five squares) was placed on the four main aspects, and all lichens inside the grid were registered. Species abundance was determined on the basis of the number of squares in which they occurred (max. 20). One hundred and forty species were identified, nine of which were classified to the genus level only (Table S2.1.1).

2.1.2.3 Biodiversity-based metrics

The taxonomic and trait-based metrics were then computed. Eight taxonomic metrics were calculated including species richness, number of rare species, the Shannon diversity index, Inverse-Simpson index, total species abundance; and, to quantify community dissimilarity, the Bray–Curtis, Jaccard, and Morisita–Horn dissimilarity indices were used. The R software (R Core Team, 2022) was used to calculate the taxonomic diversity metrics using the functions *diversity* and *vegdist* from the *Vegan* package (Oksanen et al., 2011). Regarding the trait-based metrics, indices representing both functional diversity and functional structure were computed, accounting for the abundance of species identified to the species level (Table S2.1.1). This was performed based on seven categorical traits known to respond to climate and/or air pollution (Table S2.1.2); these are growth form, main photobiont type (green algae other than *Trentepohlia*, *Trentepohlia*, and cyanobacteria), species substrate pH tolerance, tolerance to solar irradiation, tolerance to aridity, tolerance to eutrophication, and poleotolerance. Trait information was retrieved from the ITALIC database (Nimis & Martellos, 2021), and the maximum value for each species was used (trait classification is ordinal in the ITALIC database). Regarding functional diversity metrics, we calculated functional richness and Rao's quadratic entropy (RaoQ), and for functional structure, we calculated the community weighted mean (CWM). Trait-based metrics were calculated using the R software (R Core Team, 2022) function *dbFD* from the *FD* package (Laliberté, Legendre, & Shipley, 2014). See the Supporting information for details on the metrics calculation and justification of the selected metrics. Some metrics were omitted in the main text because of their high similarity with the other presented results (see Supporting information).

2.1.2.4 Environmental variables

A set of 26 environmental variables representing air pollution and climate (Harlan & Ruddell, 2011) was extracted for each sampling site (the average values per city are shown in Table S2.1.3). Both climate and pollution were derived from models working at a continental scale, thus, climate and air pollution values show little variance within each city site; this means that they represent environmental gradients working at the continental scale. In addition, in this study, scale refers to the spatial extent to which variables work (i.e. where the variance in the environmental variables between sampling sites can be found), and not to the geographical spatial extent of the study or to the quantification of variables.

For broad scale climate, 19 bioclimatic variables representing air temperature and precipitation were retrieved from the CHELSA database (Karger et al., 2018; Karger et al., 2017), (average 1979–2013) at the maximum available spatial resolution of 1 km (Table S4). For broad scale air pollution, redN and OxN deposition ($mg \cdot m^{-2}$), as well as NH_3, SO_2, NO_x, PM2.5, and PM10 concentrations in the air ($\mu g \cdot m^{-3}$) for the year 2018 were retrieved from the EMEP (Fagerli et al., 2019) at the maximum available spatial resolution (11 km). We tested pollutants data from several years (2000-2018). Because all years performed similarly, only 2018 was used for further analysis. Owing to their spatial resolution, all extracted environmental variables present minimal variation at the local scale (e.g. within each city). To account for the remaining broad scale variability not represented by climate and air pollution, an additional factor was added to the analysis. This factor is “Other”, which corresponds to city identity and is meant to represent all other potential drivers working at the broad scale, aside from air pollution and climate. This factor is expected to represent the remaining city characteristics that vary on a broad scale (i.e. differentiating cities), such as city size, air pollution legacy, and management policies.

2.1.2.5 Data analysis

All analyses were performed with R software (R Core Team, 2022). Two principal component analyses (PCAs) were performed, one on the set of 19 climate variables and another on the set of seven air pollution variables. This was done to reduce the number of variables to two dimensions each (two axes), while representing most of the variance in the original datasets. The site scores were used as climate and air pollution variables in subsequent analyses. The second axis of the climate PCA isolated Zurich in the precipitation gradient, suggesting the use of a log-transformation of precipitation variables. However, the resulting PCA was similar to the non-log-transformed variable (data not shown); thus, the transformations were discarded. The PCAs were computed using the *prcomp* function from the *stats* package. Spearman correlations were used to make a preliminary assessment of the relationships between air pollution, macroclimate PCA axes, and biodiversity metrics (Fig. S2.1.4), as well as to reduce the number of biodiversity metrics shown to prevent overcrowding. The inter-quartile range (25–75), minimum, maximum, average, and median of each taxonomic, functional diversity, and functional structure metric were calculated by city and presented as boxplots.

Linear regressions were used to model the response of each biodiversity metric to the four climate and air pollution variables (the site scores of the first two axes of each driver's PCA). To ensure that the linear model approach was adequate, we checked the residual distribution (normality) and homogeneity of distribution (quantile-quantile and density plots) of the linear models (Fig. S2.1.5 to S2.1.9). The residuals showed good distribution in terms of normality and homogeneity, thus confirming the adequacy of the linear models. The potential interactions between the four broad scale pollution and climate variables (by means of multiplicative linear models) were not tested here, as our focus was on the individual effect of each broad scale driver on each lichen biodiversity-based metric.

A categorical variable named "Other", coded as the city name, was also tested both as a fixed and random factor in the linear models. First, it was tested as a fixed factor, along with the four climate and air pollution variables, in an attempt to represent the remaining drivers other than pollution and climate acting at the broad scale (Biodiversity metric ~ Climate PCA1 + Climate PCA2 + Pollution PCA1 + Pollution PCA2 + Other). The variable "Other" was highly collinear with the remaining four variables (VIF > 150000), and the resulting model was not better in terms of fit; thus, this variable was excluded as a fixed predictor. This result suggests that all broad scale variance could be accounted for by looking only at climate and pollution, and the city did not bear any extra information. The remaining four variables (climate and pollution) presented low collinearity (VIF < 1.5) and were kept in the model (Zuur, Ieno, & Elphick, 2010). Second, "Other" was also fitted as a random term in a linear model [(Biodiversity metric ~ Climate PCA1 + Climate PCA2 + Pollution PCA1 + Pollution PCA2

+ (1|Other)]. Across all modelled metrics, the variable “Other” explained the majority of the variance that was previously explained by air pollution and climate, thereby effectively cancelling their effect without adding new information. Because doing so would not allow us to separate the effect of broad scale drivers without providing us with any new information, we proceeded with linear modelling variance partitioning. Thus, the linear models used to quantify the response of biodiversity metrics to broad scale drivers did not include “Other” [(Biodiversity-based metric ~ Climate PCA1 + Climate PCA2 + Pollution PCA1 + Pollution PCA2].

For each model, the total R^2 variance was partitioned to assess the proportion of variance explained by each predictor working at the broad scale. The remaining variance (= 1 - R^2) was interpreted as unaccounted variance associated with drivers working at a local scale, without further detail. In other words, local scale drivers were not investigated as this was not the focus of the study. Variance partitioning is presented for each biodiversity metric, and averaged by group (taxonomic, functional diversity, and functional structure for each trait) and main broad scale driver to summarise the variance explained by each, thereby facilitating clarification of the study expectations. This was done assuming that all metrics had the same value. To prevent overcrowding, metrics with very similar responses were omitted from Figure 3.3. The results of the remaining metrics are presented in the Supporting information (Fig. S2.1.10). The models were considered statistically significant at $p < 0.05$. Models were performed using the *lm* function from the *stats* package, and the variance partition corresponded to the sum of squares of each predictor divided by the total (i.e. sum of squares of all predictors). As previously stated, the remaining unexplained variance in the model, which is that not explained by any of the variables working at a broad scale, was interpreted as being most likely driven by variables working at the local scale (acting at the city spatial scale). We are confident of this interpretation of variance partitioning between the broad and local scales. The results (see above) of using "Other” as a fixed term in the model allowed us to assume that city identity represents all possible sources of variance at the broad scale (e.g. climate and pollution, but also geology, other climate variables, day length, city age or city environmental policies). Thus, although we did not include other broad scale drivers, the fact that city identity accounted for the same amount of variance in lichen biodiversity metrics as pollution and climate (data not shown) gives us confidence that the four broad scale variables used are, in fact, the most important ones acting on a broad scale. In this way, we were able to extract most of the variance that could be accounted for at the broad scale, and the remaining unexplained variance in lichen metrics could be attributed to the local scale (e.g. caused by local air pollution or surrounding land-use, park, or tree characteristics).

2.1.3 Results

2.1.3.1 Summarizing the climate and air pollution continental scale gradients

The PCA of climate variables (Fig. 2.1.1a) showed a main gradient of temperature on the first axis (58.8%) and a gradient of precipitation on the second axis (21.6%). Together, they represented most of the variance in climate (80.4%), with sampling sites clustered in cities along the temperature axis. Almada and Lisbon overlapped over the side with the warmest temperature of the continental-scale climate gradient, as expected, given their close geographic proximity. Paris and Antwerp followed in the middle part of the temperature gradient, with Zurich and Poznan next to them. Tartu stood on the opposite side of the temperature gradient as the coldest city in the dataset. In relation to the precipitation gradient (second axis), Zurich was the wettest city in our dataset, whereas the remaining cities presented similar precipitation levels that were on the driest part of the gradient.

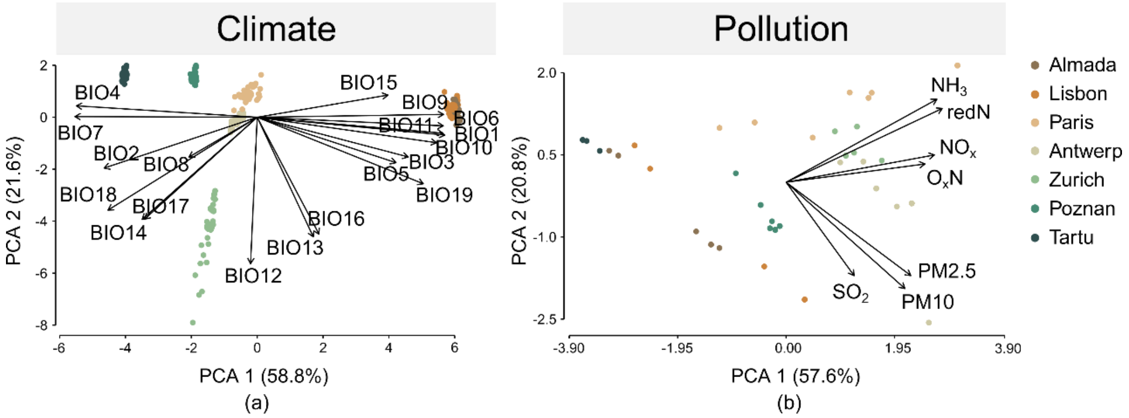

Figure 2.1.1: Principal Components Analysis showing the ordination of sampling sites (N=219) along climate (a) and air pollution (b) gradients. Decoding of pollution variables can be seen in Table S3. Decoding of bioclimatic variables can be seen in Table S4. Sites overlap in PCA (b) due to the lower spatial resolution of air pollution variables.

The PCA of air pollution variables (Fig. 2.1.1b) showed a clear main gradient of overall increasing air pollution loads on the positive side of the first axis (57.6%), and a second one (20.8%) representing a gradient of pollutant types. Together, they represented most of the variance in air pollution (78.4%). The second axis can generally be interpreted as representing a gradient of sites dominated by N-based compounds (corresponding to eutrophication), and sites dominated by sulphur dioxide and particulate matter (corresponding to acidification, although particles can also be associated with eutrophication). The cities did not appear to be as distinctly clustered by pollution as they did by climate. In terms of the overall broad scale air pollution load, Paris, Antwerp, and Zurich were the most polluted cities, followed by Poznan, Lisbon, and Almada, with Tartu representing the least polluted part of the gradient. In terms of pollutant types, Paris, Antwerp, and Zurich appeared to be more dominated by N compounds, whereas Almada, Lisbon, and Poznan seemed to have more sulphur dioxide and particulate matter.

2.1.3.2 Characterization of taxonomic and trait-based metrics in lichen

In total, 140 species of lichen were identified across all cities (Table S2.1.1). We found that Lisbon, Tartu, and Zurich harboured more species-rich lichen communities, whereas Poznan had the least rich communities (Fig. 2.1.2). The values for the Shannon diversity index was also the highest (i.e. more diverse) in Lisbon, Tartu, and Zurich; and so was the total abundance of lichens in these three cities. Meanwhile, the lowest total abundance was observed in Almada and Poznan (Fig. 2.1.2). All cities showed high spatial dissimilarity values. On average, Almada was the most dissimilar, whereas Lisbon, Paris, and Zurich showed the lowest spatial dissimilarity (Fig. 2.1.2).

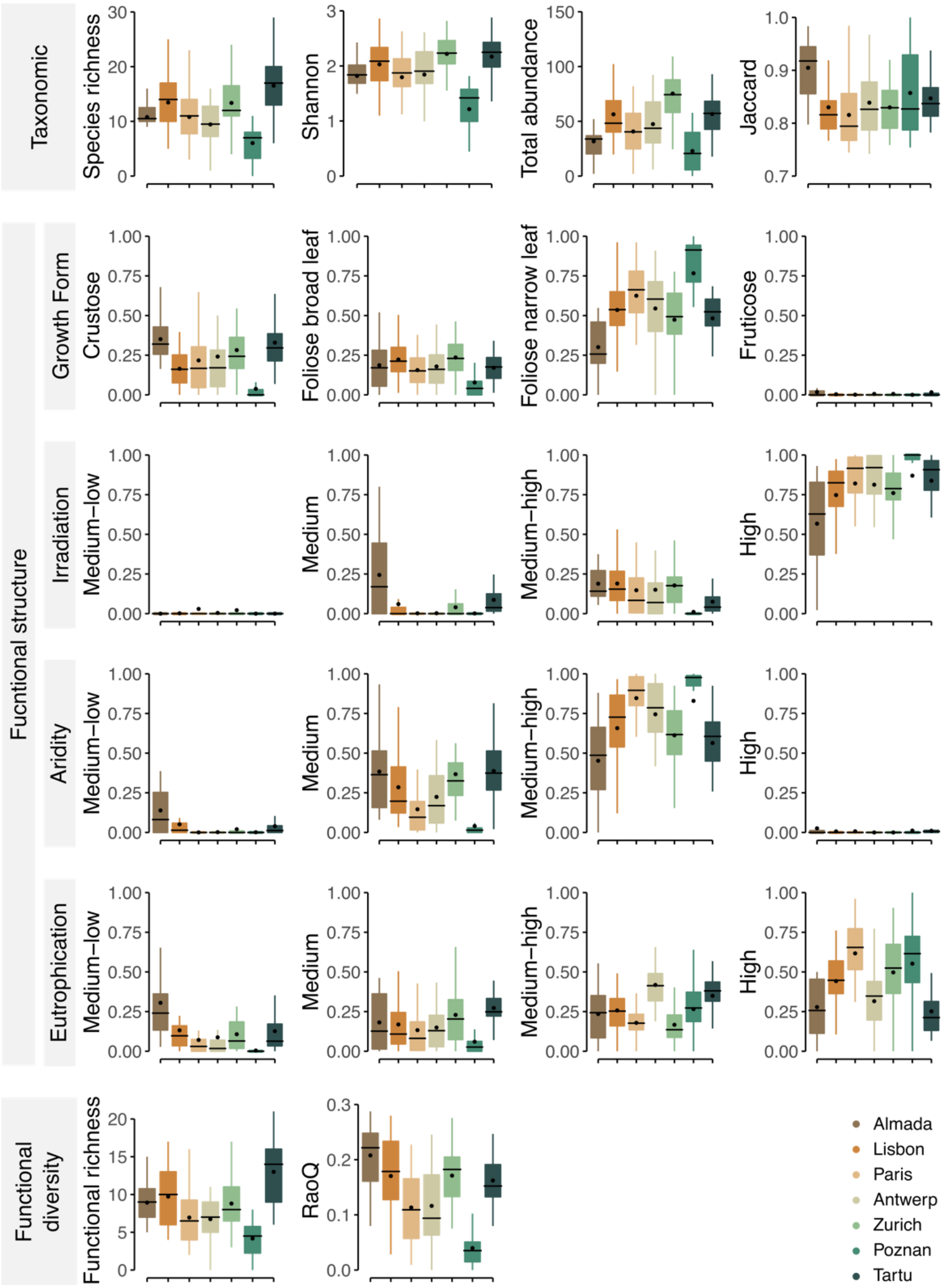

Figure 2.1.2: Boxplots representing the distribution of taxonomic, functional structure and functional diversity metrics in the seven European cities, ordered here from the warmest (Almada to the coldest (Tartu), as indicated in the first axis of the PCA. Shannon, Jaccard and Rao's Q indices values range from 0 to 1. The functional structure, represented here by the CWM of each functional group (Table S2) belonging to the same trait (growth form, tolerance to irradiation, tolerance to aridity, tolerance to eutrophication) ranges also from 0 to 1 (the sum of all functional groups from the same trait is 1 at site level). Boxes display the interquartile range (25th and 75th percentiles), the black lines the median, the dots the average, and the whiskers the maximum and minimum (N=219).

In terms of functional structure, foliose narrow-lobed lichens were the dominant growth form in all cities, whereas crustose and foliose broad-lobed lichens were present in lower and similar proportions (Fig. 2.1.2). The exception was Almada, where crustose lichens co-dominated with foliose narrow-lobed lichens, followed in abundance by foliose broad-lobed lichens. Fruticose lichens represented a small proportion across all cities (< 5% abundance). Communities were also dominated by lichens tolerant to high solar radiation in all cities, with those with the highest tolerance level accounting for more than 75% of the total lichen abundance (Fig. 2.1.2). The exception was, again, Almada, where medium-tolerant species accounted for 25% of the abundance. However, the aridity pattern was different (Fig. 2.1.2). Species less tolerant to arid conditions (i.e. those that were more hygrophytic) were nearly absent from all cities, except Almada (13%). Poznan was completely dominated by species with medium-high tolerance to aridity, with Antwerp, Lisbon, Paris, and Zurich also presenting high abundances of these species (> 60 %). In Almada and Tartu, medium-tolerant species dominated. Lichens more tolerant to eutrophication were clearly dominant (usually representing more than 50% of the total) in Paris, Zurich, Poznan, and Lisbon (Fig. 2.1.2). These were also the cities where species with lower eutrophication tolerance was scarce. However, in Antwerp, highly tolerant but especially medium-high eutrophication-tolerant lichens dominated. Tartu and Almada showed a more uniform distribution of species abundance between the different levels of eutrophication tolerance (Fig. 2.1.2). Similar to species richness, functional richness values were also highest in Tartu and lowest in Poznan, while the remaining cities ranked in the middle with similar values (Fig. 2.1.2). Regarding functional dispersion, Almada showed the highest values and Poznan showed the lowest (Fig. 2.1.2).

2.1.3.3 Partitioning the proportion of variance in biodiversity metrics explained at the broad and local scale

The vast majority of the models (Fig. 2.1.3 and S2.1.10) were found to be highly significant, showing p-values lower than 0.001, thereby strongly supporting their use for interpreting the data. Across all biodiversity metrics (Fig. 2.1.3), the four broad scale drivers explained an average of 15%. The remaining unexplained variance was on average 85%, and was assumed to represent all drivers that are not working at the broad scale. This can thus be interpreted as local scale variation (i.e. acting at the city scale). For six biodiversity metrics, broad scale drivers explained more than 20% of the variance. Only the Jaccard, low tolerance to irradiation, and high tolerance to aridity models were not significant.

Considering the main broad scale drivers (pollution and climate) and all the biodiversity metrics together, air pollution accounted for most of the variance found at the broad scale; it explained, on average, 11.3% of the variance, while climate explained 6.7% (Fig. 2.1.3b). When the metrics were grouped into taxonomic and functional structures or according to functional diversity (i.e. by the metric type, Fig. 2.1.3b), broad scale pollution surpassed climate across the three metric groups. When considering each metric individually, air pollution also surpassed climate across 14 of the 22 biodiversity-based metrics. Within the taxonomic metrics group, which is composed of the species richness, Shannon index, total abundance, and Jaccard dissimilarity index, air pollution explained more variance (7.8%) in comparison to climate (6.3%). However, not all taxonomic metrics responded equally (Fig. 2.1.3a). Within the significant models, species richness responded almost exclusively to the broad scale air pollution gradient (17.5%), the Shannon index responded similarly to both pollution and climate (7.6% and 7.5%, respectively), and the total abundance responded mainly to climate (16.3% out of 19.9%). Air pollution also explained the majority of the variance in functional diversity metrics (21.3% on average; Fig. 2.1.3b). Nevertheless, similar to the taxonomic diversity, each functional diversity metric responded differently. While the functional richness variance was almost exclusively explained by broad scale air pollution (24.3%), for the RaoQ metric, both pollution and climate were equally relevant (18.4% and 14.2%, respectively; forming a total of 32.6%).

Regarding the functional structure, the relevance of each broad scale driver was also different depending on the trait considered, and even among functional groups within the same trait (Fig. 2.1.3b). The variance explained by growth form was mostly attributed to the air pollution gradient (7.2% versus 4.3% of climate). Considering each growth form (Fig. 2.1.3a), air pollution was more important than climate for crustose and foliose narrow-lobed lichens (9.7% in 13.4%, and 12.8% in 19%, respectively), and it was the only driver explaining variance for fruticose (5.4%). For foliose broad-lobed lichens, climate was the most important driver (7.1% out of 8.1%). For the irradiance trait (Fig. 2.1.3a), the variance explained was almost equally divided between the two broad scale drivers when the average of all functional groups of the trait was considered. Within the significant models, the medium irradiance tolerance metric was mainly explained by air pollution (19.9% in 24.3%) and was one of the six models in which broad scale drivers jointly explained more than 20% of the variance. Contrarily, in both the medium-high and high metrics, variance was more satisfactorily explained by climate (19.9% in 24.3%, and 6.4% in 10.2%, respectively). For the aridity trait, air pollution explained, on average, twice as much as climate (12.6% versus 5.5%, Fig. 2.1.3b). In the medium-low, medium, and medium-high metric variances, air pollution surpassed climate (14.4% in 22.2%, 17.9% in 25.1%, and 17% in 20.4%, respectively). Finally, for the eutrophication trait, most of the variance was also explained by broad scale pollution (10.3% versus 3.8% for climate, Fig. 2.1.3b). Within the medium-low and medium metrics, air pollution was in fact the main broad scale driver (13.2% in 19%, and 8.7% in 11.8%, respectively), while in the medium-high metric, the importance of air pollution was similar with that of climate (5.6% and 6.1%, respectively).

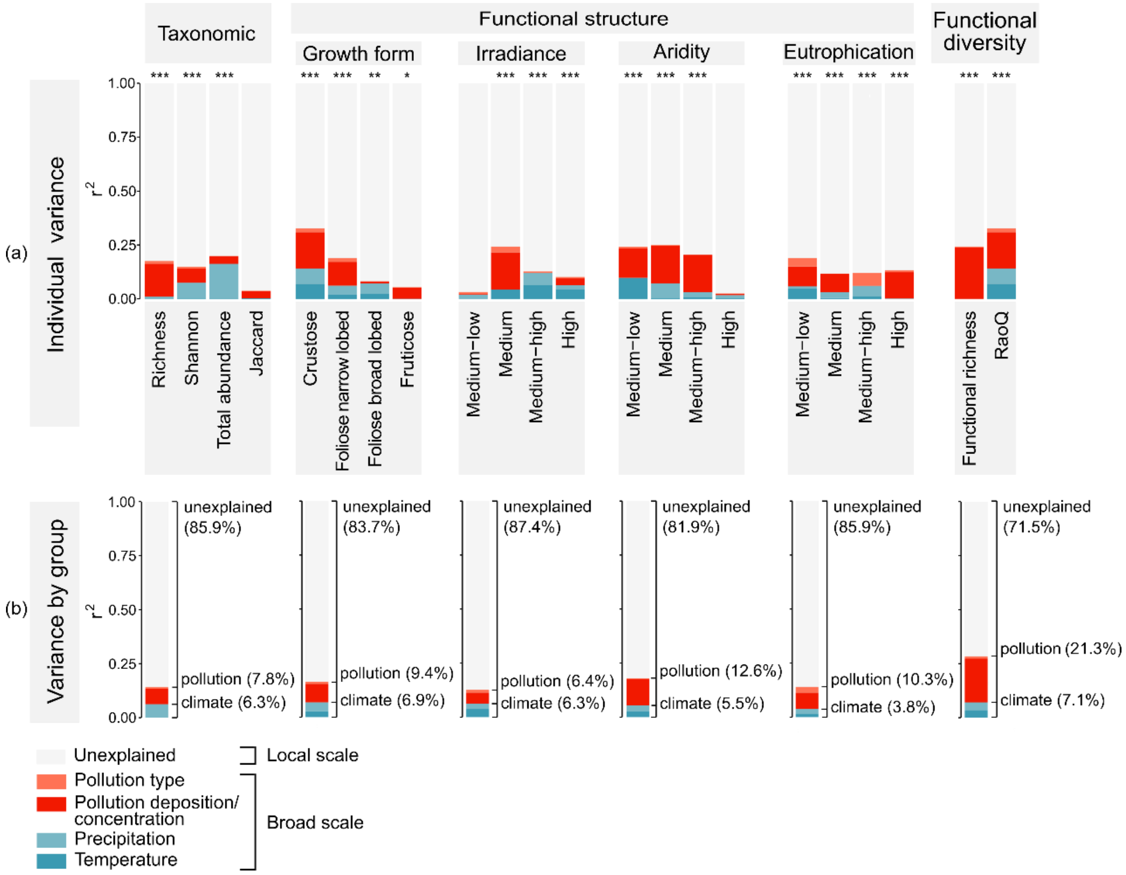

Figure 2.1.3: Variance partitioning of broad scale drivers for each (a) lichen biodiversity metrics and (b) the average variance partitioning for each group of metrics. Metrics are grouped into taxonomic and trait-based, the latter sub-divided into functional diversity and functional structure by trait (growth form and tolerance to irradiance, aridity and eutrophication). Significance of the model is indicated in superscript: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Averaged variance (%), per spatial scale and metric group, was calculated assuming all metrics to value the same when evaluating changes in lichens community structure.

2.1.4 Discussion

For city adaptation and compliance with the United Nations' Sustainable Development Goals (SDGs) (UN, 2015), we must focus on a key aspect of urban areas, namely urban ecosystems (Maes, Jones, Toledano, & Milligan, 2019). Therefore, it is fundamental to evaluate the effects of global change drivers at the ecosystem level, and this can only be fulfilled by comparing cities across large areas (i.e. continental to global) and looking at drivers working at the broad scale using EIs (Hák, Janoušková, & Moldan, 2016). To the best of our knowledge, this is the first time that lichen biodiversity-based metrics have been used as an EI to quantify the importance of broad scale drivers in an urban context. Our study found that broad scale drivers accounted for an average of 15% of all variance among lichen metrics. Furthermore, within the broad scale drivers studied, air pollution (10%) was more important than climate (5%) across all metric groups. Consequently, and as expected, taxonomic metrics were better explained by the broad scale air pollution drivers. However, contrary to our expectations, broad scale climate was less important than broad scale air pollution for trait-based metrics.

Broad scale drivers (nearly homogeneous within the city and, thus, have little variance between sampling sites) accounted for an average of 15% in lichen metric variance, and reached a maximum of 33% in the RaoQ metric. Although the relevant studies were conducted only in natural or semi-natural environments, the effects of broad scale drivers on lichen communities are well-documented (Geiser et al., 2021; Phinney et al., 2021). Therefore, we expected the variance partitioning among urban lichen metrics to reflect, to some extent, the influence of these broad scale drivers. Although broad scale drivers play a role in shaping urban lichen diversity, the unexplained variance suggests that drivers acting at the local scale may overshadow this effect. These local scale drivers are known to act as high-intensity drivers and are well-documented in cities, albeit only for single-city studies (Davies, Bates, Bell, James, & Purvis, 2007; Koch et al., 2019; Munzi, Ravera, & Caneva, 2007). Along with the urbanisation process, lichen communities are driven towards species that are more tolerant to the effects of high-intensity local drivers (Hawksworth, 1990; LiŠKa & Herben, 2008), to a point where the effects of other less intense drivers (i.e. broad scale climate) are overshadowed. Thus, one potential solution to better isolate the effects of broad scale drivers on lichen communities is to have standardisation for such local scale effects. This could be done, for example, by homogenising the characteristics of the sampling sites (e.g. green areas of the same size). Another possibility is to include more cities with broader gradients of broad scale pollution and climate. However, because the effects of broad scale drivers are statistically significant across the majority of metrics, both spatial scales must be considered simultaneously to interpret lichen data derived from multi-city studies.

Within the broad scale, and as expected, the air pollution gradient was overall more significant (10% out of 15% of the total broad scale variance) to lichen metrics than climate. Air pollution has long been a high-intensity driver in urban areas due to the presence of local industrial and traffic pollutants within nearby cities (Babiy, Kharytonov, & Gritsan, 2003; Fenger, 1999), which translates to increased pollutant concentration or deposition (Krzyzanowski et al., 2014; Riga-Karandinos & Saitanis, 2005). Although the role of specific pollutants has been observed in several local scale studies (Llop, Pinho, Ribeiro, Pereira, & Branquinho, 2017; Varela et al., 2018), our results suggest that on a broad scale, when multiple pollutants are superimposed, lichens respond to pollution overall rather than to specific pollutants. Because lichens absorb pollutants from wet and dry atmospheric deposition (Van Der Wat & Forbes, 2015), increased air pollution levels translate to an overall harmful effect on most lichen species, and result in consequent species loss, as reported by several authors in single-city studies (Gary, 2010; Koch et al., 2016; Munzi et al., 2007). Here, we confirm that this pattern is also visible at the broad scale, as the influence of broad scale air pollution largely surpassed that of climate on species richness, one of the largest across all metrics. Meanwhile, for both the Shannon index and total abundance metrics, the importance of pollution in relation to climate decreased. In contrast to our initial expectations, air pollution was also the main broad scale driver across both functional structure and functional diversity metric groups, reaching a maximum of 24% with the functional richness metric. These results reinforce the idea that high-intensity drivers, such as pollution, act as filters for lichens, promoting both species loss and the overall narrowing of functional groups. Thus, metrics based on presence (i.e. species richness or functional richness) are more suitable for tracking the effects of high-intensity drivers, regardless of them being taxonomic or trait-based in nature. Based on our results, we can also suggest that air pollution in Europe is still above the critical level and load for lichens (Cape et al., 2009); in other words, pollution levels are above the threshold for causing shifts in lichen communities. These shifts can either be due to overall negative effects of pollution or to a fertilizing effect on more tolerant lichen species (Fig. S2.1.4), although our data does not allow to disentangle their effects as they co-occur. This is in accordance with what was observed in other studies (Llop, Pinho, Matos, Pereira, & Branquinho, 2012; Llop et al., 2017), but was observed for the first time in multiple cities and with broad scale air pollution. The air pollution model used (EMEP MSC-W) includes not only pollution sources and concentrations, but also climatic information. Thus, the broad scale air pollution data are expected to be richer than the climate data, which could help explain why it surpassed climate across all metric groups. This cannot be avoided, as it represents both broad scale pollution and climate as accurately as possible. However, if climate alone had no effect on lichens, we would not have retrieved models based mostly on pollution. This reinforces the interpretation that air pollution is the main driver of lichen community composition on a broad scale, although it is very likely that climate has an indirect effect by affecting pollutant dispersion and deposition (Fiore, Naik, & Leibensperger, 2015; Kinney, 2008). Although not tested here, future studies should aim to assess the effects of the interactions between both broad scale drivers on urban ecosystems. Still, to do that, sampling must be extended to a larger number of cities, which may be unfeasible. The effects of the broad scale climate were, as expected, lower than those of pollution across all metrics and metric groups (average of 5% out of 15%). Despite the large continental gradient representing a broad range of climatic conditions (mean annual temperature and annual precipitation ranging from a maximum of 17 °C and 1071 mm to a minimum of 5 °C and 517 mm, respectively), its importance to all lichen metrics was still low. The response of lichens to broad scale climate change has been well-documented (Concostrina-Zubiri, Pescador, Martínez, & Escudero, 2014; Di Nuzzo et al., 2021; Hurtado et al., 2020; Matos et al., 2015); however, those studies were mostly conducted in natural and semi-natural environments. Nevertheless, our work suggests that, in urban contexts, air pollution is probably still the most limiting factor for lichens, overshadowing climate effects. In fact, the two least polluted cities, Almada and Tartu, showed a high abundance of lichens that were more sensitive to aridity, suggesting that climate can become an important driver of lichen diversity when air pollution is not prevalent. Such phenomenon has already been detected in a previous study in Almada (Munzi et al., 2014), where the effects of the urban heat island were only detected when air pollution was low. The effects of climate on lichens are expected to be of lower intensity than those on air pollution, primarily inducing compositional shifts in lichen communities, which should be reflected more in trait-based metrics than in taxonomic ones (Ellis & Coppins, 2006). For this reason, we expected a higher contribution of the broad scale climate across the trait-based metrics, particularly in climate-related traits (e.g. irradiance, aridity). Nevertheless, broad scale air pollution surpassed the contribution of climate in these metrics groups also, emphasising the still prevalent role of other high-intensity drivers, such as air pollution, in cities. Furthermore, low- to intermediate-intensity drivers, such as climate, are expected to lead to changes in the abundance of lichen species (Branquinho et al., 2019), rather than species loss. Here we confirm that view, as metrics based on abundance (i.e. total abundance, RaoQ) reflected a high contribution from broad scale climate. However, to develop EIs for the effects of climate in cities, future research must also focus on either proposing alternative metrics or developing statistical methodologies to disentangle the effects of the prevailing environmental drivers (e.g. air pollution) before examining the effects of climate. Such a need has already been raised (Branquinho et al., 2015), but remains unanswered. Although this problem is foreseen to decrease in the future, with the increasing importance of climate change together with a decrease in air pollution loads across Europe (Ortiz et al., 2020), disentangling climate from the remaining urban global change drivers is fundamental to establishing a baseline of effects on a broad scale (Ellis and Coppins, 2010; Nascimbene et al., 2012).

In the urban areas studied, the importance of local scale drivers across all tested metrics, assumed here to represent the proportion of variance not explained by the broad scale drivers, averaged at 85%. These could include local pollution sources (e.g. industrial, or from facilities or traffic), green urban area management and history (e.g. parks created from existing woodlands), and phorophyte species and age (although this last one was partially controlled through field sampling phorophyte restrictions), all of which have been seen to impact urban lichen communities (Matos, Vieira, Rocha, Branquinho, & Pinho, 2019; McDonald et al., 2017; Munzi et al., 2014). These results show the need to further detail the local drivers and their sources. To do so, one requires the use of local environmental data, which should be derived from common methods, rather than local information sources, such as city-specific cartography or single-city studies. Furthermore, other methods, such as lichen elemental analysis, can complement the application of lichens as ecological indicators because they possess the capacity to detail local pollution sources and origins (Jeran, Jaćimović, Batič, & Mavsar, 2002; Van Der Wat & Forbes, 2015). However, this is a more costly approach, contrary to the method of using ecological indicators, and cannot reflect the effects of environmental drivers at the ecosystem level. Thus, these findings emphasise that, for future European (EU) and global (UN - 11th SDG) efforts towards more sustainable and resilient cities, the development of indicators to monitor the effects of global change drivers on urban ecosystems must be able to detect the effects of drivers working at both scales and be applied over wide continental to global gradients (Hák et al., 2016; Klopp & Petretta, 2017). Despite all limitations and future challenges, biodiversity-based EIs are a valuable tool for quantifying the effects of broad scale drivers on the structure and properties of urban ecosystems. As they are based on biodiversity, the backbone of ecosystem functioning, they can reflect the effects of these global change drivers on urban ecosystem services and functioning, which analytical methods (i.e. pollutant concentration, air temperature, precipitation, and humidity level monitoring) alone are not capable of translating. Furthermore, their cost-effectiveness allows for extensive monitoring of these effects over multiple cities.

2.1.5 Conclusions

For the first time, we quantified the amount of variance in lichen biodiversity metrics explained by broad scale drivers associated with pollution and climate in urban areas. Overall, broad scale drivers explained 15% of variance in lichen metrics, suggesting these were overshadowed by the effects of local drivers. Thus, our work supports the need to quantify the effects of drivers working at a local scale, even in multi-city studies. On a broad scale, air pollution was more important than climate, suggesting that urban lichen communities are primarily driven by pollution. Prior to this study, this has only been shown for single-city studies, but now it is also shown here in a multi-city design. The results also suggest that the effects of climate change can be detected only when pollution decreases. In our study, the effects of broad scale drivers had a statistically significant effect on lichen metric variances, and thus must be considered alongside local drivers. From an ecological indicator perspective, the overall low contribution of broad scale drivers across most metrics suggests that the tested lichen biodiversity-based metrics and statistical approaches are difficult to apply directly, that is, independently of the local context, when characterizing the effects of broad scale drivers. However, the capacity of EIs to reflect the effects at the ecosystem level makes them valuable tools for achieving UN conventions goals in urban areas, such as those goals related to air pollution (CLRTAP) and climate change (UNFCCC). Their application can help overcome the lack of baseline characterisation of pollution and climate effects, especially in southern European cities (Reckien, Flacke, Olazabal, & Heidrich, 2015). In so doing, they can help create more resilient cities to face future climate change effects, as well as strategies to measure the efficacy of adopted measures. However, metrics must first be compared across cities, and for that, the effects of broad- and local scale drivers must be simultaneously considered. Thus, to use them to evaluate the effects of broad scale drivers in urban ecosystems, alternative sampling designs or statistical approaches must first be considered.

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Subchapter 2.2

2.2 Non-linear responses of ecological indicators to urban environmental drivers across Europe

Rocha, B., Matos, P., Giordani, P., Casanelles-Abella, J., Moretti, M., Deguines, N., Laanisto, L., Alós Ortí, M., Tryjanowski, Hallikma, T., Branquinho, C., P., Pinho, P. (2026) Non-linear responses of ecological indicators to urban environmental drivers across Europe. Journal of Environmental Management 404, 129316. doi: https://doi.org/10.1016/j.jenvman.2026.129316

Abstract

Urban ecosystems are impacted by multiple environmental drivers which can work at different spatial scales and intensities, negatively impacting biodiversity and ecological processes. Ecological indicators such as lichens are used to measure the impacts of these drivers at the ecosystem level and provide valuable information for urban policy guiding. Lichen responses interpretation is nevertheless complex, as these drivers act together in cities. Furthermore, while lichen responses are often nonlinear, studies typically ignore such complexities. This not only hinders our ability to disentangle the individual effect of each driver but also prevents evaluating the effectiveness of policies to mitigate their impact. Here, we used a continental lichen biodiversity dataset and a machine-learning approach to disentangle the individual effect of the prevailing drivers on urban lichens. Particularly, we aimed at identifying the intensity/direction of the effect of single drivers across cities and determining, for each driver, the presence of threshold-like responses shaping lichen biodiversity. We sampled epiphytic lichen biodiversity in seven European cities, from which taxonomic and functional metrics were calculated. Environmental drivers related to temperature, water availability and anthropic pressure were modelled to explain lichen metrics with Random Forest, to account for the potential collinearity and non-linear responses. Overall, temperature and water availability were the most important drivers for urban lichen biodiversity, followed by anthropic pressure, mainly related to urbanization. Partial dependence responses revealed thresholds of lichen biodiversity change along the environmental gradient considered. The quantification of the main drivers of urban lichen diversity is extremely useful to inform city-level sustainability and biodiversity policies, while the identification of thresholds is crucial to better define environmental safety limits in urban planning.

Keywords

Urban ecosystems; Biodiversity metrics; Climate; Anthropic pressure; Random Forest; Policy guiding

2.2.1 Introduction

Increasing urban sustainability and resilience requires a deep understanding on how anthropogenically modified environmental drivers shape cities, specifically when quantifying biodiversity, ecosystem change and the underlying mechanisms of those changes. Doing so is complex because in cities multiple drivers work simultaneously at both broad (i.e. with a broad spatial range of action, like climate change) and local (i.e. having a spatial scale of action at the city level, like the urban heat island or land conversion to urban uses) spatial scales and with different intensities. In this context, understanding the separate contribution of individual environmental drivers while accounting for the effects of all others remains challenging.

Ecological indicators based on biodiversity metrics are valuable tools to quantify environmental drivers' effects at the ecosystem level (Branquinho, Serrano, Nunes, Pinho, & Matos, 2019), thus helping guide European and Global sustainability goals for urban areas (Fioretti, Pertoldi, Busti, & Van Heerden, 2020; Kostetckaia & Hametner, 2022). Nevertheless, knowledge gaps remain regarding their use as ecological indicators across the world, namely in low- and middle-income countries (Rochette et al., 2019; Selomane, Reyers, Biggs, Tallis, & Polasky, 2015), despite being promising tool for environmental monitoring. Epiphytic lichen diversity has long been used as ecological indicators in cities around the world, mainly for air pollution (Boonpeng, Sangiamdee, Noikrad, & Boonpragob, 2023; Koch et al., 2016; Lawal & and Ochei, 2024; Mikhaylov, 2020; Warren et al., 2019). Due to recent trends of improved air quality in several regions, namely Europe and North America (Sicard et al., 2023; Warren et al., 2019), climate and land-use changes have become also important drivers of urban lichens biodiversity (Munzi et al., 2014; Simkin, Seto, McDonald, & Jetz, 2022), which makes data interpretation more complex. Recently, a study conducted across a broad European gradient of air pollution, climate and local urban conditions found that local scale drivers explained most of the variance across several lichen biodiversity metrics (Rocha et al., 2022). However, this focused on linear responses, and thus the importance of broad scale drivers' effects like air pollution or climate could have been overshadowed due to the existence of non-linear relationships between lichen biodiversity metrics and environmental drivers.

Lichen biodiversity responses to individual environmental drivers like temperature, precipitation and forest cover has already been found to be non-linear (Nascimbene & Marini, 2015; Phinney, Ellis, & Asplund, 2021). This is in part due to the existence of threshold-like responses, i.e., a level of environmental pressure that creates a discontinuity in species response to this pressure, producing non-linear changes (Hillebrand et al., 2020). Identification of these tipping-points is important from an ecological indicator perspective to establish environmental safety thresholds, above which ecosystem and biodiversity status decline (Andersen, Carstensen, Hernández-García, & Duarte, 2009), such as critical loads and levels for nitrogen pollution (Pinho et al., 2009). Also, identification of these thresholds is fundamental to establish envelopes of environmental conditions until or after which lichen biodiversity metrics can be applied. Building from previous work (Rocha et al., 2022), nonparametric regression analyses like Random Forest modelling could prove advantageous when dealing with non-linear responses (Breiman, 2001; Musseau et al., 2022), together with the inclusion of potentially collinear high-spatial resolution data to further help disentangle the effect of multiple drivers. Yet, these novel statistical approaches remain untested for lichens in cities.

The aim of this study was to analyse the individual effects of concurrent urban environmental drivers structuring lichen biodiversity in cities and determine if response thresholds of lichen taxonomic and functional metrics exist. This was done by testing two main hypotheses:

1. Lichen biodiversity metrics response to climate (e.g. temperature and water availability) and anthropic pressure (e.g. air pollution and land-use changes) can be non-linear. This is expected due to the large environmental gradient considered, and drivers which can have effect thresholds. This is a typical ecological response when dealing with limiting factors (e.g. water availability).

2. Identifying these non-linear responses and thresholds and making use of the partial responses of environmental variables can be used to reveal each driver's individual effects, de-facto disentangling its effects from those of other superimposed drivers.

2.2.2 Materials and Methods

2.2.2.1 Study area and field sampling

Epiphytic lichen field survey followed Rocha et al., (2022). Briefly, we intended to cover a large gradient of climate, air pollution and land-use conditions, as well as a variety of different local urban biophysical characteristics (green urban area management and history, local pollution sources, cityscape, and others). For this purpose, seven European cities were chosen (from East to West): Tartu (Estonia), Poznan (Poland), Zurich (Switzerland), Antwerp

(Belgium), Paris (France), Almada and Lisbon (Portugal), (Fig. 2.2.1). Sampling site selection was restricted to areas classified as “Green Urban Areas” in the European Urban Atlas (EEA, 2018), an extensive dataset which offers a high level of spatial and thematic detail for land cover/use maps for numerous urban areas across Europe. In each city, sampling sites selection is aimed at representing a gradient of urban density. Stratification was performed by considering both the patch size and connectivity index of each “Green Urban Area”, randomly selecting up to 36 patches per city. This resulted in a total of 219 sampling sites across all cities. For more details on site selection and stratification see Supporting information. For sites remote sensing information see (Aleixo et al., 2024) and for sites local urban biophysical characteristics, namely vegetation structure, see (Alós Ortí et al., 2022).

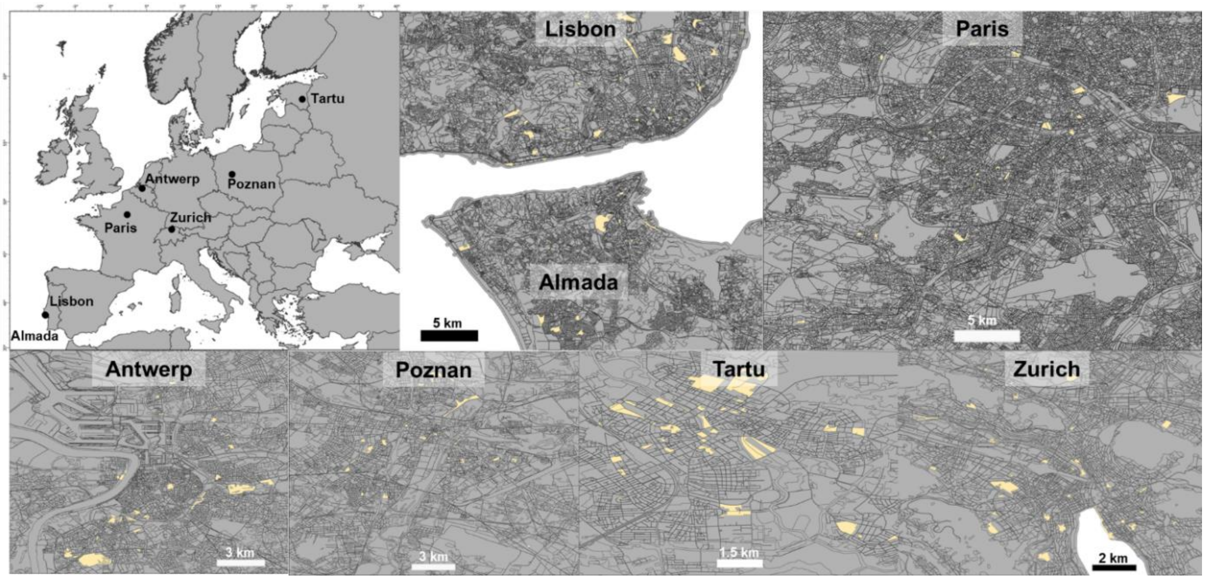

Figure 2.2.1: Map of the study area showing the urban green spaces (coloured in yellow) where epiphytic lichen field survey was conducted, in each of the seven European cities [from East to West: Tartu (Estonia), Poznan (Poland), Zurich (Switzerland), Antwerp (Belgium), Paris (France), Almada and Lisbon (Portugal)].

In each sampling site, epiphytic lichen community was sampled following the European standard method (Asta et al., 2002; Cristofolini et al., 2014). A figure summarizing the selection of sampling sites inside a green urban area patch and the sampling scheme for epiphytic lichen diversity, according to the European standard method, can be found in “Appendix A” of Rocha et al, (2022). The four trees closest to the centroid were sampled while following the method selection criteria (see Supporting information for more details). A sampling grid (50 cm × 10 cm, divided into five squares) was placed on the four main aspects, and all lichens inside the grid were registered. Species abundance was determined based on the total number of squares in which they occurred per tree (max. 20) and averaged by the number of sampled trees in each site. A total of 140 species were identified, with eight only to the genus level for which a functional characterization is thus missing (Table S2.2.1). Given that this corresponds to only 6% of the species, no bias was created. Species nomenclature and trait classification followed Nimis et al., (2018).

2.2.2.2 Biodiversity metrics

A total of 24 lichen biodiversity metrics were computed at the site level (i.e. characterizing lichens biodiversity across all sampled trees in that site), specifically three taxonomic metrics and 21 trait-based metrics. Choice of the biodiversity metrics follows the rationale of their expected response to the different nature and intensity levels of expected environmental drivers acting in urban areas, ensuring minimal collinearity between them (see Supporting information for more details on the metrics calculation and rationale of application and Table S2.2.6 for correlations between metrics). Based on existing literature (Branquinho, Matos, & Pinho, 2015), we anticipate that more intense drivers, which in cities relate to some form of anthropic pressure like air pollution or land-use changes will cause overall species loss (Paoli, Fačkovcová, Lackovičová, & Guttová, 2021), better captured by taxonomic metrics, while less intense drivers, like climate (Koch et al., 2019) will rather promote shifts in species composition, better captured by functional diversity and structure metrics (Branquinho et al., 2019). Furthermore, at the functional structure level, trait selection encompassed functional groups known to respond to specific environmental drivers' effects (Ellis et al., 2021).

Taxonomic metrics comprised three α-diversity metrics: species richness, total abundance and Shannon diversity index. Functional diversity comprised both the functional richness and Rao's quadratic entropy (RaoQ) metrics. For functional structure, the community weighted mean (CWM), representing the mean trait value in the community weighted by the abundance, i.e., the proportion of summed species abundances belonging to the same functional group of each trait was computed for four categorical traits, known to respond to the expected drivers acting in urban areas (e.g., temperature, water availability and anthropic pressure): growth form, main photobiont type and species tolerance to aridity and eutrophication. Traits information was retrieved from the ITALIC database (Nimis, 2024) and for species tolerance, the maximum value for each species was used (trait classification are ordinal in the database, see Table S2.2.1 for more details). Species that could not be identified to the species level and for which we could not retrieve specific trait classification were excluded from trait metrics calculation (Table S2.2.2). Biodiversity metrics were calculated with the *Vegan* package

(Oksanen et al., 2013) and the *FD* package (Laliberté, Legendre, Shipley, & Laliberté, 2014) from R Statistical Software (R Core Team, 2022).

2.2.2.3 Environmental drivers

For each of the 219 sites, we extracted a set of 25 variables characterizing environmental conditions at two different spatial scales (Fig. S2.2.1 and Table S2.2.3-5). Thus, all environmental variables were calculated from the lichen sampling point (i.e., corresponds to the pixel value). In this study, spatial scale refers to the spatial extent at which the driver acts (i.e. where the variance in the driver between sampling sites can be found), and not to the geographical spatial extent of the study nor the quantification of the driver variables. Broad/continental scale variables were derived from models working at a continental scale. For that reason, values show little variance within each city site, thus representing environmental gradients working at the continental scale. Contrastingly, local/city scale drivers were derived from high-resolution remote sensing measures, with values varying between sites of each city, thus representing environmental gradients working at the city scale.

Variables were classified into three main groups based on known environmental drivers of lichen diversity in cities: temperature, water availability and anthropic pressure (See Table S2.2.7 for correlations between variables). At the broad scale (i.e. having a spatial scale of action encompassing the entire city), temperature and water availability conditions (Table S2.2.3) were characterized by the 19 bioclimatic variables representing air temperature (BIO 1 to 11) and precipitation (BIO 12 to 19) annual, range and seasonal values (averages of 1981-2010). These were retrieved from the CHELSA database at the maximum available spatial resolution of 1 km (Karger et al., 2017) and its values in each sampling site extracted using the Extract Multi Values to Points tool from the ArcGIS Pro v.10.5.1 software. Bioclimatic variables were preferred over raw maximum, mean and minimum temperature and precipitation variables as the former are potentially more meaningful from an ecological perspective (Title & Bemmels, 2018). Broad scale anthropic pressure, related to air pollution conditions, was characterized by the sites score in the first two axes of the PCA comprising a set of seven air pollution variables (redN and O_xN deposition and NH_3, NO_x, PM2.5, PM10 and SO_2 air concentration) performed in Rocha et al., (2022) and were used as the only two macroair pollution variables for subsequent analyses. Pollutants' concentration or deposition values were retrieved from EMEP (Fagerli et al., 2019) at the maximum available spatial resolution (11 km), and for the year 2018 (Table S2.2.4). PCA interpretation allowed to distinguish between an overall macroair pollution gradient on the first axis (from less to more polluted sites, explaining 57.6% of the 7 variables total variation), named here “Pol_1”, and a gradient of pollutant types on the second axis (from sites dominated by N-based compounds to those dominated by sulphur dioxide and particulate matter, explaining 20.8% of the 7 variables total variation), named here “Pol_2”. Both climate and pollution data were calculated from the lichen sampling point (i.e., corresponds to the pixel value).

At the local scale (i.e. having a spatial scale of action at the city level), temperature and water availability conditions (Table S2.2.5) were characterized by the land surface temperature (LST, 30m resolution) and canopy water content (CW, 30m resolution) remote sensing indices, respectively. Values were retrieved for winter and summer from 2015 to 2019, selecting the images with less cloud cover for each year [see Casanelles-Abella et al., (2024) and its supporting material for more details]. The average (multi-annual) values were then calculated for each season. Local anthropic pressure, related to land-use, was characterized by the normalized difference built-up index (NDBI, 10m resolution) and the normalized difference vegetation index (NDVI, 10m resolution) indices, respectively. Ideally, fine-scale data on urban green spaces vegetation management would also be applied as a complement to this. However, no information across all cities was available and for that reason it was not tested. Despite representing local scale conditions, each index may present a different optimal spatial range of effects. To account for that, indices were computed at different buffers around the sampling site centroid while still ensuring variation between sites of the same city (0, 50, 100, 200, 400, 800, 1600 and 3200 meters), using ArcGIS Pro v.10.5.1. The best buffer and seasonal period for each individual index was selected to maximize its effect, based on the highest overall Spearman correlation coefficient presented, taking into consideration the 24-lichen metrics. The best combination for each local scale index was: winter canopy water content at 800 meters (CWw), summer land surface temperature at 3200 meters (LSTs), winter normalized difference built-up index at 100 meters (NDBIw) and winter normalized difference vegetation index at 200 meters (NDVIw). All remote sensing indices were extracted from either Sentinel-2 or Landsat-8 satellite imagery.

2.2.2.4 Data analysis

We modelled the responses of lichen biodiversity metrics to the selected environmental drivers using random forests. Random Forest regression modelling is a widely used machine learning algorithm which combines the predictions of multiple models, and is capable of handling non-linear relationships (Breiman, 2001), thus offering a more robust regression method in the presence of collinear variables. In our study, Spearman correlation coefficients between lichen biodiversity metrics and between environmental variables) revealed some degree of collinearity (see Table S2.2.6), confirming the need to rely on random forest regression. Random forest models were run individually for each of the 24-biodiversity metrics. In each model a set of 25 predictors were used, encompassing 12 temperature variables, 9 water availability variables and 4 anthropic pressure variables (Table S2.2.3-5). Given the large number of predictors modelled, only the five environmental variables with highest importance were displayed and mentioned in the results section, as these reasonably reflect the most important drivers for each lichen metric.

For each random forest regression model, data was randomly split in training and testing at an 80/20 ratio, corresponding to 175 and 44 sampling sites, respectively. Random splitting was done ensuring the presence of sampling sites from all cities in both the training and testing datasets. To account for the possible effect of random splitting of data, this process was repeated 100 times, each corresponding to a model with a different combination of sampling sites. All models were built using 1000 trees and with the set of environmental variables randomly sampled at each split. The root mean square error (RMSE), cross validation correlation (spearman correlation coefficient between observed and predicted values in the testing dataset), drivers variables importance and the coefficient of determination (R^2, determining the variance explained in each metric) were used to evaluate the random forest regression models performance. Models' performance presented in the Results section refers to the median of the 100 models for each lichen biodiversity metric. As each combination of metric – drivers variable was modelled, the final number of models amounted to 1050. Due to this large number of models, a prior selection was made to prevent overcrowding the results and discussion sections. The first step was to select only those explaining more than 30% of variance of the biodiversity metric. The response direction and the existence of threshold values were analysed for the top five most important variables in each model that passed the model selection procedure. For these top five drivers' variables, partial responses were plotted and the shape of the relationship between biodiversity metrics and variable (linear or nonlinear), the existence of thresholds to each individual top five variable (i.e., disentangled individual responses to each driver) and the direction of the response were visually inspected. For each partial response curve, the threshold value was determined by visual inspection and corresponds to the approximate curve inflection point. Although a statistical breakpoint detection approach could more accurately identify threshold values, as the objective of this work was mainly to identify their presence rather than the precise inflection point, we opted for a simplified approach based on visual identification.

All statistical analyses were conducted with R Statistical Software (R Core Team, 2022). Models were run using the *randomForest* package (Liaw & Wiener, 2001). Variables' importance in each random forest model was quantified using the *caret* package (Kuhn, 2008). Partial responses were built using the *pdp* package (Greenwell, 2017) and then plotted using *ggplot2* package (Wilkinson, 2011). Spearman correlation coefficients were calculated using the *Hmisc* package (Harrell Jr, 2019).

2.2.3 Results

2.2.3.1 Drivers' importance reflected across metrics

From the 24 models of lichen biodiversity metrics, eight models explained more than 30% of the variance in the response biodiversity metrics (Table S2.2.8). These eight models included three taxonomic metrics (species richness, total species abundance, and Shannon diversity), two functional diversity metrics (functional richness and RaoQ) and three functional structure metrics (CWM of nitrophytic, rather hygrophytic and mesophytic functional groups). The overall presence and importance of each driver group in each of the eight biodiversity metrics models was subsequently analysed (Fig. 2.2.2 and Table S2.2.9). Water availability variables were the most represented in the top five of taxonomic (80%) and functional diversity (70%) metrics (Fig. 2.2.2a), while temperature variables were more frequent across functional structure metrics (53%). Anthropic pressure variables registered only a small frequency across all metric types. Together with being the most frequent variables in the models, the average importance of water availability variables was the highest across all metrics types, while temperature and anthropic pressure variables shared similar importance (Fig. 2.2.2b). On average, for taxonomic metrics, the importance of the three drivers' groups was similar, with a difference of about 3 percentage points (p.p.) or less. For functional diversity and functional structure metrics though, water availability variables' average importance was around 5 p.p. or more in comparison to the other driver groups.

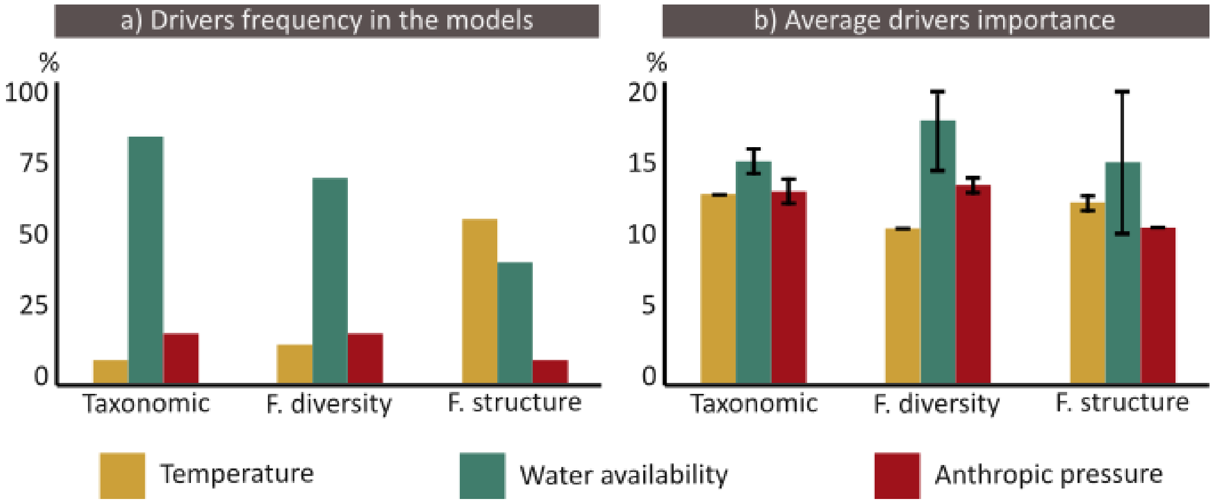

Figure 2.2.2: Analysis of the eight Random Forest regression models of lichen biodiversity metrics with explained variance above 30%, based on three main drivers' groups: temperature, encompassing both temperature-related bioclimate variables (BIO1-12) and the LSTs; water availability, encompassing both precipitation-related bioclimate variables (BIO12-19) and the CWw; anthropic pressure, encompassing both encompassing both axis of the PCA ("Pol_1" and "Pol_2"), the NDBIw and the NDVIw. a) Relative frequency (%) of all variables of each driver group in the top five variables importance, per metric type; b) Average importance (%) and standard deviation of all variables of each driver group in the top five variables importance, per metric type. Taxonomic – Species richness, Shannon index and Total species abundance; F. diversity – Functional richness and RaoQ; F. structure – CWM of nitrophytic, rather hygrophytic and mesophytic functional groups.

The five most important environmental variables explaining the eight-biodiversity metrics were analysed in more detail (Fig. 2.2.3 and Table S2.2.9). Among biodiversity metrics, functional diversity showed on average the highest variance explained (42%), followed by both taxonomic (33%) and functional structure metrics (31%). Across taxonomic metrics, water availability variables, both at the broad (precipitation - BIO12, BIO16 and BIO19) and local (Canopy water content - CWw) scale were the most important ones, with similar variables present in the top five of the three metrics. Temperature and anthropic pressure variables were present less frequently and with average less importance. In terms of functional diversity metrics, three of the top five variables were common among them (BIO12, BIO19 and CWw). Nevertheless, for functional richness, variables of the three main drivers' groups were equally present, while for RaoQ, water availability variables were more frequent. For the functional structure metrics, temperature variables were more frequent across nitrophytic and rather hygrophytic functional groups, although water availability variables were the first and second most important variables for both metrics, respectively. Mesophytic lichens exhibited an opposite pattern, with water availability variables being most frequent and more important.

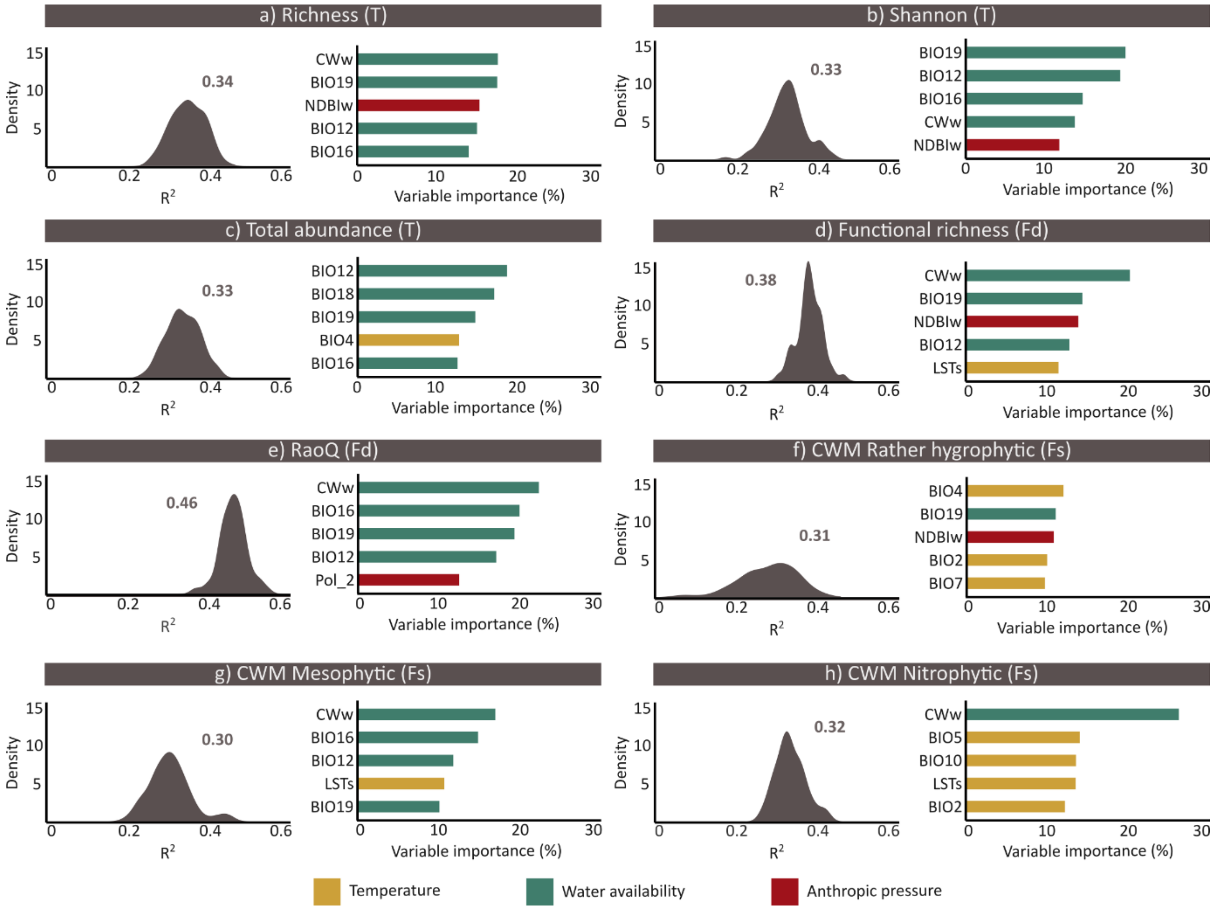

Figure 2.2.3: Analysis of the eight Random Forest regression models of biodiversity metrics by environmental variables (panels a-h). In each panel, the density curve shows the distribution from 100 models with different random training/test splitting of the coefficient of determination (R^2), with its median R^2 value displayed. The barplots in each panel show the median importance (%) of each of the top five variables in each respective model. T – taxonomic metrics; Fd – functional diversity metrics; Fs – functional structure metrics. Bioclimate variables: BIO2 – Mean diurnal range; BIO4 – Temperature seasonality; BIO5 – Maximum temperature of warmest month; BIO7 – Temperature annual range; BIO10 – Mean temperature of warmest quarter; BIO12 – Annual precipitation; BIO16 – Precipitation of wettest quarter; BIO18 - Precipitation of warmest quarter; BIO19 – Precipitation of coldest quarter. Air pollution variables: Pol_2 – sites score in the second axis of pollution PCA, representing a gradient of sites dominated by sulphur dioxide and particulate matter to N-based compounds dominated. Remote sensing variables: CWw - canopy water content during winter period and in an 800 meters buffer; LSTs - land surface temperature during summer period and in a 3200 meters buffer; NDBIw - normalized difference built-up index during winter period and in a 100 meters buffer (see Tables S2.2.3-5 for variables details). See Table S8 for the remaining metrics explained variance (R^2) and Table S2.2.9 for the median importance (%) of each variable in the model.

2.2.3.2 Shape of the environmental drivers – lichen metrics relationship

Across most of the tested metrics the response to individual drivers' variables was non-linear and monotonic, and to several of them, it was frequently associated with a threshold (Fig. 2.2.4-5). Overall, metrics response patterns were similar across variables of the same driver group. Furthermore, only ten models presented linear responses without exhibiting a threshold (Fig. 2.2.4-5). When present, these threshold values are frequently comparable across metrics, for the same driver variable (Fig. 2.2.4-5). Metrics response to increased temperature variables values was metric dependent, with the rather hygrophytic and nitrophytic functional groups exhibited dissimilar threshold values, in response to the mean diurnal range (BIO2), (Fig.

2.2.5). Contrarily, for all water availability variables, regardless of their spatial scale of action, response patterns were positive, and threshold values were similar, particularly across taxonomic and functional diversity metrics (Fig. 2.2.4). For anthropic pressure variables, all metrics declined with increasing urbanization (NDBIw), with taxonomic and functional diversity metrics sharing similar thresholds but not with the rather hygrophytic functional group (Fig. 2.2.4-5).

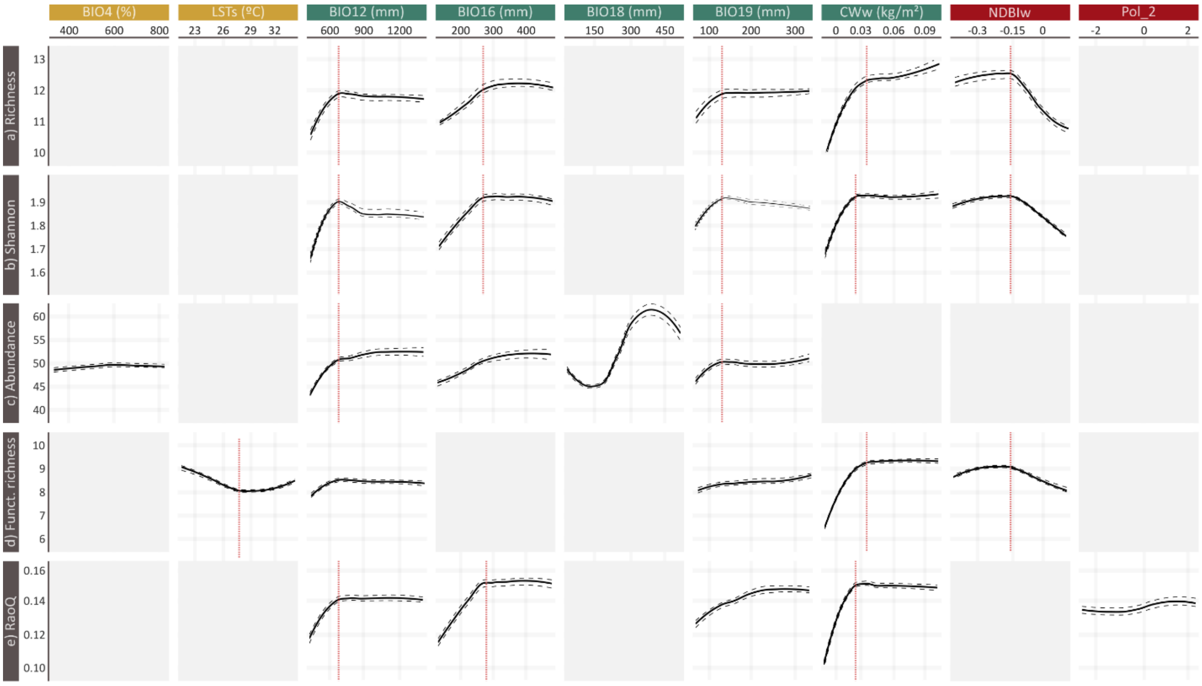

Figure 2.2.4: Partial responses of selected taxonomic and functional diversity metrics (one per line) to the five most important variables (columns) from Random Forest regression models analyses. Grey panels represent less important association between response and variables (e.g. variables outside the five most important). Dotted red lines represent the response threshold, estimated by visual inspection of the curve inflection point. Filled lines represent a loess function applied to the median of the partial predictions of 100 models with different random training/test splitting, while 25 and 75 quantiles are represented by dashed lines. Median variable importance of the models can be seen in Figure 2.2.3. Taxonomic – Species richness, Shannon index and total species abundance; Functional diversity – Functional richness and RaoQ. Bioclimate variables: BIO4 – Temperature seasonality; BIO12 – Annual precipitation; BIO16 – Precipitation of wettest quarter; BIO18 - Precipitation of warmest quarter; BIO19 – Precipitation of coldest quarter. Air pollution variables: Pol_2 – sites score in the second axis of pollution PCA, representing a gradient of sites dominated by sulphur dioxide and particulate matter to N-based compounds dominated. Remote sensing variables: CWw - canopy water content during winter period and in an 800 meters buffer; LSTs - land surface temperature during summer period and in a 3200 meters buffer; NDBIw - normalized difference built-up index during winter period and in a 100 meters buffer (see Tables S2.2.3-5 for variables details).

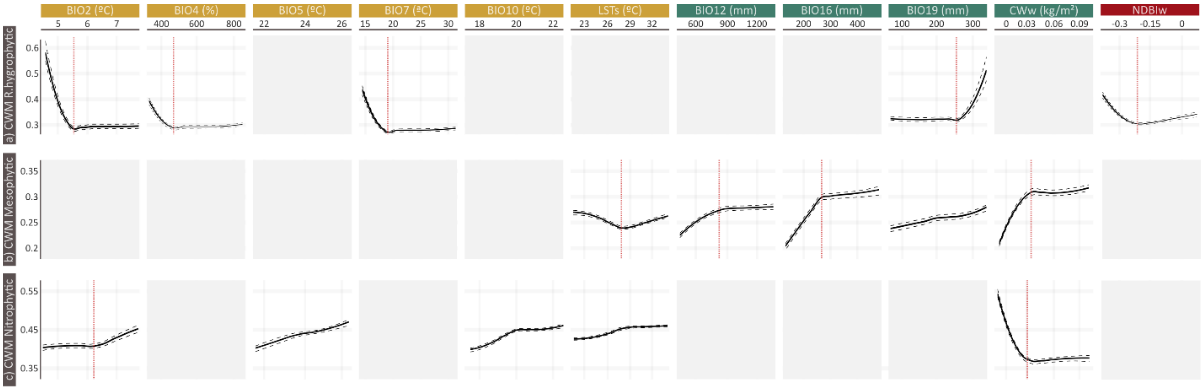

Figure 2.2.5: Partial responses of selected functional structure metrics to their five most important environmental variables from Random Forest regression models analyses. Grey panels represent less important associations between metrics and variables (e.g. variables outside the five most important). Dotted red lines represent the response threshold, estimated by visual inspection of the curve inflection point. Filled lines represent a loess function applied to the median of the partial predictions of 100 models with different random training/test splitting, while 25 and 75 quantiles are represented by dashed lines. Median variable importance of the models can be seen in Figure 2.2.3. Functional structure metrics – CWM of rather hygrophytic, mesophytic and nitrophytic functional groups. Bioclimate variables: BIO2 – Mean diurnal range; BIO4 – Temperature seasonality; BIO5 – Maximum temperature of warmest month; BIO7 – Temperature annual range; BIO10 – Mean temperature of warmest quarter; BIO12 – Annual precipitation; BIO16 – Precipitation of wettest quarter; BIO19 – Precipitation of coldest quarter. Remote sensing variables: CWw - canopy water content during winter period and in an 800 meters buffer; LSTs - land surface temperature during summer period and in a 3200 meters buffer, NDBIw - normalized difference built-up index during winter period and in a 100 meters buffer (see Tables S2.2.3-5 for variables details).

2.2.4 Discussion

2.2.4.1 Identification of non-linear responses direction and thresholds

We hypothesized that lichen biodiversity metrics response to urban environmental drivers considered could be non-linear. Our results confirm this, as several metrics presented non-linear relationships, mostly monotonic. This is likely because this study was conducted along a large continental gradient, which encompasses a wide range of abiotic conditions. Building from previous research (Rocha et al., 2022) with the same dataset, we uncovered several non-linear relationships with the application of a machine-learning approach, especially for climate variables.

The existence of thresholds in metrics response to these drivers could be responsible for that nonlinearity. The determination of thresholds is essential in ecology and the use of ecological indicators (Huggett, 2005), signalling ecological tipping points. Being able to identify tipping points for urban lichens allows us to consolidate their use as ecological indicators of environmental drivers in cities, and over broad scales. Until now, response thresholds for lichens were almost exclusively based on studies conducted in natural or semi-natural ecosystems in response to pollution (Geiser, Jovan, Glavich, & Porter, 2010; Giordani et al., 2014). Here, we were able to identify thresholds in response to several temperature, water availability and anthropic pressure variables, at different spatial scales, across several European cities.

We found that thresholds values for each driver to be generally similar across biodiversity metrics, particularly for water availability and anthropic pressure variables. The thresholds identified here, above or below which a change in response rate is seen, suggest that these drivers act as limiting factors for lichen biodiversity until the threshold is reached. Our results indicate that this is the case with water availability variables. While limiting growth and thus abundance in drier areas, above a given threshold has no effect on the biodiversity metrics (Bässler et al., 2016). Thus, these results suggest that in the driest cities across the continental gradient explored, lichen taxonomic and functional diversity are, at least partially, limited by water availability such that all metrics increased up to around 600 mm of annual precipitation. Similarly, for anthropic pressure, measured as NDBI- normalized difference built-up index, we also uncovered a threshold defined around -0.15 for most biodiversity metrics, which lies in the transition between non-built-up (e.g. vegetation, bare soil) and built-up areas (e.g. buildings, roads), (Zha, Gao, & Ni, 2003). Finally, temperature thresholds were less homogeneous across metrics at the broad but not at the local scale. At the broad scale, the threshold was lower for hygrophytic lichens, likely due to the relation between higher evaporation rates and higher temperatures, while for nitrophytic lichens this relation is less clear but possibly related to similar mechanisms driving lichen tolerance to higher temperature and air pollution alike.

2.2.4.2 Disentangling metrics response to individual urban drivers

We further hypothesized that environmental variables partial responses would allow to disentangle the individual effect of each driver on lichen biodiversity metrics. Due to the bundle of environmental drivers pressuring urban ecosystems, identifying and understanding lichen response to each individual one is key from the perspective of applying them as ecological indicators in cities (Branquinho et al., 2015).

Our results revealed that temperature and water availability variables were frequently within the top five most important predictors, and often the most important, i.e., with the highest amount of explained variance. This suggests that climate is indeed a key driver of urban lichens, at all spatial scales, reinforcing the need for further studies to encompass variables representative of both local- and broad scale climate conditions. This response was expected given that lichens are poikilohydric and poikilothermic organisms, meaning that their physiological activity responds to and depends directly on humidity conditions and air temperature (Gasulla, Del Campo, Casano, & Guéra, 2021; Gauslaa, 2014; Stanton, Ormond, Koch, & Colesie, 2023). Water availability variables explained all lichen taxonomic, functional diversity and functional structure metrics. This goes in accordance with previous studies conducted in non-urban environments, which also revealed positive relationships between higher precipitation values and taxonomic and functional diversity (Concostrina-Zubiri, Pescador, Martínez, & Escudero, 2014; Di Nuzzo et al., 2021; Hurtado, Prieto, De Bello, et al., 2020) and is now confirmed for urban environments as well. In turn, temperature variables were present in five of the eight biodiversity metrics. Several studies have reported changes both in lichen taxonomic (Alatalo, Jägerbrand, Chen, & Molau, 2017; Aptroot, Stapper, Košuthová, & van Herk, 2021; Aptroot & Van Herk, 2007; van Herk, Aptroot, & van Dobben, 2002) and functional structure metrics, related to form and photobiont type (Hurtado, Prieto, Aragón, Bello, & Martínez, 2020; Hurtado, Prieto, De Bello, et al., 2020; Phinney et al., 2021), in response to temperature changes. Although here none of the metrics related to either of these functional traits had their variance explained by more than 30%, the decrease in overall functional richness reported in these studies matches the responses observed here. These suggest that, as cities are projected to become warmer and drier (Felsche, Böhnisch, Poschlod, & Ludwig, 2024), urban lichens communities will likely tend to a biotic homogenization, filtering out dry-sensitive species.

Anthropic pressure is also seen as a major driver of urban biodiversity (Keck et al., 2025) and here also, with the NDBI appearing in top five most important predictors of taxonomic, functional diversity and functional structure metrics. Here, we characterized this driver in two main aspects: broad scale air pollution and local scale changes in land-use, both related to more urbanized (NDBI) and greener areas (NDVI). In the past, air pollution was frequently the strongest environmental filter for lichens, with critical levels and loads being established for lichens in agroforestry and forested ecosystems (Geiser et al., 2010; Pinho et al., 2009). Nevertheless, in our work, air pollution was last in the top five predictors influencing one single functional diversity metric, the RaoQ. Three factors can potentially contribute to these results. First, the application of Random Forest modelling revealed other drivers, namely temperature and water availability, whose importance was potentially underestimated due to its non-linear relationship. Second, the effect of pollutants can vary greatly depending on the pollutant nature and source distance, emissions dynamic and amount, with urban layouts also influencing pollutants diffusion (Jiang, Cheng, Zhang, & Kang, 2021). Here, a publicly available pollution model was used, and its coarse spatial resolution may influence these results. High resolution data on urban pollutants is therefore required to better quantify its effects. Third, the negative legacy effect of air pollution acts as a filter of urban lichen communities, promoting a convergence of functional traits which grants better tolerance to higher air pollution levels (i.e., protective mechanisms that are developed to increase lichen tolerance to air pollution can also grant them increased tolerance to desiccation). Thus, our results suggest that baseline pollution levels across European cities are still high enough to promote a homogenization of urban lichen functional characteristics, which then results in lichen communities less sensitive to variations in air quality. At the local scale, only the NDBI was present in the top five predictors of taxonomic and functional structure metrics, with a general decrease across metrics. Similar trends of biodiversity loss have been observed across a wide array of organisms, from flora to fauna (La Sorte et al., 2018; Palma et al., 2017; Ruas, Costa, & Bered, 2022). This index is a measure of built-up (e.g. buildings, roads), thus pointing to more urbanized areas in cities. Previous studies have already demonstrated that lichen biodiversity decreases in more urbanized areas (Piano et al., 2020; Potenza, Gerardi, Fascetti, & Rosati, 2022), reinforcing the idea that increased urban expansion will further jeopardize urban lichens biodiversity. In fact, the size of the urban green area has been found to be critical to lichens biodiversity (Matos, Vieira, Rocha, Branquinho, & Pinho, 2019; Potenza et al., 2022), highlighting the importance of improving green space areas in cities. Furthermore, this index is not only a surrogate of increased anthropic pressure (i.e., land-use changes, traffic), but also indirectly exacerbates local climate effects due to the heat island effect (Li et al., 2023), air pollution (Zhang et al., 2022) and habitat fragmentation and green urban areas encroachment and management (Koch et al., 2019; Leppik, Jüriado, & Liira, 2011). As both remote sensing based indexes reflect mostly local conditions, these results further highlight the importance of local management on urban ecosystems (Gaston, Ávila-Jiménez, & Edmondson, 2013), and the need for more detailed local environmental data, namely related to local air pollution models and green areas management, which were not directly measured in this study. This suggests that vegetation density planning and management in green urban areas may be key to mitigate functional shifts occurring due to broad and local temperature rises. As projections estimate more than 2.5 billion people will be living in cities by 2050 (Simkin et al., 2022), our results reinforce the need to reshape urbanization strategies, reconciling urban and biodiversity conservation strategies (Elmqvist, Zipperer, & Güneralp, 2016).

2.2.4.3 Future applications

Random Forest enabled us to successfully disentangle the individual effects of several main urban drivers, showing non-linear responses and thresholds for its effects. As demonstrated in our study, such an approach is particularly suitable when complex and superimposed environmental gradients are present. For example, it enables us to signal areas where urban drivers have impacts (e.g. local air pollution, green areas characteristics and management) and are working simultaneously with climate, while also allowing to quantify those impacts. Nevertheless, this modelling approach also presents its limitations. Given its machine learning nature, it requires a high number of samples and, like all modelling approaches, it has limited extrapolation power outside the range of environmental conditions found here (i.e., predicted values limited to the training set values) and for that reason, metrics response should only be considered within the abiotic envelope sampled, which is advised for all statistical predictions in ecology (Conn, Johnson, & Boveng, 2015; Pearson & Dawson, 2003).

As a toolbox to measure the effects of multiple environmental drivers in cities, the selected lichen-based ecological indicators of urban drivers' effects would include metrics with dissimilar threshold values, thus enabling a complementary approach to assess and manage urban ecosystems. Overall, most threshold values were common across metrics for each given environmental variable. Thus, from an ecological indicator perspective, and within the range of environmental conditions tested, most metrics may be applied interchangeably instead of complementarily. Still, there were exceptions. One example is the response to the precipitation of the coldest quarter (BIO19), which influences all taxonomic and functional diversity metrics only until approximately 120 mm, after which the effects above that threshold can be better monitored by lichens tolerant to rather low aridity levels (CWM rather hygrophytic). Another example is the Normalized Difference Built-Up Index (NDBI), whose effects can be tracked until the threshold using lichens tolerant to rather low aridity levels, after which taxonomic or functional diversity metric can be applied. Thus, our results also highlight the importance of complementing taxonomic and trait-based metrics when developing ecological indicators to quantify ecosystems change in response to urban environmental drivers, as the drivers and their importance differed between metrics. These results also reveal that, contrary to what previously thought, increased sensitivity of specific metric types to specific environmental drives is not necessarily accurate. Indeed, and based on existing knowledge (Branquinho et al., 2019), we expected higher intensity drivers like air pollution would better be captured by taxonomic metrics while functional metrics would capture the effects from the three groups of drivers, depending on the trait considered. Nevertheless, we found multiple temperature, water availability and anthropic pressure related variables influenced multiple lichen metrics, regardless of their type (taxonomic, functional diversity or functional structure). Future in-depth local scale studies should thus be conducted within cities to better understand the individual lichen diversity metrics responses to particular environmental drivers. At the same time, such intra-city studies focusing on lichen diversity heterogeneity across green spaces would be a valuable tool to improve policymaking efforts towards more sustainable and resilient cities. Nevertheless, in both cases, a more extensive sampling effort and the use of more high-resolution environmental data related to local pollution sources and green urban areas management, history, vegetation cover and others are required. Furthermore, our results suggest that, to act as indicators of drivers' effects in urban ecosystems, biodiversity metric selection and interpretation must be done accounting for the specific combinations of limiting factors encompassed at the spatial scale considered. Finally, it also indicates that traditional applications of taxonomic metrics as indicators of air pollution effects should thus be complemented by functional metrics as well.

2.2.5 Conclusions

The identification of the main drivers and the type of response they prompt on lichen diversity is fundamental for using ecological indicators of environmental drivers' effects in cities. By using a non-linear modelling approach, we were able to identify several limiting factors for lichen biodiversity in cities, related to water availability, temperature and anthropic pressure. Understanding the role of individual environmental drivers can guide stakeholders on the development of environmental policies to mitigate key environmental problems in cities, like air pollution, climate change or land-use. At the same time, identifying ecological thresholds is critical for management and policy as it allows the detection of breaking points beyond which ecosystem changes may become abrupt, less predictable, or harder to reverse. This information supports the definition of precautionary targets and timely interventions to avoid undesirable environmental shifts. Future efforts should nevertheless be made to improve air pollution data spatial resolution, whose effects could have been underestimated here due to the coarse resolution at which it is currently available. Furthermore, local green space management and vegetation structure data should be explored in the future to understand its role in driving lichen biodiversity. Finally, future resampling of the sites could help infer how lichen diversity metrics are changing over time in response to changing urban environmental conditions. Altogether, this work highlights the potential of using lichen diversity as a tool to improve urban planning, environmental management, and policymaking in cities. Furthermore, it supports and strengthens lichens application as ecological indicators of environmental change in urban ecosystems as a tool to help us move towards more sustainable and resilient cities, ultimately improving human living quality.

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Chapter 3

3. APPLICATION ACROSS EXTREME ENVIRONMENTS

Subchapter 3.1

3.1 Vegetation responses to macroclimate in maritime Antarctic

Rocha, B., Pinho, P., Giordani, P., Matos, P. (2025) Vegetation responses to macroclimate in maritime Antarctic. *In preparation*

Abstract

The maritime Antarctic has been experiencing rapid climate change, altering its terrestrial ecosystems and ecological processes. Across ecosystems, vegetation is dominated by three major groups, lichens, bryophytes and two locally restricted vascular plants. The abundance patterns of these vegetation groups have been changing in recent decades, suggesting their potential as ecological indicators of broad scale climate effects. However, uncertainty remains regarding the applicability of such indicators, as substrate conditions, logistical constraints, and other microscale abiotic and biotic variables may obscure climatic signals. To support the broader use of vegetation groups as ecological indicators of climate change, our objective was to determine whether vegetation group (lichens, bryophytes and vascular plants) or vegetation growth form, used here as trait-based metrics, respond to climate. To do that, we quantified the abundance of each vegetation group and each growth form, using 150 photographs from 30 sampling sites, distributed across a climate gradient associated with elevation and distance to the coast on two islands of the South Shetlands archipelago, maritime Antarctic. The nineteen traditional bioclimate variables were retrieved and their dimensionality reduced by means of a Principal Component Analysis (PCA), which mainly separated warmer and wetter sites from colder and drier ones. Each vegetation trait-based metric was then modelled with the scores of the first two axes of the PCA using Random Forest regression, and the variance explained, axes importance and type of response for each metric were determined. Broad scale climate explained a large portion of the variance for lichens abundance (59%) but less for bryophytes (44%) and much less for vascular plants (26%), with responses showing thresholds likely associated with limiting factors related to air temperature and/or water availability. Growth forms performance was overall lower, with macroclimate explaining 24% of crustose, 35% of fruticose and 23% of leprose lichens variance but lower than 20% for all other forms. Altogether, our results confirmed the potential of the three vegetation groups as ecological indicators of broad scale climate, with trait-based metrics related to the overall group performing better than life/growth forms did. In the future, studies should focus on quantifying the role of local to micro scale abiotic and biotic factors on these vegetation groups to better model these vegetation responses to climate over maritime Antarctic.

Keywords

Antarctica, Climate Change, Antarctic vegetation, Ecological indicator, Functional Diversity, Random Forest

3.1.1 Introduction

Polar regions are characterized by some of the most unfavourable environmental conditions, mainly due to the cold temperatures, scarce water availability and geographical isolation (Robinson, Wasley, & Tobin, 2003). While still adverse, conditions are shifting rapidly, with future climate scenarios pointing towards drastic changes through the end of this century (IPCC, 2022), namely in the expansion of ice-free areas (Lee et al., 2017), permafrost thawing (Tao et al., 2025), heatwaves (Robinson et al., 2020), and an overall improvement in climate conditions (Siegert et al., 2019). For that reason, maritime Antarctic terrestrial biodiversity is now faced with a rapidly changing environment which threatens to disrupt its fragile equilibrium (Convey & Peck, 2019; Convey & Smith, 2006). It is thus vital to understand the effects of climate change in polar ecosystems, at the broad scale, so that improved conservation strategies can be executed across the entire region (Hughes, Convey, & Turner, 2021).

As biodiversity is directly linked to ecosystems services and functions, it is regarded as one of the best tools to evaluate climate change effects at the ecosystem level (Branquinho, Serrano, Nunes, Pinho, & Matos, 2019). maritime Antarctic terrestrial biodiversity is mainly dominated by soil- or rock-dwelling lichens, being complemented, in some areas, by bryophytes and up to two vascular plant species (Colesie, Walshaw, Sancho, Davey, & Gray, 2023). Studies have detected changes in these main vegetation groups spatial patterns over the past decades (Roland et al., 2024; Torres-Mellado, Jaña, & Casanova-Katny, 2011), attesting their potential as biodiversity-based ecological indicators of climate change effects in the region. A recent study has shown that locally, these three vegetation groups have different ecological niches regarding microscale terrain characteristics related to water availability (Matos et al. 2024). From an ecological indicator perspective, this responsiveness to local scale water availability further points to their potential to track climate effects in a wider range of conditions. Vascular plants are usually restricted to lower areas, closer to the coast, where climate is usually warmer and nutrient availability is higher (Vera, 2011). On the other hand, lichens and most bryophytes are capable of colonizing higher elevations, with bryophytes more associated to sites with higher moisture while lichens dominating in drier areas (Matos et al. 2024). Similarly, the growth form trait has been relevant to explain vegetation response to macroclimate (Cavieres et al., 2016; Di Nuzzo et al., 2021; Löbel, Mair, Lönnell, Schröder, & Snäll, 2018). Developing ecological indicators based on easily distinguishable traits like the vegetation group or growth form, which are more easily identifiable through imagery analysis, could help bridge the gap to remote sensing techniques (Miranda et al., 2020; Pina & Vieira, 2022), therefore enabling its application at broader spatial scales. This would be particularly relevant in these extreme environments where logistic constraints limit field access for traditional ecological sampling techniques. However, the first step for its application is to determine if and how these trait-based metrics respond to broad scale climate, and this has not yet been done.

The objective of this work was to determine if vegetation groups (lichens, bryophytes and vascular plants) or vegetation growth forms, used here as trait-based metrics, respond to climate along small elevation and distance to the coast spatial gradients in the maritime Antarctic. We determined the abundance of bryophytes, lichens and vascular plants main groups and respective life/growth forms through image analysis and used Random Forest regression to model its response to macroclimate. Given the recently observed shifts in vegetation patterns across the maritime Antarctic region, we hypothesized that maritime Antarctic vegetation abundance can be used as ecological indicators of climate. Furthermore, given the different ecological requirements among vegetation groups and life/growth forms among the different vegetation groups, we further expect that each of these functional groups will respond differently to temperature and water availability, potentially exhibiting thresholds.

3.1.2 Materials and Methods

3.1.2.1 Study Area

This study was performed in Livingston and Nelson Islands, South Shetlands archipelago, maritime Antarctic (Fig. 3.1.1). This region is characterized by short summer seasons, average annual air temperature at sea-level of −2 °C and an annual precipitation ranging between 350 and 500 mm (Oliva, Pereira, & Antoniades, 2018). There, terrestrial vegetation is overall dominated by bryophytes and lichens, with the two vascular plant species occurring only in some areas closer to the shoreline (Beyer, 2002).

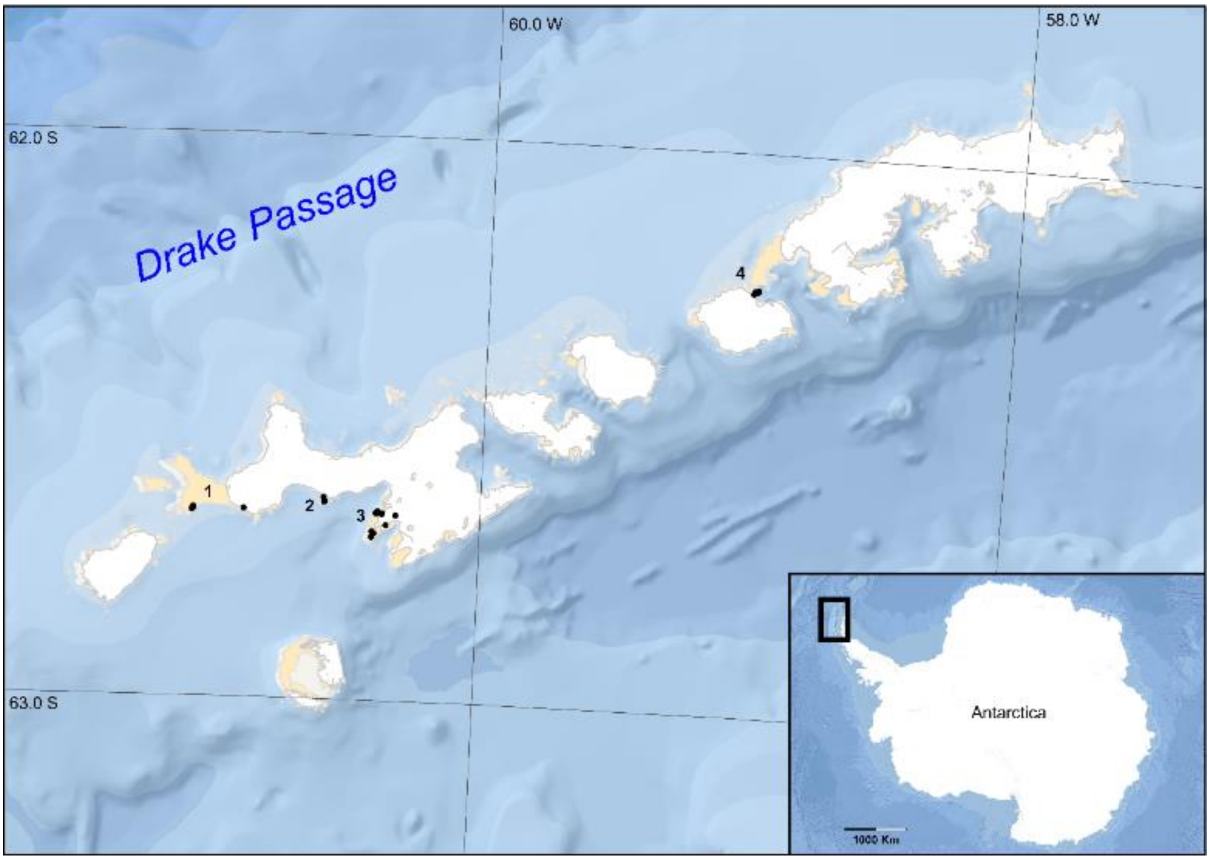

Figure 3.1.1: Location of (1) Byers Peninsula, (2) Hannah Point and (3) Hurd Peninsula, in Livingston Island and (4) Rip Point in Nelson Island in (a) Antarctica and (b) in South Shetland archipelago.

To assess the responses of the different trait-based metrics to macroclimate from the perspective of climate change, 30 sampling sites were chosen across several elevation and distance to the coast spatial gradients in four areas: Byers Peninsula, Hannah Point, Hurd Peninsula (Livingston Island) and Rip Point (Nelson Island). Sites ranged from 2 m to 370 m elevation and 12 m to 947 m from the sea, representing climatic gradients with bryophyte, lichen and vascular plants dominated communities, and possible transitions between them (Williams et al., 2017). A sampling site was defined as a 5-meter radius circular area, where at least one of the three vegetation groups (bryophytes, lichens and vascular plants) was present. A minimum distance of 50 m between sampling sites was settled.

3.1.2.2 Field sampling

Sampling sites were stratified by elevation ([0 – 23m[, [24 – 46m[, [47 – 92m[, [93 – 184m[and [185 – 367m]) and sampling area (Fig. 3.1.1). Six sampling sites were randomly chosen in each of the five elevation classes, preserving a balance in the final number of sampling sites from each of the four areas when possible. In each sampling site, and following an adaptation of the standard sampling methodology used to access vegetation abundance in arid ecosystems (Matos et al., 2024), five quadrats of 30 x 30 cm were randomly placed on top of the vegetation, ensuring that ground surface was close to horizontal (maximum slope of 20º). Each of the five quadrats was treated as an independent sample as variations at the microscale (e.g. abiotic and/or biotic conditions) are known to be critical to explain vegetation abundance in response to climate (Matos et al., 2024). Each quadrat was photographed perpendicularly to the ground surface and the coordinates, elevation and slope were recorded. Resulting images were corrected for tone, colour and distortion using Adobe Photoshop 23.0. Image J software (Schneider, Rasband, & Eliceiri, 2012) was used to overlay a grid over each image, dividing the 30 cm square into 100 small cells. The absolute abundance of each of the three vegetation groups and their respective growth forms was recorded inside each grid cell by visual inspection. The abundance values, in each quadrat, corresponds to the sum of all grid cells where that group or growth form was recorded (e.g. up to a maximum abundance of 100 in each quadrat).

3.1.2.3 Biodiversity-based metrics

A total of 14 trait-based metrics were computed based on two vegetation traits: Main group and life/growth form (Table 3.1.1). The first comprised three metrics with respect to the absolute abundance of bryophytes lichens and vascular plants. The second comprised 11 metrics with respect to the absolute abundance of each vegetation life/growth forms, based on literature review, as form is known to influence vegetation response to climate (Ellis et al., 2021; Glime, 2017). Despite several different bryophyte growth forms categories being proposed in literature (Glime, 2017; Mägdefrau, 1982; Victoria, Costa, & Pereira, 2009), three were selected for imagery analysis: cushion, mat and turf. This selection was based on the dominant bryophyte growth forms observed in previous surveys in the study area (Longton, 1979; Victoria et al., 2009) and limited to those which could be identified through image analyses alone. Lichens growth forms were divided into six groups, following the local lichen flora (Olech, 2014; Øvstedal & Smith, 2001): crustose, foliose, fruticose, leprose, placodioid and squamulose. Finally, the two vascular plant species were divided into two growth forms, *Colobanthus quitensis* (Kunth) Bartl. with a dense cushion-like form and *Deschampsia antarctica* É.Desv., characterized by a dense tussock.

Table 3.1.1: Description of bryophytes, lichens and vascular plants life/growth forms.

Trait	Growth form	Description
Bryophytes	Cushion	compact, dome-shaped clumps of shoots tightly pressed together
	Mat	creeping or prostrate shoots that grow horizontally and interweave
	Turf	upright, densely packed shoots that grow close together
Lichens	Crustose	thallus firmly and entirely attached to the substrate by the lower surface
	Foliose	thallus partly attached to the substrate, with distinct upper and lower surfaces
	Fruticose	thallus attached to the substrate by one single point and with rounded or flattened branches
	Leprose	thallus like crustose but surface with a granular mass appearance
	Placodioid	thallus like but with lobed margins
	Squamulose	thallus composed of small scales
Vascular plants	Tussock grass	dense tussocks or mats of narrow leaves and flowering culms
	Rosette	cushion-forming flowering plant

3.1.2.4 Environmental data

Climate was characterized by 19 bioclimatic variables (Table 3.1.2), covering annual and seasonal variations in air temperature and precipitation between 1970-2000. This dataset is available at WorldClim (Fick & Hijmans, 2017) and was collected at the best spatial resolution available (30 arc-seconds, equivalent to approximately 1 km resolution). This coarse resolution (i.e., smaller than the resolution of sampling in each area) implies that climate characterization was done at the broad scale, with limited variation in climate data across the spatial gradients sampled here, and for that reason, the limitations that can arise from it are considered when discussing the results. All data was retrieved for the quadrat centroid using ESRI ArcGis software (version 10.6).

Table 3.1.2: Bioclimate variables used, divided by group (temperature; precipitation) and type (annual; seasonal).

Variable group	**Type**	**Variable**	**Description**
Air temperature (Units: ºC)	annual	Bio 1	annual mean temperature
		Bio 2	mean diurnal range
		Bio 3	isothermality
		Bio 7	temperature annual range
	seasonal	Bio 4	temperature seasonality
		Bio 5	maximum mean temperature of warmest month
		Bio 6	minimum temperature of coldest month
		Bio 8	mean temperature of wettest quarter
		Bio 9	mean temperature of driest quarter
		Bio 10	mean temperature of warmest quarter
		Bio 11	mean temperature of coldest quarter
Precipitation (Units: mm)	annual	Bio 12	annual precipitation
	seasonal	Bio 13	precipitation of wettest month
		Bio 14	precipitation of driest month
		Bio 15	precipitation seasonality
		Bio 16	precipitation of wettest quarter
		Bio 17	precipitation of driest quarter
		Bio 18	precipitation of warmest quarter
		Bio 19	precipitation of coldest quarter

3.1.2.5 Data analysis

The minimum, maximum, average, median and inter-quartile range (25–75) of the total abundance of each functional metric were calculated per quadrat (N=150) and are presented as boxplots.

A Principal Component Analyses (PCA), encompassing all the 19 bioclimate variables (BIO 1 to BIO 19), was performed to reduce the dimensionality of the macroclimate dataset, thus limiting the number of explanatory variables. As the main goal here was to confirm trait-based metrics response to climate and not to determine particular biodiversity-climate variables relationships, the PCA was applied to reduce the number of models discussed. Climate variables were first normalized due to their different orders of magnitude. PCA scores in each axis were used as explanatory variables to depict the macroclimate gradients in the subsequent analysis. Only the PCA axes with an amount of variance explained equal or higher than 10% were considered for further analysis.

Random Forest regression was then used to model the response of each metric to the macroclimate gradients expressed in the PCA. By being non-parametric, this modelling approach is more capable of handling non-linear relationships, offering a more robust regression method (Breiman, 2001). Random forest regression models were run individually for each of the 14 trait-based metrics using the PCA scores of the selected axes as predictors. For each regression model, data was randomly split into a training and testing dataset, at an 80/20 ratio, thus corresponding to 120 and 30 sampling sites, respectively. Splitting ensured that both datasets comprised sampling sites from all elevation classes (see Field sampling section). All models were built using 1000 trees and with the set of predictors randomly sampled at each split. This process was repeated 100 times, each corresponding to a model with a different combination of sampling points, to account for the possible effect of random splitting of data. Root mean square error (RMSE), predictors importance and the coefficient of determination (R^2, depicting the variance explained in each biodiversity metric) were used to evaluate each random forest regression model performance. RMSE was considered optimal between 0 and 20, and acceptable between 20 to 30. Models for each metric were considered acceptable, i.e., ecological meaningful, when variance was equal or higher than 20%. For the selected models, the partial responses to each predictor were plotted to allow for the visual inspection of the shape of the biodiversity-climate relationship, to signal the existence of thresholds in the response and the show its direction. In each partial response plot presented, the solid line represents a loess function applied to the median of the partial prediction of the 100 models, and the dashed lines correspond to the 25 and 75 quantiles of the median of the 100 models.

All statistical analyses described above were performed with R software (R Core Team, 2022). The abundance minimum, maximum, average, median and inter-quartile ranges were calculated using the *quantile* function from the *Stats* package. Spearman correlation coefficients were calculated using the *cor* function from the *Stats* package. Macroclimate variables were normalized and the PCA performed with the function *PCA* from the *FactoMineR* package. Models were run using the *randomForest* function from the *RandomForest* package. Variables importance in each random forest model was quantified using the *varImp* function of *Caret* package. Partial responses were built using the *partial* function of the *pdp* package and then plotted using the *geom_smooth* function from the *ggplot2* package.

3.1.3 Results

3.1.3.1 General vegetation abundance patterns

The abundance of the 14 functional groups was assessed across the 150 quadrats (Fig. 3.1.2). Lichens were the most abundant vegetation group with an average absolute abundance of 58 (of a maximum of 100), followed by bryophytes (32) and vascular plants (11). Within bryophyte life forms, turfs and cushions were the most represented, with an average abundance of 16 and 9, respectively, with mats only 4. For lichens, the crustose form was the most abundant (36), followed by the fruticose (30). All remaining lichen forms had an abundance lower than 5. Lastly, both vascular plant forms presented low abundance, with the tussock grass being more represented than the rosette (12 and 5, respectively).

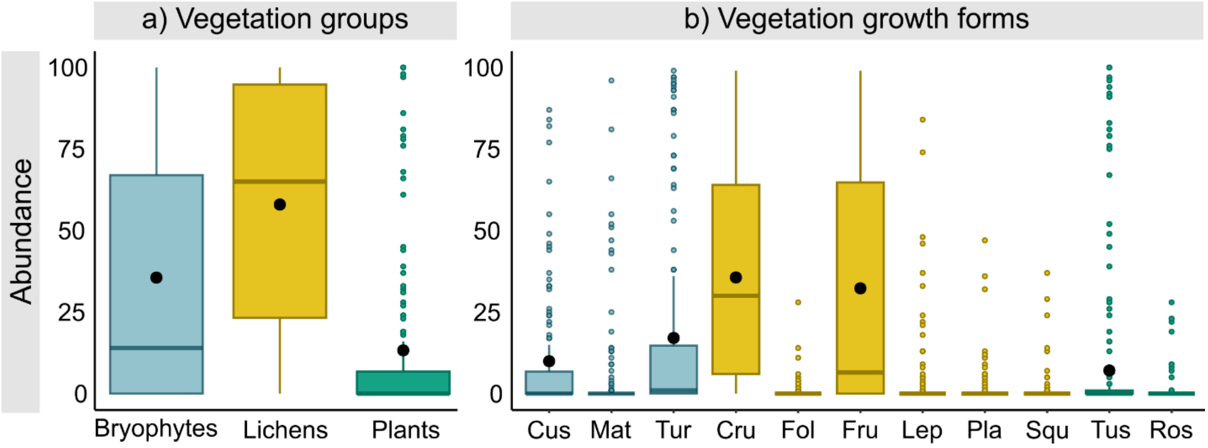

Figure 3.1.2: Trait-based metrics absolute abundances across the study area. a) Vegetation groups represented by the abundance of bryophytes, lichens and vascular plants. Vegetation growth forms of bryophytes (Cus – cushion, Mat – mat and Tur – turf), lichens (Cru - crustose, Fol - foliose, Fru – fruticose, Lep - leprose, Pla – placodioid and Squ - squamulose) and vascular plants (Tus – tussock grass, Ros – Rosette plant), coloured according to the respective vegetation group. Boxes represent first to third interquartile ranges, horizontal line the median, dots the average, whiskers the maximum and minimum abundance values and dots potential outliers (N = 150).

3.1.3.2 The main climate gradients

The first two axes of the PCA accounted for, respectively, 76.1% and 13.6% of the variance in macroclimate and thus, all subsequent analyses focus on these two axes only (total of 89.7%, Fig. 3). The PCA showed a clear macroclimate gradient across its first two axes, associated with annual and seasonal temperature and precipitation patterns (Fig. 3.1.3). The first PCA axis represented a gradient of annual and seasonal temperature and precipitation, with higher scores corresponding to overall warmer and wetter conditions and lower scores with greater annual temperature ranges, and colder and drier conditions. A gradient of temperature and precipitation seasonality was depicted in the second axis, with higher scores associated with higher temperature and precipitation seasonality and lower scores with lower diurnal range and higher isothermality.

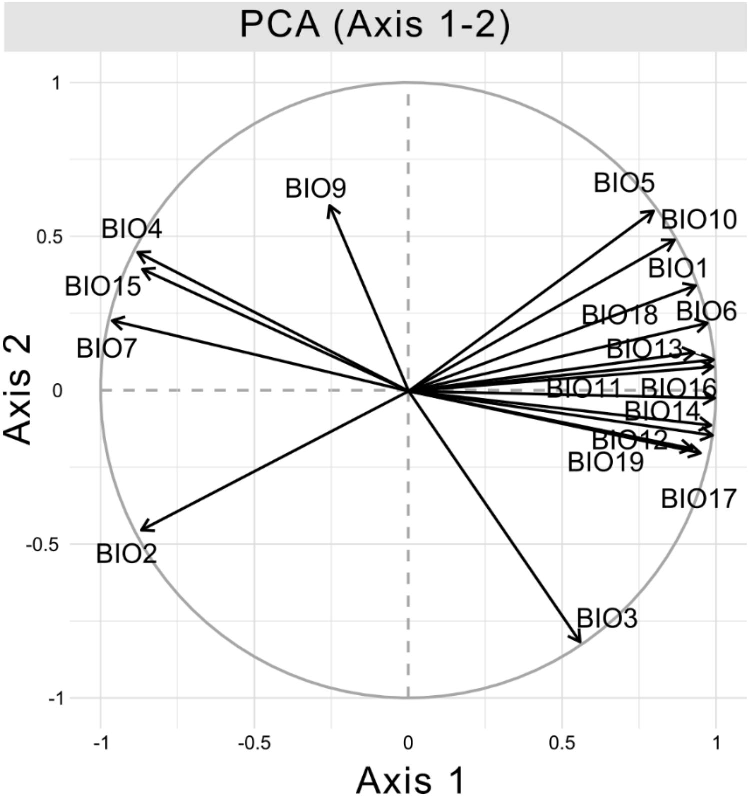

Figure 3.1.3: Principal Components Analysis (Axis one and two) showing the ordination of sampling sites (N = 150) along a macroclimate gradient of annual and seasonal temperature and precipitation. Bioclimatic variables decoding can be seen in Table 1.

3.1.3.3 Main climate drivers of vegetation functional structure

The two PCA axes, representing annual and seasonal air temperature and precipitation macroclimate gradients, were important predictors of several trait-based metrics. The variance explained by macroclimate surpassed 20% in 6 of the 14 metrics tested, and the errors were below the thresholds defined (RMSE ≤ 30). For that reason, only the three main vegetation groups and three lichen growth forms (crustose, fruticose and leprose) were considered for further analysis.

Regarding the main vegetation (Fig. 3.1.4a), macroclimate explained 43.7% of bryophyte, 59.2% of lichen and 26.3% of vascular plant abundance. The variance explained for the growth form functional groups was lower overall, and, more importantly, only macroclimate explained three growth form metrics over 20%: 24.3% of crustose lichens, 34.7% of fruticose and 22.9% of leprose lichens (Fig 3.1.4a). The importance of the two PCA axes was very similar across most metrics (Fig. 3.1.4a). The gradient of annual and seasonal temperature and precipitation (axis 1) average importance was 47,1%, while the gradient of contrasting temperature and precipitation seasonality (axis 2) was 43.4%.

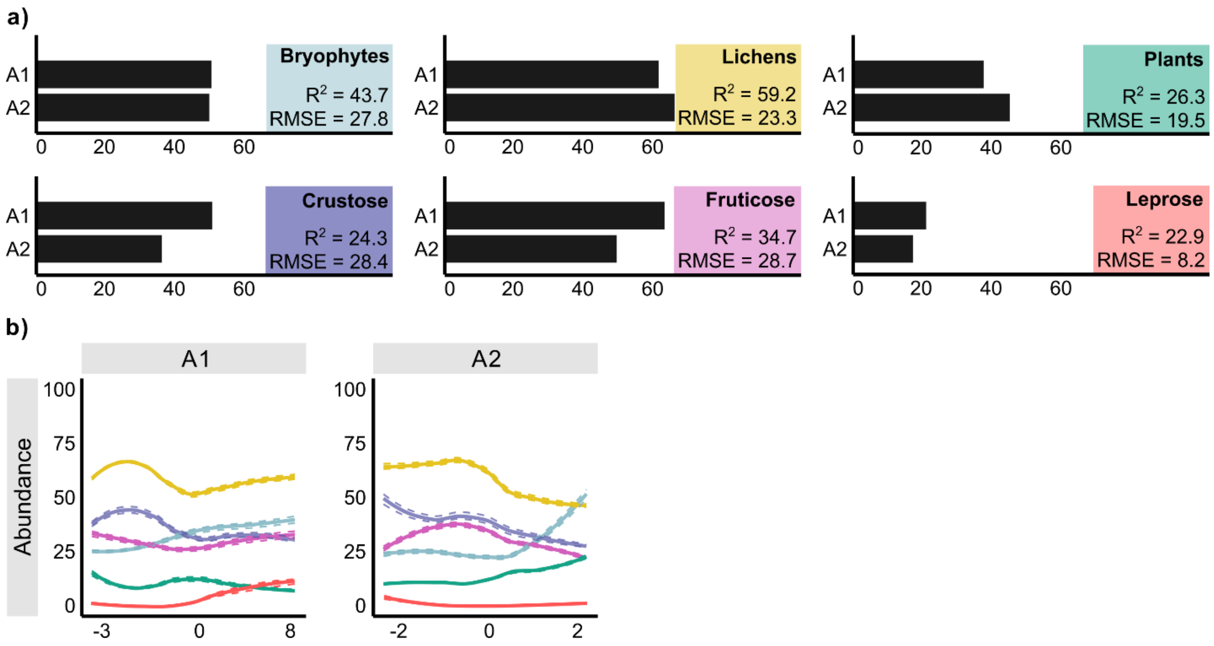

Figure 3.1.4: a) Importance of the two PCA axes representing macroclimate gradient in annual and seasonal temperature and precipitation, as predictors of maritime Antarctic vegetation trait-based metrics, related to the group and life/growth form traits. Climate variables importance refers to the median of 100 models with different random training/test splitting (for more details see Data Analysis). b) Partial response of selected metrics (R2 ≥ 20 % and RMSE ≤ 30 %) to the two macroclimate predictors. Filled lines represent a loess function applied to the median of the partial predictions of 100 models with different random training/test splitting, while 25 and 75 quantiles are represented by dashed lines.

It was possible to observe shifts in response to the different macroclimate drivers for all metrics, with shape and direction of the shift differing between vegetation groups, but not between lichen growth forms (Fig. 3.1.4b). Lichens' abundance decreased under annual and seasonal warmer and wetter areas, with bryophytes showed an opposite response trend to lichens. Vascular plants remained mainly unresponsive to the first axis but improved in abundance in areas with higher seasonal temperature and precipitation. Crustose and fruticose lichens shared similar responses along the macroclimate gradients, while leprose lichens increased in abundance in areas with higher annual temperature and precipitation. Furthermore, responses were sometimes associated with a threshold, which was shared between the lichens group metric and all its growth forms for the same macroclimate gradient. For axis one, the leprose form abundance increases after the threshold, coinciding with the warmer and humid part of the gradient. In axis two, lichens and bryophytes shared a common threshold, after which lichen abundance declined while bryophytes increased.

3.1.4 Discussion

Results revealed that broad scale climate was indeed an important predictor of the vegetation abundance across small elevation and distance to the coast spatial gradients in the maritime Antarctic. The importance was nevertheless trait dependent, as the main group trait performed better than the life/growth form trait. Regarding the main group trait, macroclimate explained over 50% of the variance in lichen abundance but relatively less for bryophytes and much less for vascular plants. The importance across life/forms was in general much lower, with climate being an important predictor of only three lichen growth forms. These findings support the application of these vegetation main groups as ecological indicators to track the effects of climate change across maritime Antarctic terrestrial ecosystems.

3.1.4.1 Macroclimate is an important determinant of maritime Antarctic vegetation

We hypothesized that ecological indicators based on maritime Antarctic vegetation could be applied in the region to track climate effects. Our results confirmed that broad scale climate gradients explained almost 60% of lichens abundance, 44% of bryophytes and 26% of vascular plants. It is widely acknowledged that climate directly influences species physiological processes (Casanova-Katny, Torres-Mellado, & Eppley, 2016; Singh, Singh, & Khare, 2018; Torres-Díaz et al., 2016) and indirectly, by extending growing seasons, increasing water availability when temperatures exceed ice melting point and due to the creation of new ice-free areas for colonization (Jin et al., 2021; Lee et al., 2017). In fact, several studies conducted over different regions of Antarctica have linked changes in both temperature and water availability to changes in vegetation cover over space and time (Cannone, Malfasi, Favero-Longo, Convey, & Guglielmin, 2022; Roland et al., 2024), highlighting these groups potential to track climate effects in the region. Our results thus corroborate the impact of climate on the vegetation abundance spatial patterns and reinforce their potential application.

Nevertheless, macroclimate explained only 50% of the variance across lichens and the contribution was around half that value for bryophytes and vascular plants. More importantly, the remaining 50% to 70% of the variance not explained by macroclimate suggests that other factors could also be important determinants of the vegetation abundance across the elevation and coast inland gradients explored. In a recent study, a series of microscale topographic variables were fitted in Random Forest models, explaining more than 60% of the variance in lichens and bryophytes abundance. Also in that study, vascular plants explained variance was substantially lower (30%), hinting that rather than climate or topographic drivers, regardless of the scale, plants abundance is mainly driven by other factors not accounted for in any of these studies. Vascular plants have higher nutrient and substrate requirements (i.e. soil depth, water holding capacity) compared to bryophytes and lichens (Park, Ahn, & Lee, 2012, 2013), thus potentially less linked to climate conditions. At the same time, biotic interactions between these vegetation groups can also play a critical role in determining their abundance patterns. Despite the simplified ecosystems of the regions, such interactions have already been seen to improve models explaining Antarctic vegetation spatial patterns (Lee et al., 2019; Matos et al., 2024).

The growth form trait performed considerably worse, with macroclimate explaining less than 30% of the variance in crustose, fruticose and leprose, and less than 20% for all bryophyte and plant forms. These results thus suggest that the underlying environmental drivers of these forms may be acting at spatial scales not contemplated here. Furthermore, all forms besides crustose and fruticose registered low abundance, hinting that the low explained variance for most forms may also be related to poor modelling fits due to low abundance values. The poor response most forms exhibited to broad scale climate also suggests that, at this scale, absolute abundance of the three main groups is sufficient to track climate effects in the maritime Antarctic and that the trade-off of higher functional characterization may not bring further information. Remote sensing studies have been able to distinguish between the three vegetation groups (Cannone et al., 2022; Roland et al., 2024), while specific vegetation forms are more difficult to differentiate. Thus, focusing on these main groups can in fact be more helpful from the perspective of a broader application in Antarctica, as remote sensing approaches allow to determine vegetation cover and abundance over larger areas with less effort, time and impact (Pina & Vieira, 2022). In that sense, the sampling approach used here can further bridge the gap between local scale studies and remote sensing, a cost-effective method capable of detecting changes in vegetation distribution across much larger areas than previously before (Miranda et al., 2020).

3.1.4.2 Individual responses to the climate gradient

We hypothesized that, due to different ecological requirements, vegetation and life/growth form responses to climate gradients would be unique. Indeed, the three main vegetation groups presented contrasting responses to annual and seasonal gradients in temperature and precipitation. From an ecological indicator perspective, these results reinforce their potential as ecological indicators in the region, as it has now been shown that lichens, bryophytes and vascular plants abundance can be applied complementarily to track macroclimate change, at least along the environmental envelope encompassed here. In a previous study (Matos et al., 2024), these three vegetation groups have been seen to dominate in areas with specific microscale topographic conditions, suggesting their complementary potential as ecological indicators. Here, we confirm this complementarity also for climate, covering a wider climatic space than each group individually.

Along the climate gradients encompassed here, increased bryophyte abundance was associated with both annual and seasonal warmer and wetter conditions. In turn, vascular plants' abundance responded positively to higher temperature and precipitation values, but seasonal changes rather than annual ones. Bryophytes and vascular plants require liquid water for hydration (Green, Sancho, & Pintado, 2011). These vegetation groups should thus be more associated with areas with higher water availability, as our results reveal. Similarly, higher temperatures should both dictate increased photosynthetic activity in bryophytes and vascular plants (Pannewitz et al., 2005; Perera-Castro et al., 2020; Sáez et al., 2018; Xiong, Ruhland, & Day, 1999), while also extending growing seasons and improving nutrient cycling. In turn, lichens abundance was higher in the opposite part of the climate gradient, associated with colder and arid sites. Lichens do not require liquid water for activation and are better adapted to cold (Armstrong, 2017), therefore able to survive in areas where the other two groups cannot (Williams et al., 2017). However, due to a competitive edge of vascular plants and bryophytes (Bao, Jia, & Xu, 2022), lichens are forced to occupy the opposite side of this macroclimate gradient. This contrasting response thus reinforces the idea that biotic interactions are also key determinants of the vegetation abundance patterns.

Contrary to what we expected, the three lichen growth forms best explained by macroclimate exhibited similar responses between themselves, and also in relation to lichens overall abundance. Growth form is a widely applied functional trait due to its responsiveness to climate (Ellis et al., 2021; Glime, 2017; Matos et al., 2015). In Antarctica, crustose and fruticose lichens have been seen to differ in water uptake, desiccation tolerance and photosynthetic activity (Ellis et al., 2021; Glime, 2017; Matos et al., 2015) but such physiological differences did not translate here into different abundance patterns. This could be explained, at least partially, due to the coarse resolution of climate variables, which can mask microhabitat differentiation or that the mechanisms which differentiate the response between the lichen forms are more noticeable at parts of the climate gradient, not encompassed here. This suggests that lichens as a whole track macroclimate change consistently, while their growth form complementarity may emerge only under different microclimatic.

We also expected that the metrics response along the macroclimate gradients explored would be defined by thresholds, as both air temperature and water availability can act as limiting factors. Despite detected for some metrics, most metrics responses along the macroclimate gradient did not show such response. When detected, these thresholds differed between lichens and bryophytes in the second axis of seasonal temperature and precipitation. These results point to the indicator potential to signal tipping-points critical for maritime Antarctic terrestrial ecosystems, but further studies are necessary to determine the values of specific temperature or precipitation-related variables after which major changes may occur. These are vital both from a conservation standpoint, as it allow to establish red lines for climate action (Barták et al., 2021; Cao, Zhang, Zheng, Liu, & Zhou, 2015; Huiskes, Gremmen, & Francke, 1997) but also from an ecological indicator perspective, as it will allow to determine a range of environmental conditions until or after which lichen functional metric can be applied (Eiswerth & Haney, 2001).

3.1.4.3 Future applications

Antarctica is forecasted to be one of the more severely impacted regions by climate change (IPCC, 2022), stressing the need for the development of ecological indicators to track its effects. Here, we showed that bryophytes, lichens and vascular plants abundance can be applied as ecological indicators of climate. Furthermore, we observed that each main vegetation group responded differently to the macroclimate gradients. This reinforces their applicability, due to their complementary ability. Nevertheless, traits response followed the main group responses, suggesting that focusing solely on the main group abundance may be more advantageous than a deeper functional characterization. Such approach would be beneficial from a monitoring perspective, as it requires less expert knowledge and is more easily detectable through remote sensing methods (Miranda et al., 2020; Pina & Vieira, 2022; Vieira, Mora, Pina, & Schaefer, 2014). Contrary to our expectations, few metrics presented thresholds associated with a response to temperature and water availability. Despite revealing the potential to signal tipping-points for Antarctic ecosystems in the future, future studies must first determine with precision which are the threshold values to particular climate variables. In addition, macroclimate alone was not able to fully explain these main groups abundance patterns, stressing the need for future studies to encompass a wider array of local environmental data and on intra- and inter-specific interactions between these vegetation groups (Matos et al., 2024). Future studies should thus determine the direct and indirect mediating effect on the climate response and understand the underlying mechanisms which define them.

3.1.5 Conclusions

In conclusion, the overall important contribution of macroclimate and the complementary potential of the three vegetation groups along the climate gradient solidifies their potential for application as ecological indicators of climate change in the region. Furthermore, it also highlights the potential use of this functional approach in broad scale studies as the use of these major functional groups, rather than the traditional species approach can be better linked to remote sensing. All together, these are crucial contributions to improve our understanding of climate effects in maritime Antarctic, although local scale abiotic and biotic drivers must be considered to improve our capacity to forecast effects and improve conservation actions.

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Subchapter 3.2

3.2 Incorporating biotic interactions to better model current and future vegetation of the maritime Antarctic

Rocha, B., Pinho, P., Giordani, P., Concostrina-Zubiri, L., Vieira, G., Pina, P., Branquinho, C. & Matos, P. (2024). Incorporating biotic interactions to better model current and future vegetation of the maritime Antarctic. Current Biology, 34(21), 4884-4893. doi:10.1016/j.cub.2024.09.011

Abstract

In maritime Antarctica, harsh abiotic conditions forge a simplified terrestrial ecosystem, where bryophytes, lichens and vascular plants constitute the bulk of the vegetation. Together with abiotic conditions, biotic interactions help shape this ecosystem, influencing species' distribution and, indirectly, mediating its response to climate. For Antarctica however, biotic interactions are often seen as of limited importance, despite the lack of studies to support this view. Thus, we need to test the importance of biotic interactions to improve our knowledge of Antarctic ecology. Here, current and future abundance patterns of maritime Antarctic main species groups were modelled accounting simultaneously for abiotic drivers and biotic interactions. For that, we quantified the three main groups abundance in 150 quadrats from 30 sites, along a climatic gradient in two islands of the South Shetlands archipelago. Influence of regional climatic and terrain drivers was modelled using structural equation models with and without the inclusion of biotic interactions. Models accounting for biotic interactions performed considerably better, revealing negative impacts of both bryophytes and vascular plants on lichens and the prominence of abiotic drivers' effects on vascular plants communities. The model with higher ecological support was used to estimate current and future spatial distributions of these groups in similar climatic areas of the archipelago, using the most pessimistic future climate change scenario. Our model suggests that plants are confined to lower elevations, where they negatively impact bryophytes and lichens communities, whereas at higher elevations both climate change and terrain drivers shape bryophytes and lichens. Our findings strongly support the use of biotic interactions to predict the spatial distribution of Antarctic vegetation.

Keywords

Antarctic ecology; Climate change; Antarctic vegetation; Plant–climate interactions; Plant–plant interactions

3.2.1 Introduction

In the South Shetland Islands, maritime Antarctic, adverse abiotic conditions, recent deglaciation history and isolation from main dispersal centres shaped simplified ecosystems (Convey et al., 2014), to the point where cryptogamic communities of bryophytes and lichens (Singh, Singh, & Khare, 2018; Øvstedal & Smith, 2001), complemented in some areas by up to two vascular plant species (Corner, 1971; Edwards, 1972), constitute the bulk of its terrestrial vegetation (Convey, 1996; Lindsay, 1971). The spatial pattern of Antarctic vegetation composition and floristic characteristics has multiple known drivers. These include spatial variations in the environmental conditions at the microscale, namely topographic features and soil conditions (Ferrari et al., 2021; Matos et al.). Also, marine vertebrate colonies are known sources of nitrogen pollution, which in turn drive changes in vegetation spatial patterns related to increased nutrient availability (Bokhorst, Convey, & Aerts, 2019; Zwolicki et al., 2015). Added to these drivers, this area experienced a warming trend of ~1.2ºC since the 1950s to the end of the century, and more recently, a cooling trend (Turner et al., 2014; Turner et al., 2016), urging the need for climate change research in the region. Climate impacts, reflected by changes in temperature, water availability and expansion of ice-free areas, are already leading to drastic shifts in these species groups (Cannone, Malfasi, Favero-Longo, Convey, & Guglielmin, 2022; Lee et al., 2017), influencing growth rates, colonization success, growing seasons, among others (Convey et al., 2014; Singh et al., 2018).

Still, terrestrial vegetation is also influenced by biotic interactions. Interspecific (biotic) interactions are ubiquitous in nature and known drivers of species distribution (Wisz et al., 2013) and communities structure (García-Girón, Heino, García-Criado, Fernández-Aláez, & Alahuhta, 2020). Despite recent evidence (Caruso et al., 2019; Chown & Convey, 2007; Lee et al., 2019) showing that, even under extreme environmental conditions, biotic interactions play a relevant role in determining several terrestrial Antarctic taxa distribution and abundance (Caruso et al., 2019; Caruso, Trokhymets, Bargagli, & Convey, 2013; Lee et al., 2019), the general belief is that these are overshadowed by abiotic drivers. Thus, biotic interactions role and relation to climate change is yet to be quantified, and models dealing with species distribution and abundance have largely failed to incorporate them (Hogg et al., 2006). Nevertheless, achieving better predictions for maritime Antarctic terrestrial vegetation, now and under future climate change, will necessarily require incorporating biotic interactions into distribution models (Araújo & Luoto, 2007; Van Der Putten, Macel, & Visser, 2010).

In this study, and for the first time for maritime Antarctica, we integrated climate (and other environmental drivers) and biotic interactions to estimate present and future abundance and spatial distribution patterns of the three main maritime Antarctic terrestrial vegetation species groups: bryophytes, lichens and vascular plants. Our main hypothesis was that the abundances of the three species groups would be better explained when biotic interactions were included, though we expect abiotic drivers to exert the strongest control. To test this, we performed structural equation modelling (SEM) to assess whether inclusion of pathways between species groups (biotic interactions) resulted in a better fitting SEM. A well-fitted SEM allows to quantitatively evaluate the strength and pathways of the direct effects of abiotic drivers and its indirect effects via biotic interactions between the three groups. As an example of applications, the SEM combining both a satisfactory goodness-of-fit and best ecological support was used afterwards to produce spatial abundance predictions of the vegetation across the ice-free areas of the South Shetlands, both for present and future environmental conditions.

3.2.2 Materials and methods

3.2.2.1 Study area

The sampling for this study was conducted in Livingston and Nelson Islands, South Shetlands, off the northwest tip of the Antarctic Peninsula. The three main species groups were surveyed along elevation and coast inland gradients (Fig. 3.2.1), in four ice-free areas encompassing different ground features (Hobbs, 1968; Smellie, Pankhurst, Thomson, & Davies, 1984): (1) Byers Peninsula, (2) Hannah Point and (3) Hurd Peninsula, in Livingston Island and (4) Rip Point in Nelson Island. Sampling followed coastal-inland and elevation gradients, from the coastline (2 m elevation and 12 m from the nearest coastline) to the highest accessible ice-free area (370 m elevation and 947 m from the nearest coastline). A sampling site was defined as a patch with the presence of at least one of the three species groups targeted (bryophytes, lichens and vascular plants), of variable dimension, along which the survey of the species groups was conducted (Fig. S1). This spatial gradient was intended to depict the shifts in the three species groups associated with the spatial climate gradients, and thus encompassing bryophyte dominated communities, lichen dominated communities, communities with vascular plants, and the possible transitions between these in response to the shifts in climatic conditions (see Supporting information S1 for details).

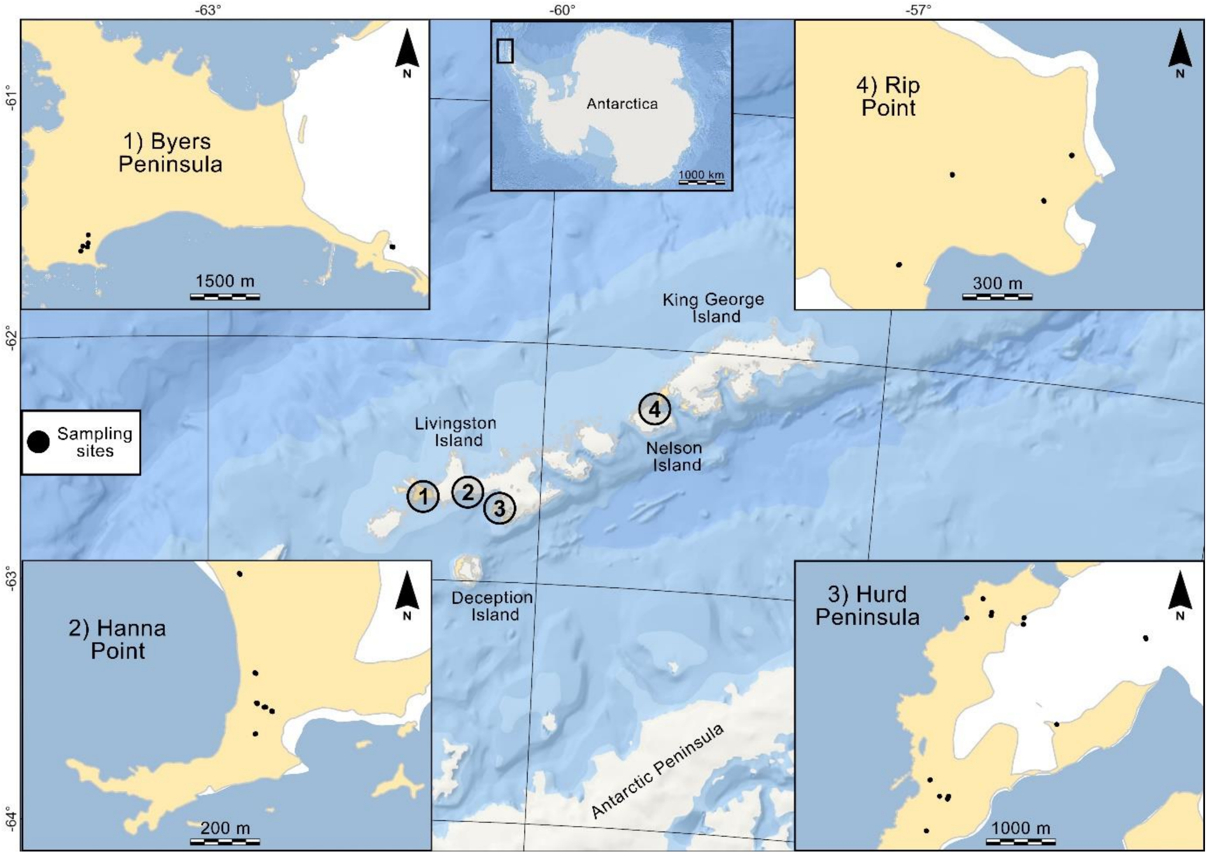

Figure 3.2.1: Sampling sites in Livingston and Nelson Islands, South Shetlands, maritime Antarctic. N = 30. Black dots represent the sampling sites and numbers represent, respectively, (1) Byers Peninsula; (2) Hanna Point and (3) Hurd Peninsula, in Livingston Island and (4) Rip Point in the area around the Eco-Nelson Czech Antarctic Base, Nelson Island, Shetland Islands, maritime Antarctic.

3.2.2.2 Vegetation sampling

The abundance of bryophytes, lichens and vascular plants was determined in 30 sampling sites (Fig. 3.2.1) in February 2019, and January and February 2020. Sampling site selection was done following an elevation stratification along the four areas in Livingston and Nelson Islands. A total of 6 sampling sites were randomly chosen from 5 elevation classes of increasing range to better capture vegetation shifts, which were greater at lower elevations ([0 – 23 m], [24 – 46 m], [47 – 92 m], [93 – 184 m] and [185 – 367 m]), distributed equally among the four areas when possible. On each of the 30 sampling sites, 5 quadrats were surveyed, resulting in a total of 150 quadrats. Each quadrat was photographed using a standard sampling methodology for biocrusts (Concostrina-Zubiri, Pescador, Martínez, & Escudero, 2014; Concostrina-Zubiri, Prieto, Hurtado, Escudero, & Martínez, 2022) (see Appendix S1 for details and Fig. S3.2.2). The abundance of each species group was posteriorly determined by image analyses. Images were first corrected for distortion, using Adobe Photoshop 23.0. Afterwards, with ImageJ software (Schneider, Rasband, & Eliceiri, 2012), a grid of 3 cm side small cells was superimposed on the image to divide the 30 cm square into 100 small cells. The presence of the three species groups inside each grid cell was recorded. The abundance of each species group, in each quadrat, corresponds to the sum of all cells where that group was recorded (e.g. up to a maximum abundance of 100 in each quadrat). The five quadrats were treated as independent samples.

3.2.2.3 Environmental data

A set of 30 regional climatic, terrain morphometry, hydrology and lighting, and natural pollution environmental variables were retrieved, as these are known to be important drivers of Antarctic vegetation patterns or their proxies (Bokhorst et al., 2019; Cannone et al., 2022). These were collected at the best spatial resolution available in the data repositories. Climate variables are here referred to as regional climatic due to their coarse spatial resolution (30 seconds or ~1 km). Although this is the best spatial resolution available, it is still not optimal and thus the limitations that can arise from it are considered in the discussion. For more information regarding each of 30 variables see Appendix S1 and S2. A set of 19 regional climatic variables were retrieved from WorldClim dataset (Fick & Hijmans, 2017; O’Donnell & Ignizio, 2012), corresponding to the period of 1970-2000 (see Appendix S2, Table S3.2.1 for details on each variable). We opted for the bioclimatic variables instead of raw temperature and precipitation variables, as the former are biologically potentially more meaningful and often used in species distributions models (Title & Bemmels, 2018). To model future vegetation patterns in response to future regional climatic projections, we retrieved the same bioclimatic variables, at the same spatial resolution, for the period 2081-2100. To characterize the terrain morphometry, hydrology and lighting variability across the study area, ten variables were retrieved (see Supporting information S2, Table S3.2.2 for details on each variable): Elevation, slope, standard curvature, flow accumulation, topographic wetness index, potential solar radiation, distance to the coast, the area occupied by sea, area occupied by ice and ice-free areas at 11 buffers of different sizes. Finally, one natural pollution environmental driver was considered, calculating the distance of each sampling site to the closest known vertebrate colony (see Supporting information S2, Table S3.2.2).

3.2.2.4 Statistical Analysis

All statistical analyses were conducted using R software (R Core Team, 2022). Vegetation species groups mean abundance, standard deviation, and interquartile range were calculated based on absolute abundances, per quadrat (N=150). The first step was to reduce the number of environmental variables for the sake of better clarity and methodological flow. We started by determining the correlations coefficients between environmental variables and the absolute abundance of each vegetation group using Spearman correlation coefficients. Environmental variables not significantly correlated with any of the groups were excluded (considered significant for $p < 0.05$). From the 11 buffers of area occupied by sea, area occupied by ice and ice-free areas, only the buffer with highest average significant correlation coefficient with the three species groups was kept. A threshold of > 0.7 in the correlation coefficient was then used to identify collinear variables. Whenever collinearity was detected among environmental variables, the one with the highest average significant correlation coefficient with the three species groups was kept. It is important to note that, for this reason, several climate variables were excluded from further analysis although presenting only slightly lower correlation values with the three vegetation groups. Finally, the four environmental variables best correlated with each species group were selected for modelling. Correlation coefficients between each species group and the set of four best environmental variables can be seen in Supporting information S2, Fig. S3.2.3.

Linear regressions were then used to determine the main abiotic drivers of abundance of the three different species groups along the elevation and coast inland the coast gradients. We followed a thorough approach, modelling each species group with all the possible combinations of their respective four best environmental variables, based on the step performed previously. We first evaluated the individual response of each species group with the set of regional climatic, terrain, and natural pollution variables. Prior to modelling, normality of response variables and linearity of the relationships between response variables and predictors were tested. The abundances of the three species groups were not normally distributed and the relationships between these and the set of environmental variables tested were not linear. For that reason, we log transformed (natural logarithm) both the response variables and predictors prior to modelling. Selection of the best model for each species group was based on the highest adjusted R^2 ($AdjR^2$) value and significance (considered significant for $p < 0.05$), while ensuring a parsimonious model. For that, all possible models were built and ranked per number of predictors and $AdjR^2$ value (Supporting information S2, Table S3.2.3-5). The model with the highest $AdjR^2$ on each level of predictors were compared, starting from the most parsimonious model (with just one predictor). The model for which including one extra predictor resulted in an $AdjR^2$ increment of more than 5% was selected. The proportion of variance in the abundance of each species group, explained by their respective best environmental predictors, was calculated using a variance decomposition analysis based on each model (Supporting information S2, Table S3.2.6). One regional climate (mean diurnal temperature range) and two terrain variables (elevation and slope) arose as the best predictors of the three species groups. Models were performed with the *lm* function from the *stats* package (R Core Team, 2022).

To evaluate these three abiotic drivers importance on vegetation patterns and the biotic effects (i.e. via interactions between species groups), we fitted a structural equation modelling (SEMs), using the *sem* function from the *lavaan* package (Rosseel, 2012). As in the prior step, response variables and predictors were log transformed prior to modelling. An initial SEM (SEM I), without biotic interactions and therefore focusing only on the relationship between the three abiotic drivers (mean diurnal temperature range, elevation and slope) and the abundance of each species group was tested (Supporting information S2, Fig. S3.2.4). Deriving from this initial model, 24 additional SEMs were tested, by adding pathways representing the biotic interactions between the three species groups, encompassing all possible biotic pathways combinations between them (Supporting information S2, Fig. S3.2.4 and Table S3.2.6). SEMs overall goodness-of-fit evaluation was tested based on the following indices cut-offs (Hooper, Coughlan, & Mullen, 2007; Kline, 2016): Satorra-Bentler chi-square statistic and its significance (the model is rejected if p-value < 0.05), robust comparative fit index (CFI > 0.9), robust Tucker Lewis index (TLI > 0.95), robust root mean square error of approximation index (RMSEA < 0.08) and the standardized root mean square residual (SRMR < 0.08). Ten models performed within the indices cut-off, with the average R^2 across the three species groups differing in less than 5% across these SEMs (Supporting information S2, Table S3.2.6). As these models were statistically similar, only the one encompassing the biotic pathways best supported by ecological literature (Model XI) was used to estimate present and future abundance spatial patterns of each species group. These estimations were performed by first retrieving the values of the three abiotic drivers present in Model XI, (ESRI ArcGis Pro 3.2), but only for the areas of the study area with the same environmental envelope as the one used to build the SEM (i.e., where values of the abiotic variables used are within the range used to build the model). In addition, both present and future abundance spatial estimations, were also limited to current ice-free areas of the Archipelago. Net effects were derived from the winning SEM (Model XI) and calculated by adding composite variables to the SEM, thus encompassing the total effect (sum of direct and indirect effects) of all environmental variables on each species group as well of each species group on the remaining groups.

We then ran the *lavPredictY* function from the *laavan* package (Rosseel, 2012), which allowed for the prediction of abundance of each species group abundance, under current (from here on referred as current abundance) and future (from here on referred as future abundance) environmental conditions given the values of predictors, and by considering the SEM built before (Model XI). Although we also intended to repeat this step for the SEM where biotic interactions are not considered, the fact that the model performed under all the goodness-of-fit evaluation indices cut-offs prevented us from doing so. Future vegetation abundance maps were estimated based on the CMIP6 regional climate variables values for the year 2100, under the more pessimist emissions scenario (SSP5-8.5). Lastly, the abundance of each species group, for both present and future environmental conditions, were used to plot their spatial patterns along an elevation and coast inland spatial gradient in the Hurd Peninsula, as a conceptual purpose. Because data fitted in the SEMs was log transformed, species groups abundance values were transformed (inverse of log transformation, i.e., exponential) prior to mapping. For more details on the several steps of the statistical analysis see Supporting information S1.

3.2.3 Results

3.2.3.1 Species groups structure and their main abiotic drivers

Our data showed that in the studied area of the South Shetlands, lichens were the dominant species group, with an average absolute abundance of 58 (of a maximum of 100), followed by bryophytes (32) and vascular plants (11). Lichens abundance increased along the elevation and coast inland spatial gradient, while both bryophytes and vascular plants abundance declined along these gradients (Supporting information S2, Fig. S3.2.5). From the initial set of 30 environmental variables, seven were selected from their higher and significant correlation coefficients with each species groups abundances (Support S2, Fig. S3.2.2) and were afterwards used in the linear models. From the linear regressions (Support S2, Table S3.2.3-5), temperature (mean diurnal temperature range), elevation and slope revealed to be the main drivers of the three species groups (Table 3.2.1). Lichens and plants were driven by elevation, positively in the case of lichens ($AdjR^2$ = 48%), and negatively in the case of vascular plants ($AdjR^2$ = 16%), whereas bryophytes were negatively driven by both slope and mean diurnal temperature range ($AdjR^2$ = 37%). These three abiotic drivers (elevation, slope and mean diurnal temperature range) were then used as abiotic predictors in the structural equation models (Appendix S2, Fig. S3.2.4).

Table 3.2.1: Summary of the best linear model selected for each response variable, unveiling the key abiotic drivers of the three species groups. The sign of the coefficients of the selected predictors, in each model, are indicated. The proportion of variance (%) explained by the regional climate and terrain variables was calculated using a variance decomposition analysis based on each model. *p < 0.05; **p < 0.01; ***p < 0.001.

	Regional climatic	**Terrain**		
	Mean diurnal temperature range	Slope	Elevation	Adj. R^2
Bryophytes	42%; (-)***	58%; (-)***		0.37
Lichens			100%; (-)***	0.48
Plants			100%; (-)***	0.16

3.2.3.2 Structural equation modelling

The initial SEM (Supporting information S2, Fig. S3.2.4), with the main abiotic drivers of each respective species group and excluding the biotic interactions, performed poorly ([RMSEA] = 0.22, X^2 = 55.26, df = 8, P = 0.000). The inclusion of the biotic interactions pathways generally resulted in a better fitting model. Ten models performed well (within indices cut-offs), all differing less than 5% in the average R^2 across species groups (Appendix S2, Table S3.2.6). From these models, model XI (Fig. 3.2.2; goodness-of-fit [RMSEA] = 0.06, X^2 = 8.01, df = 5, P = 0.156) showed the strongest ecological support, and was selected for the subsequent analysis. All pathways in this model were significant and explained 43%, 61% and 16% of the variance of bryophytes, vascular plants, and lichens, respectively. This represented an increase of 5%, 13% and 1% in the variance of each group respectively, in comparison to the initial model without biotic interactions, confirming the a priori expectations of our theoretical framework. Each species group responded independently to the set of abiotic variables present in the model, showing that their covariance is not due to similar responses to the abiotic factors, and that processes other than abiotic filtering may be responsible.

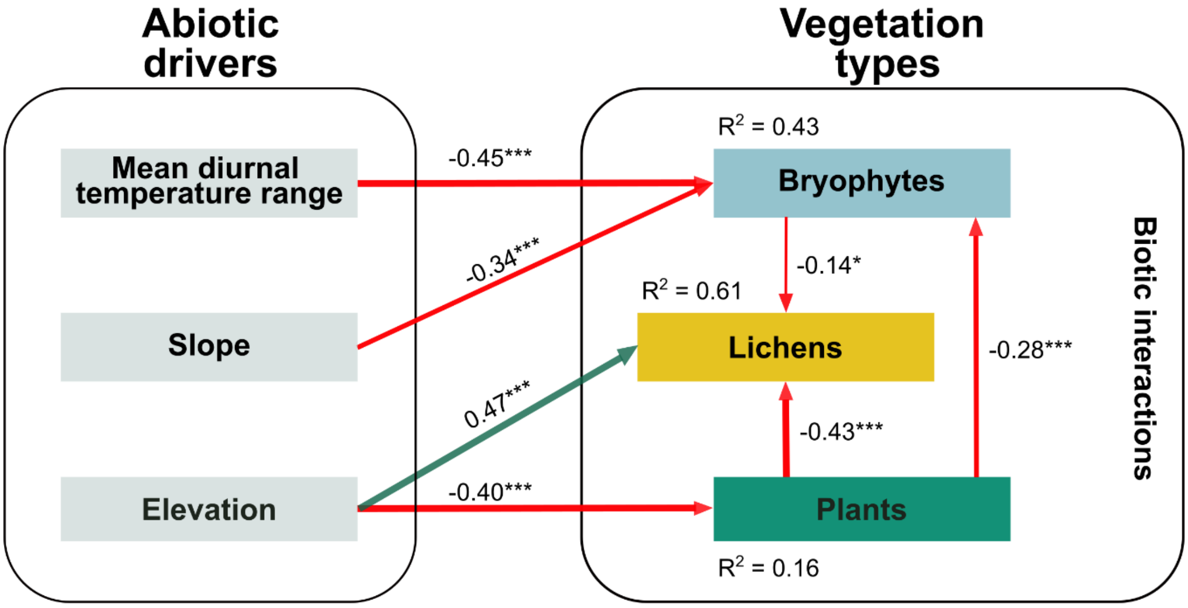

Figure 3.2.2: Structural equation model (Model XI) showing the absolute standardized path coefficients (ranging from 0 to 1), explaining Antarctic terrestrial vegetation (bryophytes, lichens and vascular plants) abundance in response to abiotic drivers (elevation, slope and mean diurnal temperature range) and accounting with the biotic interactions between species groups. Overall goodness-of-fit statistics: $\chi2 = 8.01$, df = 5, p = 0.16, robust Tucker Lewis index (TLI) = 0.98, robust comparative fit index (CFI) = 0.99, robust root mean square error of approximation (RMSEA) = 0.06 (0.000–0.13), standardized root mean Square Residual (SRMR) = 0.04. Arrow widths are proportional to the standardized path coefficients presented. The R^2 next to each response variable indicates the proportion of variance explained. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

3.2.3.3 Biotic interactions mediate abiotic effects

Total abiotic and biotic net effects (sum of all direct and indirect effects of all environmental variables and of all biotic interactions on each group, respectively) help disclosure the importance of the bulk of all biotic interactions on bryophytes, lichens and vascular plants abundance, in comparison to net abiotic effects (Table 3.2.2). For bryophytes, total biotic net effects explained 28% of bryophytes abundance patterns, less than half of the abiotic effects. For lichens, biotic net effects explained 43% of its abundance, similar to those of net abiotic drivers. For vascular plants, total biotic net effects were not calculated as the SEM does not contemplate any effect of bryophytes or lichens on this group.

Table 3.2.2: Total net effects of abiotic and biotic factors on the abundance of bryophytes, lichens and plants. Values represent the absolute standardized path coefficients (ranging from 0 to 1).

	Abiotic	**Biotic**
Bryophytes	0.66	0.28
Lichens	0.47	0.43
Plants	0.40	-

The important role of biotic interactions can also be seen by its part in mediating individual indirect effects of abiotic drivers. These not only exacerbated abiotic direct effects but also clarified the indirect abiotic effects that would have been unquantified otherwise (Table 3.2.3). The biotic interactions increased the positive effect of elevation on lichens abundance by 17%, while also mediating an indirect effect on bryophytes abundance. Similarly, these interactions also mediated the indirect abiotic effects of both elevation on bryophytes and slope and mean diurnal temperature range on lichens.

Table 3.2.3: Direct, indirect and total effects of elevation, slope and mean diurnal temperature range (BIO2) on bryophytes and lichens via biotic interactions with plants and bryophytes. Effects represent the absolute standardized path coefficients (ranging from 0 to 1). *p < 0.05; **p < 0.01; ***p < 0.001.

	Bryophytes			**Lichens**			**Plants**		
	Direct	Indirect	Total	Direct	Indirect	Total	Direct	Indirect	Total
Elevation	0	0.11**	0.11**	0.47***	0.17***	0.64***	-0.4***	0	-0.4***
Slope	-0.34***	0	-0.34***	0	0.04*	0.04*	0	0	0
BIO2	-0.45***	0	-0.45***	0	0.06*	0.06*	0	0	0

3.2.3.4 Incorporating biotic interactions to predict present and future spatial vegetation abundance patterns

Model XI was applied to estimate present and future (for 2100) vegetation abundance for the current ice-free areas of the South Shetlands (as no reliable bed elevation model is available for areas currently glaciated), and where the environmental conditions are within the range of the modelled climatic envelope (Fig. 3.2.3a and 3.2.3b).

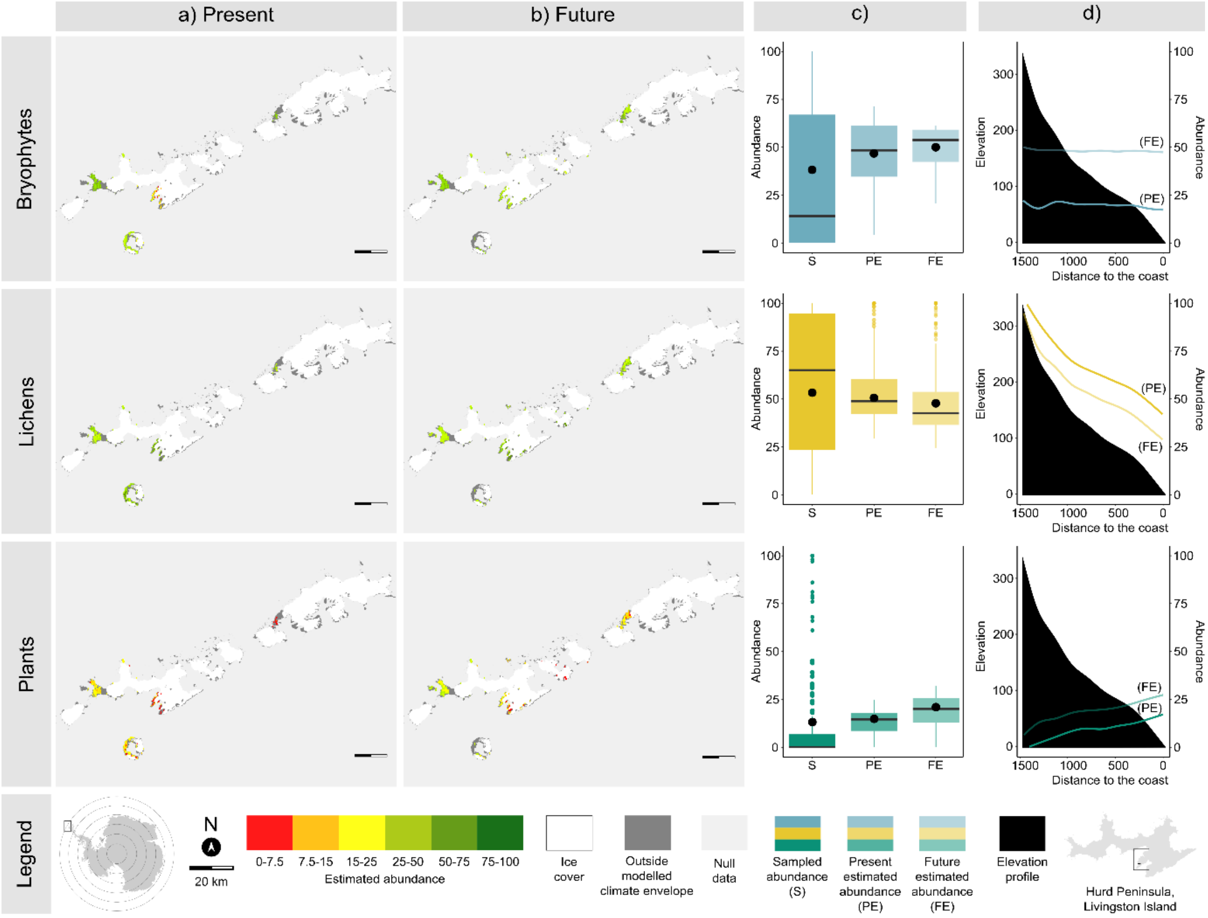

Figure 3.2.3: Estimated a) present and b) future vegetation abundance maps for the three species groups (bryophytes, lichens and plants) in the South Shetlands, maritime Antarctic. Abundance was estimated using the Model XI to model the response to abiotic drivers and integrating the biotic interactions described above. Future climate conditions are projected for 2100 under the most pessimistic CMIP6 emissions scenario (SSP5-8.5). Present and future vegetation abundance estimations are spatially limited to current ice-free areas of the archipelago and where the environmental conditions match those within the modelled climatic envelope (i.e., where values for abiotic variables used are withing the range used to build the model). White areas represent the current extent of glaciers in the Islands. Large sized, high resolution, versions of the maps are available in Appendix S2 (Fig. S3.2.6-8). c) Distribution of sampled and estimated present and future abundance values of the three species groups, considering only current ice-free areas. Boxes display first to third interquartile ranges, black lines the median, dots the average, and whiskers the maximum and minimum abundance values. d) Abundance changes along an elevation and coast inland spatial gradient, based on present and future estimated abundance values of the three species groups in Hurd Peninsula, Livingston Island, depicted here to illustrate the application of this methodology.

Overall, for both current and future (2100) conditions, the SEM estimated high abundance values for bryophytes on the western side of both Livingston Island (Byers Peninsula) and King George Island (Fields Peninsula), while for lichens, high abundance values are seen across the entire archipelago, particularly at higher elevations (Fig. 3.2.3a). Contrarily, vascular plants estimated abundance values were low across the entire archipelago. The average estimated abundance values, for present conditions, fell within the range of those sampled (Fig. 3.2.3c), particularly for bryophytes and vascular plants. When comparing the outcomes of models between present and future conditions, average abundance values were very similar (-6,3% for lichens, -2,9 for bryophytes and 0% for vascular plants). Thus, the predictions based on our models indicate only minor changes in abundance and spatial patterns of the three species groups (Fig. 3b). These spatial explicit models can also be used to zoom in specific areas. For illustration purposes, we depicted the variation of the three species groups across a elevation and coast inland transect, in Hurd Peninsula, Livingston Island (Fig. 3.2.3d). Plants decrease in abundance along the spatial gradient, until complete absence at higher elevations. This pattern is also observed when projecting for future environmental conditions. Lichens abundance increases along the gradient, although showing an overall decline when comparing present and future conditions. Finally, bryophytes do not exhibit such a clear shift along the elevation gradient but do show a clear mean increase in predicted future abundance when compared to present conditions, particularly at lower elevations.

3.2.4 Discussion

Results supported our initial hypothesis that biotic interactions must be included to better estimate present abundance patterns and to improve future predictions of maritime Antarctic vegetation spatial distribution. According to our model, the presence of vascular plants negatively impacts bryophyte and lichen communities. Similarly, bryophytes also negatively impact lichens. Furthermore, while abiotic factors are the key drivers for bryophyte and vascular plant communities, such is not the case for lichens, as biotic and abiotic factors contributed equally to their abundance. The best structural equation model (highest fitting and ecological support) was used to, for the first time, map current and future spatial vegetation patterns across most of the South Shetlands Archipelago. Even if limited by available background information of orography and ice-cover extent, it is possible to infer that, under climate change, vegetation will be promoted and expand. This is mostly based on the observation that new ice-free areas are likely to have, in the future, climate conditions that allow colonization by bryophytes and lichens (Fig. 3.2.3a/b).

Our results revealed that the incorporation of biotic interactions resulted in better-fitting SEMs compared to the one without interactions. All SEMs except one performed better than the SEM without biotic interactions (Appendix S2, Table S3.2.6), thus confirming, with empirical data, that biotic interactions help shape the distribution of each species group. Although challenging, inclusion of biotic interactions is key to improve distribution models under climate change scenarios (Araújo & Luoto, 2007; Mod, Le Roux, Guisan, & Luoto, 2015). This is now confirmed also for maritime Antarctica, and follows other recent studies for continental Antarctica, where biotic effects were identified as just as important (Caruso et al., 2019; Lee et al., 2019; Potts et al., 2020), if not prevalent (Caruso et al., 2013), drivers of several terrestrial communities. Thus, the inclusion of biotic interactions was shown to be a crucial step towards a better comprehension of the spatial abundance patterns of maritime Antarctica vegetation.

According to the stress-gradient hypothesis, the importance of positive (i.e., facilitation) species interactions in plant communities increase with increasing abiotic stress (Bertness & Callaway, 1994). In fact, recent works in maritime Antarctica show support for this hypothesis, pointing to the existence of facilitation processes between bryophytes, lichens and vascular plants (Casanova-Katny & Cavieres, 2012; Molina-Montenegro et al., 2013). Nevertheless, recent developments propose that facilitation may collapse and switch to competition at the extremes of the stress gradient (Maestre, Callaway, Valladares, & Lortie, 2009; Michalet, Le Bagousse-Pinguet, Maalouf, & Lortie, 2014). In other high latitude regions, cases of competition, commensalism and mutualism were documented for the three species groups considered here (Breen & Lévesque, 2006; Löbel, Dengler, & Hobohm, 2006). The type and mechanisms of biotic interactions in structuring maritime Antarctica and similar terrestrial ecosystems thus remain poorly understood. For that reason, no a-priori structural biotic relationship between the three species groups was tried. Rather, we opted to test all possible interaction pathways, calculating 25 SEMs. From the ten SEMs that passed the goodness-of-fit cut-offs, we selected the model with the strongest ecological support (Model XI – Fig. 3.2.2). It is known that, when environmental conditions allow for their coexistence, vascular plants can have a competitive edge over bryophytes and lichens, and bryophytes over lichens (Bao, Jia, & Xu, 2022; Löbel et al., 2006). In fact, this negative effect of plants and bryophytes on lichens was visible in all significant pathways of the 24 SEMs with biotic interactions. In the Artic (Cornelissen et al., 2001; Elmendorf et al., 2012), under warmer temperatures, an increase in vascular plants abundance was accompanied by a decline in lichens. Similar declining trends were observed for bryophytes cover in the Antarctic Peninsula, in response to increased *C. quitensis* cover (Day, Ruhland, & Xiong, 2008), and in the Arctic for lichens, in response to increased bryophytes cover (Hudson & Henry, 2010). Additionally, a study conducted along elevation gradients within the Arctic circle found a similar trend in relation to these three species groups distribution, with vascular plants dominating just above the tree line and lichens and bryophytes peaking only at higher elevations, where plants competitive strength is lowest (Bruun et al., 2006). While contradicting the positive nursing effects seen in other studies (Casanova-Katny & Cavieres, 2012; Molina-Montenegro et al., 2013) in the same region, and not allowing to discriminate the underlaying mechanisms behind these interactions, our results clearly point to the existence of negative effects between the three species groups. Despite scarce to date, field and laboratory manipulative experiments may help shed light on the underlaying mechanisms that determine the hierarchical relationships between these three species groups in maritime Antarctica (Hogg et al., 2006; Robinson, Wasley, & Tobin, 2003). This would be an important step to analyse structural relationships in maritime Antarctic ecosystems and to disclose the validity of the stress-gradient hypothesis in regions positioned at the extremes of the stress gradient.

Our model also revealed the prominence of abiotic drivers for bryophytes and vascular plants communities ((Fig. 3.2.2 & Table 3.2.2). For both species groups, regional climate (temperature seasonality) and altitude, a proxy of local climate conditions, namely temperature, explained the biggest portion of the spatial abundance patterns variance, with neither mean or seasonal temperature and precipitation variables being selected to the final SEM. Other temperature and precipitation-related variables could have been selected, although with less statistical significance. Thus, we can conclude that temperature, driven by regional and terrain factors, play a role influencing maritime Antarctica vegetation directly and indirectly. This supports the view that abiotic factors, in low-diversity ecosystems like the Antarctic region, are in fact the most important ecological filter (Convey, 1996; Convey et al., 2014; Schmitz et al., 2020), at least for bryophytes and vascular plants. Remarkably, that was not the case for lichens, for which our model revealed both biotic and abiotic factors share similar importance in determining their abundance. Numerous biotic factors driving lichen communities are documented (Lawrey, 1991). At high latitudes however, the importance of these interactions in shaping lichen communities is still poorly understood (Hogg et al., 2006). In a study conducted in the Antarctic Peninsula (Lee et al., 2019), biotic interactions importance for lichens, bundled together with other multicellular taxa, was exceeded by the importance of abiotic factors. Similar results were found in an arctic–alpine landscape (Mod, Heikkinen, Le Roux, Väre, & Luoto, 2016), where cover of three vascular plants were used as biotic predictors and amounted for less than the abiotic predictors. Our results show that for lichens, as an independent group in this region of Antarctica, the opposite is seen. We consider two potential reasons for this difference: 1) Model XI revealed negative effects of both plant and bryophyte on lichens (weighing biotic interactions importance for lichens abundance); 2) as stated before, lichens can be outcompeted by the two remaining species groups, potentially due to shading, physical displacement, between other factors. In fact, our data showed that in plant or bryophyte-dominated communities, lichens decline in abundance (Appendix S2, Fig. S3.2.5). Future studies should further investigate this pattern in other high latitude regions (i.e., sub-Artic, Artic and continental Antarctica), enlarging the climate gradient.

The addition of biotic interactions in the chosen model also helped unravel potential indirect impacts from environmental drivers not affecting the vegetation group directly, but rather by a chain of effects through other species group. Although unexplored, some studies have already pointed to the important role of these indirect effects of abiotic drivers mediated by biotic interactions (Engelhardt, Neuschulz, & Hof, 2020; Lee et al., 2019), which can potentially enhance or downplay climate change effects on maritime Antarctic vegetation. Studies conducted in maritime Antarctica (Cannone et al., 2022; Torres-Mellado, Jaña, & Casanova-Katny, 2011) suggest that plants are promoted under a climate change scenario, which according to our results will indirectly and negatively impact bryophytes and lichens communities.

Antarctica holds some of the most simplified ecosystems on Earth. For that reason, even small variations in the abundance and/or distribution of the three major species groups, due to changes in climate conditions, may imply a disproportionate increase in the biotic complexity of these systems (Cuba-Diaz, Fuentes-Lillo, Navarrete-Campos, & Chwedorzewska, 2023). Colonization by new species (Duffy et al., 2017; Duffy & Lee, 2019) is also likely to occur under changing climate conditions. As every added species can bring in a new set of interactions, this will most likely imply a shift from a primarily abiotic driven system to one with increased biotic complexity (e.g., novel, altered, or lost intra or inter-taxon interactions) with the potential to disrupt current biological composition and ecosystem structure (Cuba-Diaz et al., 2023; Lee et al., 2022). Together with future climate change effects, an increased species pool may disrupt vegetation spatial patterns and consequently the current interspecific dynamics, both in direction and intensity, established between species groups. As climate change effects are mediated by biotic interactions (Blois, Zarnetske, Fitzpatrick, & Finnegan, 2013), any changes in the biotic complexity of maritime Antarctic ecosystems will lead to changes in the way climate change will impact maritime Antarctic ecosystems, either through positive or negative feedbacks, potentially leading to greater changes in the spatial patterns of the vegetation than expected if climatic drivers would be considered alone. Furthermore, shifts in more dominant community groups are expected to drive changes in ecosystem functions and services (Lang et al., 2012; Robinson et al., 2018; Smith et al., 2020). As these three species groups dominate across terrestrial maritime Antarctic, biotic interactions will not only be key drivers of the vegetation spatial abundance patterns but also key drivers of changes to the ecosystem functioning itself.

Predicting future vegetation patterns is a crucial step in planning and designing more efficient conservation strategies (Abbott & Benninghoff, 1990; Grant, Convey, Hughes, Phillips, & Trathan, 2012). Model XI was thus used to build spatial explicit estimations of vegetation abundance patterns, for present and under future climate change (Fig. (Fig. 3.2.3a and b). Yet, the low spatial resolution of the environmental variables used, together with the uncertainty associated to future projections, demand a cautious interpretation. As such, this exercise was meant to explore its potential as an Antarctic conservation planning tool and not to produce de-facto maps for maritime Antarctic vegetation. Overall, the estimated distribution patterns (Fig. 3a), for current conditions, were similar to known patterns from past field surveys (Lindsay, 1971; Sancho, Schulz, Schroeter, & Kappen, 1999; Vera, 2011; Williams et al., 2017). This corroborates the greater potential to achieve more reliable spatial models when biotic and abiotic effects are considered together. Incorporating biotic and abiotic effects is thus an important step towards producing more accurate maps under future climate change scenarios, which so far have been almost exclusively focused on the dominant role of climate change (Araújo & Luoto, 2007; Hogg et al., 2006).

Although the constrains of environmental data seemed to have not limited the quality of the estimations for current conditions, that was not the case for the predictions of future vegetation patterns. In the last decades, an expansion trend in the spatial distribution of the South Shetlands terrestrial vegetation has been observed in response to ameliorated environmental conditions and increasing ice-free areas (Cannone et al., 2022; Torres-Mellado et al., 2011). However, such spatial trend is not observable in our estimations for 2100 (Fig. 3.2.3b), as the present ice-free extension was maintained due to the low reliability of future deglaciation predictions and elevation values (and all remaining terrain variables calculated from it) were maintained due to unreliable future bed elevation models. Furthermore, the mean diurnal temperature range is only predicted to slightly change across the Archipelago. Altogether, the uncertainty in relation to future ice-free areas and the lack of variation in the environmental variables included in model XI hinders future predictions of the three species groups. It is thus clear that, for improved future estimations, more accurate and reliable predictions of future glacier loss and consequent bed terrain models are required (Pattyn & Morlighem, 2020). Furthermore, better quality environmental data is also necessary for future predictions, as the incorporation of micro- climate and -terrain data is key to improve predictive spatial models (Lembrechts, Nijs, & Lenoir, 2019; Matos et al., 2024; Pradervand, Dubuis, Pellissier, Guisan, & Randin, 2013). The empirical data collected here reenforces this need, as clear abundance shifts in the three species groups were seen even across short spatial gradients (Appendix S2, Fig. S3.2.3). These shifts cannot be accurately explained with existing environmental datasets, which are only provided in coarser resolution. Future efforts should thus target the creation of enhanced spatial resolution environmental data that can help disclose small-scale climatic change and terrain features (e.g. areas of water accumulation in terrain depressions) leading, for example, to hotspots for bryophytes establishment at higher elevations (Williams et al., 2017). Such bryophytes hotspot can be seen in Figure 3.2.3d, where the declined trend of bryophytes with elevation was inverted due to the characteristic flat-topped hill of Reina Sofia Mountain. Despite these limitations, predictive spatial models are a fundamental tool to better understand maritime Antarctic ecosystems response to climate change impacts. Thus, it's urgent to promote bridges between remote sensing disciplines and field-based measurements to improve environmental management conservation efforts in the region (Kyker-Snowman et al., 2021; Zellweger, De Frenne, Lenoir, Rocchini, & Coomes, 2019).

In conclusion, we found that adding biotic interactions was a vital step to explain the spatial abundance patterns of these three main species groups of maritime Antarctic terrestrial vegetation. This was particularly evident for lichens, for whom biotic and abiotic effects had the same importance. Incorporating biotic interactions allowed to produce spatial explicit estimations for the current abundance of the three species groups, under present environmental conditions, that closely resemble those observed in the field. These predictive spatial models incorporating biotic interactions, if accompanied by improved environmental data spatial resolution, will boost our understanding of future climate change.

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Chapter 4

4. SPACE-FOR-TIME VALIDATION

Chapter 4

4. Challenging the space-for-time approach: evaluating its potential and limitations for climate change studies

Rocha, B., Pinho, P., Giordani, P., Serrano, H., Branquinho, C., Matos, P. (2025) Challenging the space-for-time approach: evaluating its potential and limitations for climate change studies. *In preparation*

Abstract

Epiphytic lichens are increasingly used as ecological indicators of climate change effects. As long-term lichen monitoring datasets are still scarce, most studies rely on the space-for-time substitution method, which assumes spatial biodiversity-climate relationships can predict responses over time. However, this assumption has not yet been fully tested. The aim of this study was to test the validity of the space-for-time assumption in the context of lichen diversity response to climate. Specifically, we evaluated how well predictions based on spatial models matched observed temporal changes in lichen functional metrics, both in their direction and in their magnitude. For that, we combined a large spatial lichen diversity dataset across the Iberian Peninsula and a temporal subset of seventeen years, collected at five-year intervals (2005, 2015 and 2022). We modelled the relationships between climate (several temperature, precipitation and relative humidity variables) and several trait-based metrics related to growth form and main photobiont type and then tested whether the resulting spatial models could accurately predict observed values in three years (2005, 2015, 2022), explicitly quantifying predictive bias and the direction and magnitude of change. Results showed that predictive performance varied with functional group and time period. A strong systematic bias was found for 2005, but it mostly disappeared by 2022, consistent with the existence of an ecological time lag in lichen functional response to climate change. This bias was also trait dependent, with photobionts-based metrics more closely matching observed changes while growth forms metrics were systematically overpredicted. Direction and magnitude of change was well captured for most functional groups, with squamulose and *Trentepohlia* models improving over time, while *Chlorococcoid* models became increasingly inconsistent across concurrent models, often showing overprediction photobiont in later years. These findings thus support a conditional validation of the space-for-time assumption. Spatial models captured major aspects of temporal change, particularly once time lags diminished, supporting their use in predicting climate-driven biodiversity responses. Yet, model reliability proved trait dependent: photobiont-based metrics were more robust, while growth form metrics were prone to overprediction. These results highlight both the potential and the limits of space-for-time approaches, emphasizing the need for trait-based perspectives, multiple methodological approaches (including ensemble modelling), and long-term monitoring to improve prediction reliability under ongoing climate change.

Keywords

Space-for-time; Climate Change; Ecological indicator; Lichens; Trait-based metrics

4.1 Introduction

To understand and predict how biodiversity responds to a changing climate would ideally be anchored in long-term observations (Damgaard, 2019; Estes et al., 2018). However, these long-term datasets are scarce (Lindenmayer, 2018) and despite current data collection efforts (Dornelas et al., 2018; Smith et al., 2023), we cannot afford to wait for their availability. To overcome this limitation, ecological and environmental assessment studies commonly opt to apply a space-for-time substitution method (Blois, Williams, Fitzpatrick, Jackson, & Ferrier, 2013; Pickett, 1989), based on the assumption that the spatial variations in biodiversity observed in response to climate, mimic those that will happen over time under the same climatic conditions (e.g. space-for-time equivalence). This method allows us to model both past and future biodiversity patterns based on present data alone (Blois et al., 2013; Fukami & Wardle, 2005). Despite being widely applied, studies where space-for-time is validated are far less common, challenging the confidence on the resulting predictions (Evans et al., 2025; Damgaard, 2019; Lovell, Collins, Martin, Pigot, & Phillimore, 2023). In fact, several space-for-time studies have found that, despite similar in direction, climate change impacts are often mismatched in magnitude due to a temporal biotic lag (Adler & Levine, 2007; Elmendorf et al., 2015; Sandel, 2019).

Epiphytic lichen diversity has proved to be an excellent ecological indicator of climate, with multiple studies identifying consistent functional structure metrics responses to particular climate variables (Hurtado et al., 2020; Matos et al., 2015). However, the scarcity of long-term datasets of lichen diversity means that only a handful of studies have used long-term lichen diversity changes to track climate change effects over time (Esseen, Rytterstam, Atrena, & Jonsson, 2023; Mallen-Cooper et al., 2023; van Herk, Aptroot, & van Dobben, 2002; Warren et al., 2019). Thus, most studies contributing to the development of lichens as ecological indicators of climate change have relied on the space-for-time substitution method, i.e., sampling lichen diversity over large spatial gradients to mimic potential temporal changes in climate (Bässler et al., 2016; Giordani, Brunialti, Bacaro, & Nascimbene, 2012; Matos et al., 2015; Trobajo, Fernández-Salegui, Terrón, & Martínez, 2022). Given that lichen diversity has the potential of working as a global ecological indicator to track the effects of climate change, it is crucial that the space-for-time assumption is tested, so that we can better interpret these spatial model results.

The aim of this study was to test the validity of the space-for-time assumption in the context of lichen diversity response to climate. In particular, we are interested in understanding how well predictions based on spatial models match the observed temporal trends in lichen diversity. For that, and as the space-for-time substitution method assumes that biodiversity-climate relationships observed in space mimic those over time, a two-step process is required for validation: first to model how climate is driving changes on lichen diversity metrics across space; secondly, to analyse whether predictions resulting from these models accurately match observed changes over time. Our main hypothesis is that predicted trait-based metrics values, based on the spatial models, will match those observed over time, both in direction and magnitude.

4.2 Material and Methods

4.2.1 Study area and sampling design

The study took place in the Iberian Peninsula, Southwest Europe. The spatial models of lichen diversity were built following an aridity gradient from semi-arid and to dry-subhumid climate. Climate conditions in the region range from a mean annual temperature of 7.38 ºC and mean annual precipitation of 382 mm to a mean annual temperature of 23.11 ºC and mean annual precipitation of 606 mm (mean values relative to 1980 – 2018, calculated with data from Chelsa (Karger et al., 2017). Epiphytic lichen diversity was sampled between 2010 and 2012 in 54 sampling sites across the spatial gradient of the entire study area (Fig. 4.1a; Fig. S4.1). Sampling sites selection aimed at encompassing a broad spatial climate gradient and sampled trees were always holm oak trees (*Quercus ilex* L.), to ensure that phorophyte characteristics were equal across sites. A subset of 21 sampling sites, located in the southeast of Portugal, were repeatedly sampled over the course of 17 years (Fig. 4.1b; Fig. S4.1), at an approximately five-year interval (2005, 2010, 2015 and 2022). This is one of the longest monitoring efforts, focused on epiphytic lichen diversity, that we are aware of in Europe.

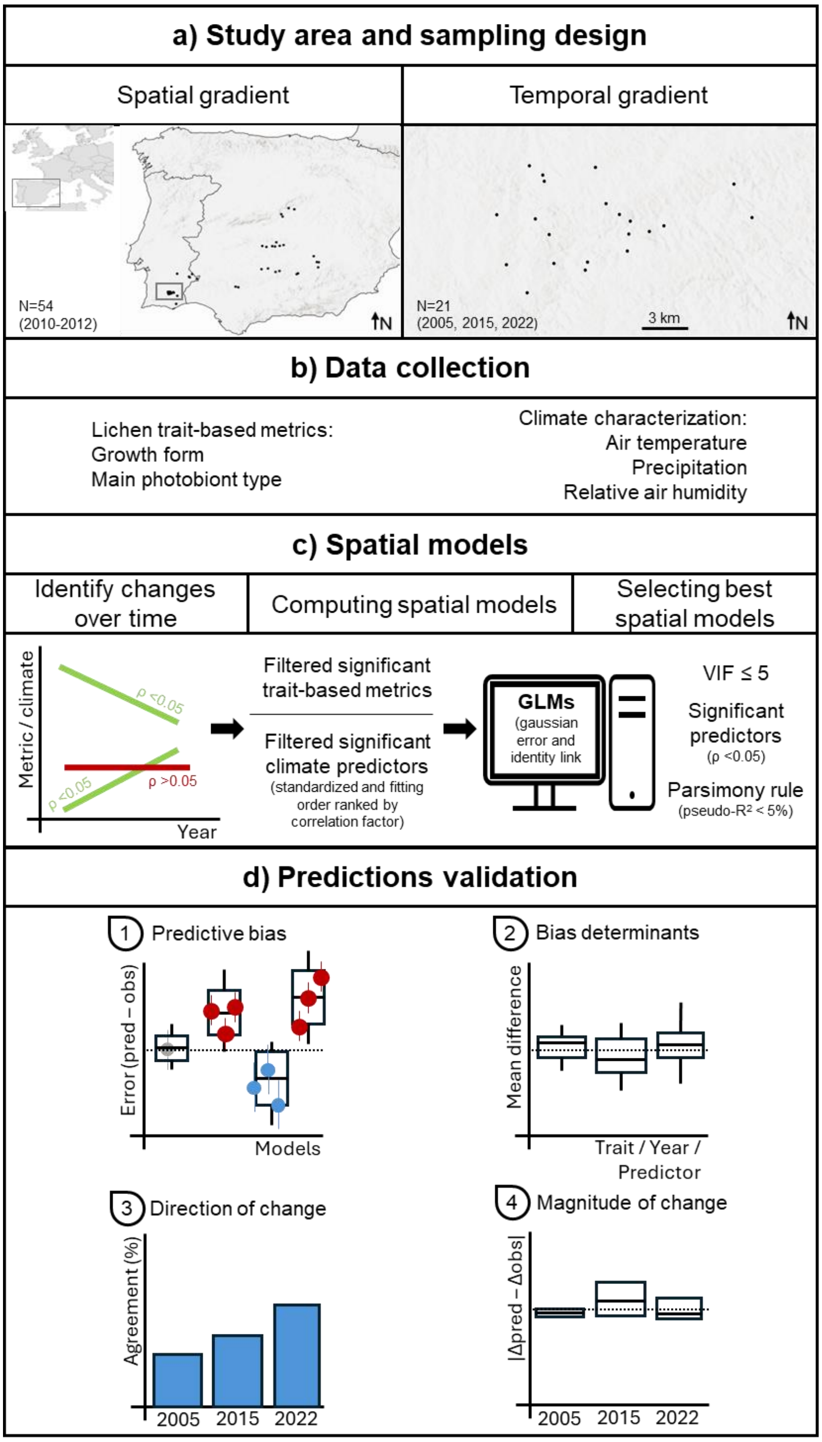

Figure 4.1: Methodological workflow for space-for-time validation.

4.2.2 Field sampling

For all sites, epiphytic lichen diversity was sampled following the European standard method (Asta et al., 2002). A minimum of one and a maximum of ten trees closest to the centroid of the sampling site were sampled following the standard method criteria. A sampling grid (50 cm x 10 cm, divided into five squares) was placed on the four main aspects of the tree trunk (North, South, East, West), and all epiphytic lichens present inside the grid were registered. Species abundance, defined as the mean frequency value of each species in all sampled trees, per sampling site, was determined based on the number of squares in which they occurred (maximum value of 20). A total of 186 species were identified (Table S4.1).

4.2.3 Lichen trait-based functional structure metrics

The vast majority of lichen diversity studies conducted so far using the space-for-time method have shown that trait-based metrics are the most responsive to climate (Di Nuzzo et al., 2021; Matos et al., 2015; Phinney, Ellis, & Asplund, 2021). We thus selected two traits already identified as responsive to climate (Marini, Nascimbene, & Nimis, 2011; Matos et al., 2015): growth form and main photobiont type (Fig. 4.1b). In total, eight metrics related to the community weighted mean (CWM) were calculated using these two traits. The CWM characterizes the communities' functional structure and represents the mean trait value in the community weighted by the abundance, i.e., the proportion of all species abundances belonging to the same functional group, within each trait. For the growth form trait, we focused on five functional groups: crustose, foliose broad lobed, foliose narrow lobed, fruticose and squamulose. For the main photobiont trait, we focused on three functional groups: *Chlorococcoid* (Green algae), cyanolichens (cyanobacteria) and *Trentepohlia* (Green algae). The CWM of each functional group was calculated with the function *dbFD* from the *FD* package (Laliberté, Legendre, & Shipley, 2014), R software (R Core Team, 2022). Trait information for each species was retrieved from the ITALIC database (Nimis, 2024) and can be seen in Table S4.1.

4.2.4 Bioclimate variables

Climate reporting to monthly average, maximum and minimum air temperature and total precipitation values were retrieved from the Chelsa timeseries database (Karger et al., 2017) at the maximum available spatial resolution of 1 km. These were extracted for each sampling site and used to calculate the 19 bioclimate variables for each year between 1989 and 2018, following the formulas in O'Donnell and Ignizio (2012) using the *stats* package of R software (R Core Team, 2022). Despite not covering the full lichen diversity temporal dataset (2005-2022), this is the broadest covering climate characterization publicly available for the study area, and for that reason the one selected for this work. As relative air humidity is also a known source of lichens hydration (Gauslaa, 2014), thus monthly average relative humidity values were also extracted for the same time period and used to calculate the equivalent of the temperature related bioclimate variables (BIO1 to BIO11), with the exception of the mean diurnal range (BIO2) and isothermality (BIO3), as these require maximum and minimum monthly relative humidity values, which are not available. A total of 28 bioclimate variables (Fig. 4.1b, Table S4.2) were calculated for each sampling site, characterizing precipitation, temperature and relative humidity annual, range and seasonal variation across the study area for the period of 1989 to 2018. We focused on bioclimate variables as these are ecologically meaningful and frequently used in ecology studies (Title & Bemmels, 2018) for better describing often overlooked seasonal climate variations affecting vegetation performance.

4.2.5 Data analysis

Validating the assumption that lichen diversity-climate relationships in space and time are equivalent requires first identifying which are the strongest relationships across space, to then confirm if the predictions based on those spatial models are similar to those observed over time. Though these two steps should ideally be followed in this order, but the large number of lichen diversity metrics and the even greater number of climate variables would result in a large number of spatial models. Thus, for clarity and simplification, and to reduce the number of spatial models presented, data analysis and results were done in the reverse order. First, we identified which lichen CWM metrics and climate variables changed significantly over time and used only those to build the spatial models. This step ensures that we validate only models that capture genuine temporal change.

4.2.5.1 Identifying climate variables and functional metrics changing over time

We started by identifying which climate variables and lichen CWM metrics changed over time (Fig. 4.1c). To test temporal trends in lichen CWM metrics, we modelled each functional group CWM as a function of the sampling years (2005, 2010, 2015, 2022) using Generalized Linear Models (GLMs) with Gaussian error and identity link. Although the CWM values range between 0 and 1, GLMs with a Gaussian assumption are robust to moderate deviations from normality given our sample size ($n = 21$). Subsequent models diagnostics (residuals vs. fitted plots) indicated that the residuals were symmetrically distributed around zero, with no evidence of heteroscedasticity or major differences from normality (Fig. S4.2). Models' goodness-of-fit was evaluated using the McFadden's pseudo-R^2 formula [$R^2McF = 1 - (Deviance/Null\ Deviance)$], where the deviance is a measure of the unexplained variance after fitting the model and the null deviance a measure of the deviance of the model with only an intercept (Allison, 2001). Models were considered significant if $p < 0.05$.

To account for the climatic conditions most likely influencing lichen diversity at the time of sampling, we summarized climate variables using 4-year moving averages over the last 30 years of existing data (1989-2018). We opted for a 30-year period as it is considered the standard minimum to define climate normals (i.e. long-term averages of climate patterns) and moving averages of four years to reduce interannual noise and thus potential model overfitting. Furthermore, as field sampling was conducted early in the year, we excluded the climate data of the sampling year itself and calculated each year average over the four preceding years (t–1 to t–4). This approach captures both the conditions experienced prior to sampling and potential legacy effects of climate on community composition, while avoiding the inclusion of incomplete or ongoing climatic influences from the sampling year itself. To test for significant changes in climate temporal trends, we modelled each climate variable as a function of the year (the 30 available years, 1989-2018) using also Generalized Linear Models (GLMs) with Gaussian error and identity link. Subsequent models' diagnostics (residuals vs. fitted plots) again indicated that the residuals were symmetrically distributed around zero, with no evidence of heteroscedasticity or major differences from normality (Fig. S4.3). We retained all CWM functional metrics and climate variables that significantly changed over time ($p < 0.05$) for further analysis. All models were run with the *glm* function of *stats* package in R software (R Core Team, 2022).

4.2.5.2 Selecting the best spatial models

Afterwards, considering only lichen CWM metrics and climate variables that changed significantly over time, we built spatial models to predict each functional group CWM based on climate (Fig. 4.1c). As the field sampling conducted along the spatial gradient was performed between 2010 and early 2012, we calculated a moving window for all climate variables encompassing the four years prior (2008-2011). As many climate variables were colinear, prior to diversity-climate models construction, climate variables were ranked by their absolute Spearman correlation with the response variable. Models were fitted using also GLMs with Gaussian errors and identity link. To allow comparison of effect sizes across predictors measured in different units, all climate variables were standardized (z-scored) prior to modelling. We followed a thorough approach, starting by modelling each CWM metric with all combinations of just one climate predictor, respecting the absolute Spearman correlation ranking. We followed by adding a second and third climate predictor to each model, again respecting the established ranking and keeping only models with no colinear predictors. Collinearity between predictors was assessed using the variance inflation factor (VIF), and only models with VIF ≤ 5 were retained. For each spatial model, we recorded the McFadden's pseudo-R^2, intercept and the standardized coefficients (± standard errors and p-values) of all climate predictors. To ensure only biologically interpretable models were retained, we applied a multi-step selection procedure. We started by only retaining models in which all included predictors were statistically significant ($p < 0.05$). Afterwards, a parsimony rule was applied: for each response variable, we identified the maximum McFadden's pseudo-R^2 across all model sizes (number of fitted climate predictors, $k = 1$ to 3) and then selected the smallest number of predictors (k) for which the best model pseudo-R^2 value was within 5% of this global maximum. Finally, all competing models within the chosen model size (k) whose pseudo-R^2 was within 5% of the best models for that k were retained. This procedure ensured that (i) all selected climate predictors have robust statistical support, (ii) parsimony is respected when model fit does not improve substantially with additional climate predictors, and (iii) model uncertainty is captured by retaining a set of equally supported models rather than a single best model. This modelling framework generated a set of candidate models (up to three climate predictors) for each response variable. Each fitted model fulfilling the above criteria was assigned a unique identifier and retained, allowing subsequent use for predictions against observed temporal datasets. Spearman correlations between climate variables were determined with the *cor.test* function of the *stats* package, all models were run with the *glm* function of *stats* package, the VIF was retrieved with the *vif* function of the *car* package, R software (R Core Team, 2022).

4.2.5.3 Evaluating predictive bias and direction agreement in metric values

To assess the predictive ability of these spatial models, we compared the predicted CWM values against independent temporal observations for three different years (2005, 2015, 2022). The space-for-time validation was carried out using a set of complementary statistical parameters that jointly evaluated models predictive bias, agreement in direction of change and magnitude of change (Fig. 4.1d). First, for each site and year, we computed models bias as the difference between predicted and observed CWM values for each functional group and model. To formally test for the existence of a significant prediction bias, we applied model-level two-way paired T-tests comparing predicted and observed CWM values for each year. From these T-tests we extracted the T-statistic, representing the standardized effect size relative to the variability; Mean difference, representing the mean difference between predicted and observed values (Md = Predicted-Observed), where positive values indicate overestimation by the models while negative values indicate underestimation, 95% confidence intervals, representing the range of plausible true average differences between predicted and observed values, p-value, representing the significance of the test. Afterwards, a Kruskal-Wallis test was used to determine if the metrics trait (growth form vs main photobiont), type of climate predictors included in the spatial models and year (here treated as a factor) of prediction had a statistically significant effect on models' predictive bias. Spearman's rank correlations were also performed to evaluate whether a predictions bias was significantly associated with the spatial models' McFadden's pseudo-R^2. Mean observed CWM values, per functional group and year, are also presented to allow comparison with the absolute bias value. Agreement in direction of change was assessed by first assuming that models without significant prediction bias between observed and predicted values matched direction. For models where a significant bias was detected, we then explicitly compared the direction of observed versus predicted changes. The agreement in direction of change was then quantified as the proportion of models where observed (Δ observed) and predicted (Δ predicted) CWM changes share the same direction of change (increase or decrease given in practice by the difference signal, positive or negative), per year. Both Δ observed and Δ predicted values were determined as the difference between each observed value and its baseline (i.e., CWM value in 2012 used to build the spatial model). Finally, we evaluated the bias in the absolute magnitude of change by computing the difference between |Δ predicted| and |Δ observed| CWM values per year. Ideally the magnitude of change depicted in the predictions should be similar to the magnitude of change observed. This was again coupled with two-way paired T-tests to check for significant differences if magnitude of change between |Δ predicted| and |Δ observed| CWM values for each prediction year. All parameters were summarized graphically: mean functional groups CWM , boxplots for models' predictive bias and for the bias in the magnitude of change and bar plots to depict the proportion of agreement in direction of change, all per year. Together, these parameters and validation approaches provide a comprehensive assessment of model performance across responses and time periods. Predictions were performed using the *predict* function of the *stats* package, R software (R Core Team, 2022). Paired t-tests were run with the *t.test* function of the *stats* package, Spearman correlations with the *cor.test* function of the *stats* package, the Kruskal-

Wallis test was run with the *kruskal.test* function of the *stats* package, R software (R Core Team, 2022).

4.3 Results

4.3.1 Identifying climate variables and functional metrics changing over time

Four of the eight trait-based metrics changed significantly over time (Fig. S4.4; Table S4.3), namely the CWM of foliose broad lobed and squamulose growth forms and the *Chlorococcoid* and *Trentepohlia* main photobiont types. The CWM of crustose, foliose narrow lobed, fruticose and cyanolichens were excluded from further analysis. Twenty-four of the twenty-eight climate variables also changed significantly over the 30-year span (1989-2018) of available climate data (Fig. S4.5-7; Table S4.4). The remaining four were also excluded from further analysis.

4.3.2 Selecting the best spatial models

A total of 22 models (Fig. 4.2; Table S4.5-8) were selected representing a unique combination of climate variables between temperature, precipitation and relative humidity which best explain the four functional structure metrics variance over space. For the foliose broad lobed growth form, only one model was selected, encompassing two seasonal precipitation variables. For the squamulose growth form four models were selected, each with one climate predictor related to annual or seasonal temperature or precipitation variables. For the *Chlorococcoid* photobiont, thirteen models were selected and encompassed combinations of two climate predictors of annual and seasonal temperature, precipitation and relative humidity variables. Finally, for the *Trentepohlia* photobiont, a total of four models were selected, each with one climate predictor only, and encompassed three relative humidity and one temperature variable.

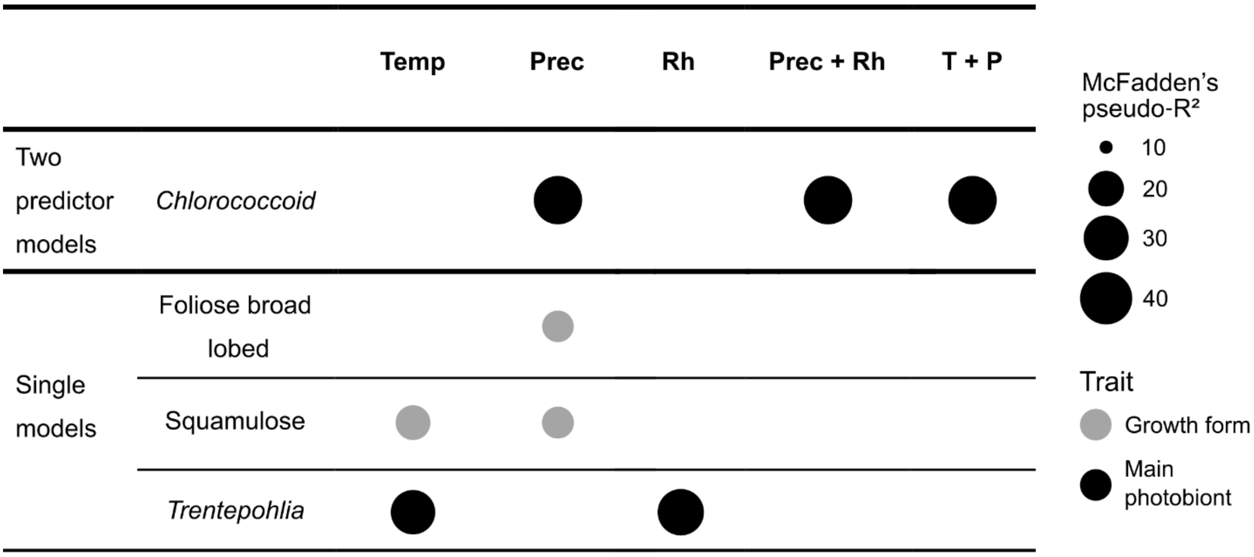

Figure 4.2: Type of climate variables selected as best predictors for each functional group spatial model. Models depicted by circles represent general linear regression, with color associated with the type of trait (growth form and main photobiont type). Size of the circles indicates the model's McFadden's pseudo-R² value. Climate predictors labels read as follow: Temp – Air temperature, Prec – Precipitation, RH – Relative humidity, Prec + Rh – Precipitation + Relative humidity, T + P – Temperature + Precipitation.

4.3.3 Evaluating the accuracy and bias of predicted metric values

The 22 spatial models' predictive ability was evaluated for three different years (2005, 2015, 2022) to validate the space-for-time method. Overall, models' performance varied strongly between CWM metrics and across years (Fig. 4.3). In the 2005 hindcast (Table S4.9), all metrics exhibited significant bias (Error = Predicted - Observed). The foliose broad model and most *Chlorococcoid* models showed significant underestimation, while squamulose and *Trentepohlia* models showed significant overestimation. In the 2015 forecast (Table S4.10), bias became less consistent. Squamulose models still significantly overestimated predictions, but the foliose broad model shifted to non-significant bias. *Chlorococcoid* models were mostly non-significant, with only a few showing a slight significant bias (two underestimating, one overestimating). Forecasts for *Trentepohlia* were also mostly non-significant, with only one slight underestimating model. By the 2022 forecast (Table S4.11), bias was mostly non-significant across all metrics. Both over- and underestimation occurred, but spreads were small, and confidence intervals generally overlapped zero. The exception was *Chlorococcoid*, which worsened relative to 2015, with more models showing significant overestimation. Overall, the strong systematic bias observed in 2005 largely disappeared by 2022, while model-to-model variability around zero increased. We tested whether prediction bias (Error = Predicted - Observed) varied with year, trait or type of climate predictor. Trait significantly explained prediction bias (Kruskal–Wallis, p = 0.021). Bias was centred around zero for the photobiont trait models, while growth form models showed systematic overestimation (Fig. 4.4). Neither year nor predictor type explained variation in bias, and bias was not correlated with models McFadden’s pseudo-R^2.

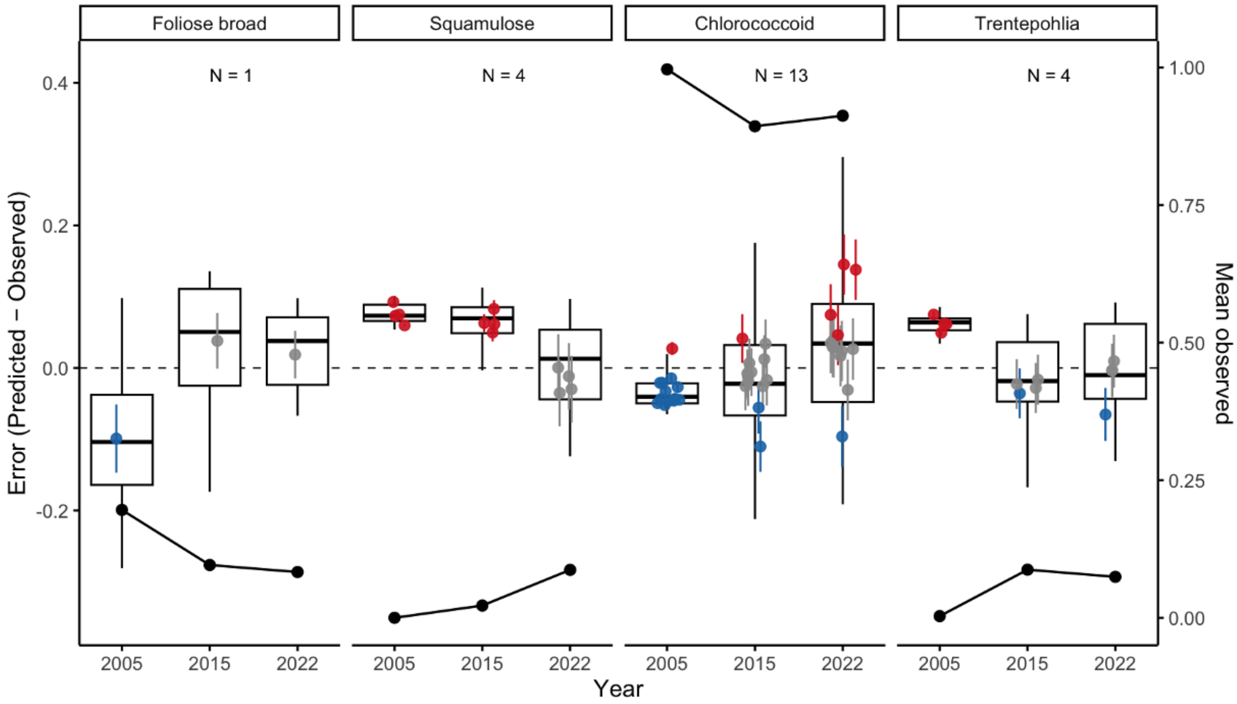

Figure 4.3: Boxplots show site-level bias (Error = Predicted - Observed) distributions by year and functional group. The box spans the interquartile range (Q1–Q3); the midline is the median; whiskers extend to the most extreme data within 1.5 × IQR (outliers, if any, are not shown). Overlaid coloured dots are model-level mean bias (mean Error), with vertical-coloured whiskers showing the 95% confidence intervals (CIs). Colours indicate bias significance and direction: red = significant overestimation (error > 0, $p < 0.05$); blue = significant underestimation (error < 0, $p < 0.05$); grey = non-significant ($p \geq 0.05$). Dots are horizontally jittered for legibility; their CIs are centred on the same jittered x-position. The dashed horizontal line at marks no bias (error = 0). The black line and points show the mean observed value per year (scaled to the right-hand y-axis). Text labels indicate the number of models per metric and year.

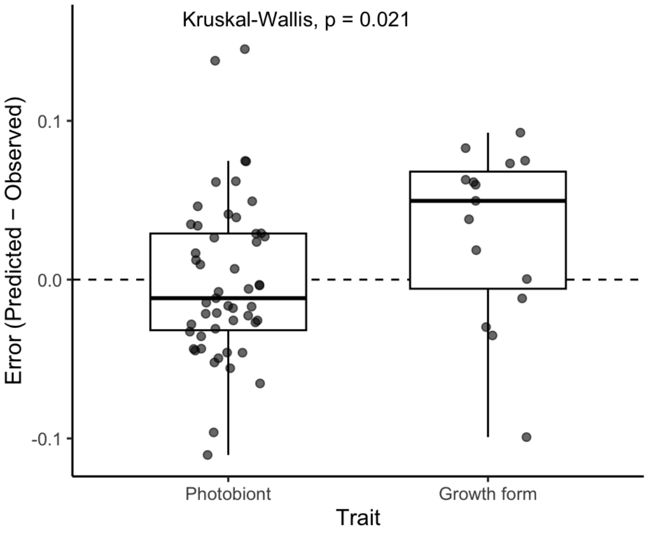

Figure 4.4: Effect of trait on the different functional group models prediction bias. Boxplots show the distribution of model bias (Error = Predicted − Observed) across models, grouped by trait. The thick horizontal line indicates the median, the box spans the interquartile range (IQR), and whiskers extend to 1.5× IQR. Points represent individual models' errors. The dashed line marks zero bias. A Kruskal–Wallis test detected significant differences among traits (p = 0.021): models based on photobiont traits were centred close to zero (lower bias), whereas models based on growth form traits showed systematic overestimation.

Agreement in direction of change between predicted and observed values also varied strongly among functional groups and years (Fig. 4.5a). In the 2005 hindcast, foliose broad model exhibited perfect agreement, whereas squamulose and *Trentepohlia* models showed poor agreement (<30%). *Chlorococcoid* models started with good agreement in 2005 and 2015 (>90%) but declined to ~70% in 2022. In contrast, squamulose and *Trentepohlia* models improved markedly over time, reaching ~75–100% agreement by 2022. In terms of magnitude of change, models performed overall well in the predictions of change (Fig. 4.5b), as magnitude bias was generally low and non-significant. The foliose broad model predicted significantly less change than observed in 2005, but it improved to non-significant magnitude bias in 2015 and 2022. *Trentepohlia* and squamulose models remained close to zero across years, indicating no significant differences between predicted and observed magnitude of change. In contrast, several *Chlorococcoid* models significantly overpredicted the magnitude of change (i.e., predicted changes bigger than observed changes) across the three years, though in most models, magnitude remained systematically unbiased (i.e., no significant differences from the observed change).

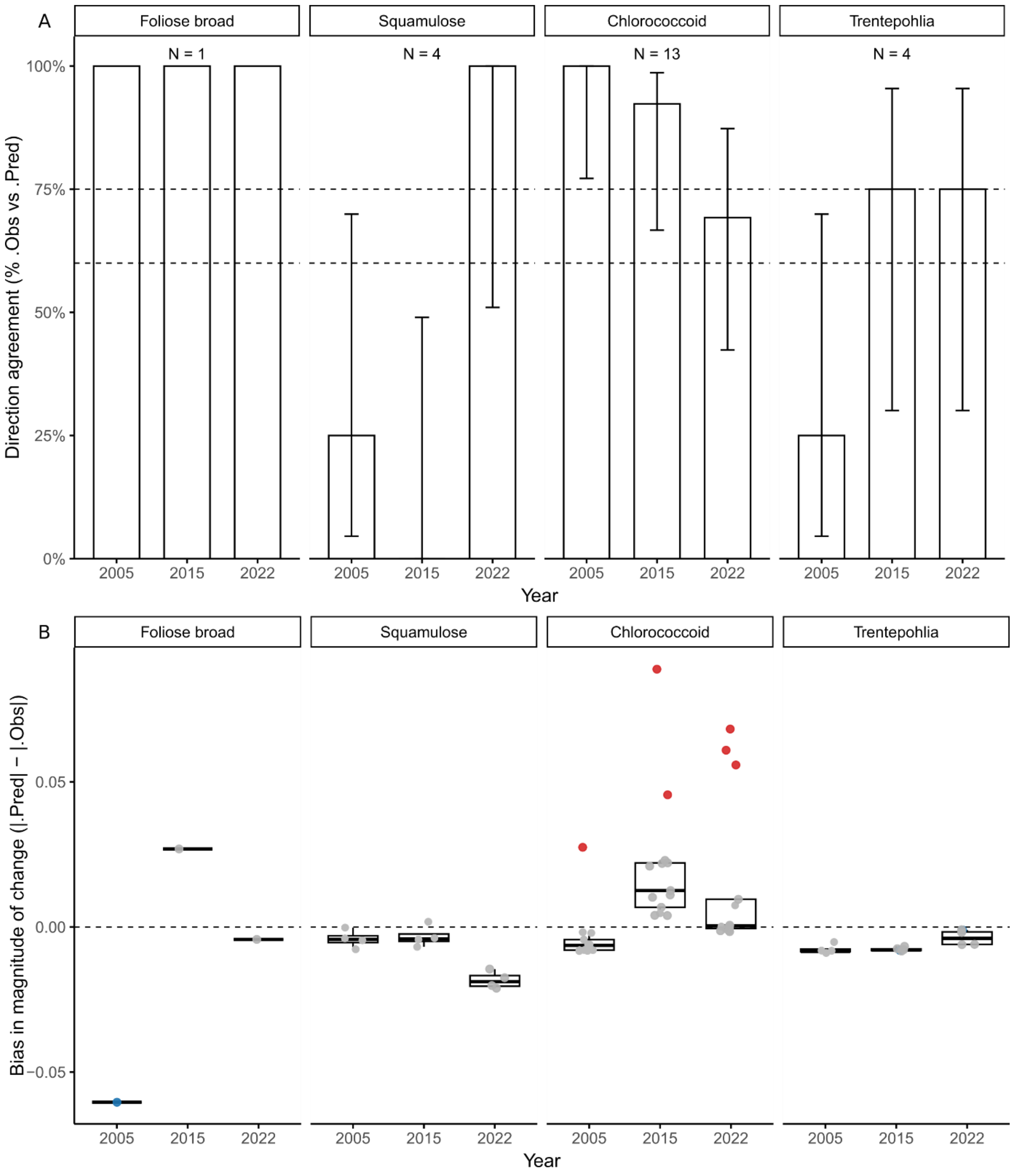

Figure 4.5: Directional agreement and bias in magnitude of change in predicted vs. observed changes (relative to the baseline). A: Boxplots show the proportion of models correctly capturing the direction of change (Δ Observed vs Δ Predictive) per functional group and year. Bars represent the proportion of agreement, with whiskers indicating 95% confidence intervals. Dashed lines mark 60% and 75% thresholds (<60% poor agreement; >=60% and <75% moderate agreement; >=75% good agreement). B: Boxplots show bias in the magnitude of change (|Δ Predicted| − |Δ Observed|) per functional group and year. The thick horizontal line indicates the median, boxes span the interquartile range, and whiskers extend to 1.5× IQR. Dots represent individual models, coloured by significance (paired t-test on differences between |Δ Predicted| and |Δ Observed|): grey = non-significant difference in magnitude of change; red = significant overprediction of change; blue = significant underprediction of change. A dashed line at zero marks perfect magnitude of change agreement.

4.4 Discussion

We hypothesized that predicted trait-based metrics values, based on the spatial models, would match those observed over time, both in their direction and magnitude of change. Our results provide partial support for this hypothesis. While spatial models often captured the magnitude of temporal change and, for several functional groups, the direction of change, they also revealed systematic biases among functional groups and years. These findings support the validity of the space-for-time assumption, a method which has been pivotal to develop an ecological indicator of climate based on lichen diversity in the absence of long-term monitoring datasets, but with caveats associated with its reliability being conditional on the type of trait, temporal horizon, and the statistical modelling approach taken.

The space-for-time method has been widely used in climate change ecology (Lovell et al., 2023), yet its validity remains debated (Damgaard, 2019; Svenning & Sandel, 2013). This work explored one of the longest available lichen diversity datasets in Europe to test the main assumption in space-for-time. A previous work with the same dataset had already identified that *Trentepohlia* responded to relative humidity in space and time (Matos et al., 2016) suggesting the potential for validation. However, this earlier analysis relied on different climate predictors for space, mean relative air humidity, and time, number of days with relative air humidity over 95%, limiting the strength of inference. In this approach we ensured the same set climate variables across both dimensions, compared predictions from spatially derived diversity–climate models with observed temporal changes to assess its accuracy and identify the underlying causes of mismatches.

4.4.1 Validity of the space-for-time assumption

Predictive accuracy improved over the years, from a strong systematic bias (i.e. predictions consistently under- or overpredicted the observed values) in the 2005 hindcast to mostly unbiased in the 2022 forecast. These results suggest that space-for-time models increasingly align with observed temporal trajectories, consistent with the idea of an ecological time lag (Gauslaa, 2014; Łubek, Kukwa, Jaroszewicz, & Czortek, 2021) resulting from a delay in communities' rearrangement in response to changing climate conditions. Similar time lags have been reported for other taxa, namely bryophytes and vascular plants, where predictions converged with observed changes but only after sufficient time had elapsed (Alexander et al., 2018; Bertrand et al., 2016; Block et al., 2022). Due to their slow growing-rate and slow colonization and recolonization rates (Armstrong & Bradwell, 2011), it is reasonable to infer that lichen functional responses to changing climate conditions can operate over longer time periods, creating an early spatial-temporal mismatch which gradually fades over the years as communities reassemble. Therefore, from the perspective of lichen diversity application as an ecological indicator of climate change, our results highlight the need to explicitly recognize temporal lags in space-for-time studies.

4.4.2 Bias in predictions

Models predictive bias was strongly trait dependent, with photobiont type showing lower bias than growth forms, which were systematically overpredicted. This aligns with previous studies that suggested that photobiont type mediates lichens physiological tolerance and climate sensitivity (Marini et al., 2011; Stanton, Ormond, Koch, & Colesie, 2023). Contrarily, several studies have shown that lichen growth forms are more intrinsically associated with other environmental drivers like air pollution (Hurtado et al., 2020; Rocha et al., 2022) and may also reflect responses more slowly due to dispersal and establishment constraints (Johansson et al., 2006; Ronnås et al., 2017; Werth et al., 2006). On the other hand, no association was found between models' predictive bias and the type of climate predictor. This seems to reinforce the notion that bias was less associated with the type of climate variable used, and more about the biological differences between the functional groups. The overestimation of squamulose and *Trentepohlia* CWM values in the hindcast likely reflects this dynamic: their observed values were close to zero in 2005, so spatial models were inferring suitability before colonization had occurred. In other words, spatial models were not incorrectly identifying favourable climate conditions for those functional groups, but rather anticipating a colonization event that only became observable later on. Altogether, these results provide direct evidence for a colonization lag where realized distributions trail behind potential climate-driven niches (Bertrand et al., 2011; Jackson & Sax, 2010). Conversely, the underestimation of *Chlorococcoid* CWM values in later years may reflect stabilizing processes, such as competitive exclusion or microhabitat limitations that constrained its realized distribution despite a predicted climatic suitability (Araújo & Luoto, 2007; Cosentino, Seamark, Van Cakenberghe, & Maiorano, 2023; Van Der Putten, Macel, & Visser, 2010).

4.4.3 Agreement in direction and magnitude of change

Results also highlighted the importance of distinguishing between predicting the direction of change in space-for-time validation studies. While the direction of change was well captured for most functional groups (e.g. consistently for foliose broad lobed, increasingly better along the years for squamulose and *Trentepohlia*), it declined for *Chlorococcoid* lichens, suggesting that their realized population dynamics diverged from the spatially inferred trend. Similarly, the magnitude of change was also well captured for most functional groups, aligning with findings from other taxa where spatial models often predict the extent rather than the exact timing of change (Gaüzère & Devictor, 2021; Lovell et al., 2023; Thuiller et al., 2008). *Chlorococcoid* lichens were again the exception, as several of its models significantly overpredicted the magnitude of change in later years. This divergence in direction and magnitude, which is more apparent for *Chlorococcoid* lichens, but was also seen in predictions for earlier years for squamulose and *Trentepohlia*, suggests that despite the potential limits set by climate, realized responses are constrained by other local scale constrains like dispersal, microclimatic refugia, or substrate availability (Marie & Bruce, 2004). Furthermore, this divergence in *Chlorococcoid* models may also reflect biotic interactions that are not represented in the spatial models. Previous works have in fact shown that biotic interaction explain a significant part of lichens spatial patterns, thus supporting for its inclusion in spatial biodiversity-climate models (Mod, Heikkinen, Le Roux, Väre, & Luoto, 2016; Rocha et al., 2024). Another important aspect to consider is the heterogeneity across concurrent models for the same functional group, with several predicting accurately both direction and magnitude and others deviating substantially. This was particularly evident for *Chlorococcoid* lichen models. Such heterogeneity likely reflects both the complexity of climate effects on functional groups and the synergistic consequences of dominance. Because *Chlorococcoid* lichens are the most abundant group, their signal is easier to detect in space, leading to a higher proportion of models identifying significant climate relationships. At the same time, their dominance makes them more prone to density-dependent constraints in time, where realized gains are limited, and to compositional trade-offs, since increases in other groups (e.g. *Trentepohlia*) inevitably reduce their relative weight in CWM. Together, these processes help explain why *Chlorococcoid* models yielded both accurate and biased outcomes, underscoring the need to account for abundance effects when validating space-for-time predictions. Such variability reinforces the value of using multiple concurrent models (i.e., models with a different set of predictors explaining the same range of variance) to capture different dimensions of uncertainty and the adoption of more complex statistical methods (e.g. ensemble forecasts, Bayesian hierarchical models, machine learning) that can better accommodate non-linear responses, hidden interactions, and biotic lags.

4.5 Conclusions and implications for future studies

Taken together, our results suggest a partial validation of the space-for-time assumption for lichen diversity. Spatial models captured the major aspects of temporal change, particularly for later years, supporting the application of the space-for-time method to infer changes over time. Nevertheless, systematic biases flagging a time-lag in response, trait-dependent variability, and inconsistent results among concurrent models for the same functional group highlight the need for caution. Thus, rather than assuming a universal applicability, space-for-time models in the context of lichen diversity as an ecological indicator of climate change should be interpreted as context-dependent, with a clear notion that predictive accuracy is dependent on the trait and the temporal scale. Our findings also reinforce the importance of adopting multiple methodological approaches. Combining concurrent models, as done here, provides a more comprehensive picture of likely responses and allows for a more explicit uncertainty assessment. Building on this, ensemble modelling approaches can further integrate information across models, weighting them according to performance or consensus, and thereby offer more robust predictions under climate change scenarios. Although not applied here due to the low sample size, this can be combined with alternative approaches such as non-parametric statistics, machine learning algorithms, and Bayesian frameworks can offer advantages over traditional regression. These methods do not rely on strict distributional assumptions, are well suited to capturing non-linear relationships, and can provide more robust inference in the presence of collinearity and complex predictor interactions. From the perspective of biomonitoring frameworks, these results imply that photobiont-based metrics may be more robust indicators of climate response over time, whereas growth form metrics require longer time scales to align with predictions. This is a particularly relevant aspect, as inaccurate predictions of the rate and extent of functional shifts under climate change can potentially lead to faulting and ineffective conservation strategies. By exploring whether spatial models accurately predicted trait-based metrics over time, our study provides a solid basis for applying and interpreting space-for-time models, emphasizing the importance of accounting for time lags in responses, for trait-based differences and to carefully consider the modelling approach when using lichen diversity as an ecological indicator of climate change.

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Chapter 5

GENERAL DISCUSSION

5.1 General discussion

Climate change is currently one of the most threatening global change drivers, dramatically altering biodiversity, the ecosystems backbone, across biomes (Pecl et al., 2017, Bellard et al., 2012). Monitoring its effects at the ecosystem level – i.e. on key components of the ecosystem, is therefore a priority. After the partial successes of the Strategic Plan for Biodiversity and its Aichi Targets, renewed efforts under the Convention on Biological Diversity promise an ambitious pathway to reach a more sustainable future. A pivotal mechanism for its success relates to the implementation of a comprehensive monitoring framework to monitor, report and review biodiversity conservation and restoration across the world. Ecological indicators based on lichen diversity can be crucial for this purpose, as their high sensitivity to surrounding environmental conditions offers an early-warning response to several global change drivers' effects, conveying an easy to interpret message which can be used to better understand how ecosystem's structure, services and functions are being impacted and the successfulness of already applied actions and policies to counter its degradation. However, though widely recognized as excellent ecological indicators of climate change with potential for global application, several unaddressed overarching knowledge gaps persist.

The aim of this thesis was to advance the global use of lichen diversity as an ecological indicator of climate change by addressing three major knowledge gaps that have so far limited its integration into research and policy applications. First, we tested if lichens can be used to track and signal the effects of climate in areas where other superimposed environmental drivers act simultaneously, which can obscure the climate signal. We found that lichen taxonomic and functional diversity can disentangle the individual effects of climate and detect thresholds for effects. This allows us to expand its application to mixed anthropic contexts (e.g. cities, high-intensity agricultural areas) where climate is rarely the dominant driver. Second, we tested the indicator's applicability in extreme environments, which differ fundamentally from the forest ecosystems where it was originally developed. In the maritime Antarctic we found that lichen abundance, together with bryophytes and vascular plants, respond predictably to broad scale annual and seasonal changes in temperature and precipitation, while also being strongly influenced by local topographic features and biotic interactions. These results broaden the ecological scope of the ecological indicator, confirming its relevance in ecosystems where lichens are dominant life forms and where monitoring is particularly challenging. Third, we evaluated the validity of the space-for-time assumption for lichen response to climate. Our results partially validated this assumption. We found that the predictive accuracy of the spatial models improved as we moved further from the baseline year (year used to build the spatial model) and lichen metric, but overall spatial models captured well the direction and magnitude of temporal trends. Rather than undermining lichens' potential as ecological indicators of climate change effects, these results disclose the importance of accounting for time lags in lichens response to climate and encourage further space-for-time validation studies in climate change ecology. By bridging these three main overarching knowledge gaps, this thesis strengthens the evidence base for the application of lichen diversity as a global ecological indicator of climate change. It both improves our understanding of how lichens respond to climate across spatial and temporal scales and across environments and provides a solid basis for their inclusion in international monitoring frameworks, as part of global strategies for climate change adaptation and mitigation towards a more sustainable future.

5.2 How to disentangle climate effects from other superimposed environmental drivers?

By applying a machine-learning statistical method, we were able to separately quantify the effects of multiple environmental drivers on lichen diversity. This was tested across a continental gradient which encompassed several European cities and a wide range of abiotic conditions. Until now, lichen diversity application in areas where multiple environmental drivers act concurrently was obscured due to the difficulty of disentangling their individual responses (Ochoa-Hueso et al., 2017), with researchers relying on complementary metrics with affinity to specific environmental drivers (Ellis and Coppins, 2010), balanced sampling designs to isolate a specific driver effects (Geiser and Neitlich, 2007) or on the selection of study areas where the driver is thought to be of the highest intensity (Munzi et al., 2014). Our results showed that metrics may not always respond strongly to particular drivers, and balanced sampling is often unfeasible.

Our initial approach was based both on a simpler linear parametric modelling method and easily obtained broad scale environmental data but failed to disentangle the individual effects of climate and revealed that broad scale climate and air pollution explained only a small proportion of the variance across several traditional taxonomic and functional metrics. These results suggested that superimposed local scale drivers could be overshadowing the effects of broad scale drivers, and, simultaneously, that lichens response, associated with such a large environmental gradient, could be non-linear. To address this, we then opted to apply a machine-learning statistical method which better dealt with the potential non-linearity and included local scale data from remote sensing. We found that the adoption of a more complex statistical method and the inclusion of environmental drivers at both spatial scales resulted in a higher lichen diversity variance explained, and that each lichen diversity response was indeed characterized by non-linear responses and thresholds. Temperature, water availability and land-use, at different intensities and spatial scales, were all important drivers of urban lichen diversity, reinforcing the importance of developing integrative approaches when applying lichen diversity based ecological indicators to track climate effects in urban ecosystems.

This work is thus a fundamental step towards the application of lichen diversity as ecological indicators of climate change at the global scale, allowing us to disentangle drivers' effects on lichen diversity based on the application of a more powerful machine learning statistical approach. This more complex approach thus allowed us, for the first time, to establish critical tipping points for urban lichen diversity associated with increasing temperature, water availability and land-use changes. Although already done previously in response to pollutants levels and loads (Geiser et al., 2021, Sutton et al., 2009, Jovan et al., 2012), this is now possible for several climate variables as well. The identification of these tipping points allows us to better understand the limiting factors for urban lichen diversity and to determine the environmental range of application for each metric. This is major step towards better informed policies to increase cities' sustainability and biodiversity as it helps to establish red lines for after which abrupt changes may occur in urban ecosystems (Samhouri et al., 2010). Furthermore, from the perspective of lichen diversity application as ecological indicators in cities, we found that various metrics, regardless of their nature, shared common threshold values for each given environmental variable. This means that, in the future, these metrics could be applied interchangeably instead of complementarily to track these drivers' effects in urban ecosystems. Still, there were exceptions, which reinforce the importance of considering and complementing taxonomic with functional metrics when developing globally applicable ecological indicators in cities, as the drivers and their importance differed between metrics.

5.3 Can lichen diversity be applied as an ecological indicator of climate change in extreme environments?

Lichen diversity, together with bryophytes and vascular plants responded to climate in a predictable way, attesting for their application as ecological indicators of climate change in extreme environments. The vegetation group (i.e. lichen, bryophyte or vascular plant) revealed to be the trait exhibiting the best response to climate, as only a few growth forms form lichens resulted in ecological meaningful models. This was tested along small elevation and cost-inland transects in maritime Antarctic, encompassing a climate gradient of annual and seasonal changes in temperature and precipitation. Our results confirmed the trait potential and showed that trait-based metrics based on the vegetation groups absolute abundance alone are suitable to track climate effects in the region. Results highlighted also that climate alone explained only a small portion of these groups spatial abundance patterns, suggesting that other underlying local scale biotic and abiotic drivers should also be considered. To address this, biotic interactions along with local scale terrain variables were incorporated and quantified through a multivariate data analysis. We found that local scale variables were important, but, more importantly, biotic interactions inclusion improved models and produced more accurate estimations of their current and future distributions under climate change. Surprisingly, net abiotic effects on lichens abundance were similar to abiotic effects, thus emphasizing biotic interaction importance in these extreme environments.

This work is thus another fundamental step towards the use of lichens as global scale ecological indicators of climate change effects. From the perspective of lichen diversity application, we found that lichens abundance, an easily measurable trait-based metric, was responsive to climate. As lichens, bryophytes and vascular plants can already be identified with satellite and drone-based imagery (Zmarz et al., 2023, Lockhart et al., 2025, Sandino et al., 2025), trait-based metrics based on their abundance can thus be a focal point to develop more cost-effective monitoring efforts to track climate change effects on Antarctic vegetation, and similar extreme ecosystems, at broader spatial scales. Furthermore, threshold like responses were sometimes observed, suggesting that these metrics can help establish tipping-points for maritime Antarctic vegetation, an important step to improve conservation actions in the region. Future studies should now be conducted to determine the limiting factors in temperature and water availability for these groups. Together, these are major steps towards improved adaptation and conservation actions in a region where climate is a severe threat to biodiversity and ecosystems (Carter et al., 2025, Hughes et al., 2020), directly answering the call from Antarctic Treaty System for standardized, long-term ecological monitoring frameworks (COMNAP, 2005).

5.4 How valid is the space-for-time substitution method for lichen diversity?

Due to the scarcity of long-term datasets, the development of lichen diversity-based ecological indicators of climate has relied heavily on the space-for-time method (Matos et al., 2015, Giordani et al., 2012, Bässler et al., 2016). Nevertheless, the lack of validation efforts has undermined the confidence in resulting predictions (Evans et al., 2025, Angert, 2024). Our approach applied spatial models of lichen diversity–climate relationships to predict trait-based metrics at three independent time points and then compared these predictions with observed temporal data. Results partially validated the space-for-time assumption in the context of lichen diversity response to climate: predictions often captured the direction and magnitude of change, but their accuracy was strongly dependent on the trait considered and the temporal horizon. Photobiont-based models showed clearly lower bias than those based on growth forms, and the overall decrease in bias across years suggests the presence of a time lag in lichen response to climate. These mismatches highlighted the role of temporal variability, potential time lags in lichen responses, and the limits of applying static spatial relationships directly to dynamic temporal processes.

These findings hold important implications. From the perspective of ecological indicators, they show that while lichens hold strong potential for detecting climate change effects, we need to be cautious when transferring spatial models into temporal predictions. Improving the robustness of this approach will require accounting for time lags in lichen responses and ensuring longer temporal datasets so that it is possible to better understand when spatial and temporal predictions align more precisely. Beyond the indicator framework, this work contributes to the broader debate on the use of space-for-time substitution in ecology. It demonstrates the value of rigorous validation and highlights the need for hybrid approaches that combine spatial modelling with repeated temporal sampling and/or experimental manipulations. Such efforts would strengthen biodiversity-based climate indicators and contribute for advancing general ecological modelling by clarifying when and how spatial patterns can reliably forecast future ecological dynamics. Ultimately, by embedding space-for-time within long-term monitoring networks, lichen indicators could serve as a cornerstone for tracking biodiversity change in the context of global climate adaptation and mitigation policies.

5.5 How can we do this at a global scale?

Altogether, our results reinforce lichen diversity potential as an ecological indicator of climate change effects and provide key guidelines for its application at a global scale. Below, we outline a set of guidelines intended to support and facilitate the application of lichen diversity as a climate indicator in global monitoring frameworks.

A) Choice of metrics is fundamental

Biodiversity-based metrics better relate to ecosystems functions and services (Zhang et al., 2022) but the choice of the most appropriate metrics to track climate effects is fundamental for the indicator correct application of the indicator. Our findings showed that easily measurable trait-based metrics related to lichens main photobiont type emerged as the most consistent under the space-for-time validation test, with an overall lowest predictive bias (Chapter 4). Conversely, growth form metrics were less reliable across the studied temporal scale (Chapter 4), and also across spatial scales in extreme (Chapter 3), semi-arid (Chapter 4), and in mixed gradients of climate and other environmental drivers across European cities (Chapter 2). This suggests their limited suitability as climate change ecological indicators. We therefore recommend prioritising photobiont-based metrics for global applications, while also exploring for other functional traits, like physiological tolerance, secondary chemistry, reflectance properties, among others, that may strengthen the indicator response to track climate effects.

B) Expand beyond well-studied regions and to other vegetation types

Applying the indicator at a global scale requires also expanding works outside well covered areas (e.g. Europe and North America). Several regions remain underrepresented and could be sampled to broaden our knowledge on the relationships between climate change and its effects on ecosystems. Tropical forests are an example of underrepresented ecosystems (Stanton et al., 2023). In addition, our results demonstrated that the ecological indicator should not be limited to classical epiphytic diversity indices. Results showed that trait- and abundance-based metrics across multiple vegetation groups (lichens, bryophytes and vascular plants) are effective ecological indicators of climate, in extreme environments like maritime Antarctica (Chapter 3). This approach could also be applied in other regions, but it is particularly relevant for extreme ecosystems (e.g., polar regions, and warm or cold deserts). In these regions, field work is particularly challenging and frequently limited by logistic constrains, which means that traditional sampling methods usually only cover smaller areas. In these treeless extreme environments, exploiting the increasing capacity of remote sensing (e.g. satellite imagery or UAV) can provide a cost-effective scalable solution to integrate multi-vegetation sampling into standardized monitoring frameworks beyond Antarctica.

C) Integrate environmental drivers across scales

As the indicator is applied over larger areas of the world, encompassed environmental gradients will equally grow and become more complex. Integrating multiple environmental drivers, at different spatial scales, is thus fundamental. Our results showed that together with broad climate drivers, local scale abiotic (local microclimate and other locals scale drivers in urban environments – Subchapter 2.2; terrain characteristics in extreme environments – Subchapter 3.2) should be incorporated into ecological indicator models. Ignoring these environmental drivers acting a different scales risks underestimating or misattributing important ecological responses. In fact, the inclusion of these drivers could even reduce the space-for-time bias, as they would account for some unexplained variance in spatial models and therefore better represent temporal dynamics (Chapter 4). We recommend that tracking climate change effects at the global scale integrate both broad- and local scale drivers to ensure a more comprehensive and trustworthy environmental characterization. In a wider perspective, these combined results suggest that multi-scale and multi-taxa monitoring systems should be encouraged, where multiple indicators can track climate effects while disentangling confounding drivers.

D) Address multi-stressor contexts

Lichen diversity revealed a clear response to climate even in complex environmental gradients. This demonstrates their indicator value even in complex, mixed anthropogenic gradients, where attributing ecological change is particularly challenging. This is critical because currently, most ecosystems are exposed to overlapping environmental pressures (Jackson, Pawar, & Woodward, 2021). We therefore encourage applying lichen indicators in urban climate–pollution studies, agricultural landscapes, among other complex environmental gradients that require robust attribution of ecological responses to specific drivers, including climate.

E) Employ advanced modelling approaches

As broader applications of the ecological indicator imply higher biodiversity and environmental complexity, statistical approaches must also follow. Traditional linear models are suitable to track high intensity drivers of change (Branquinho et al., 2019), but struggle in more complex contexts as seen in cities (Chapter 2) and even in the space-for-time validation (Chapter 4). The comparison of conventional linear models with a machine-learning approach, revealed that the methodological choice can strongly influence our capacity to detect thresholds and non-additive responses. Thus, the methodological approach is key: applying flexible modelling methods can uncover climate signals that otherwise might remain hidden by other anthropogenic pressures. Furthermore, the identification of thresholds in lichens response to temperature and water availability could eventually provide global "critical levels" for climate impacts, creating clear targets for policy action if quantified globally. Thus, we recommend the application of advanced dynamic modelling frameworks, adapted to the goals and spatial range of application.

F) Apply the space-for-time method cautiously

At a global scale, long-term datasets are scarce, making space-for-time substitution unavoidable, particularly in underrepresented areas where no lichen diversity data is available. Our results only partially validated this assumption (Chapter 4), as we observed a predictive bias which decreased over the years, consistent with an ecological time lag. Thus, we recommend that: (i) as space-for-time accuracy increases over longer temporal frames, it should preferentially be used for long-term projections; (ii) validation should be carried out whenever possible; (iii) coupling climate predictors with other local and large-scale drivers (see point C). These findings are relevant to global biodiversity projections, where time lags can also challenge predictions of species distributions or climate-driven migrations (Essl et al., 2024; Lalechère, Marrec, & Lenoir, 2025). These considerations are also relevant for biodiversity projections in ecological studies, where ecological time lags can challenge projections of climate-driven migrations or species distribution models.

G) Couple biodiversity with high-quality environmental data

Scaling the indicator globally relies on coupling the indicator data with high-quality and high-resolution environmental data. Biodiversity alone provides critical information on species distributions and ecosystem functioning, but without environmental data, the drivers underlying biodiversity patterns over space and time will remain poorly understood. Coupling biodiversity datasets with high-quality environmental data is thus essential to produce accurate, reliable, and globally relevant indicators. Integrating both allow models to better capture the complexity of ecological processes, scale indicators across regions and, consequently, improve their predictive power. Without this integration, predictions risk being oversimplified (Subchapter 3.1), biased (Chapter 4), or spatially restricted (Subchapter 3.1), reducing their usefulness for policy, conservation planning, and global assessments.

In summary, applying lichen diversity as an ecological indicator at the global scale requires prioritising robust trait-based metrics (especially photobiont type), expanding to underrepresented ecosystems, integrating multi-scale drivers and multiple vegetation groups, applying advanced modelling frameworks, validating space-for-time assumptions and ensuring access to high-resolution environmental data. Together, these guidelines provide a blueprint for next-generation indicators centred on lichen diversity, and point to the need to, and importance of, integrating them with regional to international biodiversity monitoring frameworks. Such frameworks would not only enhance our ability to detect climate impacts but also strengthen the relevance of biodiversity monitoring for global change research, conservation, and policy.

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Supporting information

2.1 Modelling the response of urban lichens to broad scale changes in air pollution and climate

Details on sampling sites selection

Sampling sites were selected within the “Green Urban Areas” class of the pan-European land-cover classification, the European Urban Atlas (EEA, 2018), ensuring land-use typology and intensity. For each patch of “Green Urban Areas”, patch size and the connectivity index (Gustafson and Parker, 1992) were calculated (see below). Sites were classified into six size classes ([0 - 0.6[, [0.6 – 1.2[, [1.2 – 2.4[, [2.4 – 4.8[, [4.8 – 9.6[and >9.6 ha) and six classes of connectivity ([0 – 18000[, [18000 – 36000[, [36000 – 72000[, [72000 – 144000[, [144000 – 288000[and >288000 – no units). From the initially available 1582 patches (across all cities), up to 36 were selected for each city, stratified by size and by the proximity index. Selection within each combination of classes (maximum of 36 combinations) was done randomly. This resulted in a total of 219 sampling sites across all cities. For some cities (Fig. S1) less than 36 patches were selected as some combinations were not available.

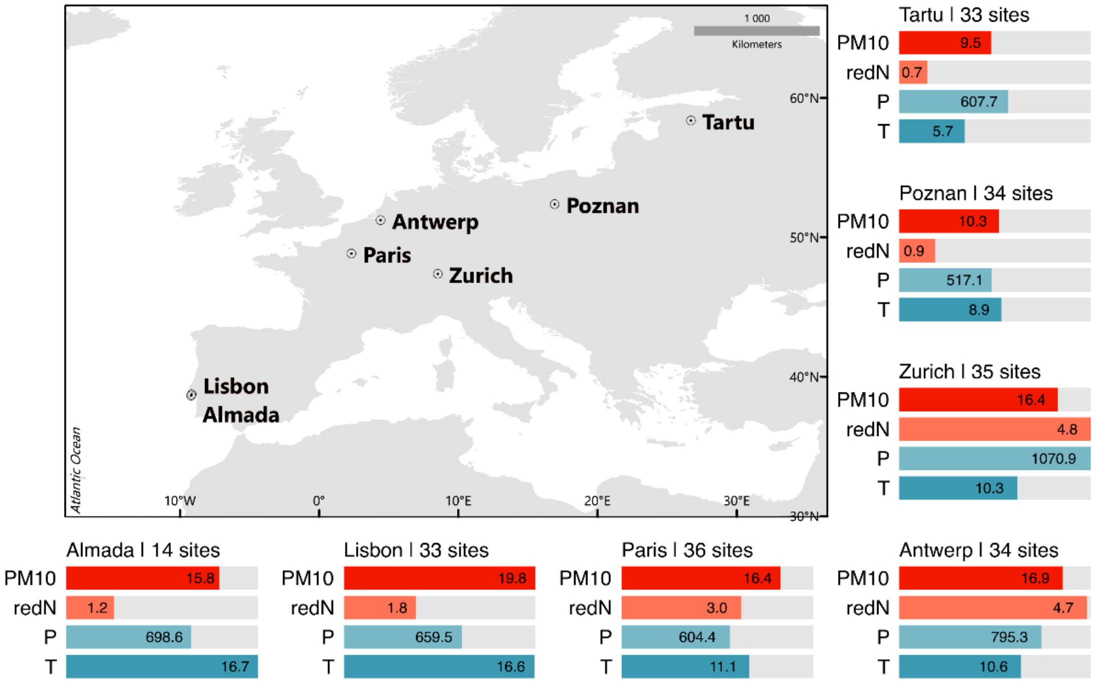

Figure S2.1.1: Map of the seven sampled European cities along a continental gradient of pollution and climate, characterized by average annual particulate matter concentration (PM10, inµg.m-3), reduced nitrogen deposition (redN, in mg.m-2), mean annual precipitation (P, in mm) and annual mean temperature (T, in °C). The number of green spaces sampled in each city was derived from the random sampling selection of parks, stratified by size and fragmentation (N=219).

The proximity index (Gustafson and Parker, 1992) is a measure of fragmentation/isolation of each focal patch. Briefly, it quantifies the amount of available habitat in neighbouring patches, each neighbour patch area being weighted by the square of the distance to the focal patch. The neighbourhood of each focal patch is defined by user-specified radius. In this case, we selected a 5000 m area to include the maximum distance for which an Urban Atlas was available. Note that because the distance to each patch is squared, nearby patches have a much higher weight on the index than those further away from the focal patch, thus, looking at longer distances would cause insignificant changes in this metric. Based on previous knowledge, we expected all neighbouring patches with trees to influence lichens in the focal patches, e.g., due to its mitigation of the urban heat-island (Munzi et al., 2014) or air pollution (Matos et al., 2019). To account for this, all land-cover types that could host a significant number of trees were included as potential habitat in the calculation of the proximity index, namely “Discontinuous Low-Density Urban Fabric” and “Discontinuous very-low-density urban fabric” (mainly small houses with gardens), “Permanent Crops” (orchards), and “Forests”. The Proximity Index takes the highest values when the focal patch is surrounded by nearby large similar habitats and takes the value of zero when the focal patch has no neighbours of the same habitat.

Details on epiphytic lichens European standard sampling methodology

Epiphytic lichen diversity was sampled following the European standard method (Asta et al., 2002; Cristofolini et al., 2014), has been used in multiple studies to quantify lichen biodiversity in gradients of air pollution, climate, land-use intensity, management and other (Lättman et al., 2014; McCarthy et al., 2009; Munzi et al., 2014), (Fig. S2).

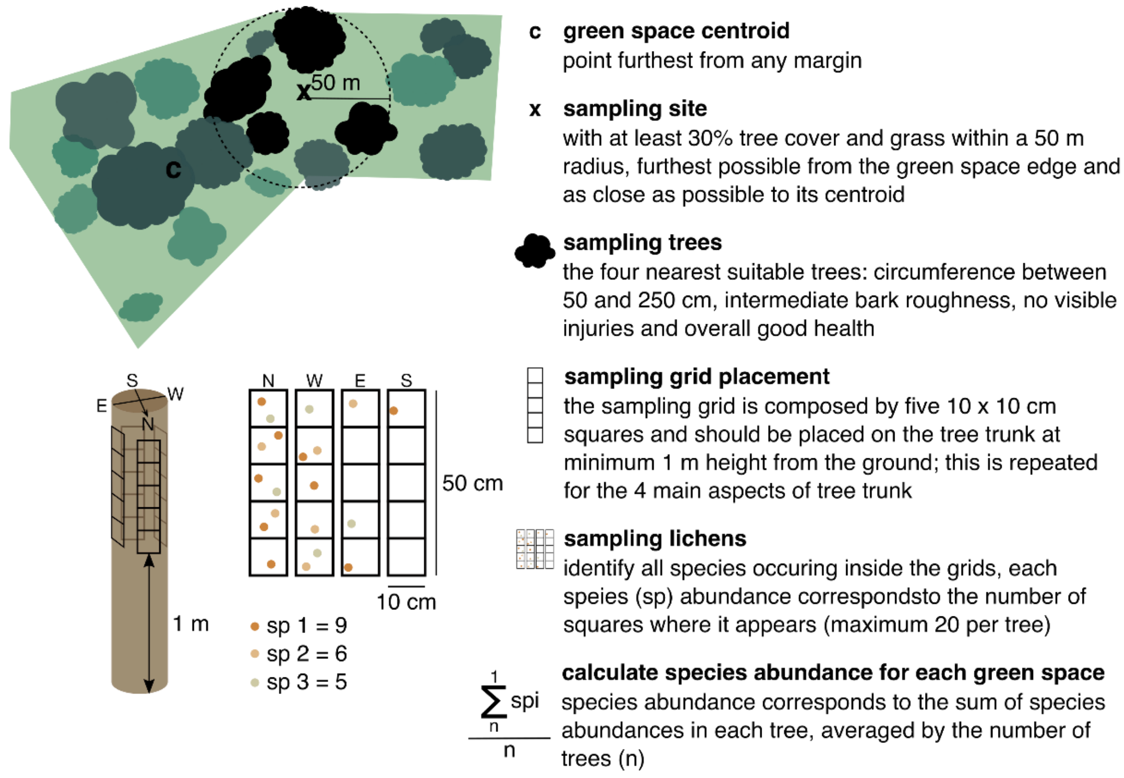

Figure S2.1.2: Selection of sampling site inside a green space patch and sampling scheme for epiphytic lichens, according to the European standard method (Asta et al., 2002; Cristofolini et al., 2014).

The use of a standard method for lichen sampling is unprecedented in urban areas, at this scale, and greatly contributed to reducing variability due to disparate sampling protocols. Within each patch, the point furthest from the margin was taken as the patch centroid. The nearest area with at least 30% cover by trees and grass, within a 50 m radius buffer, was then selected as the sampling site. On each sampling site, the four trees closest to the centroid were selected, ensuring they were within the range of conditions required by the sampling protocol: healthy trees with an upright main trunk (< 20º deviation from vertical), without branches or injuries up to two meters, with circumference at the sampling height (one to two meters from the ground) between 50 and 250 cm. A maximum distance of 50 m between trees was established.

It is important to highlight that to reduce the sampling variability to the feasible minimum, tree bark and tree size were homogenized as much as possible: as it is not possible to select a single phorophyte species across all cities, sampling was narrowed to phorophytes sharing the same bark roughness (intermediate roughness was chosen as it could be found in all cities). Additionally, tree size was limited to medium size trees (50 to 250 cm perimeter at sampling height). On each tree, a sampling grid (50 x 10 cm, divided into 5 squares) was placed on the four main aspects (N, S, E, W, total 2000 cm2 sampling area per tree). All lichens within the grid were either identified on location or collected for later laboratory identification. Species abundance was determined based on the number of squares in which they occur (maximum abundance value of 20), and then averaged by site. Species nomenclature followed (Nimis and Martellos, 2021). A total of 140 species were identified, nine of which to the genus level only (Table S1).

Table S2.1.1: List of total species identified (N=140) and respective functional characterization based on the growth form, type of algae, main reproduction type, and tolerance to different levels of substrate pH requirement, solar irradiation, aridity, eutrophication and poleotolerance. Nomenclature followed (Nimis and Martellos, 2021).

Scientific name	Growth form[1]	Photobiont[2]	Reproduction[3]	pH[4]	Irradiation[5]	Aridity[6]	Eutrophication[7]	Poleotolerance[8]
Acrocordia cavata	c	t	se	3	3	2	1	0
Alyxoria varia	c	t	se	3	3	3	2	2
Amandinea punctata	c	g	se	3	5	4	4	3
*Arthonia sp. **	-	-	-	-	-	-	-	-
Arthonia mediella	c	t	se	2	3	3	1	1
Arthonia radiata	c	t	se	3	4	3	3	3
Athallia cerinelloides	c	g	se	4	5	4	4	2
Athallia pyracea	c	g	se	4	5	4	4	2
*Arthopyrenia sp. **	-	-	-	-	-	-	-	-
Bacidia arceutina	c	g	se	3	4	3	2	1
Bacidia rubella	c	g	se	3	4	3	3	2
Bacidia subincompta	c	g	se	3	3	2	2	1
Bacidina arnoldiana	c	g	se	3	3	2	3	2
Biatoridium monasteriense	c	g	se	3	3	2	3	2
Buellia griseovirens	c	g	se	2	4	3	1	2
Caloplaca cerina	c	g	se	4	4	4	4	3
Candelaria concolor	fn	g	so	4	5	4	5	3

Candelariella reflexa	c	g	so	4	5	3	5	3
Candelariella vitellina	c	g	se	3	5	4	5	3
Candelariella xanthostigma	c	g	se	3	5	3	3	3
Catapyrenium psoromoides	sq	g	se	3	4	3	2	1
Catillaria nigroclavata	c	g	se	3	4	3	3	2
Chaenotheca chrysocephala	c	g	se	2	4	3	1	1
Chaenotheca furfuracea	c	g	se	2	3	2	1	2
Chaenotheca phaeocephala	c	g	se	2	3	2	1	1
Chaenotheca stemonea	c	g	se	2	3	1	1	1
Chaenotheca trichialis	c	g	se	2	3	2	1	1
Chaenotheca xyloxena	c	g	se	2	3	2	1	1
Chrysothrix candelaris	l	g	so	2	3	3	1	2
*Cladonia sp. **	-	-	-	-	-	-	-	-
Cladonia fimbriata	f	g	so	3	4	3	3	2
Coenogonium pineti	c	t	se	3	3	3	2	2
Coenogonium tavaresianum	c	t	se	2	3	1	1	0
Collema furfuraceum	fb	c	is	3	4	2	3	1
*Cyphelium sp. **	-	-	-	-	-	-	-	-
Dendrographa decolorans	c	t	so	2	3	2	3	2
Diploicia canescens	c	g	so	5	5	3	4	2

Evernia prunastri	f	g	so	3	5	3	3	2
Flavoparmelia caperata	fb	g	so	3	4	3	3	2
Flavoparmelia soredians	fb	g	so	3	5	3	3	2
Flavoplaca citrina	c	g	so	5	5	4	5	3
Gyalecta fagicola	c	t	se	3	3	2	1	0
Hyperphyscia adglutinata	fn	g	so	5	5	4	5	3
Hypogymnia physodes	fn	g	so	3	4	3	2	3
Hypogymnia tubulosa	fn	g	so	2	3	3	2	2
Hypogymnia tubulosa f. farinosa	fn	g	so	2	3	3	2	2
Hypotrachyna revoluta	fb	g	so	2	3	3	3	2
Lecania cyrtella	c	g	se	3	5	4	3	3
Lecania naegelii	c	g	se	3	4	3	3	2
Lecanographa amylacea	c	t	se	2	4	2	1	0
Lecanographa lyncea	c	t	se	2	4	2	1	0
*Lecanora sp. **	-	-	-	-	-	-	-	-
Lecanora albella	c	g	se	2	3	2	1	1
Lecanora allophana	c	g	se	3	5	4	3	2
Lecanora argentata	c	g	se	3	4	3	2	2
Lecanora carpinea	c	g	se	3	5	4	3	3
Lecanora chlarotera	c	g	se	3	5	4	5	3

Lecanora conizaeoides	c	g	se	2	5	3	3	3
Lecanora expallens	c	g	so	2	4	3	2	2
Lecanora populicola	c	g	se	3	4	3	4	1
Lecanora pulicaris	c	g	se	2	5	4	2	2
Lecanora strobilina	c	g	se	2	3	3	1	1
Lecanora symmicta	c	g	se	2	4	3	2	2
Lecidella elaeochroma	c	g	se	4	5	5	4	3
Lecidella euphorea	c	g	se	3	4	4	3	2
Lepra albescens	c	g	so	3	4	3	3	2
Lepra amara	c	g	so	3	3	3	3	3
*Lepraria sp. **	-	-	-	-	-	-	-	-
Lepraria lobificans	l	g	so	3	4	4	1	1
Leprocaulon quisquiliare	fn	g	is	3	5	3	3	2
Leptogium brebissonii	fb	c	is	3	3	2	2	1
*Melanelixia sp. **	-	-	-	-	-	-	-	-
Melanelixia glabratula	fb	g	is	3	4	3	3	3
Melanelixia subaurifera	fb	g	so	3	4	3	3	2
Melanohalea exasperatula	fb	g	is	3	5	3	3	3
Melanohalea laciniatula	fb	g	is	2	3	2	3	1
Micarea prasina	c	g	se	2	3	2	1	1

Myriolecis dispersa	c	t	se	5	5	5	4	3
Myriolecis hagenii	c	g	se	5	5	5	4	3
Myriolecis sambuci	c	g	se	4	5	3	3	2
Normandina pulchella	sq	g	so	3	4	3	3	2
Ochrolechia szatalaensis	c	g	se	2	3	3	1	1
Opegrapha vulgata	c	t	se	3	3	2	1	2
Parmelia saxatilis	fb	g	is	2	4	3	3	2
Parmelia sulcata	fb	g	so	3	5	3	3	3
Parmelina tiliacea	fb	g	is	2	4	3	3	3
Parmeliopsis ambigua	fn	g	so	2	5	4	1	2
Parmotrema hypoleucinum	fb	g	so	3	5	2	2	1
Parmotrema perlatum	fb	g	so	2	4	3	2	2
Parmotrema robustum	fb	g	so	2	4	2	1	0
*Pertusaria sp. **	-	-	-	-	-	-	-	-
Pertusaria heterochroa	fn	g	so	5	5	4	5	2
Pertusaria hymenea	c	g	se	3	5	3	2	2
Phaeophyscia cernohorskyi	fn	g	so	3	5	4	4	1
Phaeophyscia endophoenicea	fn	g	se	3	3	3	3	1
Phaeophyscia hirsuta	fn	g	so	4	5	4	4	2
Phaeophyscia nigricans	fn	g	is	4	5	4	4	2

Phaeophyscia orbicularis	fn	g	so	5	5	4	5	3
Phlyctis argena	c	g	so	2	3	3	2	2
Physcia adscendens	fn	g	so	5	5	4	5	3
Physcia aipolia	fn	g	se	3	5	3	4	3
Physcia caesia	fb	g	so	5	5	4	5	2
Physcia clementei	fn	g	is	3	5	3	3	2
Physcia dubia	fn	g	so	4	5	4	5	3
Physcia stellaris	fn	g	se	3	5	3	4	2
Physcia tenella	fn	g	so	4	5	4	4	2
Physcia tribacioides	fn	g	so	3	5	2	3	1
*Physciaceae sp. **	-	-	-	-	-	-	-	-
Physciella chloantha	fn	g	so	5	4	3	4	2
Physconia distorta	fn	g	se	4	5	4	4	3
Physconia enteroxantha	fn	g	so	3	5	3	4	2
Physconia grisea	fn	g	so	4	5	3	5	3
Physconia perisidiosa	fn	g	so	3	4	3	3	2
Platismatia glauca	fb	g	is	2	5	3	2	2
Pleurosticta acetabulum	fb	g	se	3	5	4	3	3
Polycauliona polycarpa	fn	g	se	3	5	4	3	2
Porina aenea	c	t	se	3	2	3	1	1

Pseudoschismatomma rufescens	c	t	se	3	3	2	1	1
Punctelia jeckeri	fb	g	so	4	4	3	3	3
Punctelia subrudecta	fb	g	so	4	4	3	3	3
Pyrenula macrospora	c	t	se	3	3	2	1	1
Ramalina calicaris	f	g	se	2	4	2	2	1
Ramalina farinacea	f	g	so	3	5	2	2	2
Ramalina fastigiata	f	g	se	3	5	3	3	2
Ramalina fraxinea	f	g	se	3	5	3	3	1
Ramalina lacera	f	g	so	3	5	2	3	1
Rinodina capensis	c	g	se	3	4	3	2	1
Rinodina ficta	c	g	se	3	5	4	4	2
Rinodina oleae	c	g	se	3	5	4	3	1
Rinodina pyrina	c	g	se	3	5	3	3	2
Ropalospora viridis	c	g	so	2	4	2	1	1
Sclerophora pallida	c	t	se	3	3	2	1	0
Scoliciosporum chorococcum	c	g	se	3	3	3	3	2
Teloschistes chrysophthalmos	f	g	se	3	5	4	3	1
Trapeliopsis flexuosa	c	g	se	2	5	4	2	2
Usnea rubicunda	f	g	so	2	4	2	2	0
Waynea stoechadiana	sq	g	se	2	4	3	3	1

Xanthomendoza fulva	fn	g	so	3	5	4	4	1
Xanthoparmelia conspersa	fb	g	is	3	5	4	3	2
Xanthoria parietina	fb	g	se	4	5	4	4	3

*Species only identified to the genus level

[1] c – crustose; fn - foliose narrow lobe; fb - foliose broad lobe; sq – squamulose; l – leprose; f – fruticulose.

[2] g – green; c – cyano; t – trentepohlia.

[3] se – sexual; so – soredia; is - isidia

[4] 2 - on acid substrata, such on non-eutrophicated bark of Quercus; 3 - on subacid to sub neutral substrata (e.g. on bark of Sambucus); 4 - on slightly basic substrata, such as dust-covered bark; 5 - on basic substrata, e.g. pure limestone.

[5] 2 - in shaded situations, such as on the northern side of boles in close-canopied deciduous forests; 3 - in sites with plenty of diffuse light but scarce direct solar irradiation, such as in rather open-canopied deciduous woodlands; 4 - in sun-exposed sites, but avoiding extreme solar irradiation; 5 - in sites with very high direct solar irradiation, e.g. on the southern side of isolated boles.

[6] 1 - hygrophytic, in sites with a very high frequency of fog; 2 - rather hygrophytic, intermediate between 1 and 2; 3 – mesophytic; 4 - xerophytic but absent from extremely arid stands; 5 - very xerophytic.

[7] 1 - no eutrophication; 2 - very weak eutrophication; 3 - weak eutrophication; 4 - rather high eutrophication; 5 - very high eutrophication.

[8] 0 - species which exclusively occur on old trees in ancient, undisturbed forests; 1 - species occurring in natural or semi-natural habitats; 2 - species occurring in moderately disturbed areas (agricultural areas, small settlements etc.); 3 - species occurring in heavily disturbed areas, incl. large towns.

Details on the calculation of biodiversity-based metrics

Taxonomic and trait-based metrics were computed. Regarding the taxonomic metrics, eight metrics were calculated. Species richness corresponds to the total number of species per site. The number of rare species corresponds to the number of rare species per site, i.e., if present in less than 5% of the total sampling sites. The Shannon diversity Index is a measure of species diversity in a community, as is the Inverse-Simpson Index. In both diversity indices, higher values correspond to higher species diversity. Total species abundance corresponds to the sum of all lichen species abundance per site (same as the Lichen Diversity Value in the lichen European Standard Method). To analyse the communities' dissimilarity, Bray-Curtis, Jaccard and Morisita-Horn dissimilarity indices were calculated. These indices were chosen to reflect changes in community composition giving both relevance to rare (Bray-Curtis and Jaccard) or dominant species (Morisita-Horn), (Barwell et al., 2015). In these three metrics, values vary from 0 to 1, with values of 1 indicating complete dissimilarity between sites, I.e., no shared species. R software (R Core Team, 2022) was used to calculate taxonomic diversity metrics, using functions *diversity* and *vegdist* from the *Vegan* package (Oksanen et al., 2011). Given the similar patterns of variation between species richness and number of rare species, between Shannon and Inverse-Simpson, between and Jaccard and Bray-Curtis and Morisita-Horn, we present only results for species richness, Shannon, total abundance and Jaccard. Results of the remaining metrics are shown in supplementary material (Fig. S3).

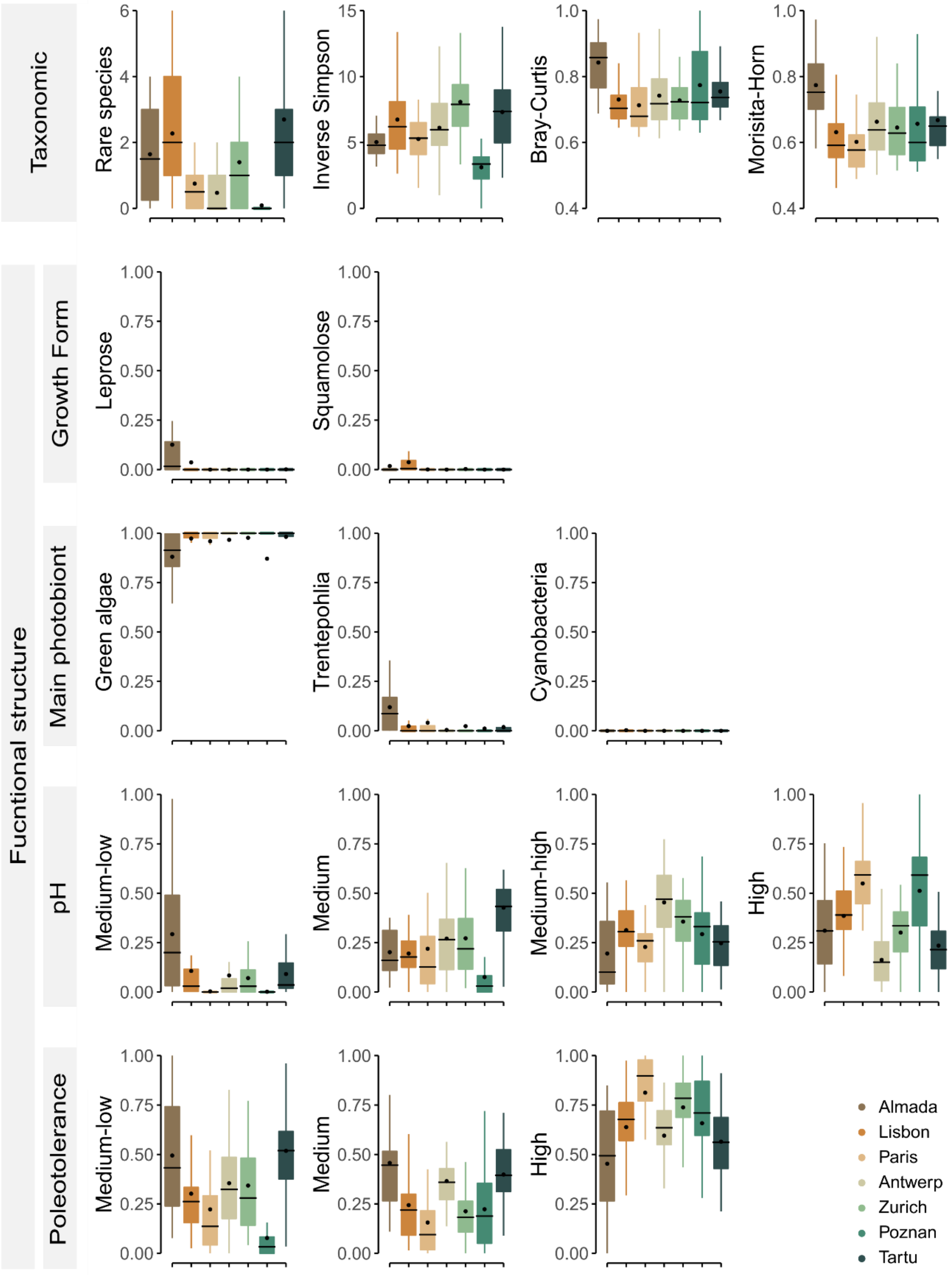

Figure S2.1.3: Boxplots representing the distribution of remaining taxonomic (rare species, inverse Simpson, Bray-Curtis and Morisita-Horn) and functional structure (Growth form, main photobiont type, species substrate pH tolerance and poleotolerance) metrics in the seven European cities, ordered here from the warmest (Almada to the coldest (Tartu), as indicated in the first axis of the PCA. Bray-Curtis and Morisita-Horn indices values range from 0 to 1. The functional structure (Table 1), represented here by the CMW of each functional group belonging to the same trait (growth form, species substrate pH tolerance and poleotolerance) ranges also from 0 to 1 (the sum of all functional groups from the same trait is 1 at site level). Boxes display first to third interquartile ranges, the black lines the median, the dots the average, and the whiskers the maximum and minimum (N=219). Only metrics not represented on the main text (Fig. 4) are shown here.

Regarding the trait-based metrics, indices representing both functional diversity and functional structure were computed based only on data from specimens identified to the species level (Table S1). This was done based on seven categorical traits known to respond to air pollution and/or climate (Table S2).

Table S2.1.2: Lichen species traits and related functional groups (with categorical and correspondent ordinal classification) used in the study, described following (Nimis and Martellos, 2021).

Trait	Functional group	Description
growth form	crustose	thallus firmly and entirely attached to the substrate by the lower surface
	foliose broad-lobed	thallus partly attached to the substrate, with distinct upper and lower surfaces and broad lobes
	foliose narrow-lobed	thallus partly attached to the substrate, with distinct upper and lower surfaces and narrow lobes
	fruticose	thallus attached to the substrate by one single point and with rounded or flattened branches
	leprose	thallus like crustose but surface thallus with a granular mass appearance and always decorticated
	squamulose	thallus composed of small scales
main photobiont type	cyanobacteria	lichens with cyanobacteria
	Chlorococcoid	lichens with green algae other than *Trentepohlia*
	trentepohlia	lichens with *Trentepohlia* (green algae)
species substrate pH tolerance	low (1)	occurs in very acid substrata
	medium-low (2)	occurs in acid substrata
	medium (3)	occurs in subacid to sub neutral substrata
	medium-high (4)	occurs in slightly basic substrata
	high (5)	occurs in basic substrata
solar irradiation tolerance	low (1)	occurs in very shaded situations
	medium-low (2)	occurs in shaded situations
	medium (3)	occurs in sites with plenty of diffuse light but scarce direct solar irradiation
	medium-high (4)	occurs in sun-exposed sites
	high (5)	occurs in very high direct solar irradiation
aridity tolerance	low (1)	Hygrophytic
	medium-low (2)	rather hygrophytic
	medium (3)	mesophytic
	medium-high (4)	xerophytic
	high (5)	very xerophytic
eutrophication tolerance	low (1)	occurs in sites with no eutrophication
	medium-low (2)	occurs in sites with very weak eutrophication
	medium (3)	occurs in sites with weak eutrophication

	medium-high (4)	occurs in sites with rather high eutrophication
	high (5)	occurs in sites with very high eutrophication
poleotolerance	low (0)	occurs in undisturbed forests
	medium-low (1)	occurs in natural or semi-natural habitats
	medium (2)	occurs in moderately disturbed areas
	high (3)	occurs in heavily disturbed areas

Growth form, main photobiont type (green algae other than *Trentepohlia*, *Trentepohlia* and cyanobacteria), species substrate pH tolerance, tolerance to solar irradiation, tolerance to aridity, tolerance to eutrophication and poleotolerance. Trait information was retrieved from the ITALIC database (Nimis and Martellos, 2021), and the maximum value for each species was used (trait classification are ordinal in the database). For species trait classification see Table S1. These traits were selected as they respond to environmental change influencing lichen growth, survival and, ultimately, fitness. Growth form and the main photobiont type traits are known to respond to both air pollution and climate (Aptroot and Van Herk, 2007; Koch et al., 2019; Matos et al., 2015). Substrate pH was used as a proxy for air pollution, as deposition of particles and gases affect natural trees bark pH, a key factor influencing lichen communities (Larsen et al., 2007). Note that substrate pH does not reflect raw bark pH values, as these were not measured, but rather lichens species tolerance to substrate pH. Eutrophication tolerance shows species' tolerance to nitrogen in the environment and has also been used to track air pollution (Giordani and Malaspina, 2017; Pinho et al., 2011) while the poleotolerance trait groups species based on its tolerance to different degrees of human disturbance, having been used in the past as an ecological indicator of the integrated effects of environmental disturbances (Rocha et al., 2019). Lastly, solar irradiation and aridity traits are known to respond to climatic conditions (Munzi et al., 2014). Species that could not be identified to the species level and for which we could not retrieve specific trait classification were excluded from trait-based metrics calculation (Table S1). In terms of functional diversity metrics, we calculated functional richness and Rao's quadratic entropy (RaoQ), both as multi-trait indices, computed only with the traits and corresponding functional groups (the different categories of each categorical trait) included in the results section. Functional richness represents the functional space occupied by the different functional groups (per sampling site), the trait diversity tally of species richness. RaoQ corresponds to the functional dispersion, reflecting the variety of functional strategies, weighted by abundance. The community weighted mean (CWM) was included to represent the communities' functional structure and represents the mean trait value in the community weighted by the abundance, i.e., the proportion of summed species abundances belonging to the same functional group of each trait. The two lower classes of the irradiation, eutrophication and poleotolerance traits were merged prior to calculations as they presented similar correlation trends with both climate and air pollution PCA axes (Fig. S4).

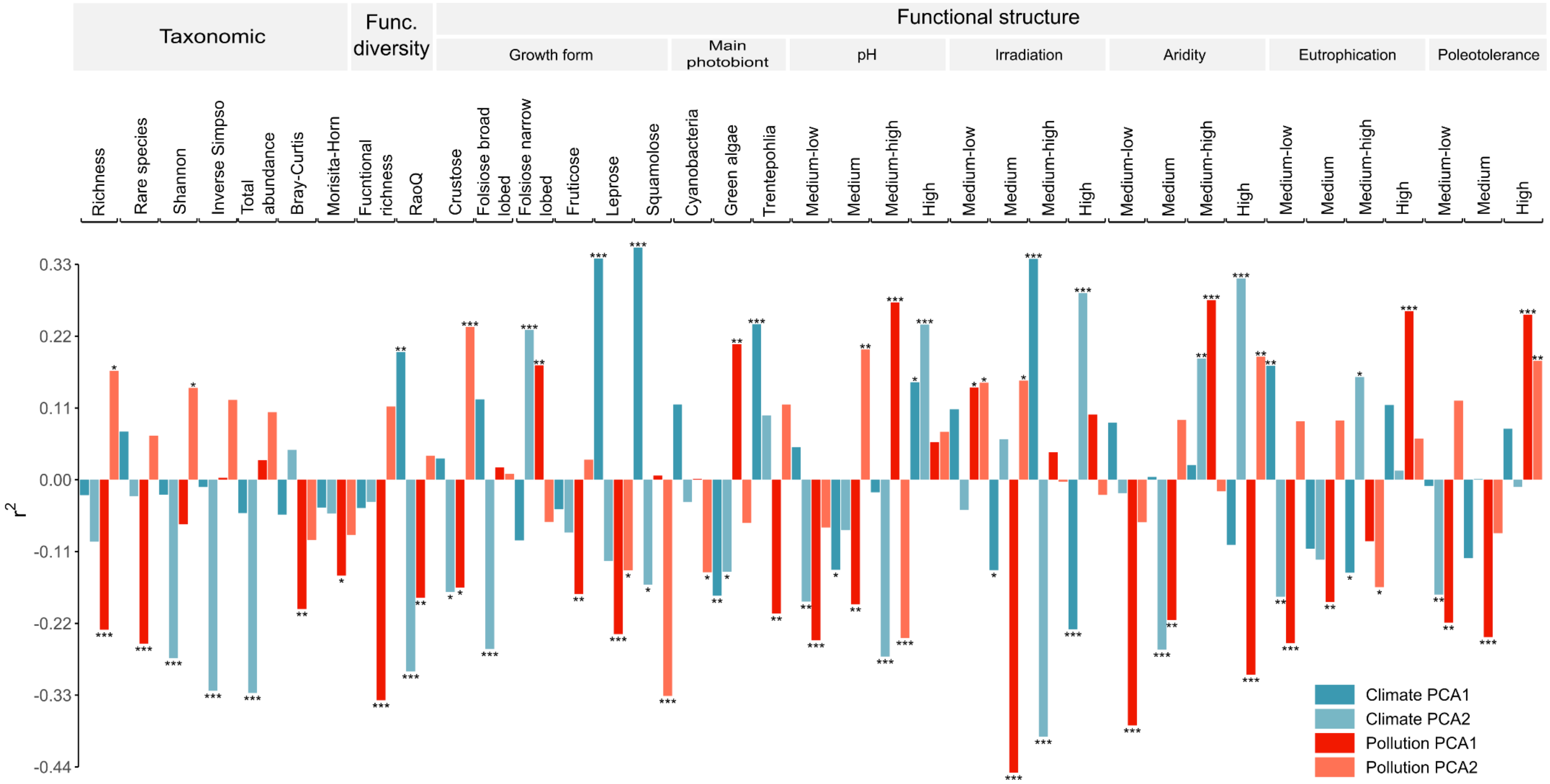

Figure S2.1.4: Spearman correlation coefficients between air pollution and macroclimate PCA axes (Climate A1, Climate A2, Pollution A1 and Pollution A2) and epiphytic lichens biodiversity metrics. N=219

Like for some taxonomic metrics, main photobiont type, pH of the substrate and the poleotolerance traits were excluded from the main results and are only presented in the supplementary material. The main photobiont showed no variation, as green algae were dominant across all cities (they represent on average 97.43% of all photobiont types). The poleotolerance and species substrate pH requirement were excluded as they showed the same patterns as the eutrophication trait (Fig. S1 and S2). Results for squamulose, leprose and cyanobacteria functional groups are also only presented in the supplementary material, as they showed very low overall abundance (less than 5 %). Trait-based indices were calculated with R software (R Core Team, 2022), function *dbFD* from the *FD* package (Laliberté et al., 2014).

Details for environmental variables

To quantify the overall importance of broad scale drivers on each biodiversity-based metric, a set of 26 environmental variables were gathered. These are intended to depict two main broad scale drivers in urban environments, air pollution and climate (Harlan and Ruddell, 2011). Note that both pollution and climate were derived from models working at continental scale, thus climate and air pollution values show little variance within each city sites, i.e., they represent environmental gradients working at the broad spatial scale. Climate and air pollution values were extracted for each of the 219 sampling sites (the average values, per city, can be seen in Table S3).

Table S2.1.3: Bioclimatic and air pollution environmental variables, averaged by city. Bioclimatic variables are coded based on (Karger D.N. et al., 2018; Karger et al., 2017).

Environmental variable	Almada	Antwerp	Lisbon	Paris	Poznan	Tartu	Zurich
BIO1	16.70	10.56	16.57	11.14	8.91	5.65	10.27
BIO2	4.81	6.37	5.10	7.30	7.00	6.70	7.70
BIO3	32.44	29.93	33.18	31.99	26.40	21.42	29.38
BIO5	24.74	22.16	24.89	23.81	23.36	22.21	24.27
BIO4	335.86	524.98	343.05	544.43	707.18	828.44	648.12
BIO6	9.90	0.89	9.51	1.02	-3.12	-8.99	-2.07

BIO7	14.85	21.28	15.38	22.78	26.49	31.20	26.34
BIO8	13.74	13.02	13.48	12.14	18.21	16.66	18.64
BIO9	21.49	8.44	21.44	7.80	-0.36	-3.08	2.02
BIO10	21.49	17.99	21.44	18.96	18.54	17.02	19.23
BIO11	12.44	3.61	12.18	4.15	-0.36	-5.68	1.74
BIO12	698.64	795.26	659.52	603.97	517.12	607.70	1070.86
BIO13	105.14	76.53	102.97	56.22	74.32	78.58	126.17
BIO14	3.07	48.85	3.15	43.33	26.15	27.42	67.31
BIO15	63.57	13.50	64.64	8.44	32.35	34.58	24.17
BIO16	314.29	224.65	305.24	165.39	208.38	234.52	364.23
BIO17	13.50	156.65	13.39	132.11	82.41	82.70	204.31
BIO18	13.50	221.12	13.39	151.31	189.50	222.58	362.89
BIO19	299.50	169.59	291.88	154.75	82.41	90.73	211.74
Ammonia (NH_3)	0.87	4.14	1.63	5.98	2.26	0.43	4.50
Reduced Nitrogen (redN)	1.18	4.68	1.75	5.68	2.83	0.70	4.75
Oxidized Nitrogen (OxN)	0.45	1.08	0.44	0.75	0.76	0.29	0.98
Particulate matter less than 2.5 µm (PM2.5)	9.59	13.35	13.95	11.66	13.92	8.38	13.39
Particulate matter less than 10 µm (PM10)	15.82	16.93	19.82	15.03	15.94	9.47	16.39
Sulfur Dioxide (SO_2)	5.26	7.02	2.39	1.23	2.90	0.40	1.36
Nitrogen Oxide (NO_x)	8.56	14.98	10.71	19.43	4.75	1.70	10.52

To characterize broad scale climatic conditions, 19 bioclimatic variables representing air temperature and precipitation annual and seasonal values, were retrieved from the

CHELSA database (Karger D.N. et al., 2018; Karger et al., 2017), at the maximum available spatial resolution of 1 km, and corresponding to modelled average values for the 1979-2013 time period (Table S4). These were preferred over raw maximum, mean and minimum temperature and precipitation variables as the former are potentially biologically more meaningful (Title and Bemmels, 2018). More detailed information on each climatic variable can be seen in (O'Donnell and Ignizio, 2012).

Table S2.1.4: Climate variables used, divided by group (temperature; precipitation), type (annual range; seasonal) and described based on (Karger D.N. et al., 2018; Karger et al., 2017).

Variable group	Type	Variable	Description
Air temperature	annual	Bio 1	annual mean temperature
		Bio 2	mean diurnal range
		Bio 3	isothermality
		Bio 7	temperature annual range
	seasonal	Bio 4	temperature seasonality
		Bio 5	maximum mean temperature of warmest month
		Bio 6	minimum temperature of coldest month
		Bio 8	mean temperature of wettest quarter
		Bio 9	mean temperature of driest quarter
		Bio 10	mean temperature of warmest quarter
		Bio 11	mean temperature of coldest quarter
Precipitation	annual	Bio 12	annual precipitation
	seasonal	Bio 13	precipitation of wettest month
		Bio 14	precipitation of driest month
		Bio 15	precipitation seasonality
		Bio 16	precipitation of wettest quarter
		Bio 17	precipitation of driest quarter
		Bio 18	precipitation of warmest quarter
		Bio 19	precipitation of coldest quarter

For broad scale air pollution, redN and O_xN deposition ($mg.m^{-2}$) and NH_3, SO_2, NO_x, PM2.5 and PM10 air concentration ($\mu g.m^{-3}$), for the year 2018, were retrieved from the EMEP (Fagerli et al., 2019) at the maximum available spatial resolution (11 km). These pollutants were selected as they have been seen to impact the diversity of epiphytic lichen vegetation across Europe (Loppi and Pirintsos, 2000; Van Herk et al., 2003). The entrapment of airborne particulate matter on lichen thallus is well known (Garty and Garty-Spitz, 2015), despite majority of works relating lichens response to particulate matter focus on fine particles (PM2.5). Still, coarser (PM10) particles can also be transported and entrapped on lichens (Massimi et al., 2019) and although only few studies have addressed PM10 effects on epiphytic lichens, these have observed a decline in lichens species richness (Matos et al., 2019) and LDV of oligotrophic and nitrophytic lichens, suggesting its role as a meaningful eutrophication pollutant (Giordani and Malaspina, 2017).

Broad scale land-cover, including total industrial or agricultural area, can be related with overall air pollution, but variables that quantify air pollution are preferable as the specific pollutant can be identified. Long-term averages of climate, contrasting with short-term (annual) air pollution values, were used as we expect lichens to respond rapidly to changes in air quality, because it is a high intensity environmental driver, capable of causing species loss (Lättman et al., 2014); while for climate the response of lichen is expected to be slower than for pollution (van Herk et al., 2002). Still, because we are dealing with macro scale gradients, we do not expect that the relative position of cities along the continental spatial gradient regarding climate would change if we used climate annual data. A common European model was used for pollution and climate to avoid the pitfalls of using national or city specific models, that would use different modelling approaches. Thus, this ensured that all values are of the same quality and can be used to compare sites and cities.

To account for the remaining broad scale variability, not represented by climate and air pollution, an additional categorical factor ("other", corresponding to city identity and meant to represent all other potential drivers working at the broad scale, aside from air pollution and climate) was added to the analysis. This factor is expected to represent the remaining city characteristics varying at the broad scale (i.e., differentiating cities), such as city size, air pollution legacy, or management policies.

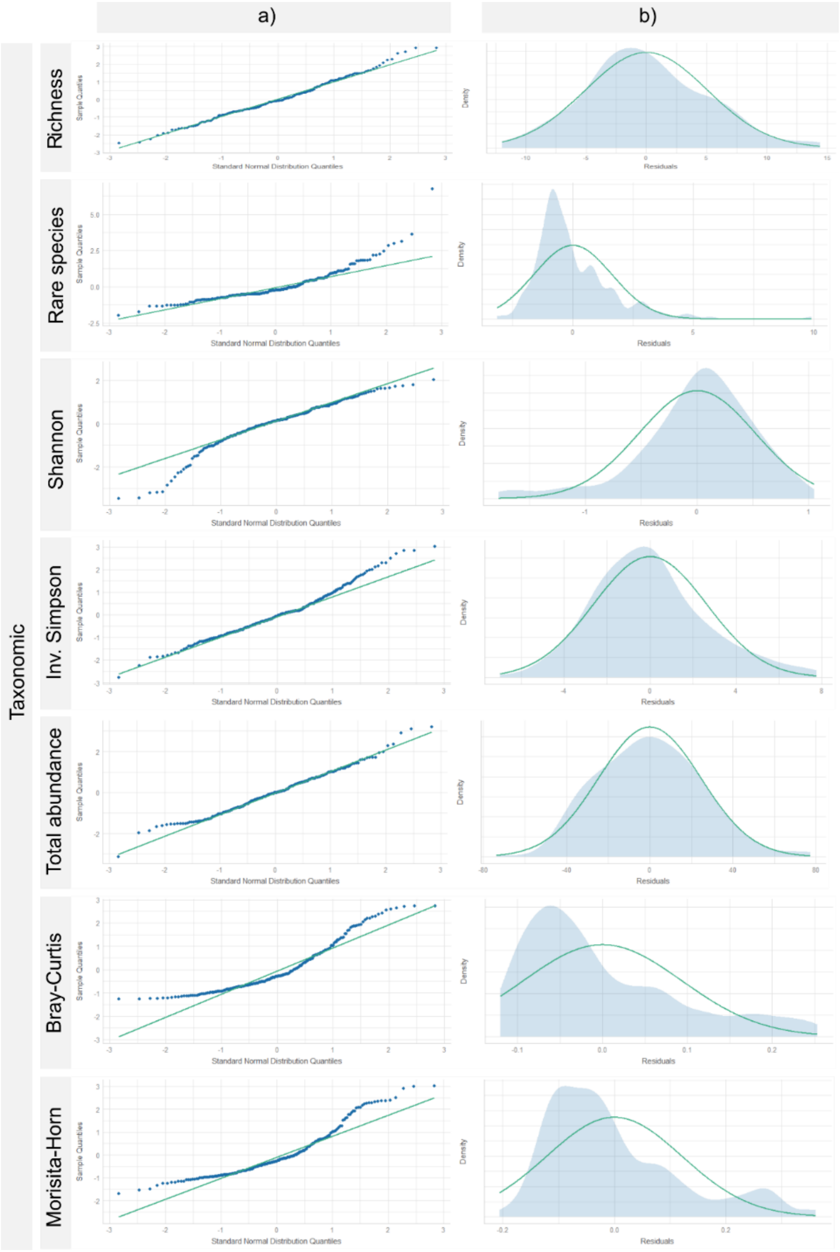

Figure S2.1.5: Linear regression model (Fig. 5) residuals normality plots for each lichen taxonomic-based metric, useful for determining if the residuals follow a normal distribution. a) Quantile-quantile plot, representing the probability of the linear regression models residuals to follow a normal distribution. The more the dots (blue) fall along the straight line (green), the highest the likelihood of the residuals to follow a normal distribution. b) Density plots, representing the distribution of the linear regression models residuals. The closest the resemble between the residuals distribution (blue) to the normal curve (green), the highest the likelihood of the residuals to follow a normal distribution.

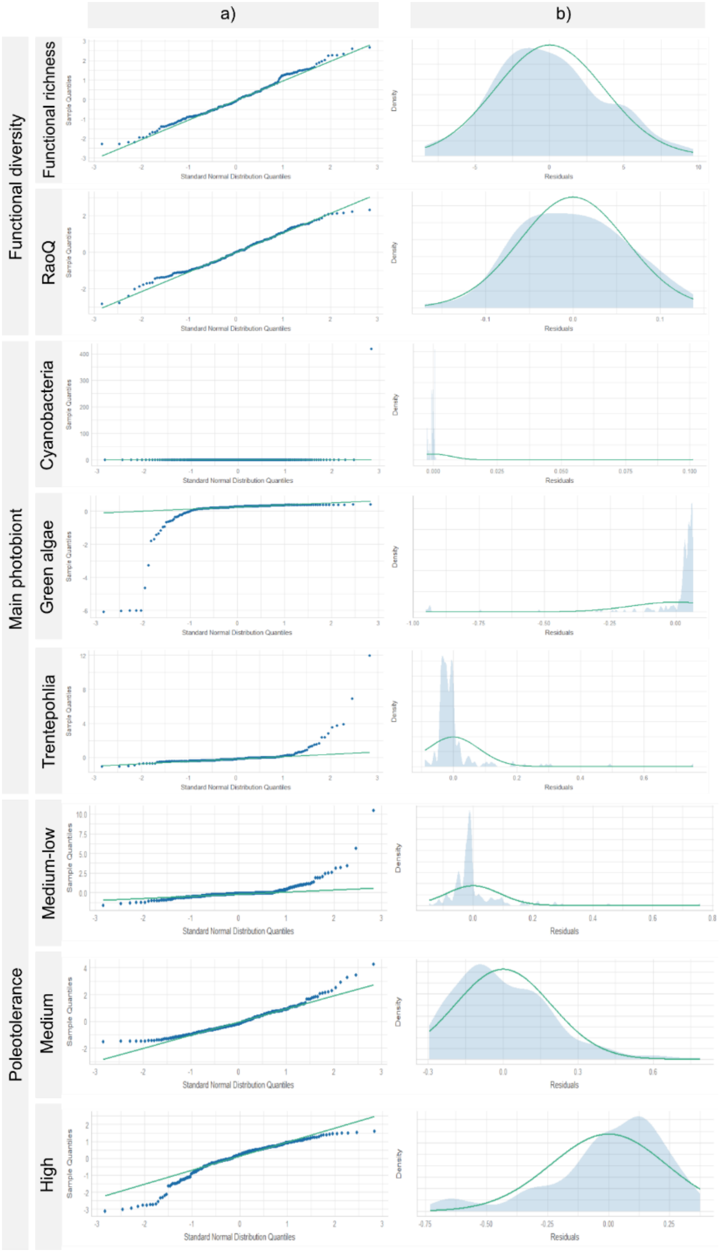

Figure S2.1.6: Linear regression model (Fig. 5) residuals normality plots for each lichen functional diversity and functional structure (main photobiont and poleotolerance traits) -based metric, useful for determining if the residuals follow a normal distribution. a) Quantile-quantile plot, representing the probability of the linear regression models residuals to follow a normal distribution. The more the dots (blue) fall along the straight line (green), the highest the likelihood of the residuals to follow a normal distribution. b) Density plots, representing the distribution of the linear regression models residuals. The closest the resemble between the residuals distribution (blue) to the normal curve (green), the highest the likelihood of the residuals to follow a normal distribution.

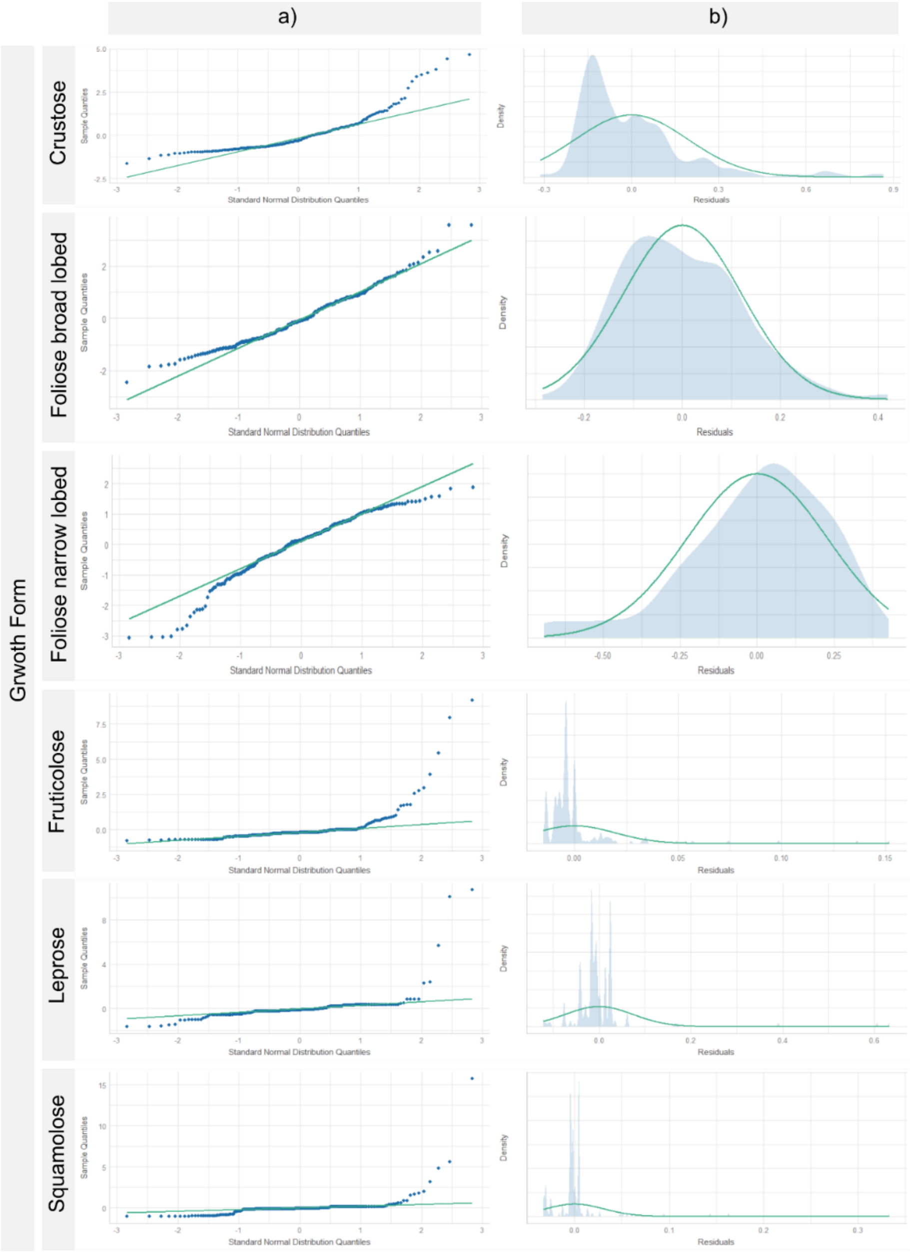

Figure S2.1.7: Linear regression model (Fig. 5) residuals normality plots for each lichen functional diversity and functional structure (growth form) -based metric, useful for determining if the residuals follow a normal distribution. a) Quantile-quantile plot, representing the probability of the linear regression models residuals to follow a normal distribution. The more the dots (blue) fall along the straight line (green), the highest the likelihood of the residuals to follow a normal distribution. b) Density plots, representing the distribution of the linear regression models residuals. The closest the resemble between the residuals distribution (blue) to the normal curve (green), the highest the likelihood of the residuals to follow a normal distribution.

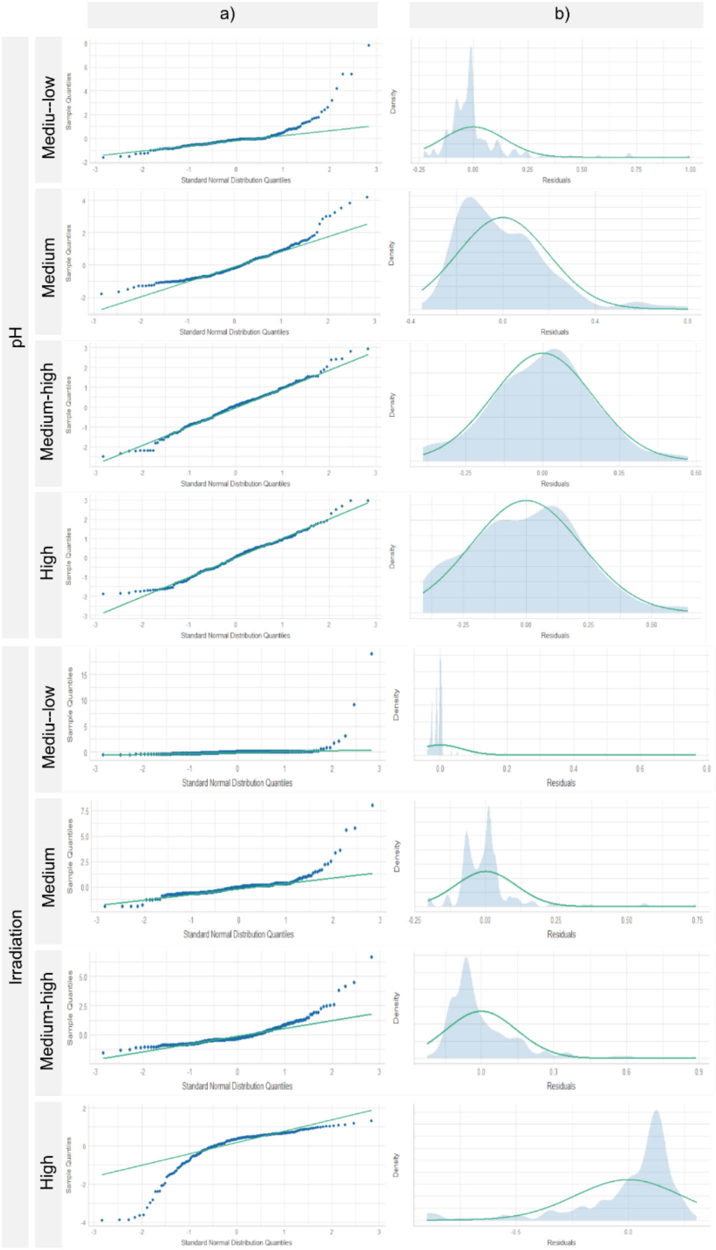

Figure S2.1.8: Linear regression model (Fig. 5) residuals normality plots for each functional structure (species substrate pH tolerance (pH) and irradiation traits) -based metric, useful for determining if the residuals follow a normal distribution. a) Quantile-quantile plot, representing the probability of the linear regression models residuals to follow a normal distribution. The more the dots (blue) fall along the straight line (green), the highest the likelihood of the residuals to follow a normal distribution. b) Density plots, representing the distribution of the linear regression models residuals. The closest the resemble between the residuals distribution (blue) to the normal curve (green), the highest the likelihood of the residuals to follow a normal distribution.

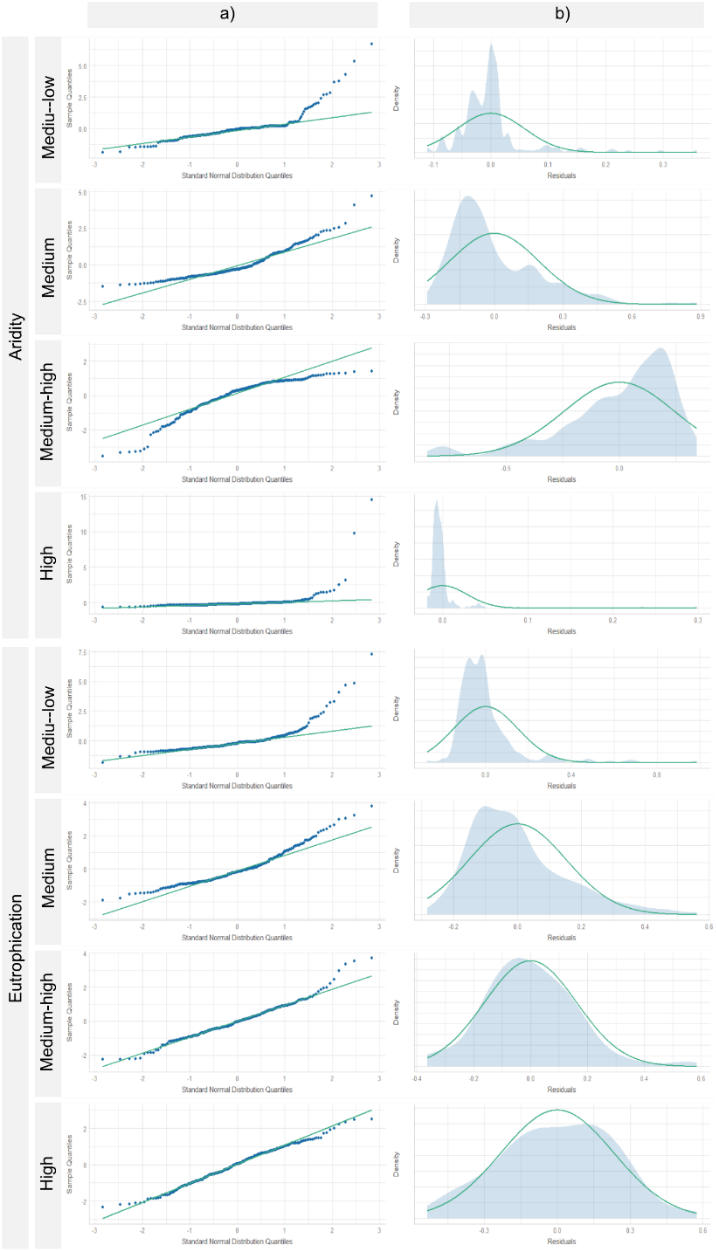

Figure S2.1.9: Linear regression model (Fig. 5) residuals normality plots for each functional structure (aridity and eutrophication traits) -based metric, useful for determining if the residuals follow a normal distribution. a) Quantile-quantile plot, representing the probability of the linear regression models residuals to follow a normal distribution. The more the dots (blue) fall along the straight line (green), the highest the likelihood of the residuals to follow a normal distribution. b) Density plots, representing the distribution of the linear regression models residuals. The closest the resemble between the residuals distribution (blue) to the normal curve (green), the highest the likelihood of the residuals to follow a normal distribution.

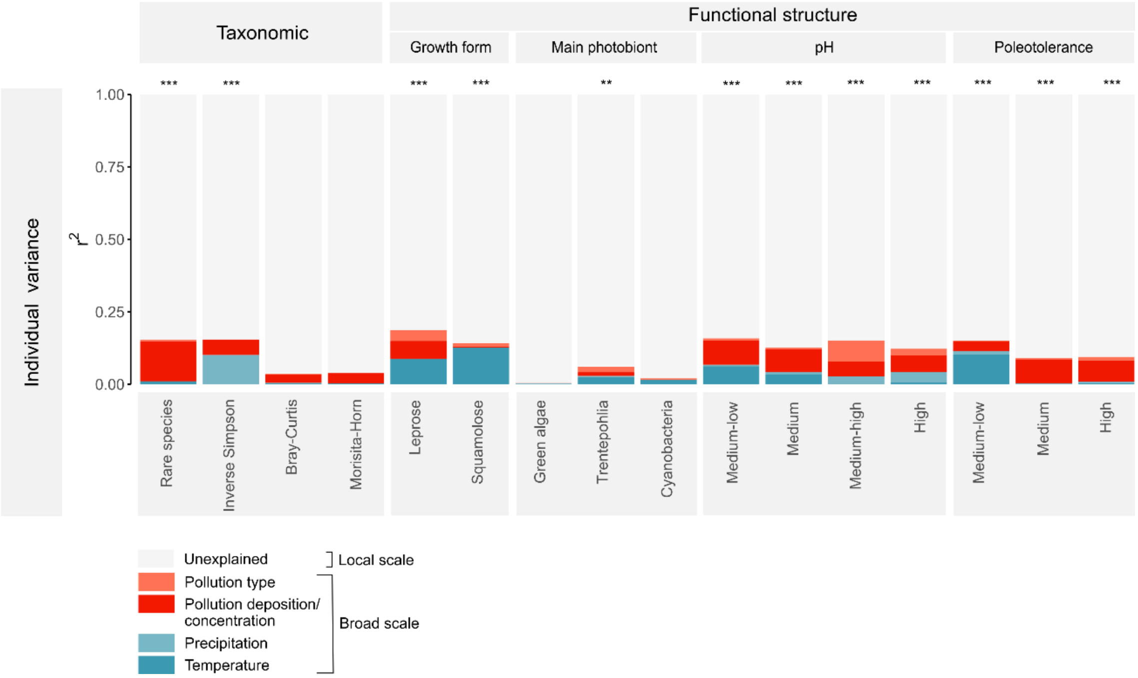

Figure S2.1.10: Variance partitioning of broad scale drivers for each lichen biodiversity metrics. Metrics are grouped into taxonomic (Rare species, Inverse Simpson, Bray-Curtis and Morisita-Horn) and functional structure (Growth form, Main photobiont type, Species substrate pH tolerance and Poleotolerance). Only metrics not represent on the main text (Fig. 5) are shown here. Temperature reflects the first climate PCA axis, related to a broad scale temperature gradient. Precipitation reflects the second climate PCA axis, related to a broad scale precipitation gradient. Air pollution deposition/concentration reflects the first air pollution PCA axis, related to a broad scale gradient of air pollution. Air pollution type reflects the second air pollution PCA axis, related to a broad scale gradient of air pollution ranging from N-based pollutants on one side, to sulfur dioxide and particulate matter compounds on the other. Significance of the model is indicated in superscript: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

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2.2 Non-linear responses of ecological indicators to urban environmental drivers across Europe

Details on sampling sites selection and stratification

Sampling sites were selected within the “Green Urban Areas” class of the pan-European land-cover classification, the European Urban Atlas (EEA, 2018), ensuring land-use typology and intensity. For each patch of “Green Urban Areas”, patch size and the connectivity index (Gustafson and Parker, 1992) were calculated (see below). Sites were classified into six size classes ([0 - 0.6[, [0.6 – 1.2[, [1.2 – 2.4[, [2.4 – 4.8[, [4.8 – 9.6[and >9.6 ha) and six classes of connectivity ([0 – 18000[, [18000 – 36000[, [36000 – 72000[, [72000 – 144000[, [144000 – 288000[and >288000 – no units). From the initially available 1582 patches (across all cities), up to 36 were selected for each city, stratified by size and by the proximity index. Selection within each combination of classes (maximum of 36 combinations) was done randomly. This resulted in a total of 219 sampling sites across all cities. For some cities (Fig. S1) less than 36 patches were selected as some combinations were not available.

The proximity index (Gustafson and Parker, 1992) is a measure of fragmentation/isolation of each focal patch. Briefly, it quantifies the amount of available habitat in neighbouring patches, each neighbour patch area being weighted by the square of the distance to the focal patch. The neighbourhood of each focal patch is defined by user-specified radius. In this case, we selected a 5000 m area to include the maximum distance for which an Urban Atlas was available. Note that because the distance to each patch is squared, nearby patches have a much higher weight on the index than those further away from the focal patch, thus, looking at longer distances would cause insignificant changes in this metric. Based on previous knowledge, we expected all neighboring patches with trees to influence lichens in the focal patches, e.g., due to its mitigation of the urban heat-island (Munzi et al., 2014) or air pollution (Matos et al., 2019). To account for this, all land-cover types that could host a significant number of trees were included as potential habitat in the calculation of the proximity index, namely “Discontinuous Low-Density Urban Fabric” and “Discontinuous very-low-density urban fabric” (mainly small houses with gardens), “Permanent Crops” (orchards), and “Forests”. The Proximity Index takes the highest values when the focal patch is surrounded by nearby large similar habitats and takes the value of zero when the focal patch has no neighbours of the same habitat.

Details on epiphytic lichens European standard sampling methodology

Epiphytic lichen diversity was sampled following the European standard method (Asta et al., 2002, Cristofolini et al., 2014), has been used in multiple studies to quantify lichen biodiversity in gradients of air pollution, climate, land-use intensity, management and other (Lättman et al., 2014, McCarthy et al., 2009, Munzi et al., 2014). The use of a standard method for lichen sampling is unprecedented in urban areas, at this scale, and greatly contributed to reducing variability due to disparate sampling protocols. Within each patch, the point furthest from the margin was taken as the patch centroid. The nearest area with at least 30% cover by trees and grass, within a 50 m radius buffer, was then selected as the sampling site. On each sampling site, the four trees closest to the centroid were selected, ensuring they were within the range of conditions required by the sampling protocol: healthy trees with an upright main trunk (< 20º deviation from vertical), without branches or injuries up to two meters, with circumference at the sampling height (one to two meters from the ground) between 50 and 250 cm. A maximum distance of 50 m between trees was established. It is important to highlight that to reduce the sampling variability to the feasible minimum, tree bark and tree size were homogenized as much as possible: as it is not possible to select a single phorophyte species across all cities, sampling was narrowed to phorophytes sharing the same bark roughness (intermediate roughness was chosen as it could be found in all cities). Additionally, tree size was limited to medium size trees (50 to 250 cm perimeter at sampling height). On each tree, a sampling grid (50 x 10 cm, divided into 5 squares) was placed on the four main aspects (N, S, E, W, total 2000 cm2 sampling area per tree). All lichens within the grid were either identified on location or collected for later laboratory identification. Species abundance was determined based on the number of squares in which they occur (maximum abundance value of 20) and then averaged by site. Species nomenclature followed (Nimis and Martellos, 2021). A total of 140 species were identified, nine of which to the genus level only (Table S1).

Table S2.2.1: List of total species identified (N=140) and respective functional characterization based on the growth form, type of photobiont and tolerance to different levels of aridity and eutrophication. Nomenclature followed (Nimis and Martellos, 2021).

Scientific name	**Growth form[1]**	**Photobiont[2]**	**Aridity[3]**	**Eutrophication[4]**
Acrocordia cavata	c	t	2	1
Alyxoria varia	c	t	3	2
Amandinea punctata	c	g	4	4
Arthonia dispersa	c	t	3	1
Arthonia mediella	c	t	3	1
Arthonia radiata	c	t	3	3
Athallia cerinelloides	c	g	4	4
Athallia pyracea	c	g	4	4
*Arthopyrenia sp. **	-	-	-	-
Bacidia arceutina	c	g	3	2
Bacidia rubella	c	g	3	3
Bacidia subincompta	c	g	2	2
Bacidina arnoldiana	c	g	2	3
Biatoridium monasteriense	c	g	2	3
Buellia griseovirens	c	g	3	1
Caloplaca cerina	c	g	4	4
Candelaria concolor	fn	g	4	5
Candelariella reflexa	c	g	3	5
Candelariella vitellina	c	g	4	5
Candelariella xanthostigma	c	g	3	3
Catapyrenium psoromoides	sq	g	3	2
Catillaria nigroclavata	c	g	3	3
Chaenotheca chrysocephala	c	g	3	1
Chaenotheca furfuracea	c	g	2	1

Chaenotheca phaeocephala	c	g	2	1
Chaenotheca stemonea	c	g	1	1
Chaenotheca trichialis	c	g	2	1
Chaenotheca xyloxena	c	g	2	1
Chrysothrix candelaris	l	g	3	1
*Cladonia sp. **	-	-	-	-
Cladonia fimbriata	f	g	3	3
Coenogonium pineti	c	t	3	2
Coenogonium tavaresianum	c	t	1	1
Collema furfuraceum	fb	c	2	3
*Cyphelium sp. **	-	-	-	-
Dendrographa decolorans	c	t	2	3
Diploicia canescens	c	g	3	4
Evernia prunastri	f	g	3	3
Flavoparmelia caperata	fb	g	3	3
Flavoparmelia soredians	fb	g	3	3
Flavoplaca citrina	c	g	4	5
Gyalecta fagicola	c	t	2	1
Hyperphyscia adglutinata	fn	g	4	5
Hypogymnia physodes	fn	g	3	2
Hypogymnia tubulosa	fn	g	3	2
Hypogymnia tubulosa f. farinosa	fn	g	3	2
Hypotrachyna revoluta	fb	g	3	3
Lecania cyrtella	c	g	4	3
Lecania naegelii	c	g	3	3
Lecanographa amylacea	c	t	2	1
Lecanographa lyncea	c	t	2	1

*Lecanora sp. **	-	-	-	-
Lecanora albella	c	g	2	1
Lecanora allophana	c	g	4	3
Lecanora argentata	c	g	3	2
Lecanora carpinea	c	g	4	3
Lecanora chlarotera	c	g	4	5
Lecanora conizaeoides	c	g	3	3
Lecanora expallens	c	g	3	2
Lecanora populicola	c	g	3	4
Lecanora pulicaris	c	g	4	2
Lecanora strobilina	c	g	3	1
Lecanora symmicta	c	g	3	2
Lecidella elaeochroma	c	g	5	4
Lecidella euphorea	c	g	4	3
Lepra albescens	c	g	3	3
Lepra amara	c	g	3	3
*Lepraria sp. **	-	-	-	-
Lepraria lobificans	l	g	4	1
Leprocaulon quisquiliare	fn	g	3	3
Leptogium brebissonii	fb	c	2	2
*Melanelixia sp. **	-	-	-	-
Melanelixia glabratula	fb	g	3	3
Melanelixia subaurifera	fb	g	3	3
Melanohalea exasperatula	fb	g	3	3
Melanohalea laciniatula	fb	g	2	3
Micarea prasina	c	g	2	1
Myriolecis dispersa	c	t	5	4

Myriolecis hagenii	c	g	5	4
Myriolecis sambuci	c	g	3	3
Normandina pulchella	sq	g	3	3
Ochrolechia szatalaensis	c	g	3	1
Opegrapha vulgata	c	t	2	1
Parmelia saxatilis	fb	g	3	3
Parmelia sulcata	fb	g	3	3
Parmelina tiliacea	fb	g	3	3
Parmeliopsis ambigua	fn	g	4	1
Parmotrema hypoleucinum	fb	g	2	2
Parmotrema perlatum	fb	g	3	2
Parmotrema robustum	fb	g	2	1
*Pertusaria sp. **	-	-	-	-
Pertusaria heterochroa	fn	g	4	5
Pertusaria hymenea	c	g	3	2
Phaeophyscia cernohorskyi	fn	g	4	4
Phaeophyscia endophoenicea	fn	g	3	3
Phaeophyscia hirsuta	fn	g	4	4
Phaeophyscia nigricans	fn	g	4	4
Phaeophyscia orbicularis	fn	g	4	5
Phlyctis argena	c	g	3	2
Physcia adscendens	fn	g	4	5
Physcia aipolia	fn	g	3	4
Physcia caesia	fb	g	4	5
Physcia clementei	fn	g	3	3
Physcia dubia	fn	g	4	5
Physcia stellaris	fn	g	3	4

Physcia tenella	fn	g	4	4
Physcia tribacioides	fn	g	2	3
*Physciaceae sp. **	-	-	-	-
Physciella chloantha	fn	g	3	4
Physconia distorta	fn	g	4	4
Physconia enteroxantha	fn	g	3	4
Physconia grisea	fn	g	3	5
Physconia perisidiosa	fn	g	3	3
Platismatia glauca	fb	g	3	2
Pleurosticta acetabulum	fb	g	4	3
Polycauliona polycarpa	fn	g	4	3
Porina aenea	c	t	3	1
Pseudoschismatomma rufescens	c	t	2	1
Punctelia jeckeri	fb	g	3	3
Punctelia subrudecta	fb	g	3	3
Pyrenula macrospora	c	t	2	1
Ramalina calicaris	f	g	2	2
Ramalina farinacea	f	g	2	2
Ramalina fastigiata	f	g	3	3
Ramalina fraxinea	f	g	3	3
Ramalina lacera	f	g	2	3
Rinodina capensis	c	g	3	2
Rinodina ficta	c	g	4	4
Rinodina oleae	c	g	4	3
Rinodina pyrina	c	g	3	3
Ropalospora viridis	c	g	2	1
Sclerophora pallida	c	t	2	1

Scoliciosporum chorococcum	c	g	3	3
Teloschistes chrysophthalmos	f	g	4	3
Trapeliopsis flexuosa	c	g	4	2
Usnea rubicunda	f	g	2	2
Waynea stoechadiana	sq	g	3	3
Xanthomendoza fulva	fn	g	4	4
Xanthoparmelia conspersa	fb	g	4	3
Xanthoria parietina	fb	g	4	4

[*] Species only identified to the genus level

[1] c – crustose; fn - foliose narrow lobe; fb - foliose broad lobe; sq – squamulose; l – leprose; f – fruticulose.

[2] c – cyanobacteria; g – *Chlorococcoid*; t – *Trentepohlia*.

[3] 1 - hygrophytic; 2 - rather hygrophytic; 3 – mesophytic; 4 – rather xerophytic; 5 - xerophytic.

[4] 1- oligotrophic; 2 - rather oligotrophic; 3 - mesotrophic; 4 - rather nitrophytic; 5 - nitrophytic.

Details on the calculation of lichen biodiversity metrics

Taxonomic and trait-based metrics were computed. Regarding the taxonomic metrics, three metrics were calculated. Species richness corresponds to the total number of species per site. The Shannon diversity Index is a measure of species diversity in a community, with higher values corresponding to higher species diversity. Total abundance corresponds to the sum of all lichen species abundance per site (same as the Lichen Diversity Value in the lichen European Standard Method). Regarding the trait-based metrics, indices representing both functional diversity and functional structure were computed based only on data from specimens identified to the species level (Table S1). This was done based on four categorical traits known to respond to temperature, water availability and anthropic pressure (Table S2). Growth form, main photobiont type (green algae other than *Trentepohlia*, *Trentepohlia* and cyanobacteria), tolerance to aridity, tolerance to eutrophication. Trait information was retrieved from the ITALIC database (Nimis and Martellos, 2021), and the maximum value for each species was used (trait classification are ordinal in the database). For species trait classification see Table S1. These traits were selected as they respond to environmental change influencing lichen growth, survival and, ultimately, fitness. Growth form and the main photobiont type traits are known to respond to both air pollution and climate (Koch et al., 2019, Matos et al., 2015, Aptroot and Van Herk, 2007). Eutrophication tolerance shows species' tolerance to nitrogen in the environment and has also been used to track air pollution (Pinho et al., 2011, Giordani and Malaspina, 2017). Lastly, the aridity trait is known to respond to climatic conditions (Munzi et al., 2014). Species that could not be identified to the species level and for which we could not retrieve specific trait classification were excluded from trait-based metrics calculation (Table S1). In terms of functional diversity metrics, we calculated functional richness and Rao's quadratic entropy (RaoQ), both as multi-trait indices, computed only with the traits and corresponding functional groups (the different categories of each categorical trait) included in the results section. Functional richness represents the functional space occupied by the different functional groups (per sampling site), the trait diversity tally of species richness. RaoQ corresponds to the functional dispersion, reflecting the variety of functional strategies, weighted by abundance. The community weighted mean (CWM) was included to represent the communities' functional structure and represents the mean trait value in the community weighted by the abundance, i.e., the proportion of summed species abundances belonging to the same functional group of each trait.

Table S2.2.2: Lichen species traits and related functional groups used in the study, described following (Nimis and Martellos, 2021).

Trait	**Functional group**	**Description**
Growth form	crustose	thallus firmly and entirely attached to the substrate by the lower surface
	foliose broad-lobed	thallus partly attached to the substrate, with distinct upper and lower surfaces and broad lobes
	foliose narrow-lobed	thallus partly attached to the substrate, with distinct upper and lower surfaces and narrow lobes
	fruticose	thallus attached to the substrate by one single point and with rounded or flattened branches
	leprose	thallus like crustose but surface thallus with a granular mass appearance and always decorticated
	squamulose	thallus composed of small scales
Main photobiont type	cyanobacteria	lichens with cyanobacteria
	green algae	lichens with green algae other than *Trentepohlia*
	trentepohlia	lichens with *Trentepohlia* (green algae)
Aridity tolerance	hygrophytic	in sites with a very high frequency of fog
	rather hygrophytic	intermediate between hygrophytic and mesophytic
	mesophytic	Intermediate tolerance to aridity
	rather xerophytic	xerophytic but absent from extremely arid stands
	xerophytic	very xerophytic
Eutrophication tolerance	oligotrophic	no eutrophication
	rather oligotrophic	very weak eutrophication
	mesotrophic	Intermediate tolerance to eutrophication
	rather nitrophytic	rather high eutrophication

	nitrophytic	very high eutrophication

Details for environmental variables

To quantify the overall importance of broad/continental and local/City scale drivers on each biodiversity-based metric, a set of 25 environmental variables were gathered. These are intended to depict three main drivers in urban environments, temperature, water availability and anthropic pressure (Fig. S1).

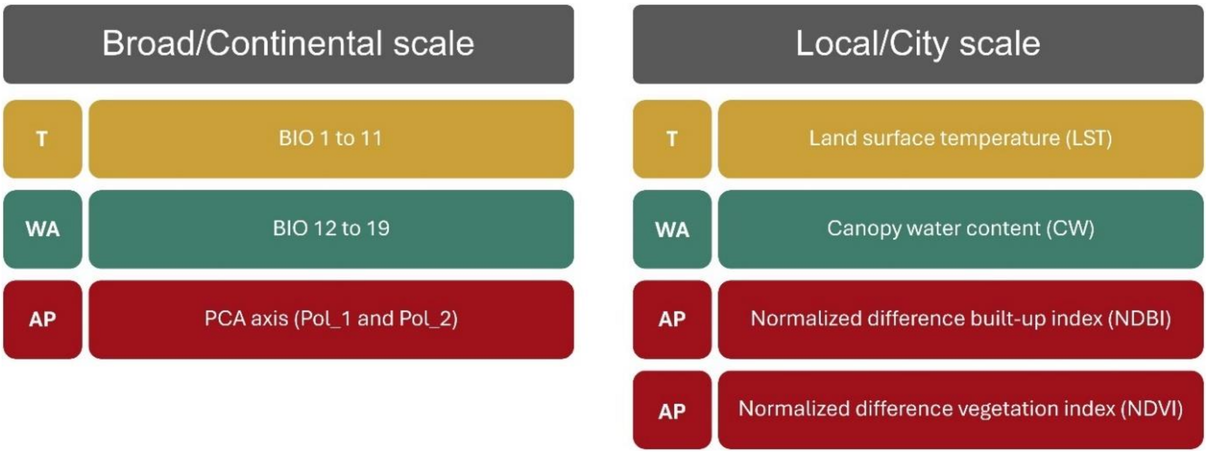

Figure S2.2.1: Overall scheme of the main urban environmental drivers tested and th (T - Temperature; WA - Water availability; AP - Anthropic pressure) and the variables related to each, depending on the spatial scale considered.

To characterize broad scale climatic conditions (Table S3), 19 bioclimatic variables representing air temperature and precipitation annual and seasonal values, were retrieved from the CHELSA database (Karger et al., 2017), at the maximum available spatial resolution of 1 km, and corresponding to modelled average values for the 1979-2013 time period (Table S3). These were preferred over raw maximum, mean and minimum temperature and precipitation variables as the former are potentially biologically more meaningful (Title and Bemmels, 2018). More detailed information on each climatic variable can be seen in (O'Donnell and Ignizio, 2012).

Table S2.2.3: Broad scale temperature and water availability related variables used, divided by group (air temperature; precipitation), type (annual range; seasonal) and described based on (Karger et al., 2017). Units: ºC – degrees Celsius; mm – millimetres).

Macroclimate main driver	Type	Code	Description
Air temperature	annual	Bio 1	annual mean temperature

(Units: ºC)		Bio 3	isothermality
	range	Bio 2	mean diurnal range
		Bio 7	temperature annual range
	seasonal	Bio 4	temperature seasonality
		Bio 5	maximum temperature of warmest month
		Bio 6	minimum temperature of coldest month
		Bio 8	mean temperature of wettest quarter
		Bio 9	mean temperature of driest quarter
		Bio 10	mean temperature of warmest quarter
		Bio 11	mean temperature of coldest quarter
Precipitation (Units: mm)	annual	Bio 12	annual precipitation
	seasonal	Bio 13	precipitation of wettest month
		Bio 14	precipitation of driest month
		Bio 15	precipitation seasonality
		Bio 16	precipitation of wettest quarter
		Bio 17	precipitation of driest quarter
		Bio 18	precipitation of warmest quarter
		Bio 19	precipitation of coldest quarter

For broad scale anthropic pressure (Table S4), redN and O_xN deposition (mg.m^{-2}) and NH_3, SO_2, NO_x, PM2.5 and PM10 air concentration (µg.m^{-3}), for the year 2018, were retrieved from the EMEP (Fagerli et al., 2019) at the maximum available spatial resolution (11 km). These pollutants were selected as they have been seen to impact the diversity of epiphytic lichen vegetation across Europe (Loppi and Pirintsos, 2000, Van Herk et al., 2003). The entrapment of airborne particulate matter on lichen thallus is well known (Garty and Garty-Spitz, 2015), despite majority of works relating lichens response to particulate matter focus on fine particles (PM2.5). Still, coarser (PM10) particles can also be transported and entrapped on lichens (Massimi et al., 2019) and although only few studies have addressed PM10 effects on epiphytic lichens, these have observed a decline in lichens species richness (Matos et al., 2019) and LDV of oligotrophic and nitrophytic lichens, suggesting its role as a meaningful eutrophication pollutant (Giordani and Malaspina, 2017).

Table S2.2.4: Broad scale anthropic pressure related variables. Units: m – meters; m^2 – square meter.

Type	**Macroair pollution driver**	**Code**	**Description**
Nitrogen based pollutant	Ammonia (Units: µg/m^3)	(NH_3)	Air concentration of Ammonia (NH_3).
	Reduced Nitrogen (Units: µg/m^2)	(redN)	Deposition of both ammonia (NH_3) and ammonium ions (NH_4^+)
	Oxidized Nitrogen (Units: µg/m^2)	(OxN)	Deposition of nitrogen oxides (NO and NO_2), nitric acid (HNO_3), and nitrate-containing particles.
	Nitrogen Oxide (Units: µg/m^3)	(NO_x)	Air concentration of both nitric oxide (NO) and nitrogen dioxide (NO_2).
Sulphur based pollutant	Sulphur Dioxide (Units: µg/m^3)	(SO_2)	Air concentration of Sulphur Dioxide (SO_2).
Particulate matter	Particulate matter less than 2.5 µm (Units: µg/m^3)	(PM2.5)	Air concentration of primary particulate matter (PM) with aerodynamic diameter up to 2.5 µm, originating directly from anthropogenic emissions.
	Particulate matter less than 10 µm (Units: µg/m^3)	(PM10)	Air concentration of primary particulate matter (PM) with aerodynamic diameter up to 10 µm, originating directly from anthropogenic emissions.

Finally, local scale temperature, water availability and anthropic pressure related variables were retrieved from remote sensing (Table S5). Values were retrieved for winter and summer from 2015 to 2019, selecting the images with less cloud cover for each year [see Casanelles-Abella et al., (2024) and its supporting material for more details].

Table S2.2.5: Local scale temperature, water availability and anthropic pressure related variables used. Units: kg/m²– kilograms per square meter; ºC – Celsius degrees.

Type	**Local drivers**	**Code**	**Description**
Vegetation Radiometric	Canopy Water Content (Units: kg/m²)	CW	The Canopy Water Content (CW) is based on specific radiative transfer model associated with strong assumptions, particularly regarding canopy architecture (turbid medium model). For forests with large crowns, or any pixel showing strong heterogeneity such as pixels at the intersection between two different vegetation patches, results may be uncertain. This applied also to pixels where the neighboring ones are very different.
	Normalized difference vegetation index (Units: none)	NDVI	The Normalized Difference Vegetation Index (NDVI) exploit the strength and the vitality of the vegetation on the earth's surface. Besides the determination between of the vegetation and other objects it allows to detect the vitality of the vegetation. NDVI > 0.5 correspond to vigorous green areas, while 0.2 to 0.5 correspond to grasslands and shrublands. Between 0.0 and 0.2 for urban areas and bare soils and negative values for water bodies. The NDVI composes a measurement for the photosynthetic activity and is strongly in correlation with density and vitality of the vegetation.
Thermal Infrared	Land Surface Temperature (Units: ºC)	LST	The Land Surface Temperature (LST) is the radiative skin temperature of the land surface, as measured in the direction of the remote sensor. It is estimated from Top-of-Atmosphere brightness temperatures from the infrared spectral channels. Its estimation further depends on the albedo, the vegetation cover and the soil moisture. Note that LST is not the same as the air temperature that is included in daily weather reports.
Urban Radiometric	Normalized difference built-up index (Units: none)	NDBI	The Normalized Difference Built-Up Index (NDBI) was developed to differentiate build-up areas from bare soils (Zha et al., 2003). NDBI values for built-up areas varies between 0.1 and 0.3, while values > 0.3 tend to be bare soils.

Table S2.2.6: Spearman correlation matrix between lichen biodiversity metrics. Significance of the correlation is indicated in superscript: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Srich – Species richeness; Sha – Shannon Index; Tabun – Total abundance; FR - Functional richness; Crust – Crustose; Frut – Fruticolose; FBL - Foliose broad-lobed; FNL - Foliose narrow-lobed; Lepro – Leprose; Squamu – Squamulose; Cyano – Cyanobacteria; GreenA – *Chlorococcoid*; Trente – *Trentepohlia*; Hyg – Hygrophytic; RHyg – Rather hygrophytic; Meso – Mesophytic; RXero - Rather xerophytic; Xero – Xerophytic; Oli - Oligotrophic; Roli – Rather oligotrophic; Meso - Mesotrophic; RNitro - Rather nitrophytic.

	SRich	Sha	TAbun	FR	RaoQ	Crust	Frut	FBL	FNL	Lepro	Squamu	Cyano	GreenA	Trente	Hyg	RHyg	Meso	RXero	Xero	Oli	ROli	Meso	RNitro
Shannon	0.94***																						
Total abundance	0.71***	0.72***																					
Functional richness	0.96***	0.88***	0.64***																				
RaoQ	0.70***	0.73***	0.41***	0.72***																			
Crustose	0.39***	0.42***	0.10	0.44***	0.63***																		
Fruticose	0.54***	0.51***	0.31***	0.55***	0.41***	0.15*																	
Foliose broad-lobed	0.52***	0.51***	0.60***	0.51***	0.44***	0.00	0.28***																
Foliose narrow-lobed	-0.33***	-0.34***	-0.08	-0.39***	-0.59***	-0.70***	-0.31***	-0.26***															
Leprose	0.14*	0.11	-0.13	0.20**	0.29***	0.17*	0.35***	0.01	-0.33***														
Squamulose	0.22***	0.22**	0.12	0.25***	0.35***	0.07	0.06	0.11	-0.14*	0.07													
Cyanobacteria	0.13	0.15*	0.02	0.14*	0.14*	0.09	0.04	0.08	-0.11	-0.03	0.27***												
Chlorococcoid	-0.03	0.01	0.22**	-0.06	-0.18**	-0.26***	-0.11	0.22**	0.37***	-0.33***	-0.10	-0.17*											
Trentepohlia	0.14*	0.10	-0.12	0.17*	0.29***	0.37***	0.15*	-0.13	-0.27***	0.37***	0.13	0.17*	-0.91***										
Hygrophytic	0.56***	0.50***	0.24***	0.63***	0.56***	0.31***	0.52***	0.21**	-0.43***	0.50***	0.25***	0.18**	-0.33***	0.16*									
Rather hygrophytic	0.56***	0.50***	0.24***	0.63***	0.56***	0.31***	0.52***	0.21**	-0.43***	0.50***	0.25***	0.18**	-0.33***	0.38***	0.69***								
Mesophytic	0.60***	0.63***	0.36***	0.63***	0.77***	0.60***	0.43***	0.30***	-0.60***	0.29***	0.24***	0.11	-0.10	0.21**	0.48***	0.77***							

Rather xerophytic	-0.47***	-0.50***	-0.19**	-0.51***	-0.67***	-0.52***	-0.41***	-0.15*	0.75***	-0.36***	-0.21**	-0.12	0.36***	-0.27***	-0.52***	-0.52***	-0.85***						
Xerophytic	0.23***	0.16*	0.08	0.25***	0.13	0.21**	0.05	0.01	-0.04	0.01	-0.08	-0.05	-0.34***	0.40***	0.12	0.12	0.00	-0.05					
Oligotrophic	0.53***	0.51***	0.26***	0.58***	0.75***	0.65***	0.41***	0.24***	-0.66***	0.34***	0.22**	0.08	-0.28***	0.37***	0.50***	0.50***	0.73***	-0.65***	0.10				
Rather oligotrophic	0.53***	0.51***	0.26***	0.58***	0.75***	0.65***	0.41***	0.24***	-0.66***	0.34***	0.22**	0.08	-0.28***	0.37***	0.50***	0.50***	0.73***	-0.65***	0.10	0.78***			
Mesotrophic	0.64***	0.66***	0.33***	0.67***	0.68***	0.45***	0.43***	0.34***	-0.46***	0.22**	0.18**	0.13	-0.04	0.14*	0.46***	0.46***	0.74***	-0.62***	0.01	0.43***	0.43***		
Rather nitrophytic	0,04	0.03	0.16*	0.12	-0.09	0.02	-0.09	0.24***	0.14*	-0.19**	-0.13	-0.01	0.27***	-0.17*	-0.15*	-0.15*	-0.19**	0.33***	0.22***	-0.18**	-0.18**	-0,08	
Nitrophytic	-0.27***	-0.27***	-0.02	-0.37***	-0.38***	-0.49***	-0.36***	-0.15*	0.77***	-0.29***	-0.01	-0.10	0.25***	-0.15*	-0.35***	-0.35***	-0.55***	0.68***	-0.05	-0.49***	-0.49***	-0.54***	-0.19**

Table S2.2.7: Spearman correlation matrix between environmental variables. Significance of the correlation is indicated in superscript: * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

	PCA_pol1	PCA_pol2	BIO1	BIO2	BIO3	BIO5	BIO4	BIO6	BIO7	BIO8	BIO9	BIO10	BIO11	BIO12	BIO13	BIO14	BIO15	BIO16	BIO17	BIO18	BIO19	CWWin800	LSTSum3200	NDVIWin200
PCA_pol2	0.03																							
BIO1	0.36***	-0.21**																						
BIO2	0.29***	0.56***	-0.39***																					
BIO3	0.36***	-0.22**	0.96***	-0.37***																				
BIO5	0.04	-0.21**	0.67***	-0.01	0.64***																			
BIO4	-0.36***	0.38***	-0.91***	0.56***	-0.90***	-0.47***																		
BIO6	0.37***	-0.24***	0.99***	-0.44***	0.94***	0.63***	-0.93***																	
BIO7	-0.37***	0.38***	-0.92***	0.57***	-0.91***	-0.48***	0.99***	-0.94***																
BIO8	-0.01	-0.02	-0.59***	0.51***	-0.62***	-0.12	0.58***	-0.59***	0.58***															
BIO9	0.38***	-0.30***	0.97***	-0.47***	0.94***	0.60***	-0.94***	0.97***	-0.95***	-0.58***														
BIO10	0.17*	-0.28***	0.78***	-0.08	0.74***	0.97***	-0.62***	0.75***	-0.63***	-0.19**	0.72***													
BIO11	0.35***	-0.21**	0.99***	-0.41***	0.96***	0.66***	-0.92***	0.99***	-0.93***	-0.60***	0.97***	0.77***												
BIO12	0.43***	-0.07	0.24***	0.00	0.25***	0.16*	-0.41***	0.27***	-0.40***	0.03	0.31***	0.21**	0.24***											
BIO13	-0.14*	-0.35***	0.07	-0.15*	0.07	0.43***	-0.20**	0.09	-0.19**	0.21**	0.11	0.40***	0.07	0.68***										
BIO14	0.60***	0.42***	-0.27***	0.66***	-0.24***	-0.32***	0.20**	-0.26***	0.20**	0.35***	-0.24***	-0.29***	-0.27***	0.58***	0.05									
BIO15	-0.69***	-0.53***	0.03	-0.60***	0.01	0.34***	-0.07	0.04	-0.08	-0.06	0.05	0.26***	0.03	-0.11	0.55***	-0.75***								

BIO16	-0.15*	-0.32***	0.08	-0.18**	0.08	0.41***	-0.21**	0.10	-0.21**	0.16*	0.12	0.37***	0.08	0.71***	0.99***	0.05	0.55***							
BIO17	0.63***	0.37***	-0.25***	0.67***	-0.22**	-0.30***	0.18**	-0.24***	0.19**	0.37***	-0.23***	-0.26***	-0.26***	0.56***	0.04	0.99***	-0.77***	0.03						
BIO18	0.16*	0.25***	-0.70***	0.52***	-0.68***	-0.50***	0.56***	-0.67***	0.57***	0.60***	-0.64***	-0.53***	-0.70***	0.47***	0.29***	0.79***	-0.31***	0.29***	0.77***					
BIO19	0.23***	-0.28***	0.72***	-0.35***	0.73***	0.64***	-0.79***	0.73***	-0.78***	-0.34***	0.74***	0.69***	0.72***	0.75***	0.68***	0.00	0.25***	0.71***	-0.01	-0.20**				
CWWin800	-0.60***	-0.07	-0.15*	-0.40***	-0.16*	-0.03	0.06	-0.15*	0.06	-0.16*	-0.13	-0.12	-0.15*	0.16*	0.47***	-0.27***	0.60***	0.52***	-0.33***	0.08	0.22**			
LSTSum3200	0.57***	-0.34***	0.54***	-0.28***	0.56***	0.16*	-0.61***	0.57***	-0.62***	-0.24***	0.57***	0.30***	0.53***	0.23***	-0.08	0.00	-0.20**	-0.08	0.05	-0.30***	0.35***	-0.48***		
NDVIWin200	-0.02	-0.16*	-0.02	-0.04	0.03	-0.02	-0.13	-0.01	-0.13	-0.04	0.01	0.02	-0.01	0.25***	0.27***	0.10	0.05	0.24***	0.13	0.13	0.18**	0.20**	0.09	
NDBIWin100	0.38***	0.07	0.01	0.42***	-0.01	0.04	0.09	-0.01	0.10	0.33***	-0.01	0.07	0.00	-0.14*	-0.30***	0.26***	-0.43***	-0.35***	0.29***	0.06	-0.24***	-0.65***	0.14*	-0.48***

Table S2.2.8: Summary of the 24 biodiversity-based metrics variance (R^2) explained by the set of 25 environmental variables. The variance results from the median value of 100 Random Forest modelling interactions for each metric. T – taxonomic metrics; Fd – functional diversity metrics; Fs – functional structure metrics. For more details on the functional structure metrics see Table S1.

Metric	**Variance (R^2)**
RaoQ (Fd)	0.46
Functional richness (Fd)	0.38
Species richness (T)	0.34
Total abundance (T)	0.33
Shannon diversity index (T)	0.33
Nitrophytic (Fs)	0.32
Rather hygrophytic (Fs)	0.31
Mesophytic (Fs)	0.30
Leprose (Fs)	0.25
Rather xerophytic (Fs)	0.24
Fruticulose (Fs)	0.23
Foliose narrow lobe (Fs)	0.23

Metric	**Variance (R^2)**
Mesotrophic (Fs)	0.21
Rather oligotrophic (Fs)	0.17
Rather nitrophytic (Fs)	0.17
Foliose broad lobe (Fs)	0.13
Squamulose (Fs)	0.08
Crustose (Fs)	0.07
Trentepohlia (Fs)	0
Chlorococcoid (Fs)	0
Xerophytic (Fs)	0
Cyanolichens (Fs)	0
Hygrophytic (Fs)	0
Oligotrophic (Fs)	0

Table S2.2.9: Summary of the five best environmental variables importance (%) for each of the 8 biodiversity-based metrics models with explained variance (R^2) higher than 30%.

Metric	Environmental variable	Importance (%)	Metric	Environmental variable	Importance (%)
RaoQ (Fd)	CWw	21.79	Shannon diversity index (T)	BIO19	19.07
	BIO16	19.47		BIO12	18.43
	BIO19	18.86		BIO16	13.93
	BIO12	16.65		CWWin800	12.98
	Pol_2	12.18		NDBIWin100	11.11
Functional richness (Fd)	CWw	19.85	Nitrophytic (Fs)	CWw	26.16
	BIO19	14.11		BIO5	14.01
	NDBIw	13.60		BIO10	13.53
	BIO12	12.53		LSTs	13.49
	LSTs	11.21		BIO2	12.16
Species Richness (T)	CWw	16.67	Rather hygrophytic (Fs)	BIO4	12.07
	BIO19	16.60		BIO19	11.19
	NDBIw	14.49		NDBIw	10.98
	BIO12	14.21		BIO2	10.10
	BIO16	13.21		BIO7	9.71
Total abundance (T)	BIO12	18.60	Mesophytic (Fs)	CWw	17.30
	BIO18	17.03		BIO16	15.12
	BIO19	14.66		BIO12	12.02
	BIO4	12.63		LSTs	10.88
	BIO16	12.45		BIO19	10.26

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3.2 Incorporating biotic interactions to better model current and future vegetation of the maritime Antarctic

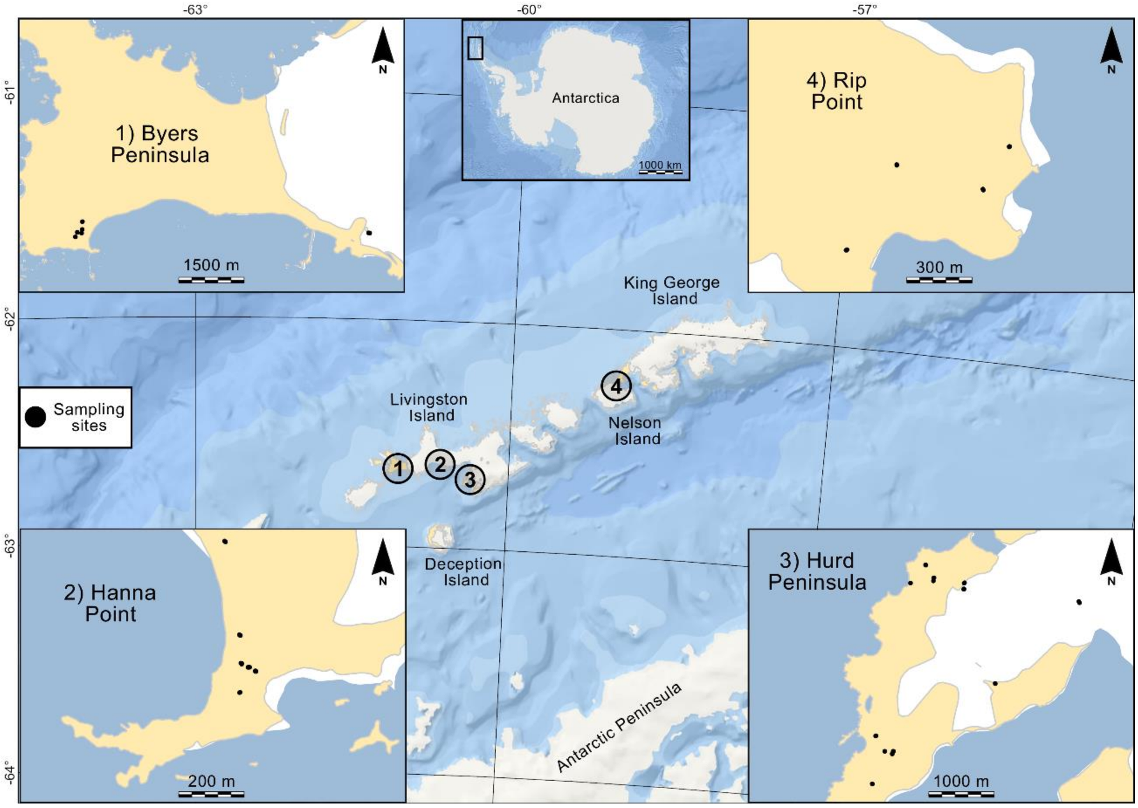


Figure S3.2.1: Sampling sites in Livingston and Nelson Islands, South Shetlands, maritime Antarctic. N = 30. Black dots represent the sampling sites and numbers represent, respectively, **(1)** Byers Peninsula; **(2)** Hanna Point and **(3)** Hurd Peninsula, in Livingston Island and **(4)** Rip Point in the area around the Eco-Nelson Czech Antarctic Base, Nelson Island, Shetland Islands, maritime Antarctic.

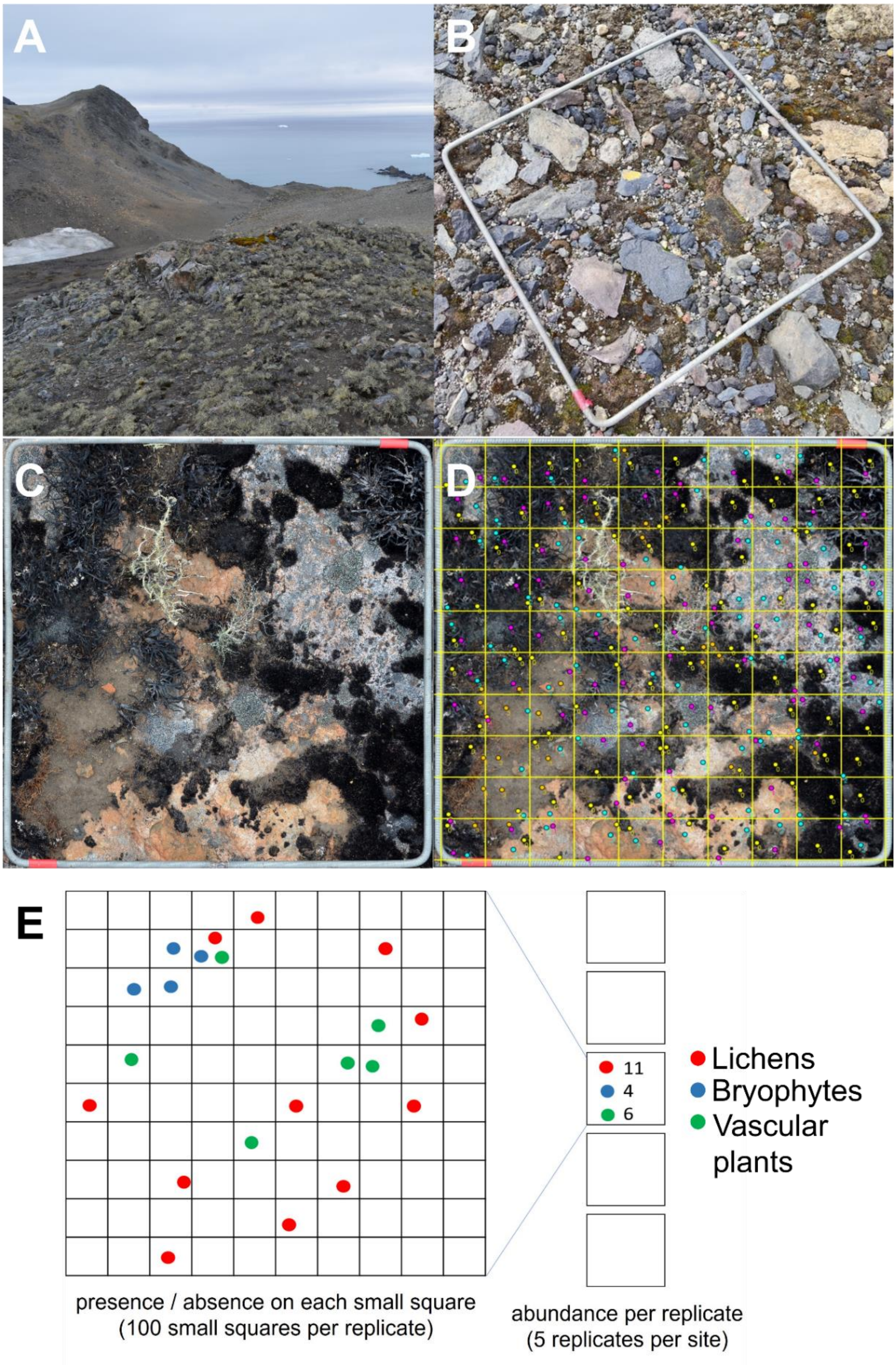
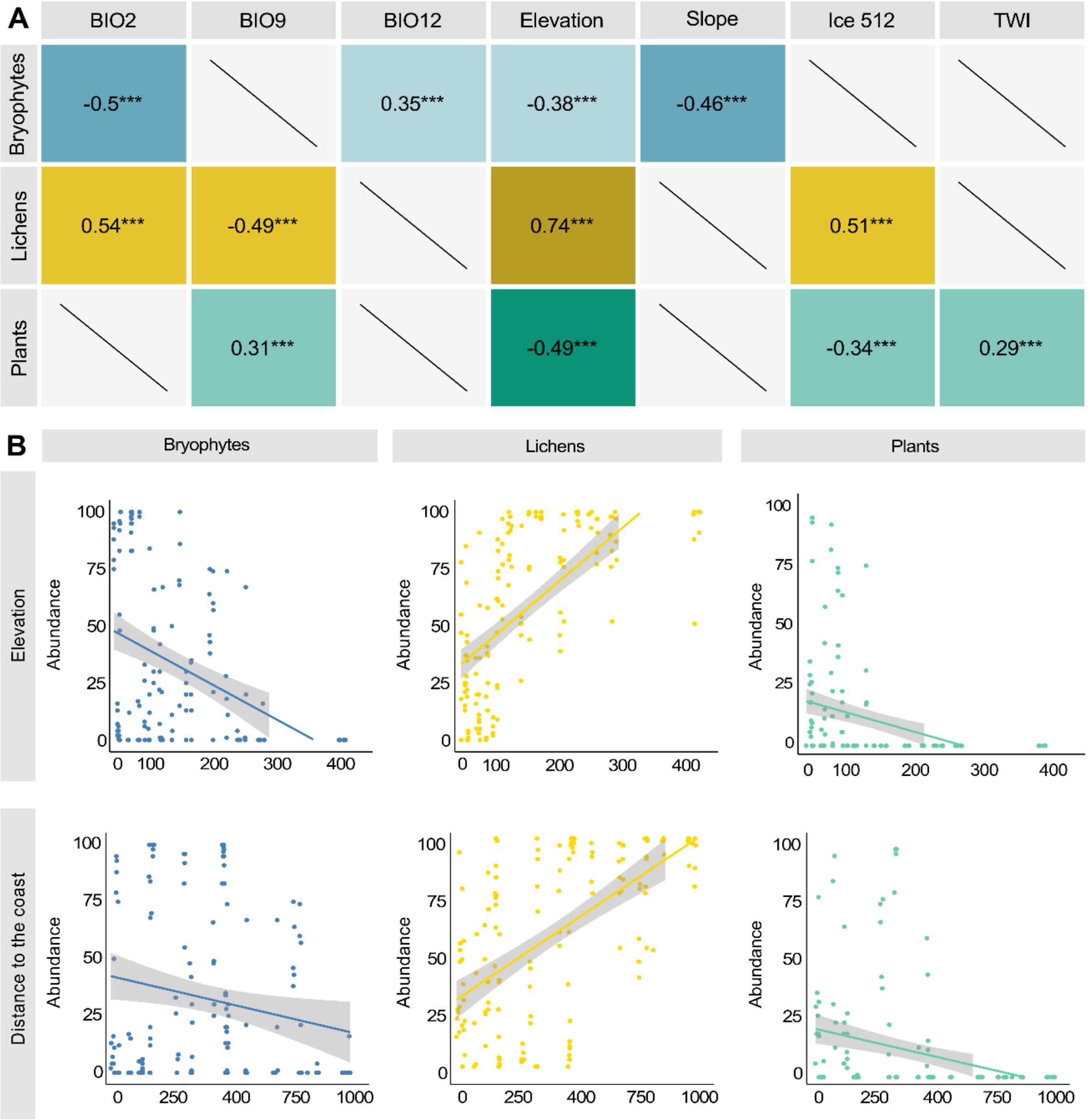


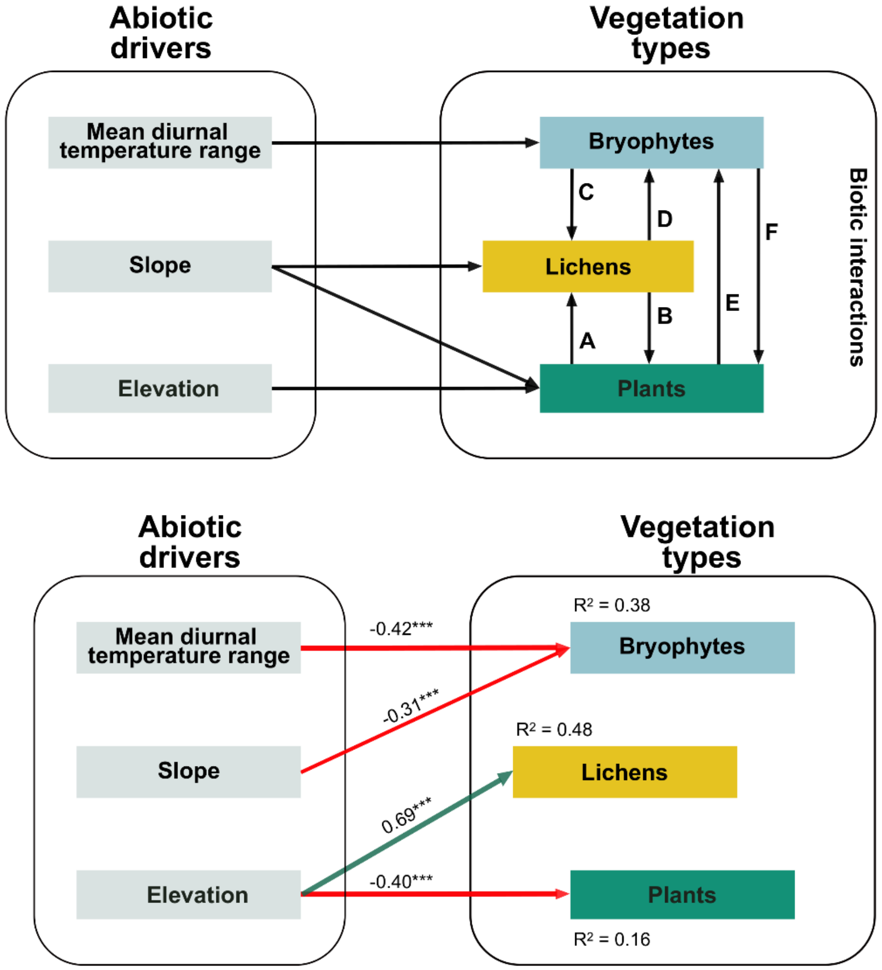
Figure S3.2.2: Determination of bryophytes, lichens and vascular plants abundance in each sampling site was done by: **A)** Example of an elevation and coast inland spatial gradient explored. adaptation of the standard sampling methodology with the **B)** quadrat of 30 x 30 cm and **C)** resulting photography of the vegetation present inside the quadrat. D) Overlayed grid of 3 x 3 cm. **E)** determination of the abundance of each vegetation group by averaging its frequency (0-100) by the five quadrats in each sampling site.



A

	BIO2	BIO9	BIO12	Elevation	Slope	Ice 512	TWI
Bryophytes	-0.5***		0.35***	-0.38***	-0.46***		
Lichens	0.54***	-0.49***		0.74***		0.51***	
Plants		0.31***		-0.49***		-0.34***	0.29***

Figure S3.2.3: A) Spearman correlation coefficients between the abundance of bryophytes, lichens and vascular plants with the respective four best correlated environmental variables of each species group. BIO2 stands for mean diurnal temperature range, BIO9 for the mean temperature of driest quarter, BIO12 to the annual precipitation, TWI to the topographic wetness index and Ice 512 to the area occupied by ice in a buffer of 512 meters around each sampling site. *p < 0.05; **p < 0.01; ***p < 0.001. **B)** Relationship between the three species groups in relation to a spatial gradient of elevation and coast inland. Fitted linear relationships of the abundance of bryophytes, lichens and vascular plants with elevation and distance to the coast represented by its respective regression line, coloured according to the species group, and 95% confidence interval, coloured in light grey. N =150.



SEM	Biotic interactions combination	SEM	Biotic interactions combination
II	A and E	**XIV**	A and D
III	E	**XV**	C and E
IV	A	**XVI**	A, C and F
V	C and F	**XVII**	B, C and F
VI	F	**XVIII**	B and F
VII	C	**XIX**	C and B
VIII	B and D	**XX**	A and F
IX	B	**XXI**	B, D and E
X	D	**XXII**	B, D and F
XI	A, C and E	**XXIII**	A and C
XII	A, D and E	**XXIV**	D and F
XIII	D and E	**XXV**	B and E

Figure S3.2.4: Structural equation model (top left) representing the initial framework upon which all 24 SEMs contemplating biotic interactions were produced. Each letter (A to F) represents a unique pathway (biotic interaction) between the three species groups. Each potential combination of these pathways, which allowed to generate the 24 SEMs contemplating biotic interactions, is summarized on the top right. Goodness-of-fit of each SEM can be seen in Table S2. On the bottom left, the structural equation model (SEM I), without considering biotic interactions, showing the absolute standardized path coefficients (ranging from 0 to 1), explaining Antarctic terrestrial vegetation (bryophytes, lichens and vascular plants) abundance in response to abiotic drivers alone (mean diurnal temperature range, elevation and isothermality). Overall goodness-of-fit statistics: $\chi 2 = 55.26$, df = 8, p = 0.00, robust Tucker Lewis index (TLI) = 0.65, robust comparative fit index (CFI) = 0.77, robust root mean square error of approximation (RMSEA) = 0.22 (0.174– 0.282), standardized root mean Square Residual (SRMR) = 0.11. Arrow widths are proportional to the standardized path coefficients presented. The R^2 next to each response variable indicates the proportion of variance explained. *p < 0.05; **p < 0.01; ***p < 0.001.

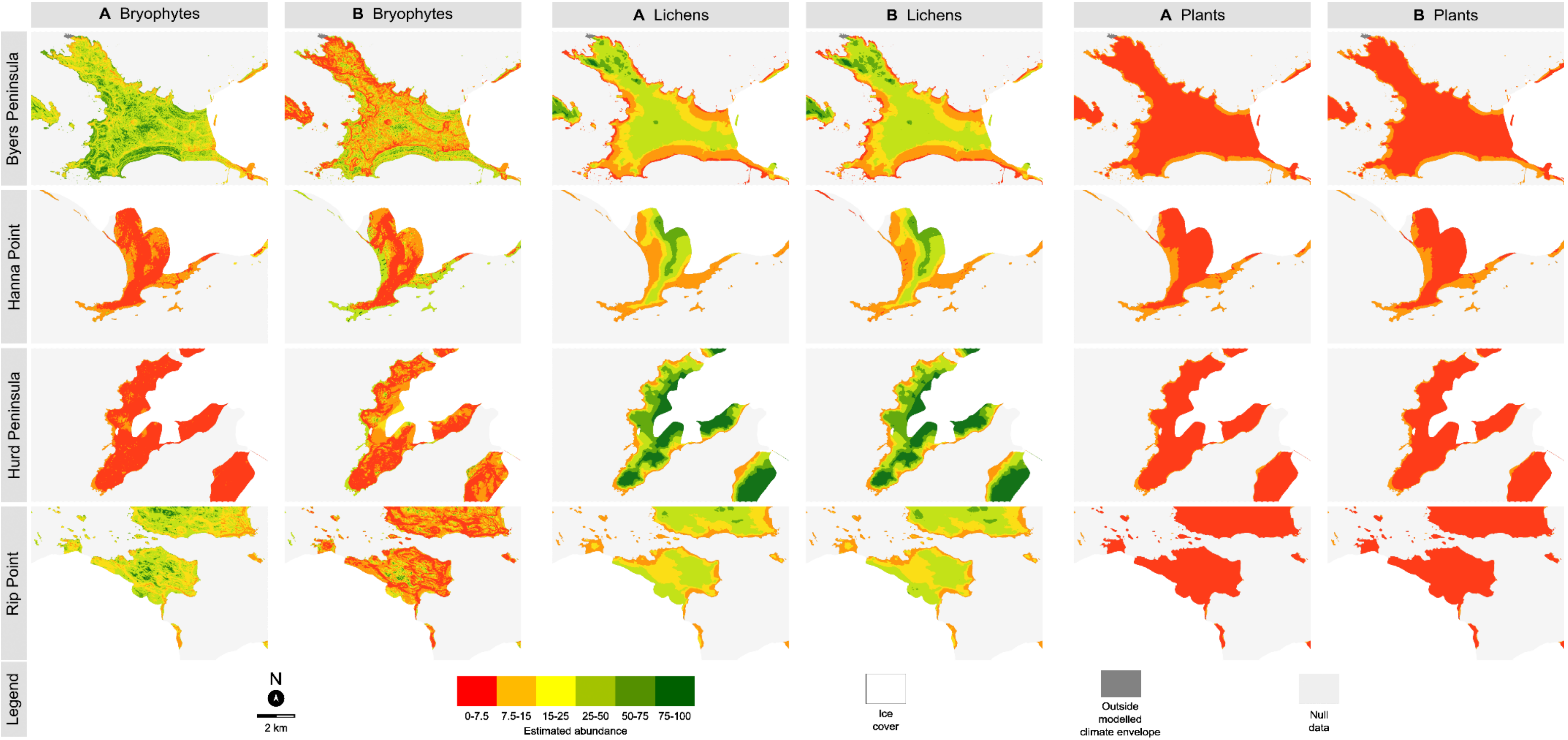


Figure S3.2.5: Estimated present and future abundance map of bryophytes, lichens and vascular plants in the four sampling areas (Fig. S1), South Shetlands, maritime Antarctic. Abundance was estimated using the Model XI to model the response to abiotic drivers and integrating the biotic interactions described above. **A)** present and **B)** future vegetation abundance estimations are spatially limited to current ice-free areas of the archipelago and where the environmental conditions match those within the modelled climatic envelope (i.e., where values for abiotic variables used are withing the range used to build the model). White areas represent the current extent of glaciers in the islands.

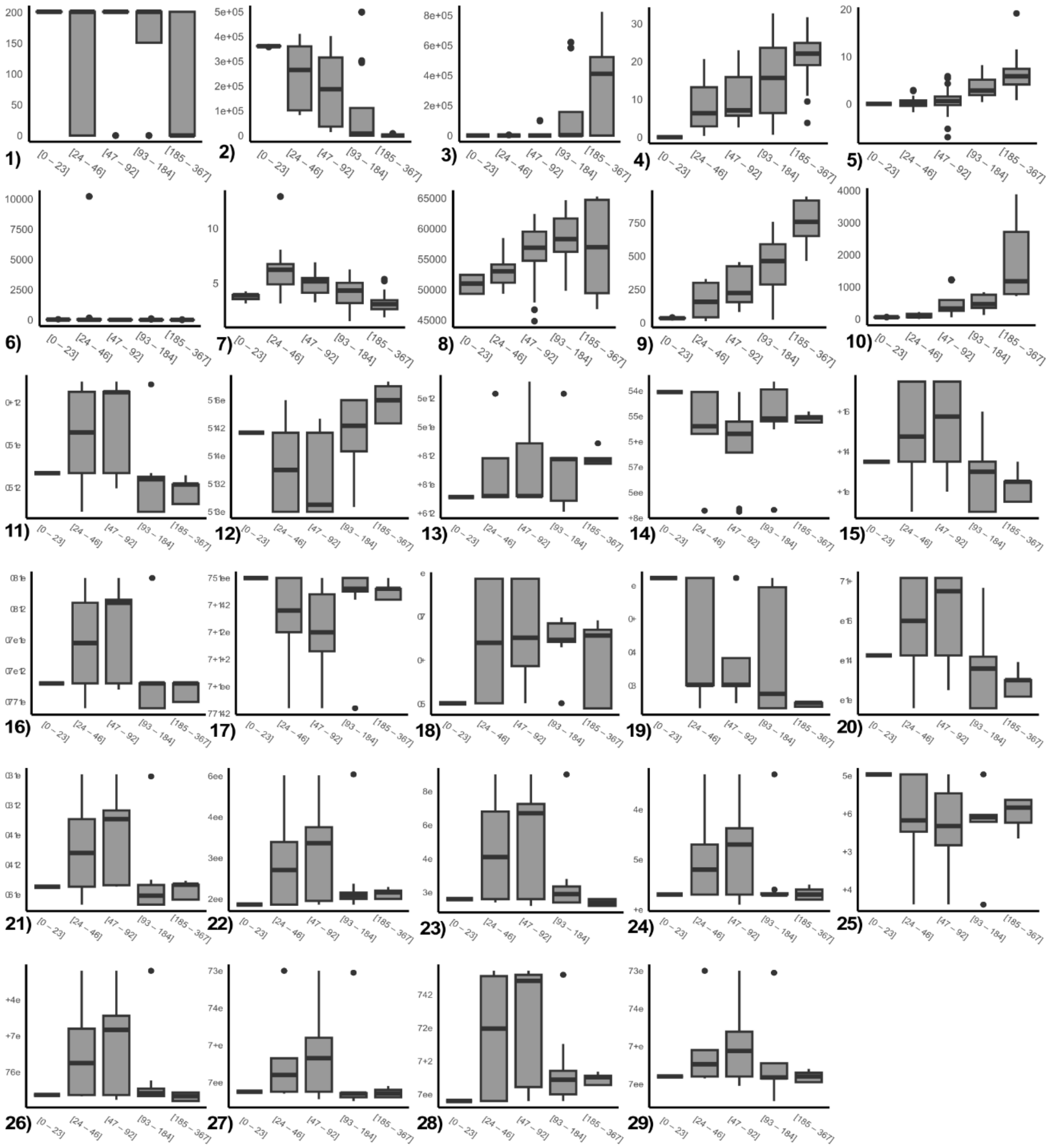


Figure S3.2.6: Boxplots showing the variation of the 10 terrain morphometry, hydrology and lighting variables, and the 19 regional climatic variables the per class of elevation ([0 – 23 m], [24 – 46 m], [47 – 92 m], [93 – 184 m] and [185 – 367 m]). Boxplots numbering correspond to: 1) Distance to the coast; 2) Distance to marine vertebrate colonies; 3) Ice-free areas (8 m buffer); 4) Area occupied by ice (512 m buffer); 5) Area occupied by sea (512 m buffer); 6) Slope; 7) Standard curvature; 8) Flow accumulation; 9) Topographic wetness index; 10) Monthly Area Solar Radiation (Appendix S2, Table S2). Boxplots numbering 11) to 29) refers to the bioclimatic variables numbering (Appendix S2, Table S1). Boxes display first to third interquartile ranges, black lines the median, dots the average, and whiskers the maximum and minimum values.

Table S3.2.1: Regional climatic variables used, divided by group (temperature; precipitation), type (annual range; seasonal) and described based on (Fick & Hijmans, 2017; Karger et al., 2017). Units: ºC – degrees Celsius; mm – millimetres).

Abiotic drivers	**Variable group**	**Type**	**Variable**	**Description**
Regional climatic	Air temperature (Units: ºC)	annual	Bio 1	annual mean temperature
			Bio 2	mean diurnal temperature range
			Bio 3	isothermality
			Bio 7	temperature annual range
		seasonal	Bio 4	temperature seasonality
			Bio 5	maximum mean temperature of warmest month
			Bio 6	minimum temperature of coldest month
			Bio 8	mean temperature of wettest quarter
			Bio 9	mean temperature of driest quarter
			Bio 10	mean temperature of warmest quarter
			Bio 11	mean temperature of coldest quarter
	Precipitation (Units: mm)	annual	Bio 12	annual precipitation
		seasonal	Bio 13	precipitation of wettest month
			Bio 14	precipitation of driest month
			Bio 15	precipitation seasonality
			Bio 16	precipitation of wettest quarter
			Bio 17	precipitation of driest quarter
			Bio 18	precipitation of warmest quarter
			Bio 19	precipitation of coldest quarter

Table S3.2.2: Terrain morphometry, hydrology and lighting environmental variables and natural pollution environmental variables used. Units: m – meters; m^2 – square meter; º - degrees; ºC – degrees Celsius; WH/m^2 – watt hour per square meter).

Abiotic drivers	Variable	Description
Terrain	Elevation (Units: m)	Relates to the ice sheet surface elevation, based on the Reference Elevation Model of Antarctica (REMA). (Howat et al., 2022)
	Distance to the coast (Units: m)	Measures the shortest distance (linear) from each quadrat and the nearest shoreline. Shoreline based on the SCAR Antarctic Digital Database (Thomson & Cooper, 1993).
	Slope (Units: º)	Measurement of the gradient or steepness of each cell of the REMA raster. Slope values range from 0º (flat surface) to 90º. Solar Radiation tool of ArcGIS's v.10.6 Spatial Analyst Toolbox.
	Standard curvature (Units: 1/100 of a z-unit)	Combines both the profile and planform curvatures of each cell, based on the REMA raster. Positive values indicate the surface is upwardly convex, while negative values indicate upwardly concave. Curvature tool of ArcGIS's v.10.6 Spatial Analyst Toolbox.
	Flow accumulation (Units: none)	Based on the accumulated weight of all cells flowing into each downslope cell in the output raster. Higher values represent higher flow accumulation(Jenson & Domingue, 1988; Tarboton, Bras, & Rodriguez-Iturbe, 1991. Flow Accumulation tool of ArcGIS's v.10.6 Spatial Analyst Toolbox.
	Topographic wetness index (Units: none)	The index is a function of both the slope and the upstream contributing area. Higher values represent increased accumulated runoff potential (Chowdhury, 2023).
	Monthly Area Solar Radiation (ASR) (Units: WH/m^2)	Takes into consideration latitude (solar declination and solar position), elevation from the REMA raster, and slope. Monthly calculations from January of 2019 to January of 2020. Solar Radiation tool of ArcGIS's v.10.6 Spatial Analyst Toolbox.
	Area occupied by ice (Units: m^2)	The area around each quadrat occupied by glaciers, within 11 buffers, ranging from 4 to 4096 m. Glaciers cover based on the SCAR Antarctic Digital Database (Thomson & Cooper, 1993).
	Ice-free areas (Units: m^2)	The ice-free area around each quadrat, within 11 buffers, ranging from 4 to 4096 m. Ice-free area based on the SCAR Antarctic Digital Database (Thomson & Cooper, 1993).

	Area occupied by sea (Units: m^2)	The area around each quadrat occupied by sea water, within 11 buffers, ranging from 4 to 4096 m. Sea cover based on the SCAR Antarctic Digital Database (Thomson & Cooper, 1993).
Natural pollution	Distance to marine vertebrate colonies (Units: m)	Measures the shortest distance (linear) from each quadrat to the nearest marine vertebrate colony. Colonies GPS coordinates were recorded during field sampling.

Table S3.2.3: Summary of the linear regression models rankings for the influence of the 4 environmental variables better related to bryophytes absolute abundance, determined from likelihood measures. BIO2 stands for mean diurnal temperature range and BIO12 to the annual precipitation. Regression models are ranked by their Adjusted R^2 value). N=150. The * symbol represents the model and predictor(s) chosen for fitting in the structural equation models.

	Model performance parameters			
Model	Rank ($AdjR^2$)	AIC	$AdjR^2$	p-value
Model with 4 explanatory variables				
Elevation + BIO 2 + BIO12 + Slope	1	259.92	38.23	< 0.001
Models with 3 explanatory variables				
BIO2 + BIO12 + Slope	1	257.92	36.96	< 0.001
Elevation + BIO 2 + Slope	2	258.08	36.89	< 0.001
Elevation + BIO 12 + Slope	3	269.02	32.12	< 0.001
Elevation + BIO 2 + BIO12	4	274.69	29.5	< 0.001
Models with 2 explanatory variables				
*BIO 2 + Slope **	1	256.11	37.31	< 0.001
BIO12 + Slope	2	268.66	31.84	< 0.001
Elevation + BIO2	3	272.76	29.95	< 0.001
BIO 2 + BIO12	4	274.29	29.23	< 0.001
Elevation + Slope	5	284.11	24.44	< 0.001
Elevation + BIO12	6	284.48	24.26	< 0.001
Models with 1 explanatory variable				
BIO 2	1	272.29	29.71	< 0.001
Slope	2	286.73	22.61	< 0.001
BIO 12	3	294.03	18.75	< 0.001
Elevation	4	303.35	13.54	< 0.001

Table S3.2.4: Summary of the linear regression models rankings for the influence of the 4 environmental variables better related to bryophytes absolute abundance, determined from likelihood measures. BIO2 stands for mean diurnal temperature range and BIO12 to the annual precipitation. Regression models are ranked by their Adjusted R^2 value). N=150. The * symbol represents the model and predictor(s) chosen for fitting in the structural equation models.

	Model performance parameters

Model	Rank (AdjR^2)	AIC	AdjR^2	p-value
Model with 4 explanatory variables				
Elevation + BIO2 + BIO9 + Ice 512	1	188.33	47.15	< 0.001
Models with 3 explanatory variables				
Elevation + BIO2 + Ice 512	1	186.41	47.48	< 0.001
Elevation + BIO9 + Ice 512	2	186.70	47.38	< 0.001
Elevation + BIO2 + BIO9	3	187.76	47.01	< 0.001
BIO2 + BIO9 + Ice 512	4	227.64	30.87	< 0.001
Models with 2 explanatory variables				
Elevation + Ice 512	1	184.22	47.56	< 0.001
Elevation + BIO9	2	185.77	47.37	< 0.001
Elevation + BIO2	3	186.22	47.21	< 0.001
BIO2 + BIO9	4	233.29	27.75	< 0.001
BIO2 + Ice 512	5	238.02	25.43	< 0.001
BIO9 + Ice 512	6	248.37	20.11	< 0.001
Models with 1 explanatory variable				
*Elevation **	1	184.22	47.56	< 0.001
BIO2	2	244.51	21.62	< 0.001
Ice512	3	253.17	16.97	< 0.001
BIO9	4	274.19	4.476	< 0.01

Table S3.2.5: Summary of the linear regression models rankings for the influence of the 4 environmental variables better related to bryophytes absolute abundance, determined from likelihood measures. BIO2 stands for mean diurnal temperature range and BIO12 to the annual precipitation. Regression models are ranked by their Adjusted R^2 value). N=150. The * symbol represents the model and predictor(s) chosen for fitting in the structural equation models.

	Model performance parameters			
Model	Rank (AdjR^2)	AIC	AdjR^2	p-value
Model with 4 explanatory variables				
Elevation + BIO9 + Ice 512+ TWI	1	206.3567	14.83	< 0.001
Models with 3 explanatory variables				

Elevation + Ice 512+ TWI	1	204.5295	15.86	< 0.001
Elevation + BIO9 + Ice512	2	206.0841	14.98	< 0.001
Elevation + BIO9 + TWI	3	208.6526	14.08	< 0.001
BIO9 + Ice 512+ TWI	4	211.451	11.89	< 0.001
Models with 2 explanatory variables				
Elevation + TWI	1	205.6007	15.81	< 0.001
Elevation + BIO9	2	206.3964	15.36	< 0.001
BIO9+ TWI	3	204.5043	15.32	< 0.001
Elevation + Ice 512	4	207.6413	14.65	< 0.001
Ice 512+ TWI	5	212.505	11.27	< 0.001
BIO9 + Ice 512	6	216.3914	8.937	< 0.001
Models with 1 explanatory variable				
*Elevation **	1	206.9996	15.57	< 0.001
Ice 512	2	214.0201	9.776	< 0.001
TWI	3	221.1154	5.406	< 0.01
BIO9	4	225.6826	2.481	< 0.05

Table S3.2.6: Summary of the 25 structural equation models goodness-of-fit, each representing a unique combination of biotic interactions. Goodness-of-fit is here represented by the following fit indices: χ^2: Model chi-square, df: degrees of freedom, P: Model p-value, TLI: Tucker Lewis Index, CFI: Comparative Fit Index, RMSEA: Root Mean Square Error of Approximation, SRMR: Standardized Root Mean Square Residual and the R^2 of each species group. SEMs overall goodness-of-fit evaluation was tested based on the following indices cut-offs: Satorra-Bentler chi-square statistic and its significance (the model is rejected if p-value < 0.05), robust comparative fit index (CFI > 0.9), robust Tucker Lewis index (TLI > 0.95), robust root mean square error of approximation index (RMSEA < 0.08) and the standardized root mean square residual (SRMR < 0.08).

Model	**$\chi 2$**	**df**	**P**	**TLI**	**CFI**	**RMSEA**		**SRMR**	**Bryophytes R^2**	**Lichens R^2**	**Vascular plants R^2**
I	55.26	8	0.00	0.65	0.77	0.22		0.11	0.38	0.48	0.16
II	13.53	6	0.04	0.95	0.97	0.09		0.05	0.43	0.60	0.16
III	44.15	7	0.00	0.71	0.83	0.20		0.08	0.43	0.48	0.16
IV	29.08	7	0.00	0.84	0.91	0.15		0.08	0.38	0.60	0.16
V	37.56	6	0.00	0.70	0.85	0.21		0.07	0.38	0.48	0.27
VI	37.84	7	0.00	0.75	0.85	0.19		0.07	0.38	0.48	0.27
VII	54.78	7	0.00	0.59	0.76	0.24		0.11	0.38	0.48	0.16
VIII	27.88	6	0.00	0.82	0.91	0.16		0.07	0.39	0.48	0.35
IX	29.08	7	0.00	0.84	0.91	0.15		0.08	0.38	0.48	0.35
X	53.38	7	0.00	0.60	0.77	0.24		0.10	0.39	0.48	0.16
XI	8.01	5	0.16	0.98	0.99	0.06		0.04	0.43	0.61	0.16
XII	9.38	5	0.10	0.96	0.98	0.08		0.04	0.44	0.60	0.16
XIII	39.05	6	0.00	0.68	0.84	0.21		0.08	0.46	0.48	0.16
XIV	27.88	6	0.00	0.82	0.91	0.16		0.07	0.39	0.60	0.16

XV	44.06	6	0.00	0.65	0.83	0.22		0.08	0.43	0.48	0.16
XVI	2.25	5	0.81	1.03	1.00	0.00		0.02	0.38	0.61	0.27
XVII	2.25	5	0.81	1.03	1.00	0.00		0.02	0.38	0.48	0.46
XVIII	2.31	6	0.89	1.03	1.00	0.00		0.02	0.38	0.48	0.46
XIX	29.45	6	0.00	0.81	0.90	0.17		0.08	0.38	0.48	0.35
XX	7.54	6	0.27	0.99	0.99	0.04		0.03	0.38	0.60	0.27
XXI	9.38	5	0.10	0.96	0.98	0.08		0.04	0.44	0.48	0.35
XXII	1.08	5	0.96	1.04	1.00	0.00		0.02	0.39	0.48	0.47
XXIII	24.48	6	0.00	0.85	0.93	0.15		0.09	0.38	0.63	0.16
XXIV	35.80	6	0.00	0.71	0.85	0.21		0.07	0.39	0.48	0.28
XXV	13.53	6	0.04	0.95	0.97	0.09		0.05	0.43	0.48	0.35

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4. Challenging the space-for-time approach: evaluating its potential and limitations for climate change studies

Lichen functional structure diversity

A total of 186 species were identified in this study, comprising both those registered within the spatial gradient of 54 sampling sites, ranging from southern and central Iberian Peninsula and the temporal gradient of 21 sites within the course of 17 years. All registered lichens were identified to the species level and their functional characterization, in relation to its growth form and main photobiont type, retrieved from the ITALIC database (Nimis, 2024. All species' respective functional characterization can be seen in Table S1.

Table S4.1: List of total lichen species identified (N=186), and respective functional characterization, retrieved from the ITALIC database (Nimis, 2024), based on the growth form and main photobiont type traits.

Scientific name	Growth form	Main photobiont type
Agonimia allobata	crustose	*Chlorococcoid* (Green algae)
Agonimia octospora	squamulose	*Chlorococcoid* (Green algae)
Agonimia opuntiella	squamulose	*Chlorococcoid* (Green algae)
Agonimia tristicula	squamulose	*Chlorococcoid* (Green algae)
Alyxoria culmigena	crustose	*Trentepohlia* (Green algae)
Alyxoria varia	crustose	*Trentepohlia* (Green algae)
Amandinea punctata	crustose	*Chlorococcoid* (Green algae)
Anaptychia ciliaris	fruticulose	*Chlorococcoid* (Green algae)
Arthonia apatetica	crustose	*Chlorococcoid* (Green algae)
Arthonia atra	crustose	*Trentepohlia* (Green algae)
Arthonia radiata	crustose	*Trentepohlia* (Green algae)
Athallia holocarpa	crustose	*Chlorococcoid* (Green algae)
Athallia pyracea	crustose	*Chlorococcoid* (Green algae)
Bacidia absistens	crustose	*Chlorococcoid* (Green algae)
Bacidia iberica	crustose	*Chlorococcoid* (Green algae)
Bactrospora patellarioides	crustose	*Trentepohlia* (Green algae)
Bacidia rubella	crustose	*Chlorococcoid* (Green algae)
Biatora beckhausii	crustose	*Chlorococcoid* (Green algae)
Bellicidia incompta	crustose	*Chlorococcoid* (Green algae)

Blastenia ferruginea	crustose	*Chlorococcoid* (Green algae)
Blastenia herbidella	crustose	*Chlorococcoid* (Green algae)
Buellia disciformis	crustose	*Chlorococcoid* (Green algae)
Buellia erubescens	crustose	*Chlorococcoid* (Green algae)
Caloplaca cerina	crustose	*Chlorococcoid* (Green algae)
Caloplaca obscurella	crustose	*Chlorococcoid* (Green algae)
Calicium viride	crustose	*Chlorococcoid* (Green algae)
Candelaria concolor	foliose narrow	*Chlorococcoid* (Green algae)
Candelariella reflexa	crustose	*Chlorococcoid* (Green algae)
Candelariella viae-lacteae	crustose	*Chlorococcoid* (Green algae)
Candelariella vitellina	crustose	*Chlorococcoid* (Green algae)
Candelariella xanthostigma	crustose	*Chlorococcoid* (Green algae)
Catillaria nigroclavata	crustose	*Chlorococcoid* (Green algae)
Catapyrenium psoromoides	squamulose	*Chlorococcoid* (Green algae)
Chrysothrix candelaris	leprose	*Chlorococcoid* (Green algae)
Collema flaccidum	foliose broad	Cyanolichen
Collema furfuraceum	foliose broad	Cyanolichen
Collema nigrescens	foliose broad	Cyanolichen
Collema subflaccidum	foliose broad	Cyanolichen
Collema subnigrescens	foliose broad	Cyanolichen
Dendrographa decolorans	crustose	*Trentepohlia* (Green algae)
Dichoporis ziziphi	crustose	*Trentepohlia* (Green algae)
Diploicia canescens	crustose	*Chlorococcoid* (Green algae)
Diplotomma alboatrum	crustose	*Chlorococcoid* (Green algae)
Diploschistes scruposus	crustose	*Chlorococcoid* (Green algae)
Evernia prunastri	fruticulose	*Chlorococcoid* (Green algae)
Flavoparmelia caperata	foliose broad	*Chlorococcoid* (Green algae)
Flavoplaca citrina	crustose	*Chlorococcoid* (Green algae)
Flavoplaca flavocitrina	crustose	*Chlorococcoid* (Green algae)
Flavoparmelia soredians	foliose broad	*Chlorococcoid* (Green algae)
Fuscopannaria ignobilis	squamulose	Cyanolichen

Fuscopannaria mediterranea	squamulose	Cyanolichen
Gyalolechia flavorubescens	crustose	*Chlorococcoid* (Green algae)
Huneckia pollinii	crustose	*Chlorococcoid* (Green algae)
Hyperphyscia adglutinata	foliose narrow	*Chlorococcoid* (Green algae)
Hypogymnia physodes	foliose narrow	*Chlorococcoid* (Green algae)
Hypogymnia tubulosa	foliose narrow	*Chlorococcoid* (Green algae)
Koerberia biformis	foliose narrow	Cyanolichen
Lecanora albella	crustose	*Chlorococcoid* (Green algae)
Lecanora allophana	crustose	*Chlorococcoid* (Green algae)
Lecanographa amylacea	crustose	*Trentepohlia* (Green algae)
Lecanora carpinea	crustose	*Chlorococcoid* (Green algae)
Lecanora chlarotera	crustose	*Chlorococcoid* (Green algae)
Lecanora conizella	crustose	*Chlorococcoid* (Green algae)
Lecania cyrtella	crustose	*Chlorococcoid* (Green algae)
Lecidella elaeochroma	crustose	*Chlorococcoid* (Green algae)
Lecania erysibe	crustose	*Chlorococcoid* (Green algae)
Lecanora expallens	crustose	*Chlorococcoid* (Green algae)
Lecanora horiza	crustose	*Chlorococcoid* (Green algae)
Lecanora hybocarpa	crustose	*Chlorococcoid* (Green algae)
Lecanora intumescens	crustose	*Chlorococcoid* (Green algae)
Lecanora lividocinerea	crustose	*Chlorococcoid* (Green algae)
Lecanographa lyncea	crustose	*Trentepohlia* (Green algae)
Lecania naegelii	crustose	*Chlorococcoid* (Green algae)
Lecidella pulveracea	crustose	*Chlorococcoid* (Green algae)
Lecanora rubicunda	crustose	*Chlorococcoid* (Green algae)
Lecanora saligna	crustose	*Chlorococcoid* (Green algae)
Lecidella elaeochroma	crustose	*Chlorococcoid* (Green algae)
Lecanora symmicta	crustose	*Chlorococcoid* (Green algae)
Lecania viridulogranulosa	crustose	*Chlorococcoid* (Green algae)
Lepra albescens	crustose	*Chlorococcoid* (Green algae)
Lepra amara	crustose	*Chlorococcoid* (Green algae)

Lepraria incana	leprose	*Chlorococcoid* (Green algae)
Lepra leucosora	crustose	*Chlorococcoid* (Green algae)
Lepra multipuncta	crustose	*Chlorococcoid* (Green algae)
Lepra ophthalmiza	crustose	*Chlorococcoid* (Green algae)
Lepra trachythallina	crustose	*Chlorococcoid* (Green algae)
Melanohalea exasperatula	foliose broad	*Chlorococcoid* (Green algae)
Melanelixia fuliginosa	foliose narrow	*Chlorococcoid* (Green algae)
Melanelixia glabratula	foliose broad	*Chlorococcoid* (Green algae)
Melanelixia subaurifera	foliose broad	*Chlorococcoid* (Green algae)
Micarea prasina	crustose	*Chlorococcoid* (Green algae)
Myriolecis dispersa	crustose	*Chlorococcoid* (Green algae)
Myriolecis hagenii	crustose	*Chlorococcoid* (Green algae)
Normandina pulchella	squamulose	*Chlorococcoid* (Green algae)
Ochrolechia balcanica	crustose	*Chlorococcoid* (Green algae)
Ochrolechia szatalaensis	crustose	*Chlorococcoid* (Green algae)
Opegrapha celtidicola	crustose	*Trentepohlia* (Green algae)
Opegrapha niveoatra	crustose	*Trentepohlia* (Green algae)
Opegrapha vulgata	crustose	*Trentepohlia* (Green algae)
Parmotrema hypoleucinum	foliose broad	*Chlorococcoid* (Green algae)
Parmotrema perlatum	foliose broad	*Chlorococcoid* (Green algae)
Parmelina quercina	foliose broad	*Chlorococcoid* (Green algae)
Parmotrema reticulatum	foliose broad	*Chlorococcoid* (Green algae)
Parmelia sulcata	foliose broad	*Chlorococcoid* (Green algae)
Parmelina tiliacea	foliose broad	*Chlorococcoid* (Green algae)
Pertusaria coccodes	crustose	*Chlorococcoid* (Green algae)
Pertusaria dispar	crustose	*Chlorococcoid* (Green algae)
Pertusaria flavida	crustose	*Chlorococcoid* (Green algae)
Pertusaria heterochroa	crustose	*Chlorococcoid* (Green algae)
Pertusaria hymenea	crustose	*Chlorococcoid* (Green algae)
Pertusaria leioplaca	crustose	*Chlorococcoid* (Green algae)
Pertusaria pertusa	crustose	*Chlorococcoid* (Green algae)

Pertusaria pruinosa	crustose	*Chlorococcoid* (Green algae)
Pertusaria pustulata	crustose	*Chlorococcoid* (Green algae)
Pertusaria werneriana	crustose	*Chlorococcoid* (Green algae)
Phaeophyscia hirsuta	foliose narrow	*Chlorococcoid* (Green algae)
Phaeophyscia orbicularis	foliose narrow	*Chlorococcoid* (Green algae)
Phaeophyscia pusilloides	foliose narrow	*Chlorococcoid* (Green algae)
Phlyctis argena	crustose	*Chlorococcoid* (Green algae)
Physcia adscendens	foliose narrow	*Chlorococcoid* (Green algae)
Physcia aipolia	foliose narrow	*Chlorococcoid* (Green algae)
Physciella chloantha	foliose narrow	*Chlorococcoid* (Green algae)
Physcia clementei	foliose narrow	*Chlorococcoid* (Green algae)
Physconia distorta	foliose narrow	*Chlorococcoid* (Green algae)
Physcia dubia	foliose narrow	*Chlorococcoid* (Green algae)
Physconia enteroxantha	foliose narrow	*Chlorococcoid* (Green algae)
Physconia grisea	foliose narrow	*Chlorococcoid* (Green algae)
Physcia leptalea	foliose narrow	*Chlorococcoid* (Green algae)
Physconia perisidiosa	foliose narrow	*Chlorococcoid* (Green algae)
Physconia servitii	foliose narrow	*Chlorococcoid* (Green algae)
Physcia tenella	foliose narrow	*Chlorococcoid* (Green algae)
Physconia venusta	foliose narrow	*Chlorococcoid* (Green algae)
Piccolia ochrophora	crustose	*Chlorococcoid* (Green algae)
Placynthiella icmalea	crustose	*Chlorococcoid* (Green algae)
Polycauliona candelaria	foliose narrow	*Chlorococcoid* (Green algae)
Porina aenea	crustose	*Trentepohlia* (Green algae)
Punctelia borreri	foliose broad	*Chlorococcoid* (Green algae)
Punctelia subrudecta	foliose broad	*Chlorococcoid* (Green algae)
Pyrrhospora quernea	crustose	*Chlorococcoid* (Green algae)
Ramalina calicaris	fruticulose	*Chlorococcoid* (Green algae)
Ramalina canariensis	fruticulose	*Chlorococcoid* (Green algae)
Ramalina farinacea	fruticulose	*Chlorococcoid* (Green algae)
Ramalina fastigiata	fruticulose	*Chlorococcoid* (Green algae)

Ramalina fraxinea	fruticulose	*Chlorococcoid* (Green algae)
Ramalina implectens	fruticulose	*Chlorococcoid* (Green algae)
Ramalina lacera	fruticulose	*Chlorococcoid* (Green algae)
Ramalina obtusata	fruticulose	*Chlorococcoid* (Green algae)
Ramalina pusilla	fruticulose	*Chlorococcoid* (Green algae)
Ramalina subgeniculata	fruticulose	*Chlorococcoid* (Green algae)
Rinodina anomala	crustose	*Chlorococcoid* (Green algae)
Rinodina capensis	crustose	*Chlorococcoid* (Green algae)
Rinodina colobina	crustose	*Chlorococcoid* (Green algae)
Rinodina efflorescens	crustose	*Chlorococcoid* (Green algae)
Rinodina exigua	crustose	*Chlorococcoid* (Green algae)
Rinodina griseosoralifera	crustose	*Chlorococcoid* (Green algae)
Rinodina isidioides	crustose	*Chlorococcoid* (Green algae)
Rinodina oleae	crustose	*Chlorococcoid* (Green algae)
Rinodina pyrina	crustose	*Chlorococcoid* (Green algae)
Rinodina roboris	crustose	*Chlorococcoid* (Green algae)
Rostania occultata	crustose	Cyanolichen
Schismatomma ricasolii	crustose	*Trentepohlia* (Green algae)
Scutula circumspecta	crustose	*Chlorococcoid* (Green algae)
Scutula effusa	crustose	*Chlorococcoid* (Green algae)
Scutula igniarii	crustose	*Chlorococcoid* (Green algae)
Scytinium aragonii	squamulose	Cyanolichen
Scytinium fragrans	foliose narrow	Cyanolichen
Scytinium subaridum	squamulose	Cyanolichen
Scytinium subtile	squamulose	Cyanolichen
Scytinium teretiusculum	squamulose	Cyanolichen
Staurolemma omphalarioides	foliose narrow	Cyanolichen
Teloschistes chrysophthalmos	fruticulose	*Chlorococcoid* (Green algae)
Tephromela atra	crustose	*Chlorococcoid* (Green algae)
Thelenella muscorum	crustose	*Chlorococcoid* (Green algae)
Trapeliopsis flexuosa	crustose	*Chlorococcoid* (Green algae)

Trapeliopsis pseudogranulosa	crustose	*Chlorococcoid* (Green algae)
Usnea ceratina	fruticulose	*Chlorococcoid* (Green algae)
Usnea esperantiana	fruticulose	*Chlorococcoid* (Green algae)
Usnea glabrescens	fruticulose	*Chlorococcoid* (Green algae)
Usnea glabrata	fruticulose	*Chlorococcoid* (Green algae)
Usnea mutabilis	fruticulose	*Chlorococcoid* (Green algae)
Usnea rubicunda	fruticulose	*Chlorococcoid* (Green algae)
Varicellaria hemisphaerica	crustose	*Chlorococcoid* (Green algae)
Verrucaria sorbinea	crustose	*Chlorococcoid* (Green algae)
Waynea adscendens	squamulose	*Chlorococcoid* (Green algae)
Waynea stoechadiana	squamulose	*Chlorococcoid* (Green algae)
Xanthoria parietina	foliose narrow	*Chlorococcoid* (Green algae)

Bioclimate characterization

Monthly maximum, minimum and mean temperature, total precipitation and maximum, minimum and mean relative humidity values were retrieved for each sampling site for the years 1989 to 2018, at the maximum available pixel resolution (30-arcsec or 1 km2). From there, based on the mathematical formulas for each bioclimate variable (O'Donnell and Ignizio, 2012), the traditional 19 annual and seasonal temperature and precipitation variables were calculated, for which we also adapted to calculate similar ones for relative humidity. For details on each bioclimate variable see Table S2. We are aware that these bioclimate variables are available at the same spatial resolution in other databases like WorldClim and even Chelsa. However, we opted for this process because in both databases, the temporal range of available data only extends to 2000 and 2010, respectively. Conversely, monthly temperature, precipitation and relative humidity values extend to 2018, allowing for a closer to the present characterization of the climate conditions in the study area.

Table S4.2: Climatic variables used, divided by group (temperature; precipitation and relative humidity), type (annual, annual range, seasonal and intra-seasonal) and described based on (Karger et al., 2017, Fick and Hijmans, 2017). Units: ºC – degrees Celsius; mm – millimetres).

Variable group	**Type**	**Variable**	**Description**
Air temperature	Annual	Bio 1	Annual mean temperature

(Units: ºC)	Range	Bio 2	Mean diurnal temperature range
		Bio 3	Isothermality
		Bio 7	Temperature annual range
	Seasonal	Bio 4	Temperature seasonality
		Bio 5	Maximum mean temperature of warmest month
		Bio 6	Minimum temperature of coldest month
		Bio 8	Mean temperature of wettest quarter
		Bio 9	Mean temperature of driest quarter
		Bio 10	Mean temperature of warmest quarter
		Bio 11	Mean temperature of coldest quarter
Precipitation (Units: mm)	Annual	Bio 12	Annual total precipitation
	Seasonal	Bio 13	Precipitation of wettest month
		Bio 14	Precipitation of driest month
		Bio 15	Precipitation seasonality
		Bio 16	Precipitation of wettest quarter
		Bio 17	Precipitation of driest quarter
		Bio 18	Precipitation of warmest quarter
		Bio 19	Precipitation of coldest quarter
Relative humidity (Units: %)	Annual	RH 1	Annual mean relative humidity
	Range	RH 7	Relative humidity annual range
	Seasonal	RH 4	Relative humidity seasonality
		RH 5	Maximum mean relative humidity of warmest month
		RH 6	Minimum relative humidity of coldest month
		RH 8	Mean relative humidity of wettest quarter
		RH 9	Mean relative humidity of driest quarter
		RH 10	Mean relative humidity of warmest quarter
		RH 11	Mean relative humidity of coldest quarter

Study Area

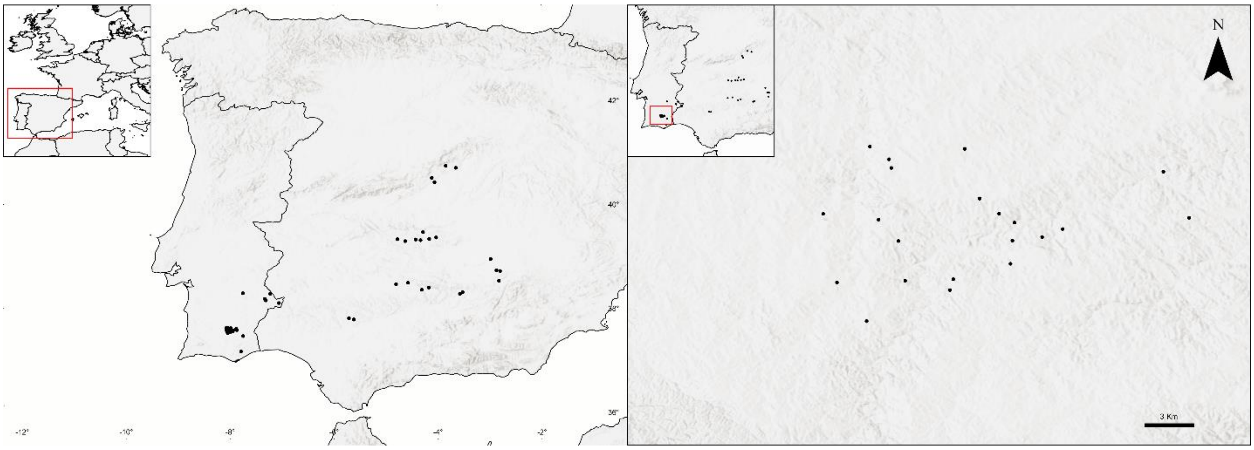

Figure S4.1: Map of the study area with sampling sites locations in (left) spatial gradient and (right) temporal gradient.

Metrics and climate variables residuals and normality

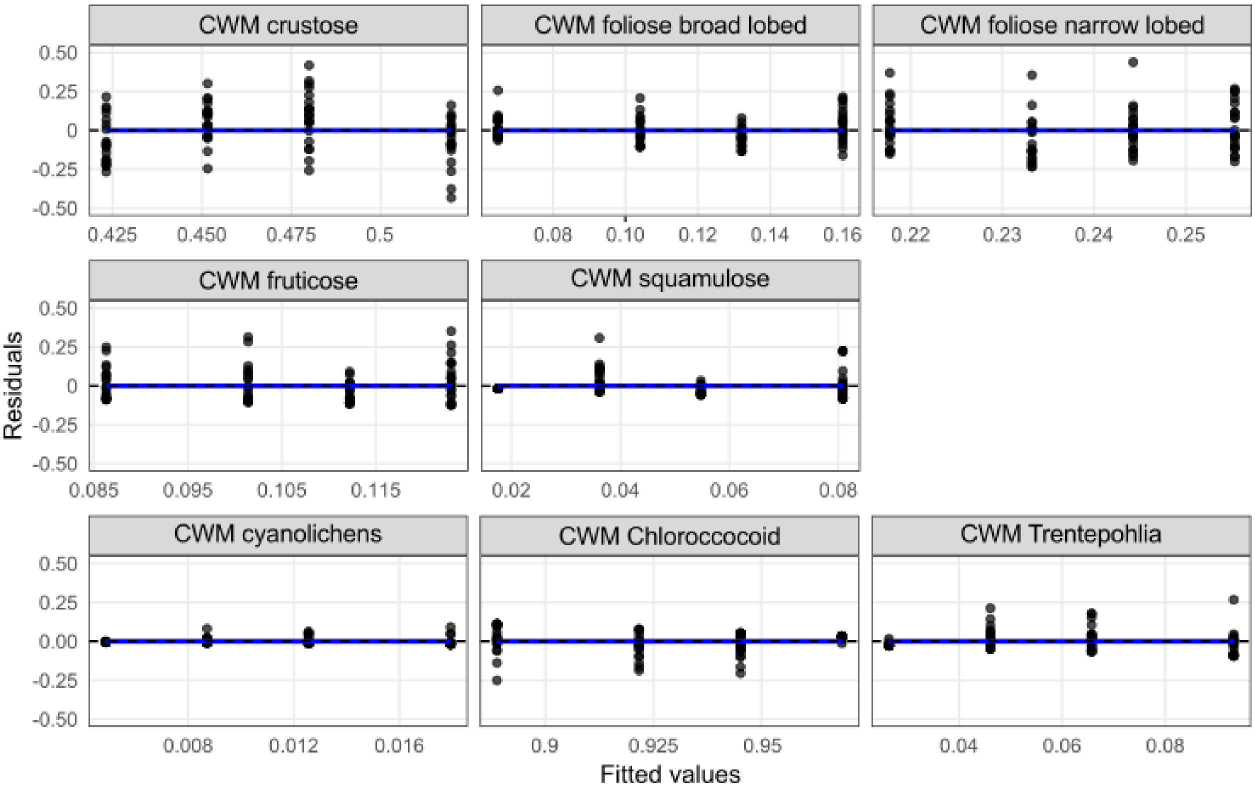

Figure S4.2: Models diagnostics (residuals vs. fitted plots). Model for each functional group community weighted mean (CWM) as a function of the sampling years (2005, 2010, 2015, 2022) using Generalized Linear Models (GLMs) with Gaussian error and identity link.

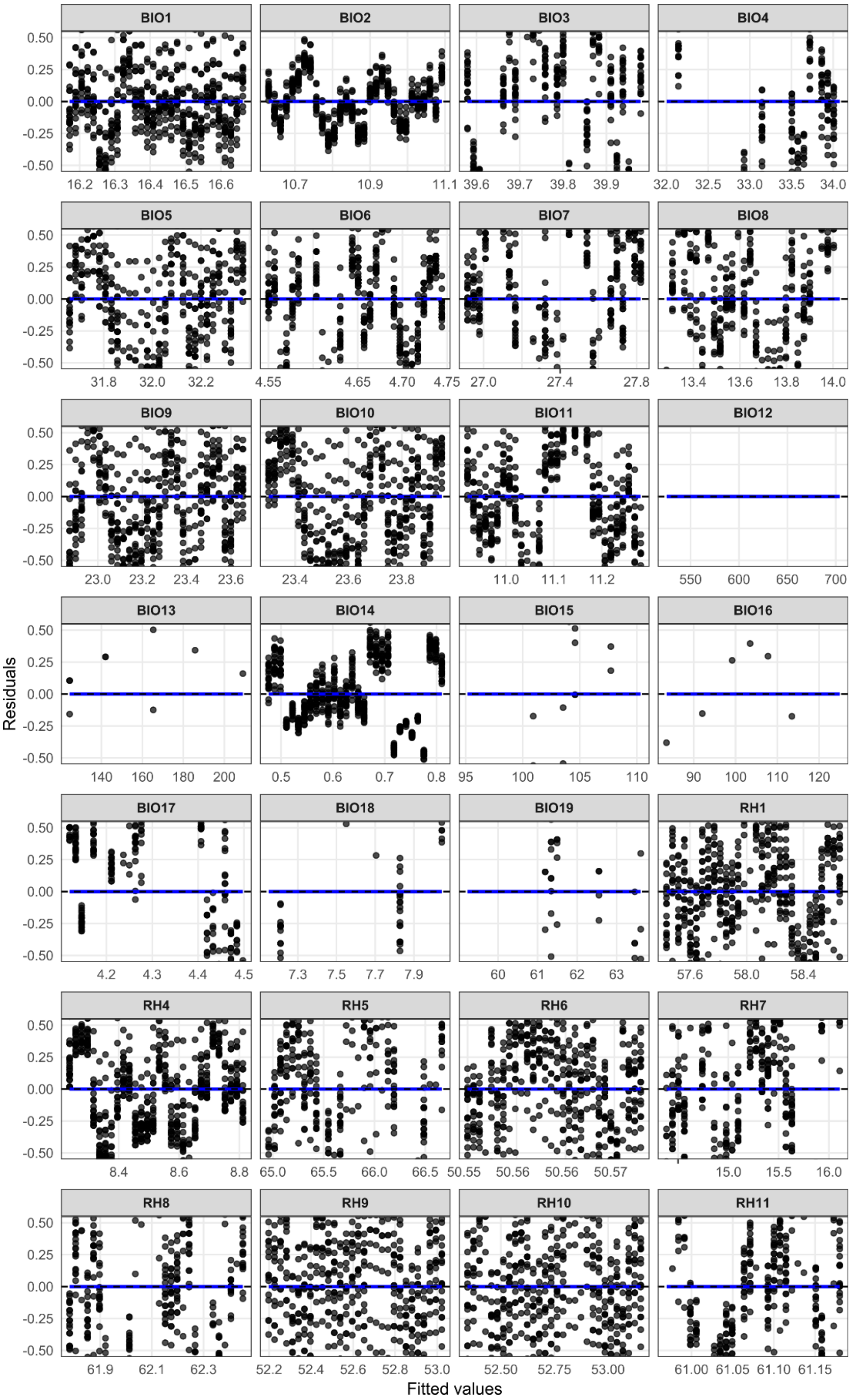

Figure S4.3: Models diagnostics (residuals vs. fitted plots). Model for each climate variable as a function of the time (1989-2018) using Generalized Linear Models (GLMs) with Gaussian error and identity link.

Lichen functional changes over time

Table S4.3: Trait-based metrics models summary over time

Functional group	**p-value**	**McFadden p-R^2**	**Estimate**	**Standard error**	**Statistic**
Crustose	0.06	4.54	0.00567	0.00287	1.975
Foliose broad lobed	0.0	14.21	-0.01	0.0015	-3.686
Foliose narrow lobed	0.38	0.92	-0.00221	0.00253	-0.873
Fruticose	0.28	1.41	-0.00216	0.00199	-1.082
Squamulose	0.0	9.34	0.00	0.0013	2.906
Cyanolichen	0.06	4.25	0.00076	0.0004	1.909
Chlorococcoid	0.0	13.85	0.0	0.0013	-3.632
Trentepohlia	0.0	11.68	0.0	0.0012	3.293

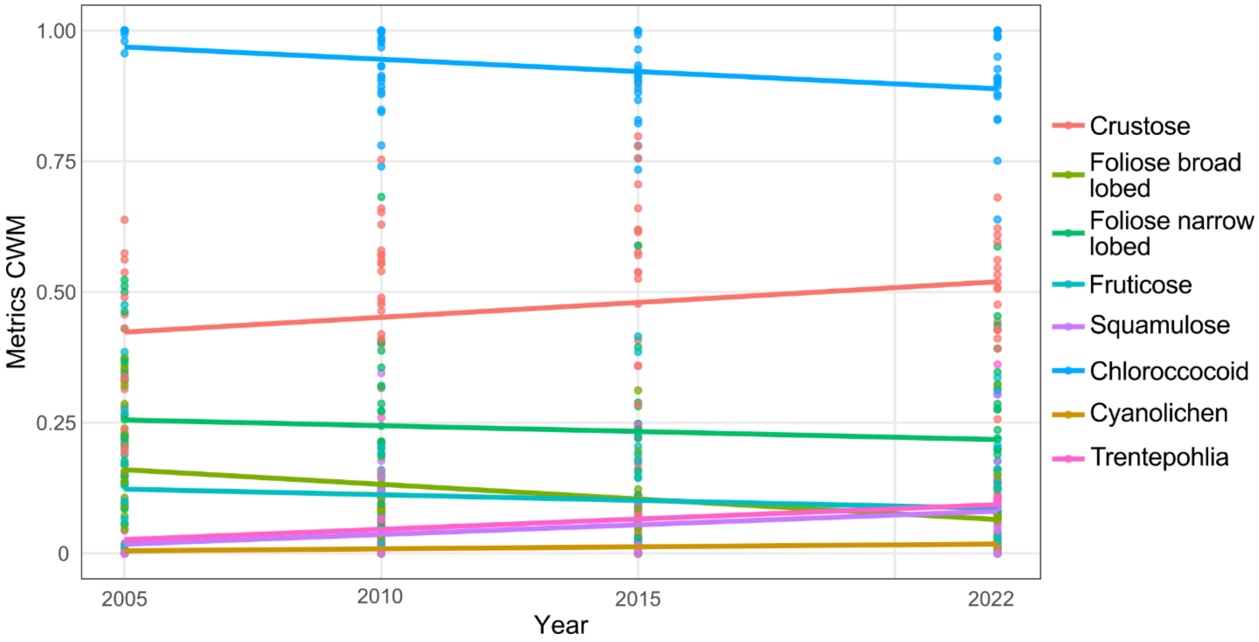

Figure S4.4: Growth form and main photobiont type trait-based metrics change over time. Dots represent each functional community weighted mean (CWM) value, in each site (N=21), for each of the four years (2005, 2010, 2015, 2022).

Climate variation across time and space

Table S4.4: Climate variables models summary over time

Climate variable	p-value	McFadden p-R^2	Estimate	Standard error	Statistic
BIO1	0	20.09	0.01693	0.00135	12.56512
BIO2	0	38.48	0.01593	0.00080	19.81778
BIO3	0	2.11	0.01380	0.00375	3.67875
BIO4	0	10.92	0.07161	0.00816	8.77283
BIO5	0	12.53	0.02454	0.00259	9.48464
BIO6	0.008	1.11	-0.00669	0.00252	-2.65217
BIO7	0	9.69	0.03123	0.00381	8.20637
BIO8	0	11.03	0.02566	0.00291	8.82267
BIO9	0	20.15	0.02693	0.00214	12.58887
BIO10	0	14.14	0.02242	0.00220	10.16870
BIO11	0	5.29	-0.01249	0.00211	-5.92401
BIO12	0	31.25	-6.17101	0.36524	-16.89573
BIO13	0	39.04	-2.91320	0.14527	-20.05360
BIO14	0	13.39	-0.01152	0.00117	-9.85422
BIO15	0	21.47	-0.52340	0.03995	-13.10130
BIO16	0	35.09	-1.43139	0.07769	-18.42417
BIO17	0.084	0.48	0.01304	0.00753	1.73270
BIO18	0.065	0.54	0.03088	0.01671	1.84853
BIO19	0.01	1.06	0.15129	0.05834	2.59325
RH1	0	35.77	-0.04216	0.00225	-18.69982
RH4	0	18.50	0.01980	0.00166	11.93889
RH5	0	25.29	-0.05881	0.00403	-14.57844
RH6	0.785	0.01	0.00061	0.00223	0.27336
RH7	0	25.50	-0.05942	0.00405	-14.66195
RH8	0	4.16	-0.02313	0.00443	-5.21953

RH9	0	15.89	-0.02843	0.00261	-10.89191
RH10	0	13.37	-0.02787	0.00283	-9.84465
RH11	0.094	0.44	0.00725	0.00433	1.67502

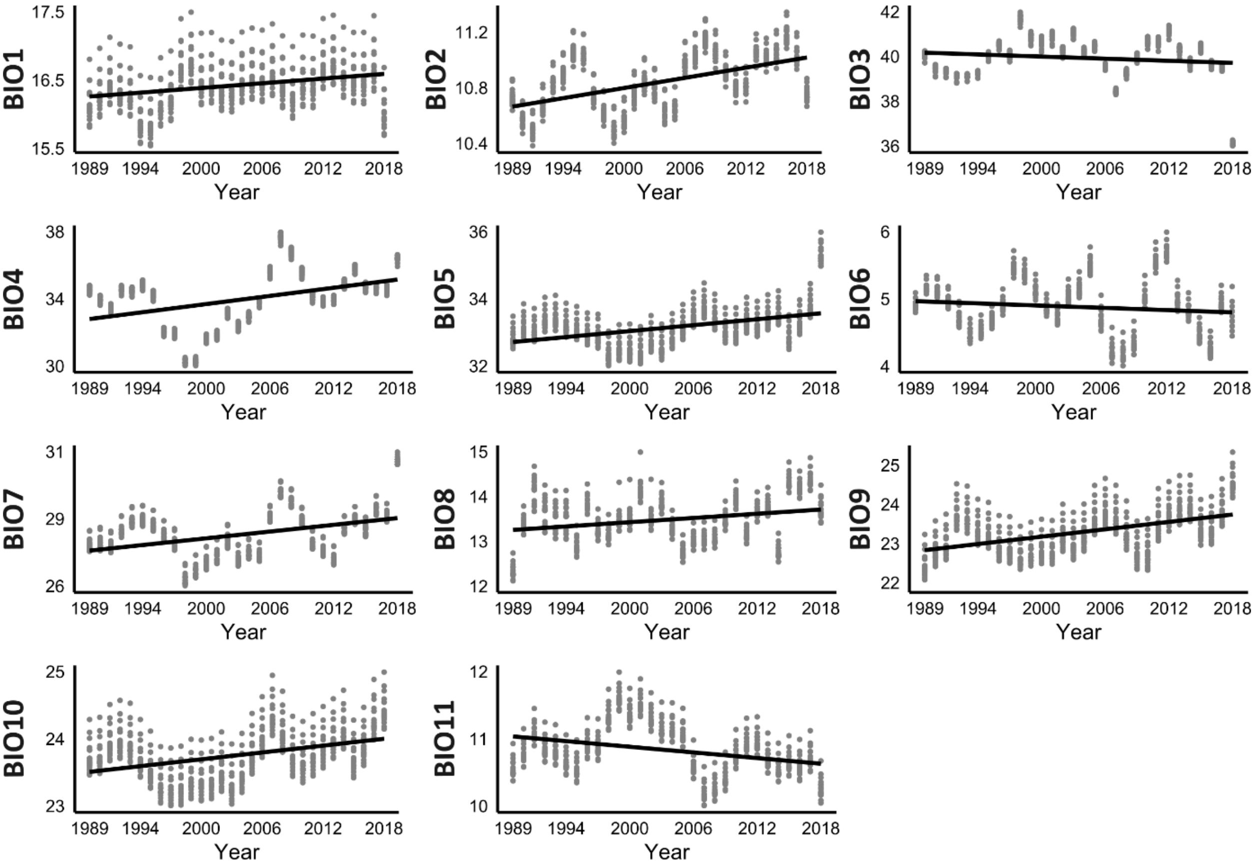

Figure S4.5: Temperature related variables evolution across time. Gray dots represent each variable value in each site (N=22) for the 30 prior to the last available year with climate data (1989-2018). A smoothed conditional mean, represented by an overlayed black line, was associated to each variable to aid in seeing its evolution across time. More details on bioclimate variables see Table S2. For more details on temperature related variables relation with time see Table S3.

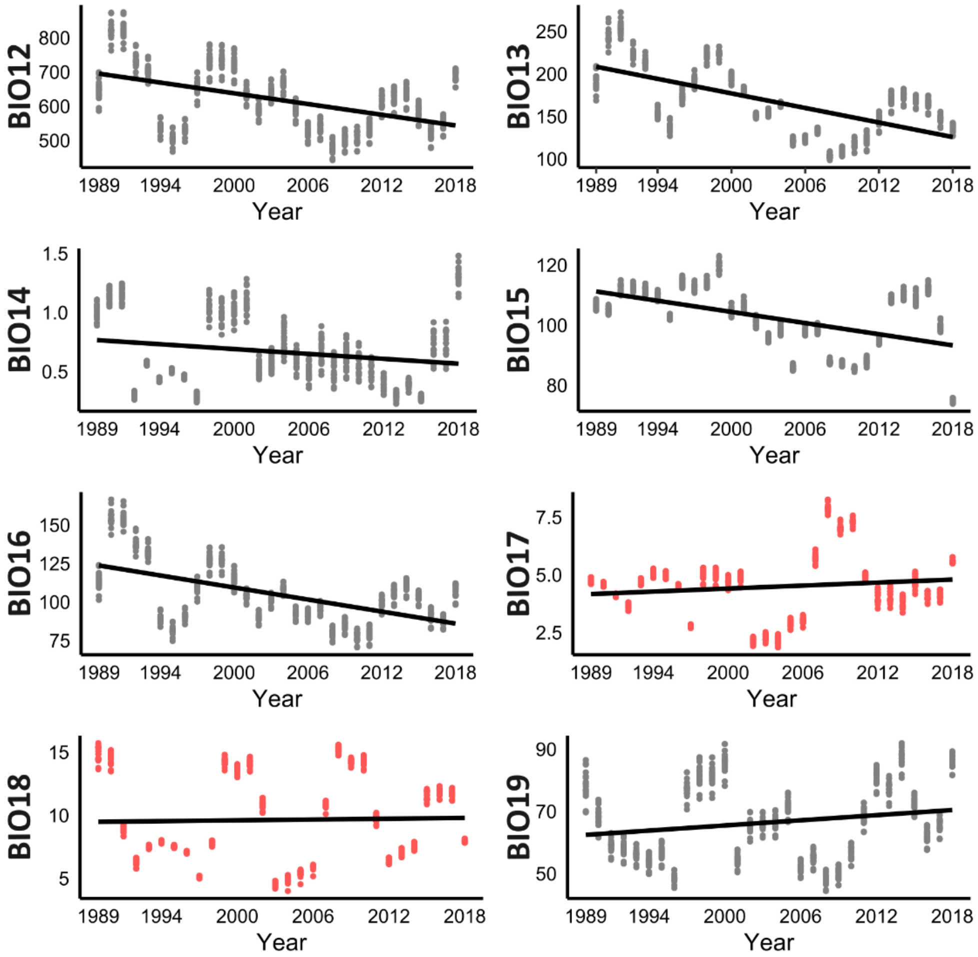

Figure S4.6: Precipitation related variables evolution across time. Gray and red dots represent each variable value in each site (N=22) for the 30 prior to the last available year with climate data (1989-2018). Red dots are associated with variables not changing significantly ($p \geq 0.05$) in time. A smoothed conditional mean, represented by an overlayed black line, was associated to each variable to aid in seeing its evolution across time. More details on bioclimate variables see Table S2. For more details on precipitation related variables relation with time see Table S3.

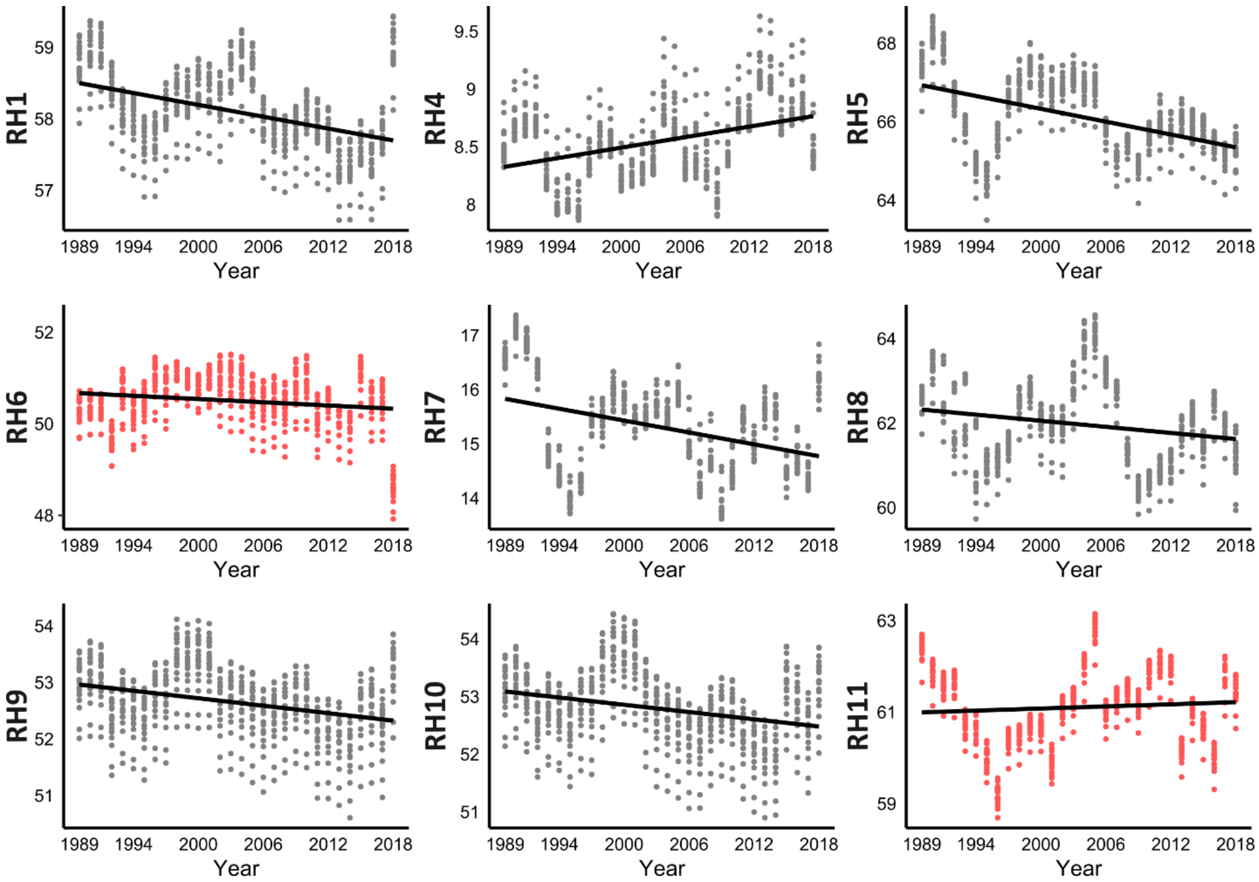

Figure S4.7: Relative humidity related variables evolution across time. Gray and red dots represent each variable value in each site (N=22) for the 30 prior to the last available year with climate data (1989-2018). Red dots are associated with variables not changing significantly ($p \geq 0.05$) in time. A smoothed conditional mean, represented by an overlayed black line, was associated to each variable to aid in seeing its evolution across time. More details on bioclimate variables see Table S2. For more details on relative humidity related variables relation with time see Table S3.

Selecting the best spatial models

Table S4.5: Summary of the selected models for the foliose broad lobed functional group across space. Regression models are ranked by their McFadden pseudo-R^2 value. N=54.

Model	**Rank**	**AIC**	**McFadden p-R^2**	**p-value**
BIO16 + BIO19	1	-135.85	16.69	< 0.05

Table S4.6: Summary of the selected models for the squamulose functional group across space. Regression models are ranked by their McFadden pseudo-R^2 value. N=54.

Model	**Rank**	**AIC**	**McFadden p-R^2**	**p-value**
BIO1	1	-132.21	21.84	< 0.05
BIO11	2	-130.45	19.24	< 0.05
BIO8	3	-129.73	18.16	< 0.05
BIO14	4	-128.94	16.95	< 0.05

Table S4.7: Summary of the selected models for the *Chlorococcoid* (Green algae) functional group across space. Regression models are ranked by their McFadden pseudo-R^2 value. N=54.

Model	**Rank**	**AIC**	**McFadden p-R^2**	**p-value**
BIO1 + BIO13	1	-112.49	37.13	< 0.05
BIO5 + BIO15	2	-111.25	35.66	< 0.05
BIO15 + RH5	3	-111.10	35.50	< 0.05
BIO5 + BIO16	4	-110.69	35.00	< 0.05
BIO10 + BIO16	5	-110.49	34.76	< 0.05
BIO2 + BIO15	6	-110.34	34.58	< 0.05
BIO13 + BIO14	7	-110.18	34.38	< 0.05
BIO11 + BIO13	8	-110.09	34.27	< 0.05
BIO10 + BIO15	9	-109.73	33.83	< 0.05
BIO13 + RH7	10	-109.39	33.41	< 0.05
BIO8 + BIO13	11	-109.07	33.01	< 0.05
BIO4 + BIO13	12	-108.96	32.88	< 0.05
BIO6 + BIO13	13	-108.83	32.71	< 0.05

Table S4.8: Summary of the selected models for the *Trentepohlia* (Green algae) functional group across space. Regression models are ranked by their McFadden pseudo-R^2 value. N=54.

Model	**Rank**	**AIC**	**McFadden p-R^2**	**p-value**
RH1	1	-176.45	32.58	< 0.05
RH9	2	-175.74	31.69	< 0.05
RH10	3	-175.65	31.58	< 0.05
BIO3	4	-173.85	29.25	< 0.05

Evaluating models' predictive bias

Table S4.9: Summary of the paired T-test analysis, between observed and predicted CWM metric values, for the hindcast of 2005. Test significance in the model ($^{NS}\rho > 0.05$; $^{*}\rho < 0.05$; $^{**}\rho < 0.01$; $^{***}\rho < 0.001$).

Functional trait	**Functional metric**	**t-statistic**	**Mean difference**	**95% CI**	**RMSE**
Growth form	Foliose broad lobed	-4.31	-0.10***	[-0.15; -0.05]	0.14
	Squamulose	66.56	0.06***	[0.06; 0.06]	0.06
		45.59	0.07***	[0.07; 0.08]	0.07
		146.69	0.09***	[0.09; 0.09]	0.09
		143.81	0.07***	[0.07; 0.08]	0.07
Main photobiont type	*Chlorococcoid*	-6.82	-0.03***	[-0.04; -0.02]	0.03
		-5.09	-0.02***	[-0.03; -0.01]	0.03
		6.19	0.03***	[0.02; 0.04]	0.03
		-4.32	-0.01***	[-0.02; -0.01]	0.02
		-16.63	-0.05***	[-0.05; -0.04]	0.05
		-15.56	-0.04***	[-0.05; -0.04]	0.05
		-14.98	-0.04***	[-0.05; -0.04]	0.05
		-19.41	-0.05***	[-0.05; -0.04]	0.05
		-9.21	-0.02***	[-0.03; -0.02]	0.02
		-23.39	-0.05***	[-0.06; -0.05]	0.05
		-22.51	-0.05***	[-0.05; -0.04]	0.05
		-19.62	-0.04***	[-0.05; -0.04]	0.04
		-14.09	-0.03***	[-0.04; -0.03]	0.03
	Trentepohlia	22.82	0.05***	[0.04; 0.05]	0.05
		27.95	0.06***	[0.06; 0.07]	0.06

		28.07	0.06***	[0.06; 0.07]	0.06
		33.46	0.07***	[0.07; 0.08]	0.08

Table S4.10: Summary of the paired T-test analysis, between observed and predicted CWM metric values, for the forecast of 2015. Test significance in the model ($^{NS}\rho > 0.05$; $*\rho < 0.05$; $**\rho < 0.01$; $***\rho < 0.001$).

Functional trait	**Functional metric**	**t-statistic**	**Mean difference**	**95% CI**	**RMSE**
Growth form	Foliose broad lobed	2.02	0.02^{ns}	[0.00; 0.08]	0.09
	Squamulose	13.99	0.00^{ns}	[0.07; 0.10]	0.09
		8.22	-0.03^{ns}	[0.04; 0.06]	0.06
		10.41	-0.01^{ns}	[0.05; 0.07]	0.07
		10.65	-0.04^{ns}	[0.05; 0.08]	0.07
Main photobiont type	*Chlorococcoid*	-1.06	0.07**	[-0.05; 0.02]	0.08
		-0.99	0.03^{ns}	[-0.05; 0.02]	0.08
		-3.20	0.14***	[-0.09; -0.02]	0.10
		-6.51	0.15***	[-0.15; -0.08]	0.13
		0.42	0.04^{ns}	[-0.03; 0.04]	0.07
		2.08	-0.03^{ns}	[0.00; 0.07]	0.08
		2.51	-0.10***	[0.01; 0.08]	0.08
		-0.48	0.05*	[-0.04; 0.03]	0.07
		-1.59	0.02^{ns}	[-0.06; 0.01]	0.08
		-0.36	0.03^{ns}	[-0.04; 0.03]	0.07
		-0.73	0.02^{ns}	[-0.05; 0.02]	0.07
		0.76	0.03^{ns}	[-0.02; 0.05]	0.07
		-1.60	0.03^{ns}	[-0.06; 0.01]	0.08

	Trentepohlia	-2.13	-0.07**	[-0.07; 0.00]	0.08
		-0.98	0.00^{ns}	[-0.05; 0.02]	0.08
		-1.35	0.00^{ns}	[-0.06; 0.01]	0.08
		-1.68	0.01^{ns}	[-0.06; 0.01]	0.08

Table S4.11: Summary of the paired T-test analysis, between observed and predicted CWM metric values, for the forecast of 2022. Test significance in the model ($^{NS}\rho > 0.05$; $^{*}\rho < 0.05$; $^{**}\rho < 0.01$; $^{***}\rho < 0.001$).

Functional trait	**Functional metric**	**t-statistic**	**Mean difference**	**95% CI**	**RMSE**
Growth form	Foliose broad lobed	1.16	0.04^{ns}	[-0.01; 0.05]	0.07
	Squamulose	0.02	0.08***	[-0.05; 0.05]	0.10
		-1.33	0.05***	[-0.08; 0.02]	0.10
		-0.53	0.06***	[-0.06; 0.03]	0.10
		-1.57	0.06***	[-0.08; 0.01]	0.11
Main photobiont type	*Chlorococcoid*	3.61	-0.02^{ns}	[0.03; 0.12]	0.12
		1.28	-0.02^{ns}	[-0.02; 0.07]	0.10
		6.79	-0.06**	[0.10; 0.18]	0.17
		7.16	-0.11***	[0.10; 0.19]	0.17
		1.91	0.01^{ns}	[0.00; 0.08]	0.10
		-1.51	0.03^{ns}	[-0.07; 0.01]	0.10
		-4.70	0.04*	[-0.14; -0.05]	0.13
		2.28	-0.01^{ns}	[0.00; 0.09]	0.10
		0.82	-0.03^{ns}	[-0.03; 0.06]	0.09
		1.42	-0.01^{ns}	[-0.01; 0.07]	0.10
		1.17	-0.01^{ns}	[-0.02; 0.07]	0.09

		1.71	0.01^{ns}	[-0.01; 0.08]	0.10
		1.45	-0.03^{ns}	[-0.01; 0.07]	0.09
	Trentepohlia	-3.66	-0.04*	[-0.10; -0.03]	0.10
		-0.21	-0.02^{ns}	[-0.04; 0.03]	0.08
		-0.18	-0.02^{ns}	[-0.04; 0.03]	0.08
		0.53	-0.03^{ns}	[-0.03; 0.05]	0.08

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