Impacts of global changes on biodiversity through the lens of epiphytic bryophytes.

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cover picture :epiphytic bryophytes on a branch, Mormont (Wallonia), picture from Lea Mouton, April 2024

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Department of Biology, Ecology and Evolution

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« Je suis choqué que les biologistes ou les écologues n'aient jamais un mot sur la beauté. Ça me choque parce que la nature c'est beau. »

« I am shocked that biologists and ecologists never mention beauty. It shocks me because nature is beautiful. » (personal translation)

Francis Hallé

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Abstract

The Anthropocene is characterized by unprecedent environmental transformations driven by human activities, including industrialization, agricultural intensification, and deforestation. These changes have deeply altered biogeochemical cycles, climate, and air quality, resulting in a dramatic global decline in biodiversity and disastrous impacts on human health. Among the major contemporary threats, climate change and air pollution are two of the main drivers of species loss. However, their respective effects are difficult to assess, as they both originate from similar anthropogenic sources and interact across spatial and temporal scales.

In this thesis, we aim to document and disentangle the respective impacts of air quality changes and climate change on epiphytic bryophyte communities across space and time. We first assess how improvements in air quality and concomitant climate change between 1980 and 2020 have shaped the spatio-temporal turnover of epiphytic bryophyte communities, evaluating the relative roles of major air pollutants (e.g., SO_2, NO_2, O_3, particulate matter) and climatic conditions. We further investigate whether air pollution continues to affect current epiphytic bryophyte distribution, identifying the specific pollutants involved—including particulate matter, black carbon, ammonium, heavy metals, and pesticides—and disentangling their effects from background environmental factors such as climate, topography, and forest structure.

The spectacular expansion of epiphytic bryophytes in southern Belgium since 1980 is characterized by increasing species frequency, species richness and the emergence of newcomers. Temporal beta diversity was twice higher as extant spatial beta diversity, indicating that community composition differed more at different time periods within the same site than among sites. The spatio-temporal variation in species composition reflected a clear time-series and not a geographic clustering. Variance partitioning analyses demonstrated that this temporal turnover was almost exclusively driven by the improvement in air quality and not by climate change. The sharp decline in SO_2 and NO_2 concentrations since the pollution peaks of the 1970s–1980s allowed acid-sensitive species to recolonize formerly polluted areas, while acidophilous species declined.

Although major pollutants such as SO_2 and NO_2 no longer appear to affect epiphytic bryophyte communities, other pollutants may have emerged. O_3 was identified as the main driver of extant species distributions. Its concentrations are, however, strongly correlated with climatic and land-use conditions, suggesting that it may not necessarily have an actual ecotoxicological impact but instead reflects an urban to rural gradient. The second most important pollutant identified was NH_3. Although the concentrations of the latter are slightly decreasing, NH_3 acts as a base, potentially counter-acting the acidifying effect of historical pollutants and contributing to the decrease of acidophilous species and the increase of nitrophilous species. The potential effects of other pollutants, such as pesticides or heavy metals, remain insufficiently documented, emphasizing the need for improved environmental monitoring.

The lack of any signature of climate change in the recovery of epiphytic bryophyte floras is surprising given the reliance of bryophytes on precipitation for water uptake and the sensitivity of temperate species to moderately warm temperatures. While climatic conditions are commonly assessed at a regional scale from standardized weather stations, organisms, however, actually experience local conditions shaped by topography, vegetation structure,

solar radiation, and wind exposure. This is particularly true for small-size organisms like bryophytes, calling for a shift from a macro to a microclimatic perspective. The difference between macroclimate (open-field) and (*in-situ*) microclimate is called the microclimatic effect. In forest ecosystems, canopy cover typically generates a buffering effect that stabilizes temperature and humidity. Temperature and relative humidity were recorded *in-situ* for one year at 42 stands of *Quercus-Fagus* forests in southern Belgium. The microclimatic effect was characterized at the level of each sensor through the “slope and equilibrium” approach and its variation across sensors was modelled using spatially explicit variables of topography and vegetation structure obtained by satellite imagery. The slope of the linear relationship between macro and microclimate accurately characterized the microclimatic effect, evidencing both buffering and amplification for temperature and only buffering for relative humidity. The models to map the microclimatic effect using topography and vegetation structure as predictors performed, however, poorly. Increasing the number of dataloggers measuring microclimate *in-situ* to calibrate the models and adding new predictors, such as vegetation structure variables derived from LiDAR, appear as the next steps to increase model performance. Species response curves to the microclimatic effect allowed for a quantitative characterization of species indicator values for forest microclimates and their drivers. Overall, this preliminary study sets the premises towards a fine-scale mapping of forest microclimates and the characterization of species responses to microclimatic effects, opening the door to the identification of microrefugia and the formulation of recommendations for forest management under climate change.

Résumé

L'Anthropocène se caractérise par des transformations environnementales sans précédent causées par les activités humaines, notamment l'industrialisation, l'intensification de l'agriculture et la déforestation. Ces changements ont profondément modifié les cycles biogéochimiques, le climat et la qualité de l'air, entraînant un déclin alarmant de la biodiversité à l'échelle mondiale et des conséquences désastreuses sur la santé humaine. Parmi les menaces actuelles qui pèsent sur la biodiversité, le changement climatique et la pollution atmosphérique sont deux des principaux facteurs de disparition des espèces. Cependant, leurs effets respectifs sont difficiles à évaluer, car ils proviennent de sources anthropiques similaires et interagissent à différentes échelles spatiales et temporelles.

Dans cette thèse, nous visons à documenter et à démêler les impacts respectifs des modifications de la qualité de l'air et du changement climatique sur les communautés de bryophytes épiphytes à travers l'espace et le temps. Nous évaluons d'abord comment l'amélioration récente de la qualité de l'air et le changement climatique concomitant entre 1980 et 2020 ont façonné le *turnover* spatio-temporel des communautés de bryophytes épiphytes, en évaluant les rôles relatifs des principaux polluants atmosphériques (par exemple, SO_2, NO_2, O_3, les particules fines) et des conditions climatiques. Nous déterminons ensuite si la pollution atmosphérique continue d'affecter la distribution actuelle des bryophytes épiphytes, en identifiant les polluants spécifiques impliqués (notamment les particules fines, le noir de carbone, le NH_3, les métaux lourds et les pesticides) et en dissociant leurs effets des facteurs environnementaux tels que le climat, la topographie et la structure forestière.

L'expansion spectaculaire des bryophytes épiphytes en Wallonie depuis 1980 se caractérise par une augmentation de la fréquence et de la richesse des espèces, ainsi que par l'apparition de nouvelles espèces. La diversité bêta temporelle est deux fois plus élevée que la diversité bêta spatiale actuelle, ce qui indique que la composition des communautés diffère davantage entre différentes périodes au sein d'un même site qu'entre les sites. La variation spatio-temporelle de la composition des espèces reflète un gradient chronologique clair et non un regroupement géographique. Les analyses de *variance partitioning* ont démontré que ce renouvellement temporel est presque exclusivement dû à l'amélioration de la qualité de l'air et non au changement climatique. La forte baisse des concentrations de SO_2 et de NO_2 depuis les pics de pollution des années 1970-1980 a permis aux espèces sensibles à l'acidité du milieu de recoloniser les zones autrefois polluées, tandis que les espèces acidophiles ont décliné.

Bien que les principaux polluants (SO_2 et NO_2) n'affectent plus les communautés de bryophytes épiphytes, d'autres polluants peuvent leur avoir succédé. L'O_3 a été identifié comme le principal facteur déterminant la répartition actuelle des espèces. Ses concentrations sont toutefois fortement corrélées aux conditions climatiques et à l'utilisation du territoire, ce qui suggère qu'il n'a pas nécessairement un impact écotoxicologique direct, mais reflète plutôt un gradient entre les zones urbaines et rurales. Le deuxième polluant identifié comme le plus déterminant de la distribution des bryophytes épiphytes est le NH_3. Bien que les concentrations de ce dernier soient en légère diminution, le NH_3 agit comme une base, pouvant potentiellement contrebalancer l'effet acidifiant des polluants historiques et contribuer à la diminution des espèces acidophiles et à l'augmentation des espèces nitrophiles. Les effets potentiels d'autres polluants, tels que les pesticides ou les métaux lourds, restent insuffisamment documentés, ce qui souligne la nécessité d'améliorer la surveillance environnementale.

L'absence de contribution évidente du changement climatique dans le rétablissement de la flore bryophyte épiphyte est surprenante, compte tenu de la dépendance des bryophytes à l'égard des précipitations pour leur approvisionnement en eau et de la sensibilité des espèces tempérées aux températures modérément chaudes. Si les conditions climatiques sont généralement évaluées à l'échelle régionale à partir de stations météorologiques standardisées, les organismes sont toutefois soumis à des conditions locales déterminées par la topographie, la structure de la végétation, le rayonnement solaire et l'exposition au vent. Cela est particulièrement vrai pour les organismes de petite taille comme les bryophytes, ce qui nécessite de passer d'une perspective macroclimatique à une perspective microclimatique. La différence entre le macroclimat (en milieu ouvert) et le microclimat (*in-situ*) est appelée « effet microclimatique ». Dans les écosystèmes forestiers, la couverture forestière génère généralement un effet tampon sur la température et l'humidité. La température et l'humidité relative ont été enregistrées *in situ* par des capteurs pendant un an dans 42 peuplements de hêtraies-chênaies en Wallonie. L'effet microclimatique a été caractérisé au niveau de chaque capteur à l'aide de l'approche « *slope and equilibrium* » et sa variation entre les capteurs a été modélisée à l'aide de variables spatialement explicites de topographie et de structure de la végétation obtenues par imagerie satellite. La pente de la relation linéaire entre le macroclimat et le microclimat a permis de caractériser avec précision l'effet microclimatique, mettant en évidence à la fois un effet tampon et un effet d'amplification pour la température, et uniquement un effet tampon pour l'humidité relative. Les modèles permettant de cartographier l'effet microclimatique à l'aide de la topographie et de la structure de la végétation comme prédicteurs ont toutefois une faible performance. L'augmentation du nombre de capteurs mesurant le microclimat *in situ* afin de calibrer les modèles et l'ajout de nouveaux prédicteurs, tels que les variables de structure de la végétation dérivées du LiDAR, semblent être les prochaines étapes pour améliorer les performances des modèles. Les courbes de réponse des espèces à l'effet microclimatique ont permis une caractérisation quantitative des valeurs indicatrices des espèces des microclimats forestiers. Cette étude préliminaire pose donc les bases d'une cartographie à fine échelle des microclimats forestiers et de la caractérisation des réponses des espèces aux effets microclimatiques, ouvrant la voie à l'identification de micro-refuges et à la formulation de recommandations pour la gestion forestière dans le contexte du changement climatique.

List of contributions

The thesis is based on the following articles:

I. Virginie Hutsemékers, **Lea Mouton** , Hannah Westenbohm, Flavien Collart and Alain Vanderpoorten, 2023. Disentangling climate change from air pollution effects on epiphytic bryophytes. Global Change Biology. 29, 3990–4000. https://doi.org/10.1111/gcb.16736.

II. **Lea Mouton**, Virginie Hutsemékers, Flavien Collart and Alain Vanderpoorten, 2025. Does air pollution still impact epiphytic bryophytes in the post acidic rain era? Insights from spatial variation of community composition in southern Belgium. Environmental Pollution. 379, 126495. https://doi.org/10.1016/j.envpol.2025.126495.

III. **Lea Mouton**, Virginie Hutsemékers, François Jonard, Flavien Collart and Alain Vanderpoorten, 2026. In search of forest microrefugia: an analysis of the microclimatic effect. *in prep*.

and the contribution of the following articles (in appendix):

IV. Ting Shen, Richard Corlett, Flavien Collart, Thibault Kasprzyk, Xin-Lei Guo, Jairo Patiño, Yang Su, Olivier Hardy, Wen-Zhang Ma, Jian Wang, Yu-Mei Wei, **Lea Mouton**, Yuan Li, Liang Song, and Alain Vanderpoorten, 2022. Microclimatic variations in tropical canopies: a glimpse into the processes of community assembly in epiphytic bryophyte communities. Journal of Ecology. 10, 3023–3038. https://doi.org/10.1111/1365-2745.14011

V. **Lea Mouton**, Jairo Patiño, Marc Carine, Fred Rumsey, Miguel Menezes de Sequeira, Juana María González-Mancebo, Rosalina Maria de Almeida Gabriel, Olivier J. Hardy, Manuela Sim-Sim, J. Alfredo Reyes-Betancort, Flavien Collart, and Alain Vanderpoorten. (2023). Patterns and drivers of beta diversity across geographic scales and lineages in the Macaronesian flora. Journal of Biogeography. 50, 858–869. https://doi.org/10.1111/jbi.14580

VI. Ting Shen, Liang Song, Richard Corlett, Antoine Guisan, Jian Wang, Wen-Zhang Ma, **Lea Mouton**, Alain Vanderpoorten and Flavien Collart, 2023. Disentangling the roles of chance, abiotic factors and biotic interactions among epiphytic bryophyte communities in a tropical rainforest (Yunnan, China). Plant Biol. 25(6):880-891. https://doi.org/10.1111/plb.13570

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1.General Introduction

cover picture :epiphytic bryophytes on a tree, Châtelet (Wallonia), picture from Lea Mouton, Decembre 2025

1. General Introduction

The contemporary geological era Anthropocene has been marked by various changes caused by human activities (Steffen *et al*., 2011). During the last couple of hundred years, many aspects of the environment have changed through time and space (Wolkovich *et al*., 2014) resulting from increasing human population and consecutive activities such as industrialization, the intensification of agriculture, deforestation, and the growing intake of natural resources. Consequently, human activities have drastically modified climate, ecosystems and therefore, species living conditions (Winter *et al*., 2009). Indeed, 95% of Earth have undergone modifications by human-hand (Kennedy *et al*., 2019), 40% of the Earth's land surface has been transformed into cropland and pasture (Foley *et al.*, 2005) and 178 million ha of forest have been lost worldwide since 1990 (FAO, 2020). As a result, 163,040 of living species are threatened with extinction (Union for Conservation of Nature, 2024) and the number of threatened species is expected to increase (Asafu-Adjaye, 2003). Barnosky *et al.* (2011) predict an extinction of 75% of species in 240–540 years.

Among the environmental consequences of global change, the Anthropocene is marked by unprecedent changes in air quality and climate (Steffen *et al*., 2011). Industrial activities massively released greenhouse gases and pollutants in the air, contributing to global warming (IPCC, 2013). Affecting element cycle, human health and other species living conditions (Steffen *et al*., 2011), climate change has become a primary concern.

For this thesis, I focus on the responses of biodiversity in face to changes in air quality and climate change. Climate change and pollution account together for 14% of the global biodiversity decline and are the third and fifth major threat to species loss, respectively (Prakash and Verma 2022;TRS, 2021). Air pollution and climate change are closely related and it is difficult to separate their effects (Marcantonio *et al*., 2021) because they both result from similar human activities. Understanding how these two threats are impacting biodiversity over space and time is essential to implement effective policies to improve living conditions on earth.

1.1 Air quality: 200 years of changes in air pollution

During the past century, especially since the industrial era, a range of major pollutants of different origins (Box 1) have impacted both human health and ecosystem functioning (*L'Agence wallonne de l'Air et du Climat (AwAC) et la Cellule Qualité de l'Air de l'Institut Scientifique de Service Public (ISSeP), 2025;* Govindapyari *et al*., 2010).

Box1 -What are the main air pollutants in Europe and where do they come from?

Sulfur dioxide (SO_2) comes from impurities present in fossil fuel sources (oil, coal and natural gas). Sulfur impurities are released during the combustion of fossil fuels and oxidize in the air.

Nitrogen dioxide (NO_2) and **nitrogen monoxide** (NO) result from the oxidation at high temperature of atmospheric nitrogen N_2 by combustion. NO_x are unstable and quickly oxidize into NO_2. Although NO_x can have natural origins, they are mostly resulting from fossil fuels combustion for energy production, industrial processes and transport.

Ozone (O_3), which is naturally present in the stratosphere, is a secondary inorganic aerosol formed from volatile organic compounds (VOC, C and H resulting from combustion of fossil fuels or the evaporation of biomass, agriculture and solvents) and nitrogen oxides (NO_x) under sunny conditions in the troposphere. O_3 is mainly produced during daylight and destroyed at night. This equilibrium can be disturbed by the overproduction of primary pollutants, notably NO. High NO concentrations promote the destruction of O_3 to form NO_2, much more stable. As a result, O_3 concentrations are higher in rural than in urban areas, wherein O_3 is largely degraded into NO_x due to the high concentrations of NO resulting from traffic (Alvim-Ferraz *et al*., 2024).

Ammonium, NH_4^+, is largely used in agriculture as crop fertilizer, either under the form of industrial fertilizers or animal manure. Ammonium is converted to **ammoniac** NH_3 by volatilization. NH_4^+ is industrially produced by combining atmospheric nitrogen with hydrogen usually derived from natural gas, a process that requires a lot of energy. (Del Moro *et al*., 2017).

Particulate Matter (PM) are solid or liquid entities suspended in the air from various origins, with various composition, size and density. They are issued mostly from biomass and fossil fuel combustion (Byčenkienė *et al*., 2022), such as industrial and natural fires. The largest particles contain elements from the Earth's layer (such as rock-forming elements or metals) while the smallest ones contain sulfates, nitrates, ammonium, carbon, organic compounds and metals. PM are usually divided and studied in two fractions, based on the aerodynamic diameter: PM_{10} and $PM_{2.5}$ refer to Particulate Matter with a diameter smaller than 10 and 2.5 µm, respectively (Byčenkienė *et al*., 2022).

Black carbon (BC) is a particular $PM_{2.5}$, mainly composed of pure carbon, resulting from an uncompleted combustion of hydrocarbons such as fossil fuels for transport, coal for domestic heating and agricultural wastes. Black carbon is also released naturally during forest wildfires.

Heavy metals are non-biodegradable pollutants (Govindapyari *et al*., 2010) present in traces or PM in the air. In Europe, they were mainly emitted by the steel industry in the past. Today, it is the transport and industry sectors that are the main emitters.

Pesticides come mainly from the agricultural sector and are released into the air either directly, by aerial spraying, which can carry pesticides quite far (diffuse pollution), or by granules or powders. Solid and liquid pesticides can also end-up in the air through volatilization or erosion (Huang and Li, 2024).

History of air pollution in Europe

The first serious estimation of pollutant concentration in the air dates back to 1860-1880 (Mylona, 1996). Back then, the primary energy source was coal, whose combustion emits massive quantities of SO_2 and NO_2 into the air. Coal combustion has increased, due to industrialization, by a factor 10 between 1880 and 1970 (Figure 1), resulting in an increase of NO_2 and SO_2 (Fenger, 2009). These concentrations peaked in the 1970s and the 1980s for SO_2, and in the 1980s-1990s for NO_2 (Figure 1) (Mylona, 1996).

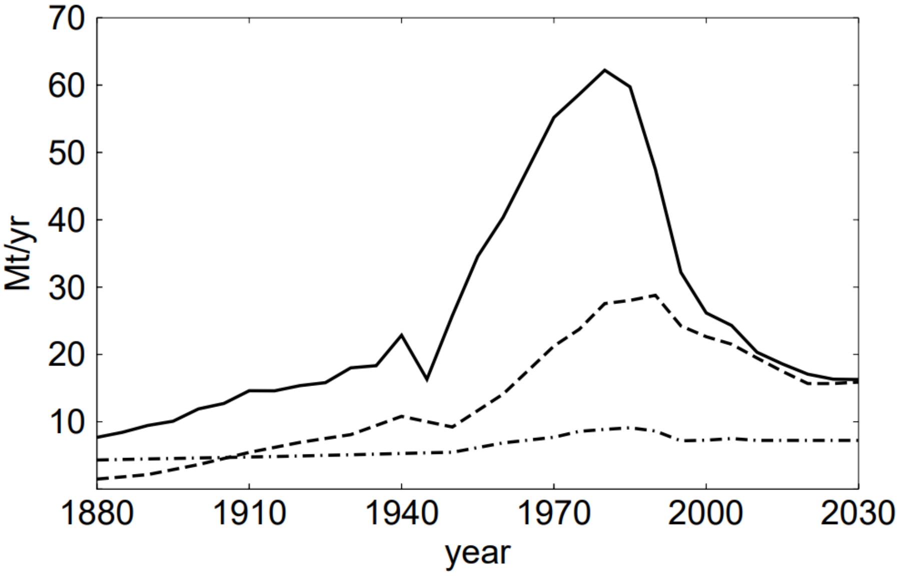

Figure 1. Measured and predicted emissions of major atmospheric pollutant (---- for SO2, - - - for NO2 and -·-·-· for NH3) from 1880 to 2030 in Europe, from Schöpp et al. (2003).

Air pollution has had dramatic consequences for public health. The great smog of London in 1952 killed several thousand people due to respiratory illnesses caused by air pollution (Polivka, 2018). The industrial pollution crisis in the 1980s caused extremely high rates of respiratory diseases and reduced life expectancy in Poland (Slocock and Sowinski, 1996; Milczanowski, 1990; Kramer, 1987). Lead (Pb) in fuels, released into the air throughout Europe by transport, has caused numerous cardiovascular diseases (von Storch *et al*., 2003). These same pollutants have also had disastrous consequences on the environment such as forest decline caused by acidic rains (Pitelka and Raynal, 1989). SO_2 emissions have drastically contributed to the acidification of soil, water and tree bark (Dittrich, Leuschner and Hauck, 2016; Hauck *et al*., 2011). In Germany and Scandinavia in particular, acidification of lakes caused the demise of the aquatic fauna and the pollution of rivers and soils (Menz and Seip, 2004; Munton *et al*, 1999).

Since the 1950s, the intensification of agriculture in Europe has led to a sharp increase in the use of nitrogen inputs, particularly in the form of fertilizers and manure (de Vries *et al*., 2021; Sutton *et al*., 2011). A significant proportion of this nitrogen (nearly 40% according to Leip *et al.,* 2011) is not absorbed by crops and leaks into the environment, with potentially harmful consequences for terrestrial and aquatic ecosystems, causing general eutrophication, which peaked in the late 1980s (Figure 1). In addition to reducing species diversity by eutrophication, leached nitrates pollute water and air, (de Vries *et al.*, 2021).

With increasing concerns about the negative effects of air pollution on human health and the issue of ecosystems eutrophication, several environmental policies have been implemented globally, such as the Convention on Long-Range Transboundary Air Pollution (1979), and in the European Union to improve air quality, notably the National Emissions Ceiling Directive (2001/81/EC) fixing emission targets for NH_3 and nitrogen oxide (NO_x), the Ambient Air Quality Directive (2008/50/CE), the Nitrates Directive (1991) and the Water Framework Directive (2000), which fix upper limits for nitrogen and phosphorus (P) concentrations in water bodies.

These policies, together with changes in fuel usage, combustion technology and relocation of industries, resulted in a significant decrease of SO_2, NO_2 and NO_3 concentrations in the air after the 1990s (Figure 1) (Syrek-Gerstenkorn *et al.*, 2024 ;de Vries *et al.,* 2021; Jyethi, 2016; Pescott *et al.*, 2015; Stern, 2005). While the progressive reduction of the use of coal since the 1980s resulted in a substantial decrease of the emissions of SO_2, the decrease in NO_2 was somewhat lower due to increasing road traffic (Figure 1) (Hilboll *et al.*, 2013; Castellanos and Boersma, 2012; Schöpp *et al.*, 2003). NH_3 concentrations, however, keep on slightly increasing due to the intensification of agriculture (Figure 1).

These policies, however, mainly focused on SO_2 and NO_x. Other pollutants, which previously attracted less attention (O_3 or NH_4^+), raise now new public health concerns (Fenger, 2009). In the meantime, new sources of pollution emerge such as pesticides (Brüggemann *et al.*, 2024; Kruse-Plaß *et al.*, 2021), plastic components (Shah and Saiyad, 2025; Amato-Lourenço *et al.*, 2021), and endocrine disruptors (Darbre, 2018).

Pesticides began to be used at a massive scale during World War II, including by aerial spraying. Awareness of the harmful effects of DDT on health and the environment, triggered by the publication of Rache Carson's book Silent Spring in 1962 (Fenger, 2009), led the USA to ban DDT in 1972. Europe also began banning certain molecules in the 1970s. Following the discovery of diffuse pesticide pollution (far from the place of their application) in 1980-1990, the European Union introduced the placing of plant protection products on the market Directive (91/414/EEC), which aims, among other things, to harmonize plant protection products on the market in Europe and remove many substances judged too dangerous. From 2000 onwards, authorities and scientists focused on the regulation and study of pesticides, notably with the adoption of the Registration, Evaluation, Authorization and restriction of CHemicals (REACH) regulation in 2006. Since 2006, EU member states have been encouraged to reduce pesticides and monitor their concentration, notably with the Directive 2009/128/EC establishing a framework to achieve the sustainable use of pesticides. Surprisingly, no specific standards for pesticides in ambient air have yet been imposed by EU, unlike for water, food or soil (Huang and Li, 2024; Storck *et al.*, 2017).

Despite these policies, some pollutants, such as $PM_{2.5}$, Black Carbon, NH_4^+ and O_3, are still responsible for poor air quality. Indeed, even if the concentrations of PM_x and BC have been decreasing significantly since the 2000s, notably thanks to the EURO 6 European Emission Standards (Commission Regulation (EU) 2016/646 2016), the NEC Directive (2016/2284/EU), and programs to reduce the use of polluting cars in cities (such as low emission zones), air concentrations in large cities remain higher than the recommendations established by the World Health Organization (Byčenkienė *et al.*, 2022; Stafoggia *et al.*, 2022). In addition, O_3 is a major cause of premature mortality linked to air pollution in Europe, especially in summer, and its impact is worsening with global warming. More than 95% of European citizens are still exposed to O_3 levels above WHO guidelines, increasing health risks in a context of increasing urbanization and an aging population (Achebak *et al.*, 2024; Byčenkienė *et al.*, 2022).

1.2 Climate change

Intense human activities released, notably by burning fossil fuels (Fakan, 2020; Steffen *et al.*, 2015), several chemicals into the atmosphere. These pollutants, in addition to hurting human and ecosystem health, are responsible for changes in Earth climate (Jeffry *et al.*, 2021; Steffen *et al.*, 2015). Some of such chemicals are known as greenhouse gases. Carbon dioxide (CO_2) and methane (CH_4) are the two main greenhouse gases. The concentration of net greenhouse gases increased in 2024 by 151% as compared to pre-industrial times for CO_2 and 265% for CH_4 (WMO, 2025). This trend is still continuously increasing with the burning of fossil fuels for the increasing needs of energy for modern human activities (Jeffry *et al.*, 2021). By trapping heat in the atmosphere, greenhouse gases contribute drastically to the rise of temperature globally.

The first mention of climate change dates back to 1896, with the « greenhouse warming theory » of the Swedish chemist Svante Arrhenius. Arrhenius predicted that CO_2 would increase due to burning coal and with it, a global rise in temperatures (Charlson, 1998). In 1960, an increase of CO_2 concentration in the atmosphere was evidenced (Luterbacher and Sprinz, 2001; Keeling *et al*, 1976). This report, including the Keeling curve (Figure 2), set off the alarm inside the scientific sphere in the late 1960's early 1970's (Luterbacher and Sprinz, 2001). Since then, greenhouse gases have increasingly become an area of concern. In the year 1988, two United Nations agencies, the World Meteorological Organization (WMO) and the United Nations Environment Programme (UNEP), launched the Intergovernmental Pannel on Climate Change (IPCC) in order to communicate on climate change issues with decision-makers. IPCC includes also a large number of experts in climate change effects on human and environment health (Luterbacher and Sprinz, 2001; Agrawala, 1997).

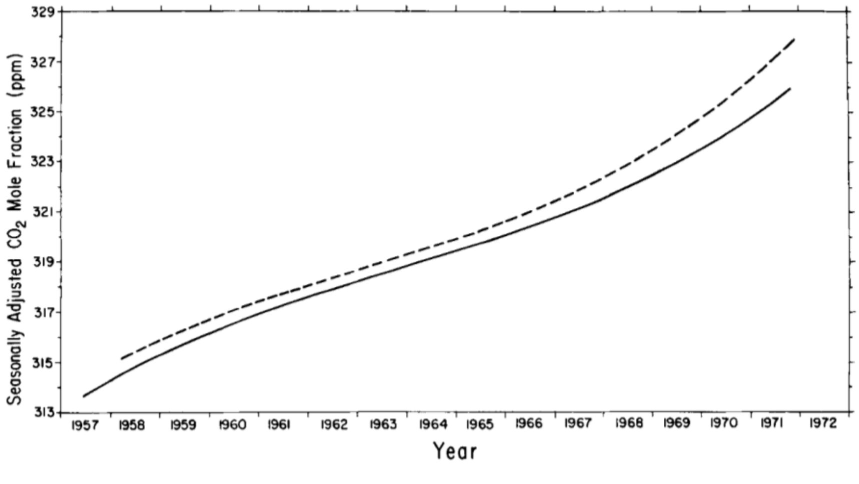

Figure 2. Long term variation in concentrations of atmospheric CO2 at Mauna Loa Observatory (for the dashed line) and the South Pole (for the plain line). Concentrations are expressed as the CO2 mole fraction of dry air in ppm. From: Keeling et al, 1976.

The last report of the Intergovernmental Panel on Climate Change (IPCC, 2024) points to a global temperature increase of approximately 1.19°C for 2014–2023, reaching an increase of 1.31°C in 2023 relative to 1850–1900. This Human-induced warming corresponds to a rate of 0.26°C per decade. IPCC estimates that the 1.5°C increase will be reached by 2031 and 2.0°C by 2050, i.e., a faster rate than initially anticipated. For instance, IPCC estimated in 2018 that the 1.5°C would be reached by 2040 and 2.0°C by 2100. Human activities affecting climate change keep on developing with increasing intensity despite IPCC warnings.

Climate change is not only about a rise in global temperature, but also about changes in weather extremes, which are most impacting for human health and biodiversity. As an example, the number of temperature anomalies has drastically increased, snowpacks are dramatically reducing, flooding and wildfire are getting increasingly frequent and intense (Bolan *et al.*, 2024).

The global rise of temperatures and the increase in extreme weather events lead to a change of habitat conditions, and hence, to a change of species composition. Consequently, the number of threatened species is expected to increase with global warming (Asafu-Adjaye, 2003) and its consequences. In 2019, Nunez *et al.* predicted that on average across plant and animal taxa, habitat suitability will be reduced by 35% with only 2°C increase in global temperature.

To survive, species have three options. They can migrate to maintain suitable climatic conditions. An expansion of species distribution toward cold areas, such as poles and high altitudes, has already been evidenced (Zu *et al.*, 2021; Lenoir *et al.*, 2020; Pauchard *et al.*, 2016; Lenoir and Svenning, 2015), but involves that species migration rates are at least equally fast as the velocity of climate change. In addition, characteristics of the environment, such as fragmentation, topography and spatial discontinuity, can also limit species to migrate. Species unable to track climate change must adapt to new climatic conditions or persist in microrefugia to not perish.

1.3 Microclimate for micro-organisms

Climatic conditions are typically measured by weather stations located in flat open field, in conditions of air mixing. These conditions are standardised to harmonise the measurements taken all over the world (Kemppinen *et al.*, 2024) and are used to predict future climatic scenarios, depending on potential political decisions (Viríssimo and Stainforth, 2025). These standardised and large-scale conditions, also called the **macroclimate**, are, however, not the ones actually experienced by organisms in their immediate environment (Kemppinen *et al.*, 2024). Indeed, the combination of topography, vegetation structure, solar radiation, wind, and water availability locally generates a **microclimatic effect** (Gril, Laslier, *et al.*, 2023; Gril, Spicher, *et al.* 2023) that modifies the macroclimate into local climatic conditions, called **microclimate**, that are actually experienced by organisms.

The microclimate determines at fine scale the distribution and the richness of species, and therefore influences species responses to climate change (Kemppinen *et al.*, 2024). To better understand how macroclimatic changes are affecting or will affect species distribution, microclimate science has progressively developed (Kemppinen *et al.*, 2023). Ignoring the microclimatic effect leads to a misrepresentation of the range of species niche (Haesen *et al.*, 2023b), which, consequently, can lead to an over-representation or a disparity (Lembrechts *et al*., 2019; Franklin *et al*., 2013) of the actual suitable area for species under climate changes. In mountain ecosystem for example, the variability of the mean temperature can reach 6° C within 1km² (Haesen, *et .al*, 2023a), biasing species distribution models based on climatic data at a resolution >=1 km². Taking into account the microclimatic effect is, therefore, crucial to develop conservation programs and identify appropriate areas to protect, such as microrefugia (Haesen *et al*., 2023a,b; Man *et al*., 2022; Meineri and Hylander, 2017).

Forests are a key example of ecosystems experiencing a striking microclimatic effect (De Frenne *et al*., 2019). Under forest canopy, the climatic conditions are more stable than outside the forest. It is called the "**buffering effect**". The canopy of forest trees prevents direct light to reach the soil and radiate forest species, but also provides protection against wind. Temperature and humidity variations are therefore smoothed in understories. The canopy absorbs and reflects solar radiations, cooling the temperature by up to 4.1° C on average compared to open fields (De Frenne *et al*., 2019). Indeed, wind is reduced by 80% and light by 93% at 80 m inside the forest compared to an open area at the edge of the forest (Davies-Colley *et al*., 2000). In the future, these buffered forest areas could, therefore, serve as microrefugia for forest-specific species (Kemppinen *et al*., 2024; Haesen *et al*., 2023a). The microclimatic conditions of forests are called to play a crucial role in protecting species from extreme macroclimatic events, such as those predicted with global climate change (Bolan *et al*., 2024).

Although forests are recognised as highly buffered environment, their microclimates can also, however, be exacerbated as compared to macroclimates, especially in forest edges or forest gaps (Gril, Spicher, *et al*. 2023; Vandewiele *et al*., 2023; Carnicer *et al*., 2019). It is called the "**amplifying effect**". Forest edge environments are accentuated by deforestation, which reduces the area of forest and thus increases the proportion of forest edges, as well as by forest fragmentation. Forest gaps are the result of natural tree falls or macrofauna grazing or forest management. Through forestry activities such as clear-cutting and thinning, direct light easily penetrates the forest. In addition, the absence of wind due to the presence of surrounding trees prevents air mixing, unlike in open fields. Direct light and the lack of wind thus create a microclimate that is exacerbated compared to the macroclimate.

Under forest canopies, wind and solar radiation decrease proportionally to the complexity of vegetation structure and topography, whose variation shapes a variety of microclimates (Man et al., 2022; De Frenne *et al*., 2021; Frey *et al*., 2016) (Figure 3).

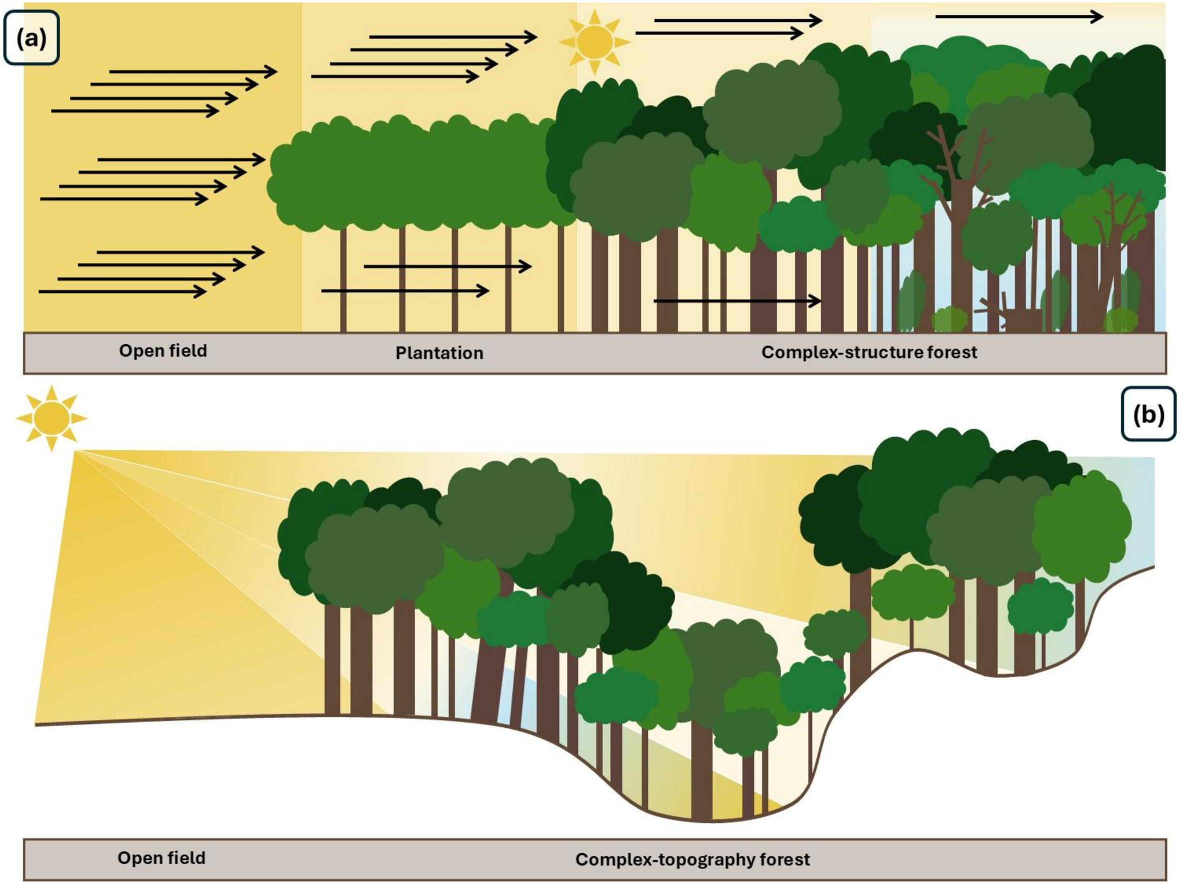

Figure 3. Microclimatic effect variations depending on (a) vegetation structure and (b) topography. Arrows represent the intensity and the speed of the wind, the brightness of yellow represents the intensity of solar radiations and blue represents water retention.

Since forest structure itself varies depending on forest management, one of the biggest challenges at the present time is to adapt forest management and production to increase forest resilience to climate warming in the future. Identify forests with high buffering capacity and protect these areas is therefore essential to help species better cope with climate change and preserve forest health and functions.

1.4 Bryophytes, witnesses of fast environmental changes

In forest micro-habitats, characterised by microclimate and vegetation structure, live micro-species. These small species are extremely adapted to their micro-habitat and depend on its stability. Therefore, these micro-species have turned out to be an excellent tool for tracking and predicting changes in microclimatic conditions. Micro-species can also be used to identify high buffered forest patches that should be protected to anticipate forest changes in the future.

Among these micro-species, I have chosen to focus my thesis on epiphytic bryophytes. Because of their small size and their extreme sensitivity to their environment, they provide an excellent tool to witness global change impacts on biodiversity over space and time, and as such, appear as promising bioindicators in the context of climate change.

What are bryophytes?

Bryophytes are non-vascular archegoniates characterized by a haplodiplontic life cycle with a dominant gametophytic phase (Figure 4), which is unique among land plants (Tuba *et al.*, 2011). Sexual reproduction depends on water, as the male antherozoid has to swim to a female archegonium in a continuous film of water. The result of sexual reproduction is the growth of a sporophyte (2n) on the gametophyte (n). The sporophyte releases spores, resulting from meiosis in the capsule. The spore (n) is the sexual dispersal unit and measures usually between 10 and 30 µm (see examples in Figure 5). Under suitable conditions, the spore germinates into a protonema that develops into a gametophyte (Figure 4). Most bryophytes are also capable of asexual reproduction through the production of vegetative propagules. Bryophytes exhibit the highest morphological diversity of propagules among all plant groups (see examples in Figure 5).

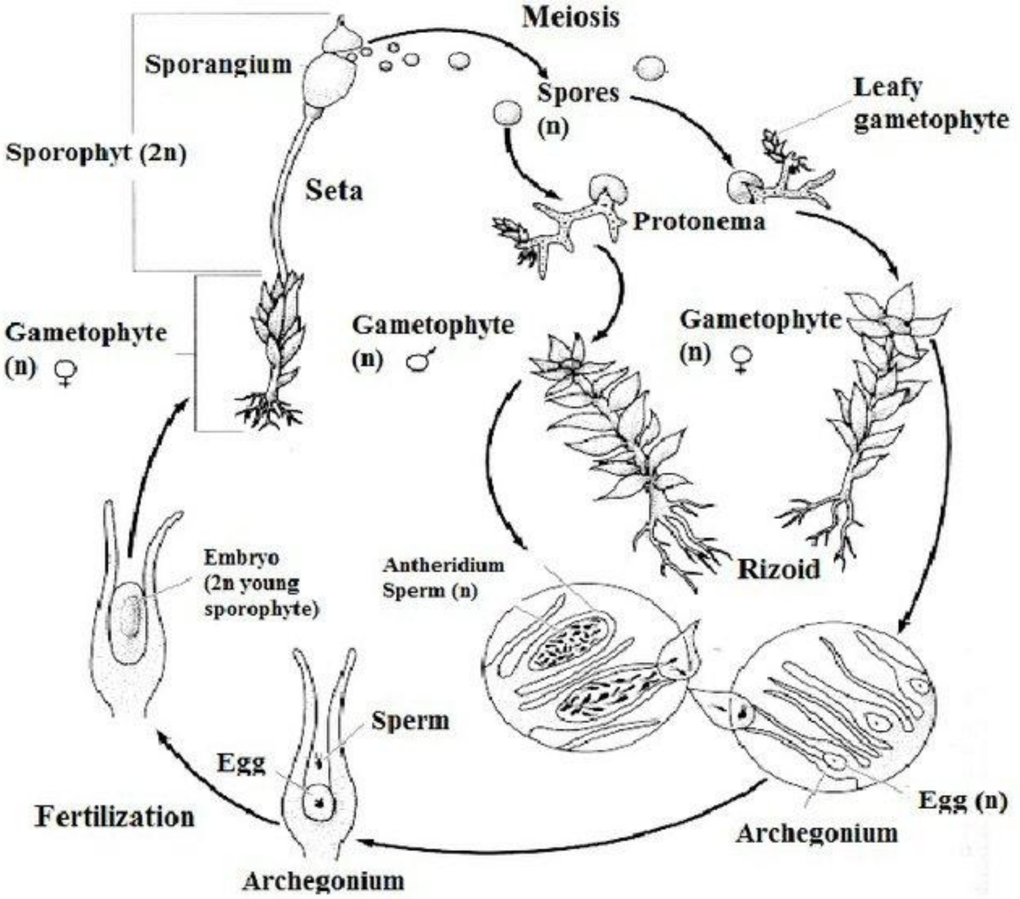

Figure 4. Typical bryophyte live cycle, from Ursavaş et al.(2013).

Bryophytes are the second most diversified group of land plants (the first group being angiosperms), with approximately 20 000 species in the world (Patiño and Vanderpoorten, 2018), 1 800 in Europe (Hodgetts, 2019) and 720 in Belgium (Sotiaux and Vaderpoorten, 2015). Bryophytes are divided into three groups: mosses (bryophytes in the strictest sense), liverworts (Marchantiophyta) and hornworts (Anthocerophyta) (Figure 5) (Renzaglia *et al.*, 2007). Bryophytes can be found in all terrestrial and freshwater ecosystems with the only exception of permanent ice. Bryophytes grow on every substrate, soil, rocks, trees and even on leaves.

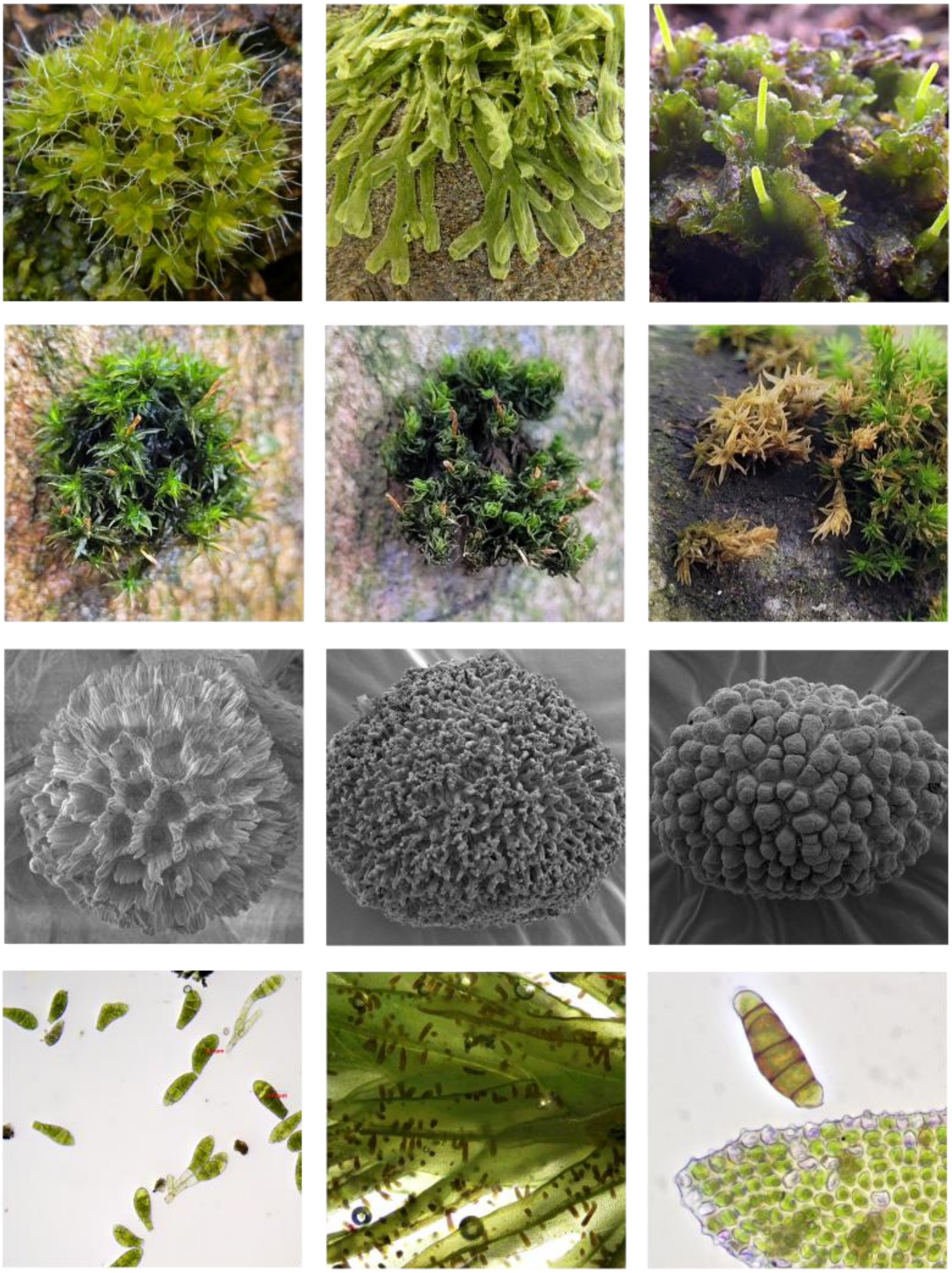

Figure 5. Bryophyte diversity. From the top left corner to the bottom right corner,: Syntrichia laevipila is an acrocarp moss (bryophyte in the strictest sense), Metzgeria furcata is a simple-looking thalloid liverwort (Marchantiophyta) and Anthoceros punctatus is a hornwort (Anthocerophyta), picture from The British Bryological Society (2021). A moisturised Ulota, a dried Ulota and a dead Orthotrichum spp, pictures from the author, Lea Mouton (2026). The spores belong to Bruchia flexuosa, Archidium alternifolium and Ulota bruchii, pictures from Alix Millis (2024). The propagules belong to Zygodon viridissimus, Pulvigera lyellii and Nyholmiella obtusifolia, pictures from The British Bryological Society (2021).

Why are bryophytes good bioindicators?

Bryophytes have no vascular system, no roots and no functional stomata. Therefore, bryophytes uptake water and nutrients from rainfall directly though their tissues. They are poikilohydric, which means that they cannot regulate their water content. They enter dormancy when they dry out due to lack of humidity in the air and, when moisturized, resume their physiological activities (He *et al*., 2016; Vanderpoorten and Goffinet, 2009).

In the context of climate change, bryophytes have been qualified as the “canaries in the coal mine” by Tuba *et al*. (2011) because their ecophysiological characteristics make them extremely sensitive to their environment, so that their distribution and the composition of their communities change quickly after environmental change. In particular, bryophytes are known to largely exhibit a temperature optimum colder than angiosperms and weak tolerance for hot weather. For instance, Furness and Grime (1982) showed that many temperate species die under continuous temperatures above 30° C when kept hydrated. This, together with the dependence of bryophytes to precipitation for water uptake (He *et al*., 2016), make them extremely sensitive to climate change (Tuba *et al*., 2011; Glime, 2007).

In addition to being sensitive to climate change and for the same reasons, bryophytes are sensitive to pollution, especially SO_2 (Rao, 1982). Having mostly one-cell thick leaves, thereby maximizing the surface of exposure, bryophytes indistinctively absorb nutrients and pollutants through their tissues. Because they have no possibility to regulate gas exchanges, bryophytes tend to accumulate pollution, which make them more sensitive than angiosperms. As bryophytes generally have low nutrients requirements, they do not benefit, unlike angiosperms, from the artificial addition of nitrogen and phosphorous to the environment. Indeed, because of their small size, they tend to suffer from competition with angiosperms in eutrophic environments (Vanderpoorten and Goffinet, 2009). The increase of nitrate concentrations in the environment largely explains why the diversity of bryophyte communities has severely decreased since the early 20th century (Dittrich *et al*., 2016; Pescott *et al*., 2015; Govindapyari *et al*., 2010).

Epiphytism, a sensitive lifestyle

Epiphytes are organisms growing structurally, non-parasitically and directly on the surface of a plant (for example: trees, shrubs, herbs or even on an epiphytic plant), without attachment to the ground (Zotz, 2016). Many epiphytic plants are obligatory epiphytic, strictly restricted to growing on other plants, mostly on tree bark. Other species are more generalist, thriving on bark but also found on rocks or soil (Smith, 1982). Living unconnected to the ground provides the two unique advantages of space and light access but comes also along with a series of constraints requiring specific adaptations, such as tolerance to drought, attachment to the plant substrate and tolerance to extremely nutrient-poor substrates (Benzing, 1987).

Because epiphytic bryophytes have no contact with the soil, they rely entirely on atmospheric moisture for water and nutrient uptakes, which makes them extremely sensitive to global warming and drought waves. In addition, located at the interface between air and forest, epiphytic bryophyte survival and reproduction depends heavily on host trees and micro-environmental conditions (Werner *et al*., 2011; Larrea and Werner, 2010; Köster *et al*.,2009), generated by the trees themselves. Box 2 provides details on the impact of microclimatic variations in forests on epiphytic bryophytes, to illustrate the small scale at which environmental variations can affect the epiphytic bryoflora (based on the article in Appendix I).

Finally, tree bark, and especially, the acidic bark of trees such as that of birch, beech and oak, has a very low buffering capacity against acidifying compounds such as SO_2, making epiphytes more vulnerable to air pollutants than, for instance, ground-dwelling species that can benefit from the buffering capacity of their substrate.

Box2 - Microclimate for micro-epiphytes

The plant organism used as a substrate by epiphytes is called a phorophyte (Barkman, 1958). The phorophyte provides to epiphytes a heterogeneous habitat with a strong vertical structure (Cornelissen and Steege, 1989; Annaselvam and Parthasarathy, 2001). This vertical gradient translates into a variation of micro-environmental variables, such as light, humidity, temperature, exposure and orientation, from the base of the trunk to the top of the canopy. Evaporation, temperature range, exposure to solar radiation, and wind speed increase with tree height (Petter *et al*., 2016; de Oliveira *et al*., 2009; Barkman, 1958), while debris accumulation and humidity decrease (Shen *et al*., 2018). For example, between 2 and 59 m above ground along a tropical skytree, relative humidity and temperature can vary as much as 30 % and 7.96° C, respectively (Shen *et al*., 2022) at 2pm in February. Species living at tree base are limited by the lack of light and have therefore adapted to shade, while canopy species are limited by drought (de Oliveira and ter Steege, 2015). In addition, tree architecture differs from the tree base to the canopy, due to the presence of branches, leaves, changes in diameter, and changes in bark roughness. The differentiation of habitats along the trunk generates a succession of epiphytic communities inhabiting distinct ecological niches. This has been evidenced, notably in Appendix I, where we aimed to identify the response of epiphytic bryophytes to microclimatic variations. We took advantage of one of the world's 22 canopy cranes, which gives access to 1.1 ha of tropical rainforest in Yunnan (China), to record epiphytic bryophytes from the entire height of 42 trees and to deployed 54 climatic loggers at different heights (Figure 6). Dataloggers recorded hourly temperature and relative humidity during 30 months from July, 2017 to December, 2019.

We showed that the species richness patterns of liverworts are different from that of mosses. Indeed, the number of liverwort species increases with height while the richness of mosses shows the reverse pattern. The vertical turnover of moss and liverwort communities strongly varies depending on microclimatic variation, reflecting a strong shift of species along the tree. Indeed, in tropical forests, almost all epiphytes are specific to a class of height. Therefore, almost no species is shared between the top of the canopy and the tree base.

The sensitivity of epiphytic bryophytes is such that their vertical distribution is driven by average variations of 2.4° C for temperature and −16.7 % for relative humidity during the day between 2 m and 50 m. Finally, we modelled the species turnover according to different variables, such as climatic variables, tree height and tree diameter. We found out that microclimatic variation explains 33 and 18 % of the vertical moss and liverwort turnover, respectively.

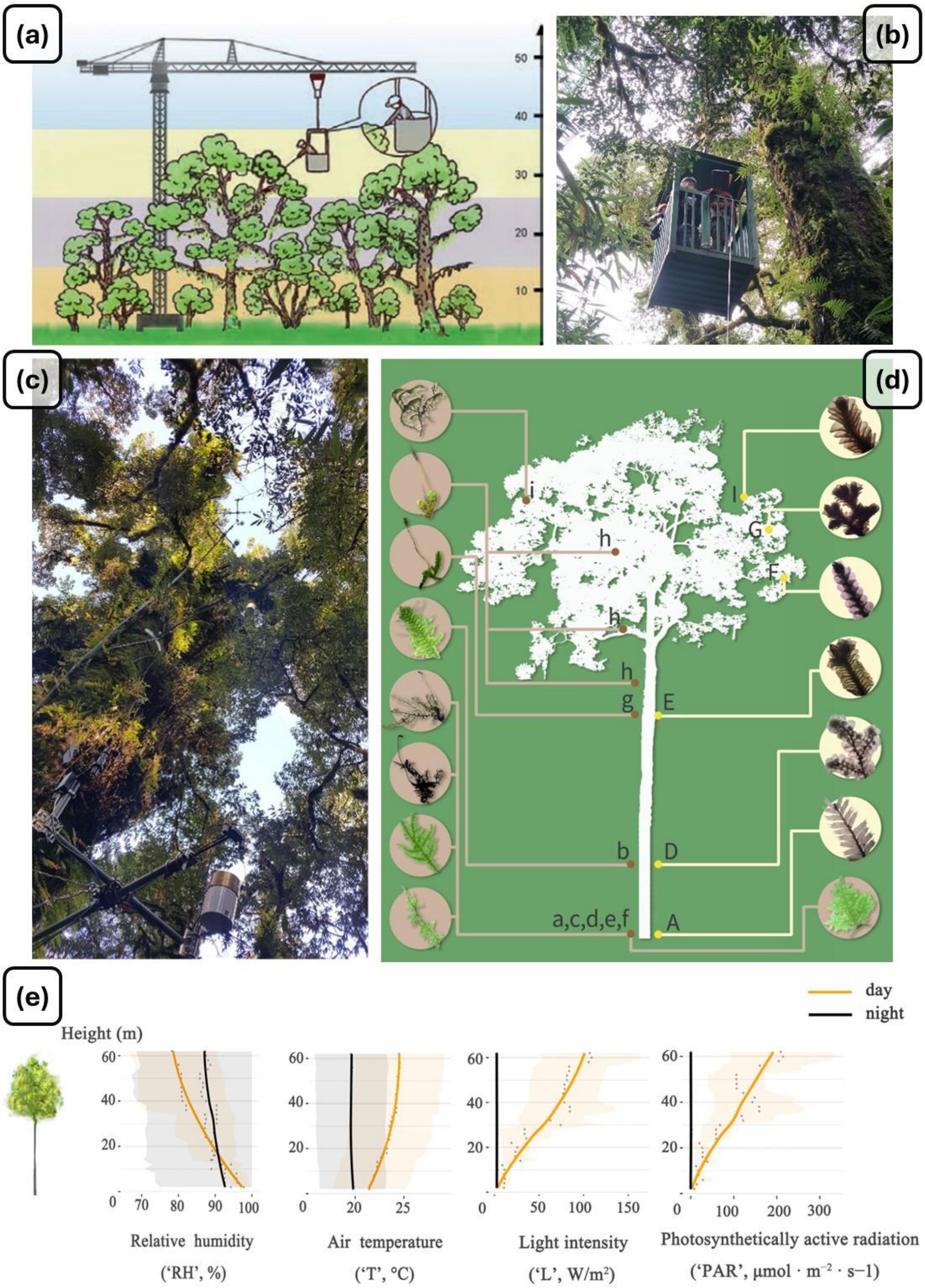

Figure 6. Representation of the canopy crane. (a) Diagram of the canopy crane in a subtropical forest with a scale in meters, (b) canopy crane basket allowing access to the canopy (Ailao Mountains, Yunnan), (c) air temperature and humidity sensors placed from the base to the top (Ailao Mountains, Yunnan), (d) vertical distribution of mosses and liverworts along a tree and (e) day and night climatic variation along a tree. Figure (a) reproduced from Shen, Guo, and Corlett (2018), photo (b) is by Ting Shen (2019), photo (c) is by the author, Lea Mouton (2019), figure (d) is reproduced from Ting Shen's thesis (2023), and figure (e) is reproduced from Shen et al. (2022).

Due to the high sensitivity of epiphytic bryophytes to environmental variations, they have been reacting massively to current global changes. Epiphytic bryophytes have declined dramatically, more than other groups (Pescott *et al*., 2015), with industrialisation in Europe, where large amounts of pollutants have been released since the 50's. SO_2 and NO_x, having a strong acidifying power when dissolved in water, their emission increase has changed the composition of species, from acid-sensitive species to acidophilous species (Pescott *et al*., 2015; Bates *et al*., 1997). Over the past two decades, a spectacular increase in epiphytic bryophyte species richness and frequency has been reported. Figure 7.a, b illustrates for example the distribution of *Cryphaea heteromalla* and *Lewinskya affine* in Belgium between 1980-1095 and 2015-2020. This expansion, recurrently reported elsewhere (Blockeel and Fisk, 2018), has been commonly interpreted as a witness of improved air quality, as evidenced for epiphytic lichens as well (Pescott *et al*., 2015). Indeed, acid-sensitive, but also nitrophilous bryophyte and lichen species have been back-colonising areas formerly exposed to high SO_2 concentrations (Ellis and Coppins, 2009; Pakeman *et al*., 2022).

The impact of air quality improvement is, however, difficult to disentangle from impacts of concomitant climate change (Ellis and Coppins, 2010; Bates and Preston, 2011). For example, *Myriocoleopsis minutissima* reached, until 1980, its northern limit in France in Normandy. It then began to spread northward and was first reported from Belgium in 1985 (Hoffmann, 1985). In 2016, it was found as far north as Denmark (van der Pluijm, 2019). Some species, such as *Myriocoleopsis minutissima* (Hoffmann, 1985) and *Plenogemma phyllantha* (De Zuttere, 1992), only known in Belgium along the coast in the 1980s-1990s, have since expanded their distribution inland. The northward and inland expansion of these species suggests that they may have benefitted from milder winters (Hoffmann, 1985)

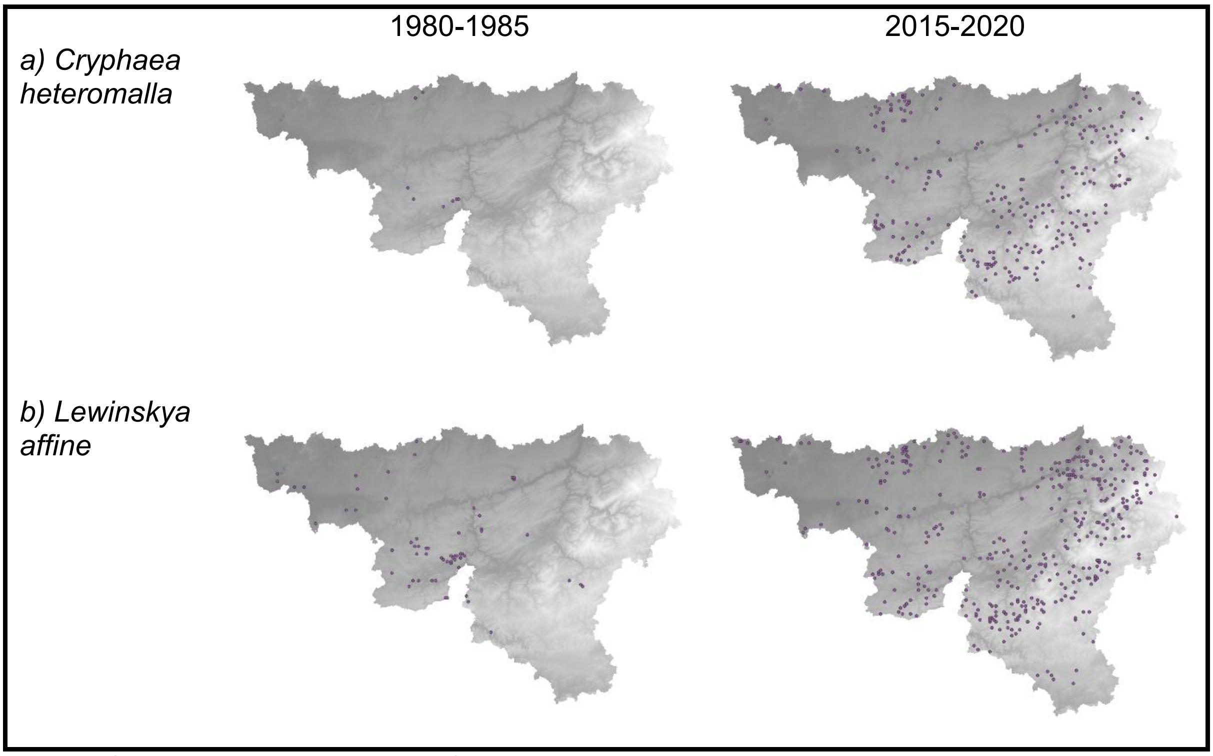

Figure 7. Comparative distribution maps of two moss species in 1980-1985 and 2015-2020 in southern Belgium.

1.5 Objectives

The primary goal of the present thesis is to document and disentangle the impact of changes in air quality and climate on epiphytic bryophyte communities. Analyzing spatio-temporal changes in bryophyte species communities and physical variables of their environment, we determine the main drivers of a regional epiphytic bryophyte flora across space and time. We finally focus on the impact of microclimatic variation in forest environments to formulate conservation guidelines under changing climate conditions.

In chapter I, we disentangle the role of air quality improvement and the concomitant climate change on epiphytic bryophyte community changes between 1980 and 2020. In this chapter, we investigate the dynamics of past and present floras in relation to past and present climatic conditions and past and present concentrations of major air pollutants (SO_2, NO_2, O_3, and PM_{10}). More precisely, 1a) we investigate whether changes in species composition correspond to an increase of nitrophilous species and a decrease of acidophilous species, 1b) we assess whether the change in species composition is more important over space or over time, 2) we disentangle the relative contribution of climatic change and improvement of air quality on the temporal turnover of epiphytic bryophytes and 3) we determine the relative contribution of past and present climatic conditions and air pollution concentration on the current spatial turnover of epiphytic bryophytes.

In chapter II, we address the question of whether air pollution still impacts the distribution of epiphytic bryophyte species, and if so, which pollutants are involved. In this chapter, we study the major pollutants mentioned in chapter I and add $PM_{2.5}$, BC, NH_4, heavy metals, and, for the first time, pesticides. More precisely, 1) we disentangle the relative contribution of the current air pollutant loads from background environmental factors (such as climatic conditions, topography and forest vegetation) to the variations in epiphytic community composition at the present time and, 2) we determine which source of pollutant accounts for variations in community composition and species distribution, among heavy metals, pollutants of agricultural origin and other major pollutants.

Finaly, in chapter III, we investigate the impacts of climate change on epiphytic bryophytes at the scale of microclimate, anticipating future climatic changes. We identify forests patches characterized with a high buffered effect and species characteristics of mature forest. To do so, we first 1) relate microclimatic to macroclimate variation to characterize the microclimatic effect, and model the spatial variation of the microclimatic effect. This relation is then projected spatially using topographic and vegetation structure variables to create a map of microclimatic effect intensity at a resolution of 25 m. Secondly, 2) we identify indicator species of the microclimatic effect in forest.

2. Chapter I

Disentangling climate change from air pollution effects on epiphytic bryophytes

cover picture :epiphytic bryophytes on a branch, Liège (Wallonia), picture from Lea Mouton, January 2026

Chapter I – Disentangling climate change from air pollution effects on epiphytic bryophytes

The authors' contribution to the article.

Virginie Hutsemékers : field work, interpolation and writing

Lea Mouton : field work, data analysis

Hannah Westenbohm : participation to field work and variance partitioning analysis

Flavien Collart : macro-climatic data analysis and statistical support

Alain Vanderpoorten : field work, conception, first draft and writing

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Disentangling climate change from air pollution effects on epiphytic bryophytes

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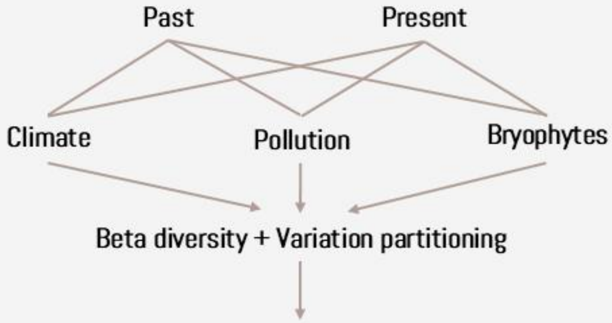
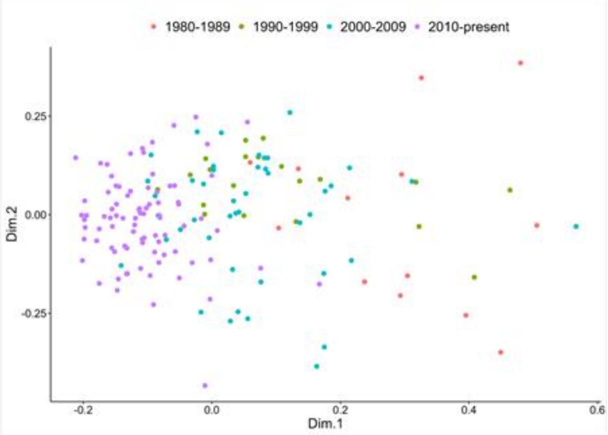
Introduction

For few decades, a general change in **epiphytic flora** has been noticed among bryologists in Europe. In Belgium, this change in taxonomic composition and richeness is concomitant with the amelioration of **air quality** after the pollution peak of the industrial era in late 1980s. Epiphytic bryophytes are largely known as good **bioindicators** for air quality. However, disentangling the impact of air quality from **climate change**, that occures meanwhile and also affects bryophytes flora, is a contemporary challenge.

Method

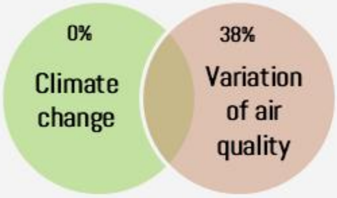


Past
Present
Climate
Pollution
Bryophytes
Beta diversity + Variation partitioning



MDS ordination of sites based on a Sorensen distance matrix of their composition in epiphytic bryophytes.

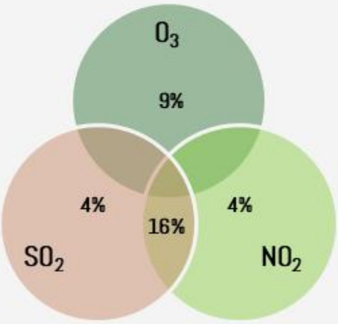
Results



Variance percentage of epiphytic bryophyte richness explained by the temporal variation of climate (t° and precipitation) and air pollutants concentrations (SO_2, NO_2, O_3 combined).

Through time :
richness increase > change in taxonomic composition

The increasing richness:
with time > among sites



Variance percentage of epiphytic bryophyte richness explained by the temporal variation in air pollutants concentrations.

Conclusion

Epiphytic bryophyte communities experienced strong **increase of species richness** in the course of the past four decades, which is larger than their actual spatial patterns of variation. While the global warming rises attention, our analyses revealed that the **temporal shifts** in epiphytic communities result mostly from variation of **air pollution loads**.

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RESEARCH ARTICLE

Disentangling climate change from air pollution effects on epiphytic bryophytes

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Abstract

At the interface between atmosphere and vegetation, epiphytic floras have been largely used as indicators of air quality. The recovery of epiphytes from high levels of SO_2 pollution has resulted in major range changes, whose interpretation has, however, been challenged by concomitant variation in other pollutants as well as climate change. Here, we combine historical and contemporary information on epiphytic bryophyte species distributions, climatic conditions, and pollution loads since the 1980s in southern Belgium to disentangle the relative impact of climate change and air pollution on temporal shifts in species composition. The relationship between the temporal variation of species composition, climatic conditions, SO_2, NO_2, O_3, and fine particle concentrations, was analyzed by variation partitioning. The temporal shift in species composition was such, that it was, on average, more than twice larger than the change in species composition observed today among communities scattered across the study area. The main driver, contributing to 38% of this temporal shift in species composition, was the variation of air quality. Climate change alone did not contribute to the substantial compositional shifts in epiphytic bryophyte communities in the course of the last 40 years. As a consequence of the substantial drop of N and S loads over the last decades, present-day variations of epiphytic floras were, however, better explained by the spatial variation of climatic conditions than by extant pollution loads. The lack of any signature of recolonization delays of formerly polluted areas in the composition of modern floras suggests that epiphytic bryophytes efficiently disperse at the landscape scale. We suggest that a monitoring of epiphyte communities at 10-year intervals would be desirable to assess the impact of raising pollution sources, and especially pesticides, whose impact on bryophytes remains poorly documented.

KEYWORDS
air quality, ammonia, bryophytes, climate change, epiphytes, fine particles, nitrogen oxides, ozone, sulfur dioxide

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Flavien Collart and Alain Vanderpoorten contributed equally.

1 | INTRODUCTION

Since the beginning of the industrial era, terrestrial ecosystems have experienced anthropogenic disturbance, involving complex factors such as land use changes, pervasive levels of air pollution from fossil fuel combustion, and climate change. Disentangling how these factors, or the combination thereof, affect ecosystem functioning and diversity remains, however, challenging (Esseen et al., 2022; Mathias & Thomas, 2018; Suleiman et al., 2022).

Anthropogenic emissions of the main air pollutants, including oxidized (NO_X) and reduced (NHy) forms of nitrogen, fine particulate matter, and sulfur oxides (SO_X), contribute to air quality problems, with damaging effects on human health, vegetation, and ecosystems (Bobbink et al., 2010; Castellanos & Boersma, 2012; Hilboll et al., 2013; Stern, 2005).

In Europe, changes in fuel usage and combustion technology, as well as commitments to reducing air pollution under the Convention on Long-Range Transboundary Air Pollution (1979) and, subsequently, the European Union National Emissions Ceiling Directive (Directive 2001/81/EC), have resulted in a substantial decline of SO_2 concentrations following a peak in the late 1980s (Stern, 2005) and, subsequently, of NOx concentrations in the late 1990s (Castellanos & Boersma, 2012; Hilboll et al., 2013). NHy deposition, which mostly originates from fertilizer and manure and thus peak in rural areas, experienced a less sharp decrease (about 20% since the 1980s in the UK, Pescott et al., 2015) and even increased in some areas, potentially offsetting the benefits of NOx deposition reduction (Tan et al., 2020).

While the spectacular forest decline in industrial countries during the 1980s and the 1990s focused the attention on the dramatic impact of acidic mists and rains (Pitelka & Raynal, 1989), the impact of global change on less obvious, but highly sensitive organisms such as cryptogams was already recognized since the 19th century, when bryophytes and lichens disappeared from the immediate vicinities of industrial areas during the Industrial Revolution (Lee et al., 1998; Pescott et al., 2015). Bryophytes are in fact the “canaries in the coal mine” for exhibiting a series of traits that make them particularly vulnerable to pollution (Slack, 2011), and hence, sensitive indicators of air quality (see Pescott et al., 2015 for review). In particular, bryophytes absorb water and nutrients directly through leaf surfaces from the immediate environment, resulting in an equilibrium with their water environment that makes them particularly susceptible to atmospheric pollution (Pescott et al., 2015). Bryophyte leaves further tend to be one-cell thick, thereby enhancing exposure to pollutants, while the absence of stomata in leaf tissues prevents the regulation of gas exchanges, especially under stress conditions (Bates, 2000). At the interface between atmosphere and vegetation, epiphytic floras are a case in point. They have, by a considerable margin, undergone the most dramatic change out of any of the groups analyzed in the United Kingdom (Pescott et al., 2015).

Since the 1990s, a massive back-colonization of acid-sensitive and a decline of acidophilous species have been recurrently reported (Bates et al., 1997; Duckett & Pressel, 2010; Pescott et al., 2015; Purvis et al., 2010; Sérgio et al., 2016; Stebel & Fojcik, 2016). Identifying which factors, or combination of factors, account for this back-colonization is, however, not straightforward. In fact, changes in air quality have been largely concomitant with climate change, which is likely to affect epiphytic bryophytes in two ways. On the one hand, milder winters may benefit to species that previously had a hyper-oceanic range (e.g., *Myriocolea minutissima*, *Plenogemma phyllantha*) and that have expanded inland for the last few decades (Bates & Preston, 2011). On the other hand, bryophytes rely on atmospheric precipitation for water uptake and temperate species are highly sensitive to moderately warm temperatures (He et al., 2016). Even relatively slight temperature increase (1.5°C) and relative humidity decrease impact on growth rates and photosynthetic performance (Hao & Chu, 2021; Song et al., 2012). Many temperate species die when kept hydrated continuously at temperatures above 30°C (Furness & Grime, 1982). Although the vulnerability to climate change varies across species and habitats (Barbé et al., 2020; Hespanhol et al., 2022), increased summer temperature and drought are therefore expected to impact the composition of epiphytic communities, as suggested by dramatic projections of future species distributions under climate change in Europe (Zanatta et al., 2020).

While the relative importance of extant climate conditions and pollution loads on epiphytic bryophyte and lichen distributions has been assessed (Ellis & Coppins, 2009), disentangling the contribution of the historical variation of climatic conditions and air pollution on the observed recolonization of epiphyte floras during the last decades has been challenging. Bates and Preston (2011) concluded that “there are problems in attributing changes to climate change as opposed to other causal factors, always assuming that the changes are actually the result of single factors (rather than of interactions between factors). In particular, the recovery of epiphytes and possibly other species from high levels of SO_2 pollution, has resulted in major range changes, which may be masking the effects of climate change.”

Furthermore, the extent to which extant species composition reflects extant conditions, suggesting that species are at equilibrium with their environment or reflect past pollution loads due to delays in recolonization caused by dispersal limitations, remains to be assessed. In epiphytic lichens, historical (19th century) woodland structure significantly contributed to explain extant distribution patterns, pointing to a strong habitat specialization and a time lag between changes in habitat conditions and extant species distributions caused by dispersal limitations (Ellis & Coppins, 2009). Bryophytes disperse by spores and specialized vegetative diaspores (Figure 1c), which exhibit high long-distance dispersal capacities (see Patiño & Vanderpoorten, 2018 for review). This is especially true for epiphytes, which need to track patches of suitable trees in a dynamic landscape for persistence (Snäll et al., 2005). Despite this, epiphytic bryophyte distributions are typically clustered (Löbel et al., 2006a, 2006b) and some exhibit strong fine-scale genetic structures (Snäll et al., 2004), pointing to dispersal limitations. This raises the question whether current epiphytic bryophyte distributions reflect current patterns in air quality and climatic conditions or are still constrained by past pollution events.

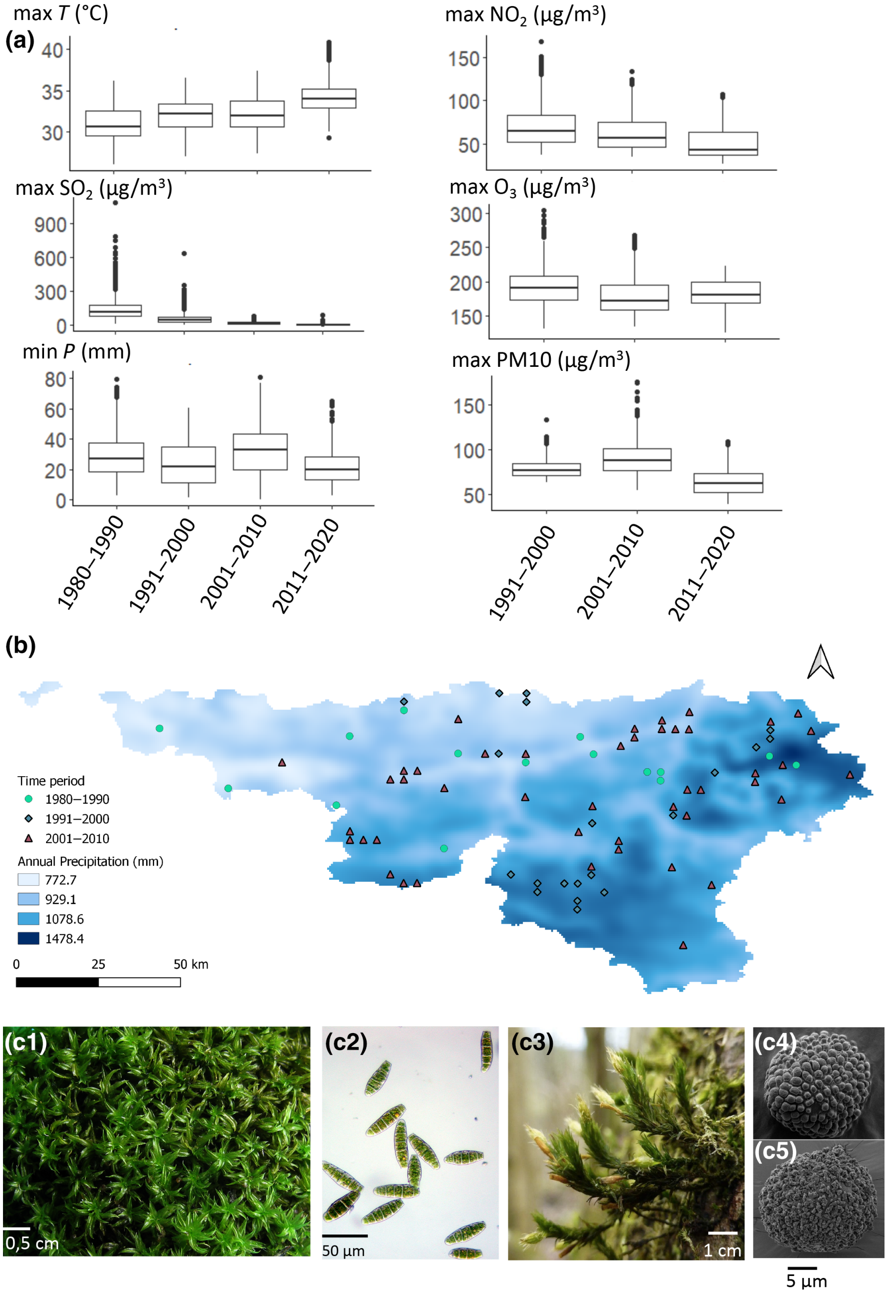

FIGURE 1 Spatiotemporal variation of the epiphytic bryophyte flora, climatic conditions, and air pollution in southern Belgium. (a) Boxplots (showing the first and third quartiles (upper and lower bounds), second quartile (center), 1.5* interquartile range (whiskers), and minima–maxima beyond the whiskers) of the spatiotemporal variation of annual maximal temperature (max *T*), minimum precipitation (min *P*), and maximum air pollutant loads (max NO_2, SO_2, O_3, particle matter with an aerodynamic diameter smaller than 10 µm PM10) across 81 16 km^2 pixels in southern Belgium per decade (see Figure S1 for annual average variations). (b) Map of the survey area, location, and decade of first survey of epiphytic bryophytes in the 81 16 km^2 selected pixels. (c). Epiphytic mosses: *Zygodon conoideus* (c1) and its vegetative gemmae (c2); *Lewinskya speciosa* (c3); spores of *Ulota bruchii* (c4); and *Syntrichia laevipila* (c5). c1. photo A. Mora. c2–3 photo A. Sotiaux. c4–5 photo M. De Haan.

Here, we take advantage of the availability of epiphytic bryophyte species distributions, climatic data, and an air pollution monitoring program since the 1980s in southern Belgium to characterize temporal shifts in species frequencies and community composition in the epiphytic bryophyte flora. In particular, we assess whether (i) shifts involve a decrease of the acidophilous flora and an increase of the nitrophilous flora (Q1a) and (ii) changes in species composition have been more important within the same community through time, as a response to environmental change, or among communities under present conditions due to the spatial variation of environmental conditions (Q1b). We then disentangle the relative impact of climate change and temporal variation of air pollution on temporal shifts in species composition (Q2). We finally determine the relative contribution of past and present climatic conditions and pollution loads on present patterns of species composition to assess whether species distributions are at equilibrium with present conditions or reflect past conditions, pointing to a recolonization delay (Q3).

2 | METHODS

2.1 | Study area and data collection

This study took place in southern Belgium, thanks to the availability of spatiotemporal data of air quality and climatic conditions, and of complete floristic inventories of the bryophyte flora since the 1980s. The atlas of bryophyte species distributions in southern Belgium (Sotiaux & Vanderpoorten, 2015) originated from the systematic survey, during two complete days at different seasons to take potential phenological differences into account and by the same team of three recorders, of all 1139 pixels of 16 km^2 constitutive of the area. In the framework of the present study, we resurveyed 81 of those pixels between 2016 and 2020 using the same protocol (Figure 1b). Initially, we focused on pixels that include one of the 23 stations of the network of measuring stations of air quality in southern Belgium. We then extended the sampling to 81 pixels, selecting additional pixels to maximize the spatial cover of the study area and have comparable numbers of pixels surveyed before and after 2000 ($n = 36$ and 45, respectively).

Epiphytes were recorded up to 2.5 m, which, in temperate forests, is sufficient to capture the bulk of epiphytic bryophyte diversity. Boch et al. (2013) in fact reported that, after thorough plot sampling, only 4% of the bryophyte species were overlooked if the tree crown was not surveyed. All analyses were based on a dataset (Hutsemékers et al., 2022) documenting the distribution of 51 species (nomenclature of Hodgetts et al., 2020), which are either strict epiphytes or are preferentially found on living trees in southern Belgium, in the 81 selected pixels.

Data on the variation of air pollutant concentrations through time were obtained for NO_2, SO_2, O_3, and fine particles (Particle Matter, hereafter PM) with an aerodynamic diameter smaller than 10 µm (PM10), which are among the major pollutants in southern Belgium. Data have been collected across the area since 1980 for SO_2, since 1990 for NO_2 and O_3, and since 1996 for PM10, and stored by the Belgian Interregional Environment Agency (IRCEL—CELINE). These data have been recorded hourly from the 23 measuring stations in southern Belgium, complemented by data from an additional 73, 18, 26, and 51 measuring stations of the Belgian official network (thus including stations from the Brussels area and Flanders) for NO_2, SO_2, O_3, and PM10, respectively. These data served to calibrate the RIO model. RIO is an interpolation model based on land use, a semivariogram based on the distances to the nearest measuring stations and the levels of air pollution, which was employed to compute, on an hourly basis, the background concentrations at the centroid of all the investigated pixels. Based on the interpolated data, the maximum hourly concentration and annual average concentration of each pollutant were computed every year for each pixel. For NH_3, only concentrations recorded by 32 passive samplers were available between mid-April and mid-May 2021, that is, during the peak season of fertilizer spreading in the area. Using a simple inverse distance weighting method, we interpolated these data to generate values at each of the 81 pixels.

Climatic data were provided daily since 1980 at the centroid of each pixel by the Royal Meteorological Institute of Belgium (RMI). Precipitations were recorded daily from the manual rain gauges of the climatological network and from the automatic rain gauges from the RMI network of automatic weather stations. Temperature data include daily extreme observations from the climatological network and from the RMI network of automatic weather stations at 10′ intervals. These data are subsequently spatially interpolated by kriging methods, taking geographic distance, elevation, and remote sensing data into account (https://opendata.meteo.be). These daily data served to compute total annual precipitation, minimum precipitation of the driest month and maximum precipitation of the wettest month, average annual temperature, annual maximum temperature of the warmest month, and annual minimum temperature of the coldest month.

All air pollution loads and climatic data are available from Hutsemékers et al. (2022).

2.2 | Data analysis

To summarize the temporal variation of species frequencies, we computed species frequencies for three time periods (i.e., 1980–1999, $n = 36$; 2000–2015, $n = 45$; 2016 to present, i.e., the present re-survey, $n = 81$), as the number of occupied pixels divided by the number of pixels surveyed during that time period. To visualize whether the observed temporal changes of species frequencies involve a decrease in acidophilous species and an increase in nitrophilous species (Q1a), we characterized the observed changes in species frequencies by shifts in the proportions of acid-sensitive to acidophilous species and N-sensitive to nitrophilous species, as defined by species Ellenberg indicator values (Hill et al., 2007 with updates for N from Simmel et al., 2021). We thus computed, for each time period, the proportion of species depending on their Ellenberg indicator values for pH (indR, ranging in the southern Belgian flora from acid (3) to strongly basic (7)) and the proportion of species depending on their Ellenberg indicator values for N (indN, ranging from infertile (2) to richly fertile (8)) substrates.

Differences among epiphytic communities within the same pixel at two time periods (temporal variation between the initial survey and the resurvey) and between pairs of pixels under present conditions (spatial variation based on resurvey between 2016 and 2020) were characterized by beta diversity. Beta diversity can be split into two components reflecting different phenomena (Baselga, 2010). Nestedness occurs when the poorest assemblages are subsets of the richest assemblages, reflecting the orderly loss (or gain) of species that may occur, for example, along chronosequences due to dispersal limitations or changes in environmental conditions. Species turnover reflects the replacement of some species by others among communities, which typically occurs along ecological gradients (Baselga, 2010). Here, we employed Baselga's nestedness-resultant dissimilarity (βsne) and Simpson's dissimilarity index (βsim) among pixel pairs:

$$\beta\text{sim} = \frac{\min(b, c)}{a + \min(b, c)}$$

$$\beta\text{sne} = \frac{\max(b, c) - \min(b, c)}{2a + \min(b, c) + \max(b, c)} \times \frac{a}{a + \min(b, c)}$$

where a is the number of species common to both pixels, b is the number of species that occur in the first pixel but not in the second one, and *c* is the number of species that occur in the second pixel but not in the first one.

These metrics were computed with the command beta.pair in the R package betapart (Baselga et al., 2021) in R version 4.2.1. We focused on βsne as a means to characterize spatiotemporal differences among pixels, because this metric best correlated with variations in air quality through time, and presented results based on βsim as an appendix.

To determine whether changes in species composition have been more important within the same community through time, as a response to environmental change, or among communities under present conditions due to environmental variation at the landscape scale (Q1b), we compared the spatial differences among communities between pairs of pixels for the 2016–2020 period (βsne-spat and βsim-spat) with the differences among communities from the same pixel at two time periods (βsim-temp and βsne-temp). This comparison involves the inclusion of the same observation multiple times (the same species occurrences in a pixel serving to compute the pairwise βsim-spat and βsne-spat with all the other pixels), violating the assumption that the observations are independent from each other. We therefore computed, for each pixel, the average βsim-spat and βsne-spat values with all the other pixels. We thus obtained 81 comparisons, each involving the average values of βsim-spat and βsne-spat of a pixel and all the other pixels for the 2016–2020 period on the one hand, and the temporal variation (βsim-temp and βsne-temp) between communities of the same pixel at the two time periods on the other. Because the distributions of average values of βsim-spat and βsne-spat significantly departed from a normal distribution (Shapiro test, $p < .001$ in both cases), we employed nonparametric paired Wilcoxon tests.

To help visualizing the spatiotemporal changes in species composition, a classical (metric) multidimensional scaling (MDS) based on the Sørensen distance matrix was performed.

To determine the extent to which temporal changes in climatic conditions and pollutant loads contributed to variations of species composition through time (Q2), we implemented variance partitioning as implemented by the varpart function of the vegan package (Oksanen et al., 2022). The dependent variable was either βsne·temp or βsim.temp. Predictors included two matrices: M1 included the difference in average and maximum concentrations in O_3, PM10, SO_2, and NO_2 between the two time periods of floristic survey for the same pixel. M2 included the difference in minimum, maximum, and average temperature; annual precipitation; precipitation of the driest and of the wettest month between the two time periods of floristic survey for the same pixel. Because NO_2 and O_3 concentrations were available from 1990, and PM10 concentrations from 1996, the analyses were performed three times, first with all floristic data but using only SO_2 in the M1 matrix ($n=81$); second with the floristic data starting in 1990 and including SO_2, NO_2, and O_3 in the M1 matrix ($n=68$); and third with the floristic data starting in 1996 and including SO_2, NO_2, O_3, and PM10 in the M1 matrix ($n=64$). To further disentangle the impact of the main pollutants, we repeated the analyses, using average and maximum concentrations in O_3, SO_2, and NO_2 as predictors.

Finally, to determine whether extant or historical variation in climatic conditions and air quality shape current species composition patterns (Q3), we implemented variation partitioning extended to several predictor matrices with the rdacca.hp package (Lai et al., 2022). The Y matrix documented the presence/absence of all 51 species in each pixel. X matrices included two matrices describing the climatic conditions and pollution loads, respectively, which prevailed in 1990–1994, and two matrices describing the climatic conditions and pollution loads, respectively, which prevailed in 2016–2020. Extant climatic conditions included the average annual temperature and precipitation, maximum temperature of the warmest month and minimum temperature of the coldest month between 2016 and 2020. Extant pollution loads included the annual average and hourly maximum concentrations of SO_2, NO_2, and O_3 between 2016 and 2020. Past climatic conditions and pollution loads involved the average annual temperature and precipitation, maximum temperature of the warmest month and minimum temperature of the coldest month, annual average and hourly maximum concentrations in SO_2, NO_2, and O_3 between 1990 and 1994. To analyze the impact of extant NH3 concentrations among other pollutants, we performed a last variation partitioning analysis, using the same predictors as above, but also including average NH_3 concentrations during the Spring 2021.

3 | RESULTS

The annual maxima and average temperature, precipitation, NO_2, O_3, SO_2, and PM10 concentrations at the investigated sites in southern Belgium are represented in Figure 1a and Figure S1, respectively. There was a clear trend for an increase of average temperature over the past four decades, from 8.56 ± 1.08°C during the 1980–1990 period to 9.86 ± 1.14°C after 2010 (Figure S1). Maximum temperatures followed the same trend (Figure 1a), while precipitation did not exhibit any temporal trend. SO_2 concentrations exhibited a continuous decrease, from annual average and hourly maximum concentrations of 14.1 ± 10.8 and 1083.0 $\mu g/m^3$ during the 1980–1990 period to 1.1 ± 0.1 and 87.0 $\mu g/m^3$ after 2010, respectively. NO_2 concentrations tended to decrease from annual average and hourly maximum concentrations of 16.7 ± 7.8 and 168.0 $\mu g/m^3$ during the 1991–2000 period to 10.3 ± 5.8 and 107.5 $\mu g/m^3$ after 2010, respectively. O_3 annual average concentrations increased from 45.4 ± 7.0 $\mu g/m^3$ during the 1991–2000 period to 49.8 ± 5.8 $\mu g/m^3$ after 2010, but hourly maxima exhibited the reverse trend, decreasing from 303.8 $\mu g/m^3$ in 1991–2000 to 223.2 $\mu g/m^3$ after 2010. Minimum, average (±SD), and maximum NH_3 average concentrations across pixels in Spring 2021 ranged between 7.71, 11.29 ± 1.31 and 16.12 $\mu g/m^3$, respectively.

On average, species frequencies increased since 1980, from 0.29 ± 0.25 in 1980–1999, 0.37 ± 0.11 in 2000–2015 to 0.49 ± 0.09 in 2016–2020 (Figure 2a). These trends reflect the sharp increase in frequency for the bulk of the species (81%), while three species (*Ptychostomum moravicum*, *Ptilidium pulcherrimum*, *Sanionia uncinata*) exhibited the reverse trend, four species (*Dicranoweisia*

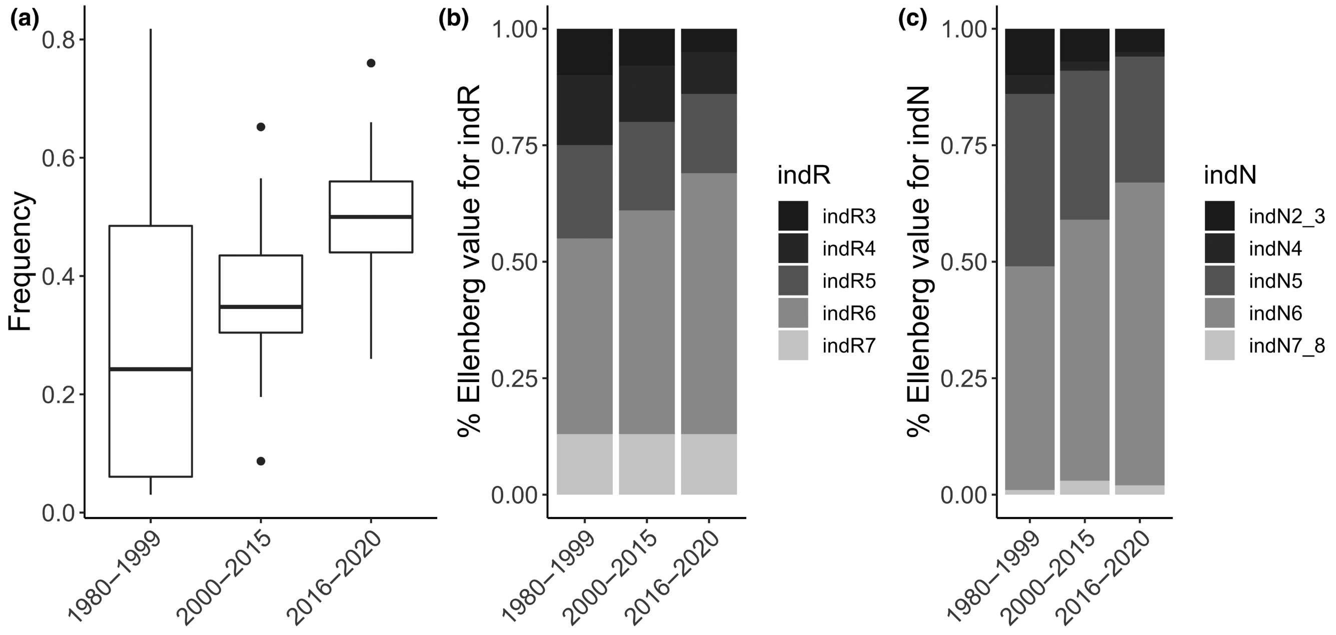

FIGURE 2 Temporal variation of (a) the frequency of epiphytic bryophyte species, (b) the proportion of species depending on their Ellenberg indicator values for pH (indR, ranging from acid (3) to strongly basic (7)) and (c) for N (indN, ranging from infertile (2) to richly fertile (8)) substrates. The frequencies are computed for three time periods (1980–1999, $n=36$; 2000–2015, $n=45$; 2016–2020, $n=81$). The boxplots (showing the first and third quartiles (upper and lower bounds), second quartile (center), 1.5* interquartile range (whiskers), and minima–maxima beyond the whiskers) show the frequencies of all 51 species (see Table S1 for species-specific trends) per time period.

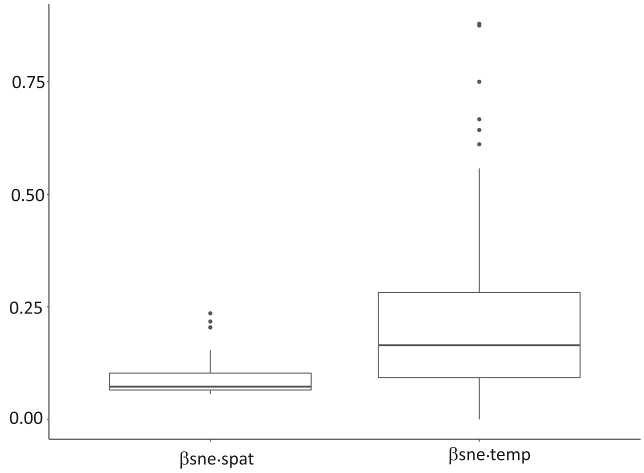

FIGURE 3 Spatiotemporal differences among communities of epiphytic bryophyte floras in southern Belgium. Boxplot (showing the first and third quartiles (upper and lower bounds), second quartile (center), 1.5* interquartile range (whiskers), and minima–maxima beyond the whiskers) represent the (spatial differences under present conditions, βsne·spat) across 81 16 km^2 pixels and the temporal variation (comparisons among assemblages of the same pixel at two time periods, βsne·temp).

cirrata, *Dicranum tauricum*, *Microlejeunea ulicina*, *Orthotrichum diaphanum*) exhibited a peak of frequency during the end of the 20th century, and the frequency of two species (*Frullania tamarisci*, *Leskea polycarpa*) did not exhibit any marked temporal trend (Table S1).

These changes in species frequencies were paralleled by shifts in the proportion of acidophilous and nitrophilous species through time. Thus, the proportions of acidophilous (Figure 2b) species decreased, while the proportions of acid-sensitive species (Figure 2b) and nitrophilous (Figure 2c) species increased.

Differences in community composition were significantly larger among communities from the same pixel through time than among communities from different pixels at present time (βsne·temp $=0.22 \pm 0.19$, βsne·spat $=0.09 \pm 0.08$, $p<.001$; Figure 3; see Figure S2 for differences among communities expressed as βsim).

These spatiotemporal variations among epiphytic bryophyte assemblages are illustrated by the MDS ordination of the 81 investigated 16 km^2 pixels based on a Sorensen distance matrix of their composition in epiphytic bryophytes under present and past (<2010) conditions, showing that floristic assemblages tend to segregate depending on the period of record (Figure 4). Some pixels resurveyed during the 2016–2020 period (see arrows in Figure 4), however, had coordinates, and hence, floristic compositions that were more similar to those of pixels surveyed during previous decades.

The variation partitioning analysis showed that temporal changes in air pollution loads accounted for 38% of the observed temporal differences among communities from the same pixel at different time periods (βsne·temp). Climate change did not contribute to this pattern (Figure 5a). Among pollutants, NO_2 and SO_2 together explained 25% of the variance of βsne·temp against 9% for O_3 alone (Figure 5b). Analyses based on climatic variation and SO_2 concentrations since 1980, and including PM10 after 1996, revealed the same

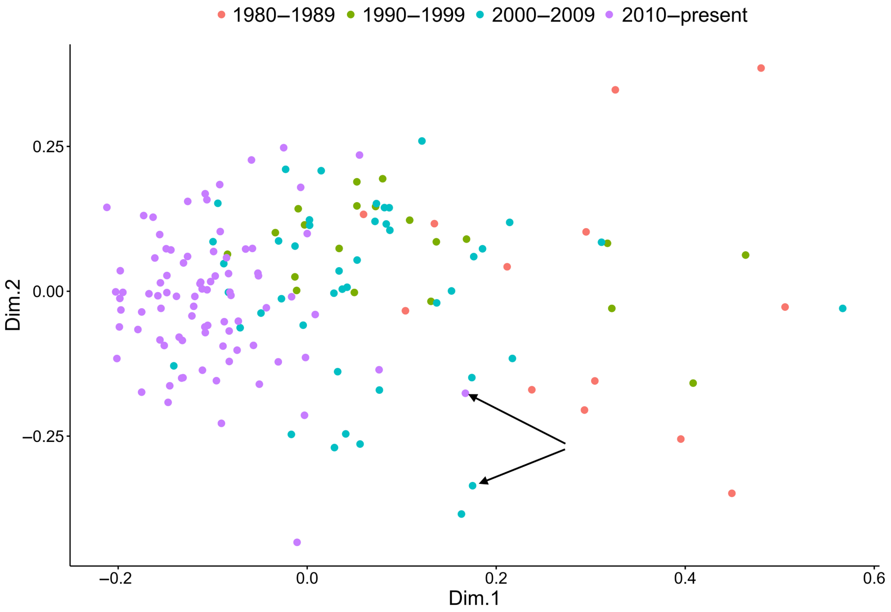

FIGURE 4 MDS ordination of 81 $16\,\text{km}^2$ pixels in southern Belgium based on a Sorensen distance matrix of their composition in epiphytic bryophytes in 1980–1990, 1991–2000, 2001–2010, and 2016–2020. Each dot represents an individual pixel, with a color reflecting the time (decade) or record. Arrows identify the position of a specific pixel (G447) at two different time periods, the dot corresponding to the present record (in mauve) being closely located to the dot corresponding to the 2000–2009 record (in turquoise), indicating little floristic change between the two records and pointing to locally persisting high air pollution loads.

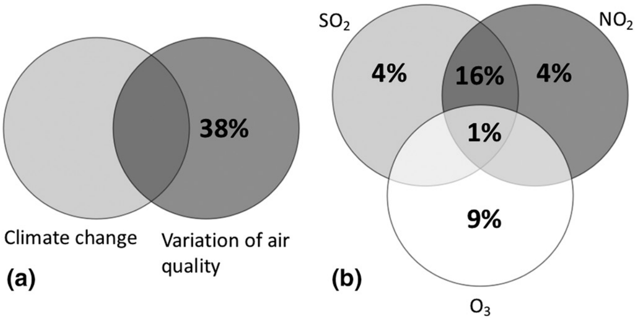

FIGURE 5 Venn diagram representing the contribution (in %variance explained) of the temporal variation in air pollutant concentrations (SO_2, NO_2, O_3) and climate change (precipitation and temperature; a) and of each pollutant individually (b) to temporal differences among epiphytic bryophyte communities (expressed as βsne·temp) since 1990 in southern Belgium.

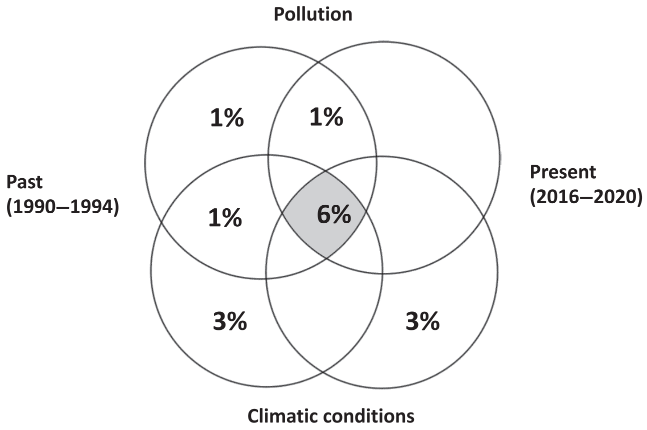

FIGURE 6 Venn diagram representing the contribution (in %variance explained) of past (1990–1994) and present (2016–2020) variation in climatic conditions (annual minimum, maximum, and average temperature *T* and precipitation *P*) and air pollutant loads (hourly maximum and average SO_2, NO_2, O_3) on the present variation of the epiphytic bryophyte species composition in 81 $16\,\text{km}^2$ pixels in southern Belgium.

trend (Figures S3 and S4), although climate change and temporal changes in pollution levels jointly contributed to 7% of the variance of βsne·temp in the analysis involving SO_2 concentrations and climate change since 1980 (Figure S3). Similar trends were observed for turnover, but with a lower proportion of variance explained (Figure S3–S5).

Fifteen percent of the species composition of the present epiphytic bryophyte floras could be explained in terms of past and present climatic conditions and pollution loads. The factors that, alone, most contributed to this explained floristic variation were past (1990–1994) and present (2016–2020) climatic conditions (6%), with a marginal contribution of past pollution loads and no contribution of current pollution loads (Figure 6).

Analyses including NH_3 on extant patterns of species composition revealed that NH_3 alone marginally further contributed to the variation in species composition at 0.1%.

4 | DISCUSSION

4.1 | Temporal shifts in epiphytic bryophyte communities and their relationships with changes in climatic conditions and air pollution loads

The comparative analysis of epiphytic bryophyte floras in southern Belgium since 1980 revealed that on average, species frequencies increased from 0.29 ± 0.25 in 1980–1999, 0.37 ± 0.11 in 2000–2015 to 0.49 ± 0.09 in 2016–2020, contributing to a growing bulk of evidence for the massive recolonization of formerly polluted areas by epiphytes since the end of the 20th century (Bates et al., 1997; Duckett & Pressel, 2010; Pescott et al., 2015; Purvis et al., 2010;

Sérgio et al., 2016; Stebel & Fojcik, 2016). The increase in species frequencies was paralleled by an increase of species richness through time, with the emergence of "newcomers" in the flora (*Habrodon perpusillus*, *Lewinskya shawii*, *Myriocoleopsis minutissima*, *Orthotrichum consimile*, *O. rogeri*, *O. scanicum*, *Plenogemma phyllantha*, *U. rehmannii*) since the late 1990s and the beginning of the 21th century (Sotiaux & Vanderpoorten, 2015) that represent 15% of the current flora of strict and preferential epiphytes. These shifts were also characterized by a decrease of acidophilous species (e.g., *Dicranoweisia cirrata*, *Dicranum* spp.) and an increase of acid-sensitive (e.g., *Pylaisia polyantha*) and of nitrophilous (e.g., *Syntrichia* spp.) species.

The differences between communities from the same pixel through time were such that they were, on average, more than twice larger than the differences observed today among communities scattered across the study area. Therefore, epiphytic communities are more similar among pixels recorded at the same time period than among geographically close pixels recorded at different periods of time. Similar patterns were observed in the aquatic bryophyte flora (Vanderpoorten, 1999), emphasizing the strength of the temporal variation in species composition as a response to the dramatic changes in their environmental conditions since the past four decades. Locally, however, present-day communities exhibited a composition that was closer to that observed during previous decades than to other extant communities (see, e.g., pixel G447 in Figure 4, which, with 82 and 209 $\mu g/m^3$ of NO_2 and O_3, respectively, is among the most polluted pixels), suggesting that pollution loads may still, in some areas, prevent a complete back-colonization of the epiphytic flora.

Our spatiotemporal analysis of the variation in species composition, climate conditions, and pollutant loads allowed us to determine that the main driver, contributing alone to 38% of the temporal variation of differences in epiphytic bryophyte communities, is the variation of air quality. Among pollutants, SO_2 and NO_2 contributed to about three times as much as O_3 to the observed changes in bryophyte communities. Beginning in the late-18th and early-19th century, large-scale SO_2 and NO_2 pollution resulting from the industrial and residential burning of fossil fuels and associated acidification of substrata decimated epiphytic floras (Pescott et al., 2015). If fully grown gametophytes proved to tolerate SO_2 concentrations beyond 2500 $\mu g/m^3$, which were reached only during the most acute pollution waves, such as the dramatic fog in London of 1952, younger developmental stages, and in particular, protonemata, are killed beyond concentrations of 500 $\mu g/m^3$ (see Lee et al., 1998 for review). In lichens, fumigation experiments revealed that SO_2, interacting with low bark pH, may play a role at even much lower concentrations (Bates et al., 1996). Annual means of 30 $\mu g/m^3$ are sufficient to eradicate the most sensitive taxa (World Health Organization, 2000), and community changes were observed at average concentrations below 10 $\mu g/m^3$ around a newly established rural point source (Will-Wolf, 1981). Hence, even modest SO_2 levels have still inhibited recolonization of trees by epiphytes in major European cities in the early 2000s (Batty et al., 2003). In southern Belgium, concentration peaks higher than 200 $\mu g/m^3$ have not been reported since the early 1990s. The substantial decrease in SO_2 concentrations, currently of about 1–2 $\mu g/m^3$, have allowed SO_2-sensitive species to back-colonize areas where they previously had suffered extinction, in line with the decrease of acidophilous species and increase of acid-sensitive species mentioned above. In the United Kingdom similarly, the rise and fall of acidophilous epiphytic bryophytes has been clearer, or larger in magnitude, in areas exposed to high SO_2 pollution, consistent with the interpretation that these species were favored by acidic air pollution until the 1980s (Pescott et al., 2015).

In the context of decreasing NO_2 pollution, however, the global increase in nitrophilous species since the 1980s, also reported in epiphytic lichens (Ellis & Coppins, 2009) and non-epiphytic bryophytes (Pakeman et al., 2022), is more puzzling. One interpretation is that other N sources than NO_2 have played a role in the increase of nitrophilous species. In particular, it has been suggested in lichens that atmospheric NH_3 caused the increase of the nitrophilous flora, while the rise of bark pH due to NH_3 pollution has caused the decrease of the acidophilous flora (Van Herk, 2001). This would invalidate the notion that the decline of acidophilous species mirrors an improvement of air quality, while in fact, an increase of NH_3 may be the main cause (Van Herk, 2001). Such an interpretation is, however, not consistent with the fact that, in southern Belgium, NH_3 emissions have, like NO_2, decreased, dropping by 25% between 1990 and 2020 (http://etat.environnement.wallonie.be/contents/indicatorsheets/AIR%202.html). Furthermore, although a long-term monitoring of NH_3 concentrations in southern Belgium would be desirable, our preliminary analyses based on NH_3 concentration measurements in Spring 2021 suggest that extant NH_3 concentrations do not account to differences in species composition among pixels.

Altogether, these results therefore suggest that the observed differences in species composition are linked with decreasing air pollution loads. In this context, past SO_2 pollution levels could have masked the impact of eutrophication, which is now progressively revealed as SO_2 concentrations have been decreasing, leading to the observed spread of nitrophilous species. Past NO_2 concentrations themselves could have been toxic, even for the nitrophilous flora. In fact, while bryophyte growth is initially boosted by moderate N supplies (Armitage et al., 2012), wet N deposition beyond 10–15 kg/ha/year is sufficient to impact epiphytic bryophyte community composition and richness (Mitchell et al., 2005; Song et al., 2012; Shi et al., 2017; Wilkins & Aherne, 2016). In this context, nitrophilous species may have increased in frequency since the 1990s as N levels would have shifted from toxic to enhancing growth. It is tempting to see in the most recent decline of the highly nitrophilous *Orthotrichum diaphanum* (Simmel et al., 2021), the signature of a forthcoming global decline of nitrophilous species as N deposition continues to decrease.

Another pollutant that could have played a role in the temporal variation of epiphytic floras is O_3, whose average (but not maxima) concentrations have been increasing. Reduction of photosynthetic activity and membrane leakage was reported in four of 22 species exposed to concentrations of 300 $\mu g/m^3$ (Lee et al., 1998), which are almost in the range of the maxima currently recorded in southern

Belgium. The ecophysiological impact of O_3 on cryptogamic epiphytes remains, however, poorly known (Ellis & Coppins, 2009), calling for further experimental work. In fact, this pollutant could have a growing impact on epiphytic floras in the future, given that O_3 concentrations tend to increase with global warming and with the decrease of NO_x emissions.

The contribution of climate change alone to the observed temporal shifts in community composition ranged between 0 and 7% depending on the analysis performed. This suggests that the dramatic compositional shifts in epiphytic bryophyte communities observed in the study area, and recurrently reported across Europe, cannot be attributed to climate change. Experimentally increased temperatures up to +3°C, combined with various moisture regimes, similarly showed modest impacts on the bryophyte layer of limestone grasslands (Bates et al., 2005). The impact of climatic conditions in our study may even be overestimated, as the climatic conditions employed in the present analyses were obtained from meteorological stations, which are typically located in open areas. Epiphytes, the bulk of which occur on the lower trunk and in areas exposed to high air humidity (Ellis, 2020; Ellis & Eaton, 2021), experience microclimatic conditions under the canopy that are substantially buffered as compared to the ones prevailing in open areas (see De Frenne et al., 2021 for review), so that their variation since the 1980s is likely to have been even less important than that documented from available meteorological data.

4.2 | Factors accounting for the extant composition of epiphytic communities

Present-day variations of epiphytic floras were better explained by the spatial variation of climatic conditions than by extant air pollution loads. This suggests that, as a result of air quality improvement in southern Belgium, spatial variation in climatic conditions is now the main driver of the composition of epiphytic communities. No signature of past pollution events could be detected in modern floras, as suggested by the marginal contribution of past pollution loads to extant patterns of species composition. Although epiphytes need to track patches of suitable trees for persistence (Snäll et al., 2005), epiphytic bryophytes typically exhibit spatially clustered distributions (Löbel et al., 2006a, 2006b) and their fine-scale patterns of genetic variation are significantly spatially structured (Ledent et al., 2020; Vanderpoorten et al., 2019), pointing to dispersal limitations. The lack of any signature of recolonization delay of formerly polluted areas reported here thus suggests that efficient dispersal occurs at the landscape scale, in line with similar observations on the efficient colonization of newly available substrates by bryophytes within a few decades (Hutsemékers et al., 2008). This does not undermine the value of some species, such as *Antitrichia curtipendula*, *Frullania fragilifolia*, *F. tamarisci*, or *Microlejeunea ulicina*, which did not increase or even decreased in frequency during the past decades in southern Belgium, as bioindicators of ancient forests. Such species, which are long-lived and seldom produce sporophytes, can only colonize suitable habitats quickly if they are found in the immediate vicinity (Baumann et al., 2022), and their distribution patterns are mostly explained by forest continuity (Wierzcholska et al., 2020).

5 | CONCLUSION

Epiphytic bryophyte communities experienced dramatic compositional shifts in the course of the past four decades, larger than their extant spatial patterns of variation. While the concomitant global warming and shifts in air pollution have long blurred the interpretation of the dramatic changes in epiphytic floras (Bates & Preston, 2011), our analyses revealed that the temporal shifts in epiphytic communities result from variation of air pollution loads. The minimal role played by climate change to the observed patterns was unexpected because bryophytes are globally sensitive to moderately high temperatures and drought.

The lack of climatic impact on epiphytic floras reported here does, however, not suggest that such floras are resilient to climate change. While, in line with the inconclusive trends reported across central Europe (Gudmundsson & Seneviratne, 2016), no temporal trends in the precipitation regime were observed in the course of the past four decades in southern Belgium, anthropogenic climate change has already increased drought risk and number of dry days in southern and northern Europe, respectively (Gudmundsson & Seneviratne, 2016). Furthermore, Europe has been experiencing its worst drought in at least 500 years in the course of the past couple of years (European Drought Observatory, https://edo.jrc.ec.europa.eu/edov2/php/index.php?id=1000). This, together with record-breaking temperatures in Europe during the 2022 summer (Witze, 2022), suggests that climate change impacts on bryophytes will start to become apparent during the next decades. Epiphytic floras proved to globally efficiently recover from past pollution events, but these have a local to regional impact. In contrast, climate change occurs at a much larger scales, especially in lowland areas that, as southern Belgium, are characterized by high velocities of climate change (Dobrowski & Parks, 2016), raising the question of the ability of species to efficiently track the shift of climatically suitable areas across large distances (Zanatta et al., 2020).

In addition, other pollutants than those investigated here, and especially pesticides, whose impact on bryophytes remains poorly documented, may play a potentially increasing role. Given the fast response of epiphytic communities to environmental change, we suggest that a monitoring of epiphyte communities at 10-year intervals would be desirable to accurately assess the threats that they will face in the course of the next decades.

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DATA AVAILABILITY STATEMENT

The data employed in the present study are openly available in Figshare at DOI: 10.6084/m9.figshare.21630179.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Supplementary material for Chapter I

Table S1. *Variation of the frequency of 52 epiphytic bryophyte species in southern Belgium per decade since 1980.*

	1980-1990	**1991-2000**	**2001-2010**	**2016-2020**
Antitrichia_curtipendula	0.00	0.00	0.04	0.05
Brachythecium_salebrosum	0.08	0.20	0.39	0.48
Myriocoleopsis_minutissima	0.00	0.00	0.00	0.10
Cryphaea_heteromalla	0.08	0.35	0.39	0.90
Dicranoweisia_cirrata	0.69	0.90	0.86	0.69
Dicranum_montanum	0.46	0.85	0.82	0.82
Dicranum_tauricum	0.23	0.70	0.51	0.51
Frullania_dilatata	0.46	0.90	0.94	0.98
Frullania_fragilifolia	0.00	0.15	0.18	0.13
Frullania_tamarisci	0.23	0.20	0.25	0.24
Isothecium_alopecuroides	0.46	0.47	0.49	0.69
Isothecium_myosuroides	0.46	0.60	0.63	0.88
Leskea_polycarpa	0.31	0.40	0.31	0.40
Leucodon_sciuroides	0.15	0.00	0.12	0.26
Lewinskya_affine	0.62	0.90	0.96	1.00
Lewinskya_speciosa	0.00	0.05	0.10	0.25
Lewinskya_striata	0.00	0.45	0.39	0.76
Metzgeria_furcata	0.69	0.80	0.78	0.98
Microlejeunea_ulicina	0.08	0.25	0.08	0.13
Neckera_pumila	0.00	0.00	0.02	0.06
Nyholmiella_obtusifolia	0.15	0.05	0.22	0.45
Orthotrichum_diaphanum	0.69	0.90	0.88	0.85
Othotrichum_pallens	0.00	0.10	0.22	0.20
Othotrichum_pulchellum	0.00	0.45	0.49	0.74
Orthotrichum_pumilum	0.08	0.20	0.06	0.23
Orthotrichum_scanicum	0.00	0.00	0.02	0.04
Orthotrichum_stramineum	0.00	0.45	0.31	0.70
Orthotrichum_tenellum	0.08	0.35	0.33	0.71

Platygyrium_repens	0.31	0.40	0.27	0.52
Plenogemma _phyllantha	0.00	0.00	0.02	0.05
Pterygynandrum filiforme	0.00	0.00	0.00	0.02
Ptilidium_pulcherrimum	0.23	0.25	0.04	0.08
Ptychostomum_moravicum	0.31	0.10	0.02	0.11
Pulvigera_lyelii	0.23	0.70	0.59	0.92
Pylaisia_polyantha	0.23	0.05	0.41	0.50
Radula_complanata	0.31	0.75	0.90	0.96
Rhynchostegium_confertum	0.23	0.25	0.47	0.76
Sanionia-uncinata	0.15	0.05	0.08	0.10
Syntrichia_laevipila	0.15	0.30	0.25	0.50
Syntrichia_latifolia	0.15	0.00	0.12	0.35
Syntrichia_papillosa	0.00	0.05	0.24	0.92
Syntrichia_virescens	0.00	0.05	0.29	0.67
Ulota_bruchii	0.38	0.95	0.96	0.95
Ulota_crispa	0.15	0.60	0.67	0.89
Zygodon_conoideus	0.00	0.35	0.22	0.55
Zygodon_rupestris	0.00	0.05	0.12	0.30
Zygodon_viridissimus	0.08	0.00	0.18	0.49

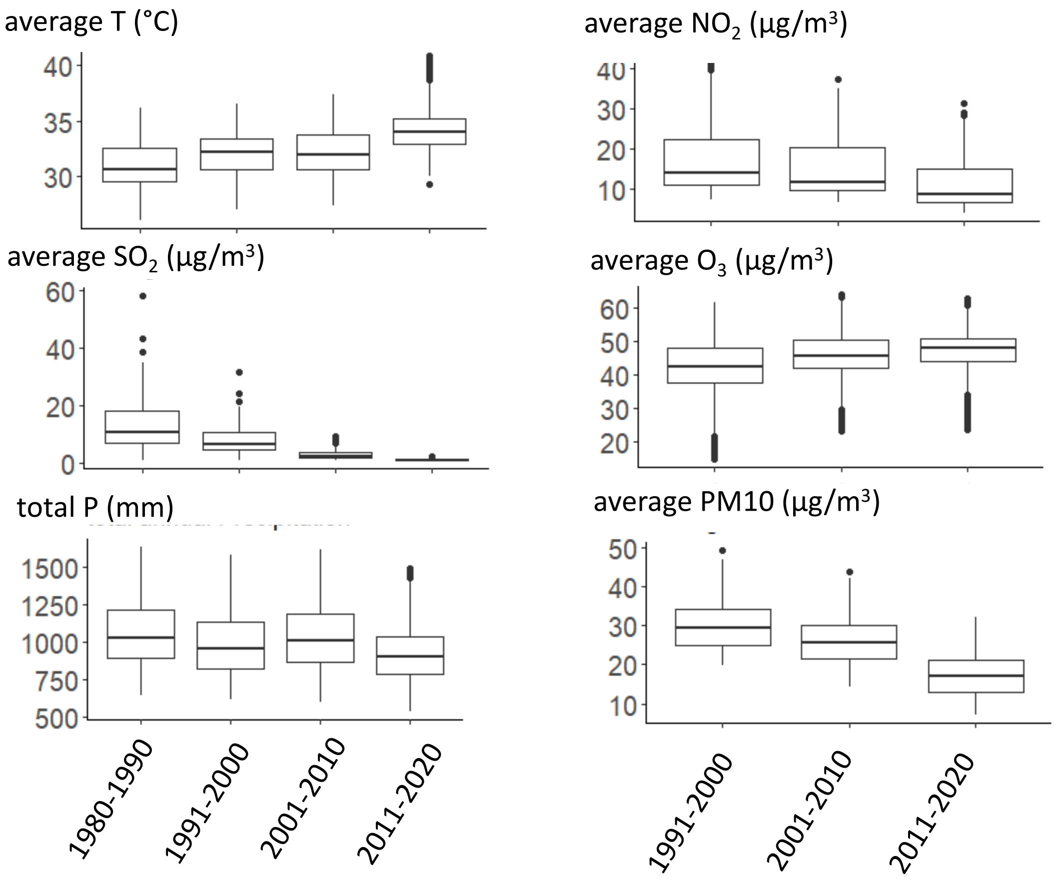

Fig. S1. *Box-plots (showing the 1st and 3rd quartiles (upper and lower bounds), 2nd quartile (centre), 1.5* interquartile range (whiskers) and minima-maxima beyond the whiskers) of the spatio-temporal variation of annual average climate conditions and air pollutant loads across 81 16 km^2-pixels in southern Belgium per decade since 1980.*

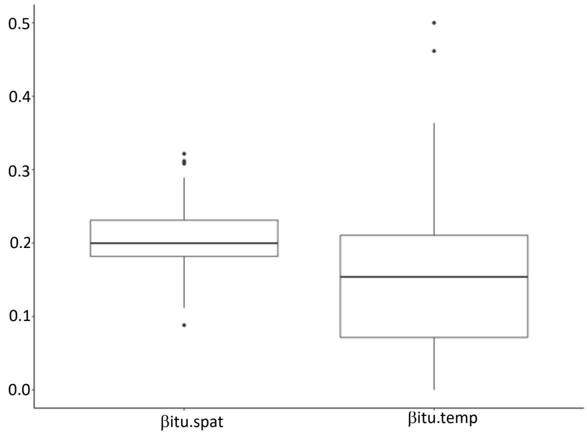

Fig. S2. *Spatio-temporal differences (expressed as species turnover) among communities of epiphytic bryophyte floras in southern Belgium. Box-plot (showing the 1st and 3rd quartiles (upper and lower bounds), 2nd quartile (centre), 1.5* interquartile range (whiskers) and minima-maxima beyond the whiskers) represent the spatial differences under present conditions, bitu.spat) across 81 16km2-pixels and the temporal variation (comparisons among assemblages of the same pixel at two time periods, bitu.temp).*

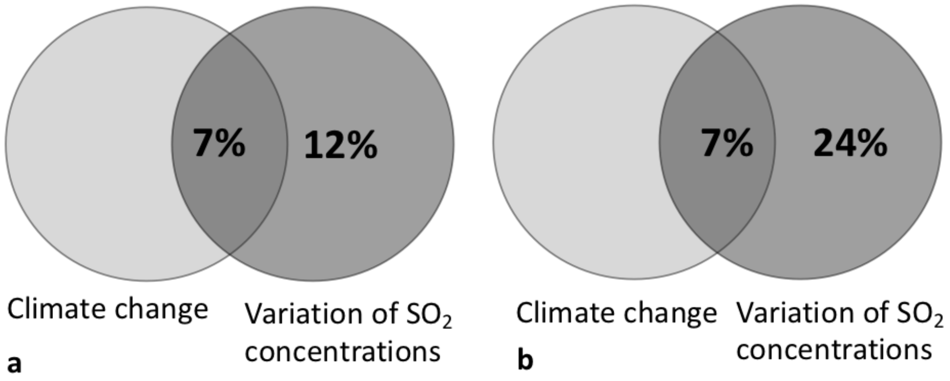

Fig. S3. Venn diagram representing the contribution (in % variance explained) of the temporal variation in SO_2 and climate change (precipitation and temperature) to temporal changes of the composition of epiphytic bryophyte communities, expressed as the species turnover (a) and nestedness (b) components of beta diversity, since 1980 in southern Belgium.

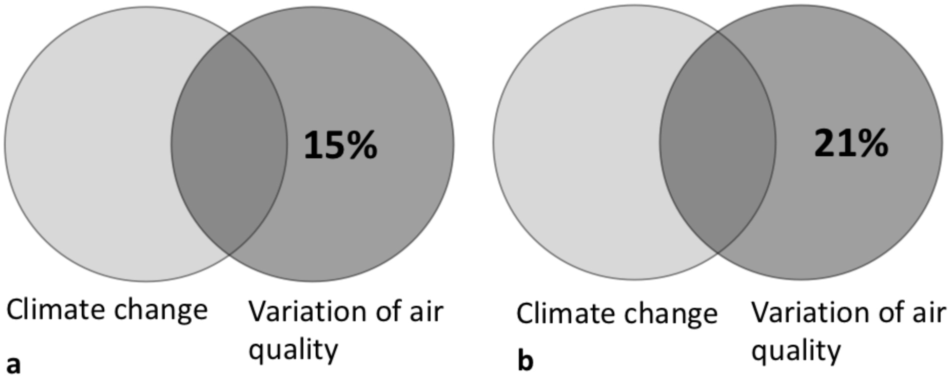

Fig. S4. Venn diagram representing the contribution (in %variance explained) of the temporal variation in air pollutant concentrations (SO_2, NO_2, O_3, PM10) and climate change (precipitation and temperature) to temporal changes of the composition of epiphytic bryophyte communities, expressed as the species turnover (a) and nestedness (b) components of beta diversity, since 1997 in southern Belgium.

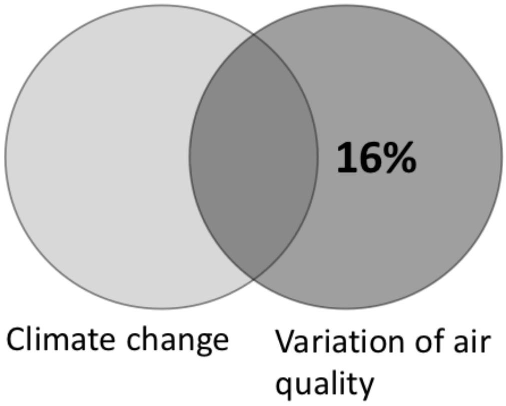

Fig. S5. Venn diagram representing the contribution (in %variance explained) of the temporal variation in air pollutant concentrations (SO_2, NO_2, O_3) and climate change (precipitation and temperature) to temporal changes of the composition of epiphytic bryophyte communities, expressed as the species turnover component of beta diversity, since 1990 in southern Belgium.

3. Chapter II

Does air pollution still impact epiphytic bryophytes in the post acidic rain era?

cover picture:epiphytic bryophytes (*Ulota*) on a branch, Mormont (Wallonia), picture from Lea Mouton, April 2024

Chapter II – Does air pollution still impact epiphytic bryophytes in the post acidic rain era?

The authors' contribution to the article.

Lea Mouton : field work, data analysis and writing

Virginie Hutsemékers : field work, interpolation and review

Flavien Collart : data analysis and statistical support

Alain Vanderpoorten : field work, conception and writing

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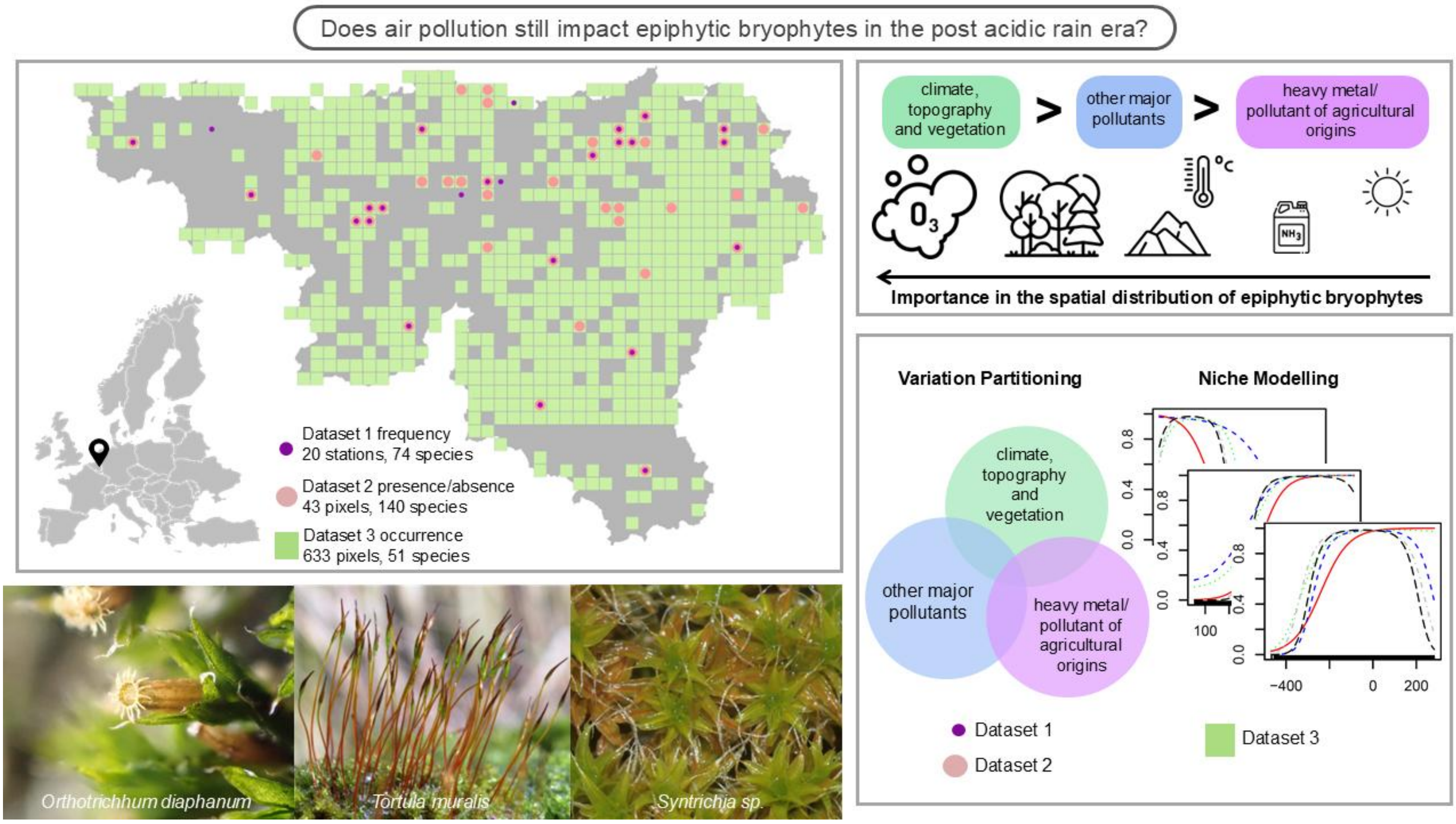

Graphical abstract

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Does air pollution still impact epiphytic bryophytes in the post acidic rain era? Insights from spatial variation of community composition in southern Belgium☆

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ABSTRACT

Since the dramatic air pollution peaks that prevailed in the course of the 20th century in Europe, effective environmental policies, along with major shifts in fuel usage, resulted in the substantial decrease of SO_x and NO_x pollution. At the interface between atmosphere and vegetation, epiphytic bryophyte floras responded by massive back-colonisation of formerly polluted areas. Whether extant concentrations of these pollutants are indeed too low to impact species distributions, and whether other pollutants today play a more important role remains, however, an open question. Taking advantage of an air quality monitoring network for a wide range of pollutants in southern Belgium, we implement here variation partitioning and embedded covariate selection to assess the contribution of current air pollutant loads to variations in epiphytic community composition relative to that of background environmental factors. Factors accounting for variation in species composition included, by decreasing order of importance, background environmental factors, major air pollutants (SO_x, NO_x, O_3, fine particle matter), pollutants of agricultural origin (NH_3, pesticides), and heavy metals. The substantially larger role played by background environmental factors over air pollution points to the efficiency of air pollution reduction policies, even for such sensitive organisms as epiphytic bryophytes. Ozone was the most important pollutant. Its ecophysiological impact on cryptogamic epiphytes remains poorly known, and the difference of its concentrations between urban and rural areas suggests that it may actually be interpreted as a land-use marker. Pesticides, whose impact on epiphyte floras was not previously assessed, marginally contributed to community composition and species distributions.

1. Introduction

In the course of the industrial era in Europe, air concentrations in oxidized forms of nitrogen (NO_x) and sulfur (SO_x), largely resulting from coal and fuel combustion, peaked in the 1950s–1980s (Stern, 2005; Castellanos and Boersma, 2012; Hilboll et al., 2013). These pollution peaks had a substantial impact on the environment, such as the spectacular forest decline caused by acidic rains (Pitelka and Raynal, 1989), and on public health, as best exemplified by the great smog of London in 1952 (Polivka, 2018). The global eutrophication trend that peaked in the 1990s, with 79 % of the ecosystem area having exceedances (i.e., the difference between the deposition loads of acidifying and/or eutrophying airborne pollutants and the critical loads) (European Environment Agency, 2014), resulted in shifts in plant species community composition (Bahr et al., 2012; Karlsmo et al., 2023). Westwards shifts in plant distributions caused by eutrophication took place at faster rates than northward range shifts in response to climate change (Sanczuk et al., 2024).

Several environmental policies aiming at improving air quality, such as the Convention on Long-Range Transboundary Air Pollution (1979), the Ambiant Air Quality Directive (2008/50/CE) and the European Union National Emissions Ceiling Directives (Directive, 2001/81/EC), have therefore been implemented. These policies, together with concomitant changes in fuel usage and combustion technology, resulted in a substantial and global decrease of NO_x and SO_x pollution since the 1990s onwards (Stern, 2005; Jyethi, 2016; Chen et al., 2024;

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Syrek-Gerstenkorn et al., 2024). Five pollutants, namely NO_x, non-methane volatile organic compounds, SO_2, NH_3, and fine particulate matter with a diameter <2.5 μm (hereafter, $PM_{2.5}$) have, however, been identified for still contributing to poor air quality and leading to negative impacts on human health and the environment. It is further estimated that 96 % of the EU's urban population remains exposed to unsafe concentrations of black carbon (here after BC) (Byčenkienė et al., 2022; Chen et al., 2024), and ozone, whose long-term concentrations even tend to increase in Europe, especially in the south (Chen et al., 2024). In 2022, almost one third of Europe's agricultural lands were exposed to ground-level ozone concentrations above the threshold value set for protection of vegetation in the EU's Ambient Air Quality Directive (https://www.eea.europa.eu/en/analysis/publications/impacts-of-air-pollution-on-ecosystems-in-europe). The NEC Directive (2016/2284/EU) therefore sets 2020 and 2030 emission reduction commitments for these five main pollutants to reduce the health impacts of air pollution by half compared with 2005. This Directive also introduces a number of reporting requirements on emissions of a number of pollutants including, in addition to the five precited ones, PM_{10} (fine particulate matter with a diameter <10 μm), BC, total suspended particulate matter, several heavy metals, and persistent organic pollutants.

At the interface between atmosphere and vegetation, epiphytic bryophyte floras are particularly exposed to air pollution. Their ecophysiological features, and in particular, direct reliance on atmospheric precipitation for water and nutrient uptake and lack of stomates to regulate gas exchanges, make them particularly vulnerable to variations in environmental conditions (Bates, 2000). As a consequence, they have undergone the most dramatic change out of any of the groups analysed as a response to global change since the past decades by a considerable margin (Pescott et al., 2015). Since the 1990s, a massive back-colonisation of acid-sensitive and a decline of acidophilous species have been recurrently reported (Bates et al., 1997; Duckett and Pressel, 2010; Pescott et al., 2015; Purvis et al., 2010; Sérgio et al., 2016; Stebel and Fojcik, 2016). The temporal shift in species composition due to changes in air pollutant concentrations was such that it was, on average, more than twice larger than the spatial change in species composition observed today among communities (Hutsemekers et al., 2023). The extent to which major pollutants such as NO_x and SO_x still drive extant species distributions, and whether other pollutants, either emerging or whose effects were historically masked by the impact of major pollutants, play a role in contemporary species distribution patterns, remain, however, open questions. This is particularly the case for pesticides, whose extensive application has led to growing concerns about their impact on ecosystems and population health, but whose impact remains difficult to assess due to the lack of quantitative data on their spreading and concentrations in the environment (Habran et al., 2022).

Here, we take advantage of the official network of air quality monitoring stations for a wide range of pollutants and spatially-explicit estimates of agricultural pesticide use (Habran et al., 2022) in southern Belgium to address the following questions: what is the contribution of current air pollutant loads to variations in epiphytic community composition relative to that of background environmental factors of climatic conditions, topography and vegetation (Q1)? Which pollutants, if any, still account for variations in community composition and species distribution patterns (Q2a)? In particular, what is the relative role played by heavy metals, pollutants of agricultural origin and other major pollutants (Q2b)?

2. Materials and methods

2.1. Data collection

We assembled three datasets to analyse the relationships between pollution loads, environmental factors and epiphytic bryophytes (Table 1). Datasets 1and 2 were based on complete species inventories of selected areas at two spatial resolution levels, suitable for analyses of the environmental drivers of community composition. Dataset 3included species occurrence data across the entire study area, suitable for analyses of the environmental drivers of individual species distributions.

Table 1
Datasets of epiphytic bryophyte species, air pollutant concentrations, and environmental background (climate, topography, vegetation) in southern Belgium.

	Dataset 1	Dataset 2	Dataset 3
Sampling units	Air pollution measuring stations (n = 20)	16 km^2 pixels (n = 43)	16 km^2 pixels (n = 633)
Floristic data	frequency of all epiphytic bryophyte species recorded on 20 phorophytes surrounding the station (n = 74)	presence/absence of all epiphytic bryophyte species per pixel (n = 140)	occurrence of only strict epiphytic bryophyte species per pixel (n = 51)
Topographic data	Elevation, northness, slope	Elevation, northness, slope, averaged across each 16 km^2 pixel	idem
Climatic data	Annual mean air temperature (mean T), annual range of air temperature (range T), annual total precipitation, mean relative humidity of the driest month, (meanRH_driest), annual range of radiations at 1 km^2 resolution (Rangersrad)	Idem, averaged across each 16 km^2 pixel	idem
Pollutants	Measured concentrations at n = 20 stations: - Heavy metals (arsenic, cadmium, chromium, nickel, lead, zinc) - Other major pollutants (NO_2, O_3, SO_2, black carbon (BC), fine particles with an aerodynamic diameter <10 μm (Particle Matter, PM10) and 2.5 μm (PM2.5)	Interpolated concentrations - Pollutants of agricultural origin (NH_3 and modelled loads of herbicides, insecticides, fungicides, all pesticides) - Other major pollutants (NO_2, O_3, SO_2, black carbon (BC), fine particles with an aerodynamic diameter <10 μm (Particle Matter, PM10) and 2.5 μm (PM2.5)	idem
Forest	Frequency of host tree species, % broadleaf forest cover (% BLForest), % mixed forest cover (% MixForest) in a 50m (1ha) and 200m (10ha) radius around the station	% broadleaf forest cover (%BLForest), % mixed forest cover (%MixForest) per pixel	idem

The factors that affect epiphytic bryophyte distributions vary depending on the degree of spatial resolution and extent (Medina et al., 2014). At the level of individual trees, tree identity, age, and size (Mitchell et al., 2021) drive bark texture and physico-chemistry, and hence, the composition of epiphytic communities (Tyler & Olsson, 2016; Kovářová et al., 2022; Shao et al., 2023). At increasing spatial resolution and geographic extent, site characteristics, such as microclimate, air quality, and vegetation type, increasingly prevail (Medina et al., 2014; Hutsemekers et al., 2023). The present data were collected at the level of epiphyte communities and therefore included site-specific characteristics rather than features of individual trees. The dataset at the finest spatial resolution (dataset 1) included, however, information on host-tree identity, an integrative factor accounting for bark texture and physico-chemistry shown to explain more the variation in epiphytic species distributions than bark pH (Spier et al., 2010).

Dataset 1included local species frequencies of all epiphytic bryophytes recorded between 2016 and 2020 across the 20 nearest phorophytes with a DBH (diameter at breast height) $>$ 20 cm surrounding the 20 air quality monitoring stations. Data on atmospheric pollutants originated from actual hourly measurements at the 20 stations and stored by the Belgian Interregional Environment Agency (IRCEL—CELINE).

For each pollutant, we computed annual mean and maximum concentrations for the same period of time as the floristic data, i.e., 2016–2020. These pollutants included heavy metals (arsenic, cadmium, chromium, nickel, lead, zinc) and other major pollutants (SO_2, NO_2, O_3, PM_{10} and $PM_{2.5}$). Climatic data included annual mean air temperature, annual range of air temperature, annual total precipitation, mean relative humidity of the driest month, annual range of solar radiation, derived from CHELSA 1.2 (Karger et al., 2017, 2018) at 30 arc-second ($\sim$1 km) resolution for the 1981–2010 time period. Topographic data included elevation, northness and slope at 30 arc-second resolution from Amatulli et al. (2018). Vegetation data included species identity of the 20 selected trees, as well as % forest cover, % broadleaf forest cover, % mixed forest cover in a 50m (1ha) and 200m (10ha) radius around the station, retrieved from Bolyn et al. (2022). Altogether, dataset 1included 37 variables (appendix 1).

Dataset 2included complete inventories of all epiphytic bryophyte species recorded between 2016 and 2020 in 43 pixels of 16 km^2 (Hutsemekers et al., 2023), background environmental factors, and air pollutant concentrations. Background environmental factors also included percentages of broadleaf forest cover (%BLForest) and of mixed forest cover (%MixForest) per pixel. These variables at 30 arc-second resolution were averaged to 16 km^2 resolution using the raster package (Hijmans, 2024) in R 4.2.1. Pollutants included major pollutants (SO_2, NO_2, O_3, PM_{10} and $PM_{2.5}$) as well as pollutants of agricultural origin. Concentrations of the major pollutants were obtained for each pixel of 16 km^2 resolution by interpolation. The interpolation was performed using the RIO model based on land use, a semivariogram based on the distances to the nearest measuring stations and the levels of air pollution, which was employed to compute, on an hourly basis, the background concentrations at the centroid of all the investigated pixels. Pollutants of agricultural origin included NH_3, which originates at $>$90 % from agriculture in the study area (Regional Air Pollutant Inventories, 2023), but to which traffic emissions also contribute (Cape et al., 2004; Manninen et al., 2023) and pesticides. NH_3 was measured at 32 passive samplers during the peak season of fertilizer spreading (between mid-April and mid-May 2021) and interpolated using a simple inverse distance weighting method. Data for pesticides included estimated amounts of total active ingredients (in kg/ha/year) for herbicides, insecticides, fungicides and all active substances (composed of herbicides, insecticides, fungicides and growth regulators) across the period 2015–2017 (Habran et al., 2022). These estimates were obtained by quantifying the amount of pesticides yearly applied to particular crops based on a reference sample (around 4 % of farms in southern Belgium) and then extrapolating these figures for each crop field across the area depending on crop type (Habran et al., 2022). Based on these estimated application loads per crop field, we derived mean and maximum values for each 16 km^2 pixel. Heavy metals, which are characterized by limited airborne spread (Kováčik et al., 2023) and whose concentrations cannot be interpolated, were excluded from this analysis. Altogether, dataset 2included 30 variables (appendix 2).

Dataset 3 included occurrence data for 51 strict epiphytic bryophyte species recorded after 2016 from the atlas of bryophyte species distributions in southern Belgium (Sotiaux and Vanderpoorten, 2015, updated in www.Biogeonet.ulg.ac.be) across 633 pixels of 16 km^2 and the same set of pollutants and environmental factors as in Dataset 2.

2.2. Data analysis

Variation partitioning, as implemented by the varpart function of the vegan package (Oksanen et al., 2022), was used to disentangle the role of pollution loads from that of background environmental factors on the composition of bryophyte communities (Q1). For dataset 1, the Y matrix included species frequencies across 20 nearest phorophytes to the measuring stations. We also implemented a second analysis where species frequencies were converted into presence/absence data to assess the relevance of frequency vs presence-absence data in such analyses. Predictor matrices included heavy metals (X1), other major pollutants (X2) and background environmental factors (X3) listed in Table 1. To reduce the number of predictors and avoid multicollinearity, a Principal Component Analysis (PCA) was performed to summarize the information included within each of the three predictor matrices. For each PCA, the first two axes were retained. For Dataset 2, the Y matrix included species presence/absence per 16 km^2 pixel. Predictor matrices included interpolated concentrations of pollutants of agricultural origin (X1), other major pollutants (X2) and background environmental factors (X3) listed in Table 1. To visualize how species respond to these factors, we presented the species x environment plots of the Redundancy Analyses (RDA) underlaying the variation partitioning analyses for both datasets. To help interpreting the gradients in species composition, species scores on the first two RDA axes were correlated with species ecological traits, including ecological indicator values and species habitat preference (van Zuijlen et al., 2023). Ecological indicator values included light (indL), temperature (indT), continentality (indK), moisture (indF), acidity (indR), nutrient availability (indN), as well as an additional indicator value for heavy metals (indHM). Habitat preference traits included whether species are classified as epiphytes, epixylic (on rocks), how strongly species are bound to forest habitats (forest), and hemeroby, i.e., whether a species is largely restricted to close-to-nature habitats, largely restricted to man-made habitats, or indifferent.

To determine which factors account for variations in species distribution patterns (Q2), we implemented the embedded covariate selection procedure of Adde et al. (2023). Based on three algorithms (Generalized Linear Model (GLM), Generalized Additive Model (GAM) and Random Forest), the embedded covariate selection procedure was used to identify the best predictors of each of 51 strict epiphytes (dataset 3) among 30 predictors at 16 km^2 resolution. We implemented this analysis for each species in two ways: (i) keeping only variables jointly selected by the three algorithms, and (ii) fixing the total number of variables in the model to 4. This number of four variables was determined, based on the rule-of-thumb according to which the addition of a variable in a model requires the addition of $>$10 observations (Peduzzi et al., 1996; Harrell et al., 1996), to the size of the datasets with the lowest numbers of occurrences. We then assessed the importance of each variable as a function of the proportion of models, in which it was included, weighted by its ranking during the selection procedure. For the analysis consistently keeping the four best variables, the first variable to be selected had a weight of 1, the second variable a weight of 0.75, 0.5 for the third variable, 0.25 for the fourth and 0 for the variables that have not been selected. The variable importance corresponded to the sum of its weights across species. For the analyses retaining the variables selected by all three algorithms, the number of variables retained varied among species, and the weight was thus rescaled between 0 and 1 depending on the number of variables selected (total number of selected variables – variable rank + 1/total number of selected variables). To help visualizing and interpreting the results, and in particular, take the correlation among variables into account, we computed the correlation among variables and generated a dendrogram of similarity using Ward's algorithm (Murtagh and Legendre, 2014).

3. Results

Background environmental conditions, measured concentrations of heavy metals and other major pollutants uniquely accounted for 30 %, 1 % and 9 %, respectively, of the total explained variance in epiphytic bryophyte species frequencies at the level of air pollution measuring stations (dataset 1) (Fig. 1a). When species presence-absence instead of frequencies were employed as dependent variables, these proportions dropped to 8 %, <1 % and 3 %, respectively (Fig. 1b). At the level of 16 km^2 pixels (dataset 2), background environmental conditions, interpolated concentrations in pollutants of agricultural origin and other major pollutants uniquely accounted for 7 %, <1 % and 2 %, respectively, of the total explained variance in species composition (Fig. 1c).

The results of the Redundancy Analyses between epiphytic bryophyte community composition, air pollution and background environmental factors using datasets 1and 2 are displayed in Fig. 2. In both analyses, the main gradient involved a shift from forest oligotrophic specialist epiphytes (e.g., *Frullania tamarisci, Tetraphis pellucida*) to nitrophilous (*Syntrichia* spp., *Orthotrichum diaphanum*), primarily epixylic species (*Grimmia pulvinata*, *Tortula muralis*) (see Fig. S1 for the correlation between species ecological traits and their scores on the RDA axes).

The results of the embedded covariate selection procedure determining the importance of individual variables accounting for species distributions (dataset 3) yielded similar results when keeping the four best variables per model (Fig. 3) vs all the variables consistently selected by the three algorithms per model (Fig. S2). The three first most important variables were maximum O_3 concentrations, mixed forest cover, and elevation. These variables belonged to a cluster of highly correlated variables that, except for O_3, characterize background environmental conditions. The fourth most important variable was mean annual temperature. Mean annual temperature belonged to a cluster of highly correlated variables that mostly included major air pollutants. The fifth most important variable was NH_3, which belonged to a group of correlated pollutants of agricultural origin (group3).

4. Discussion

The results from different datasets at different spatial resolutions and based on different analytical approaches congruently evidence that current epiphytic bryophyte distribution patterns in southern Belgium are, in an increasing order of importance, explained by heavy metals and pollutants of agricultural origin, then by other major pollutants, and finally by background environmental factors. While the importance of macroclimatic variation on species distribution patterns is indeed expected to increase with spatial extent (Siefert et al., 2012), change in air pollutant concentrations has been, in the course of the past decades, the major factor of the temporal variation of epiphytic bryophyte community composition at regional scales (Pescott et al., 2015). This change was such, that the temporal shift in species composition has been, on average, more than twice larger than the spatial change in species composition observed today (Hutsemekers et al., 2023). The larger role played by background environmental factors over air pollution reported here thus points to the efficiency of air pollution reduction policies and their impact on biodiversity patterns, even for such highly sensitive organisms as epiphytic bryophytes. This pattern is consistent with previous reports on the partitioning of environmental drivers of lichen species composition at a regional scale, highlighting the role of climatic variation over air pollution loads (Ellis and Coppins, 2010). Several studies reported, however, departures from this pattern. Concentrations in major air pollutants still significantly impact epiphytes in large cities (Rocha et al., 2022; Sebald et al., 2022) and in central Europe (Procházková et al., 2025), highlighting that, despite ongoing overall improvements in air quality, current EU standards are still not met across Europe (European Environment Agency, 2024).

SO_x and NO_x, which have driven the spectacular shifts in species composition in the course of the past decades (Hutsemekers et al., 2023), marginally contributed to explain spatial variation in community composition and distribution, suggesting that these pollutants reached concentrations levels below critical levels for epiphytic floras. In fact, SO_2 concentrations between 2015 and 2020 reached, on average across the study area, 1 $\mu g/m^3$, i.e., well-below the concentrations shown to impact bryophytes and lichens (Lee et al., 1998; Bates et al., 1996). In urban epiphytic lichens, the contribution of contemporary concentrations of SO_2 to current patterns of diversity and composition was similarly shown to be minimal (Sebald et al., 2022). Regarding NO_x, the shift from species with low to high (*Syntrichia* spp., *Orthotrichum diaphanum*)

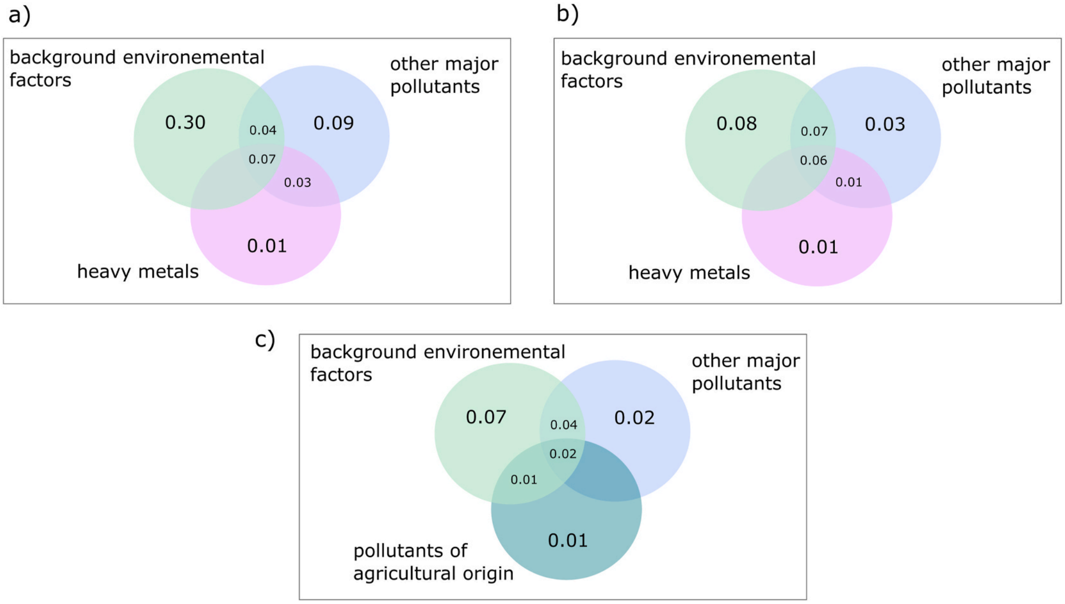

Fig. 1. Venn diagrams of the variation partitioning analysis between epiphytic bryophyte species composition, air pollutant concentrations and background environmental factors. (a). Species frequencies at 20 trees surrounding the measuring stations vs measured concentrations of heavy metals (X1), other major pollutants (X2, including SO_2, NO_2, O_3, PMs), and background environmental factors (X3, including topography, climate, vegetation). (b). idem, but with species frequencies converted into presence/absence. (c). Species presence/absence at 43 pixels of 16 km^2 vs interpolated measured concentrations of pollutants of agricultural origin (X1, including NH3, herbicides, insecticides, fungicides), other major pollutants (X2), and background environmental factors (X3).

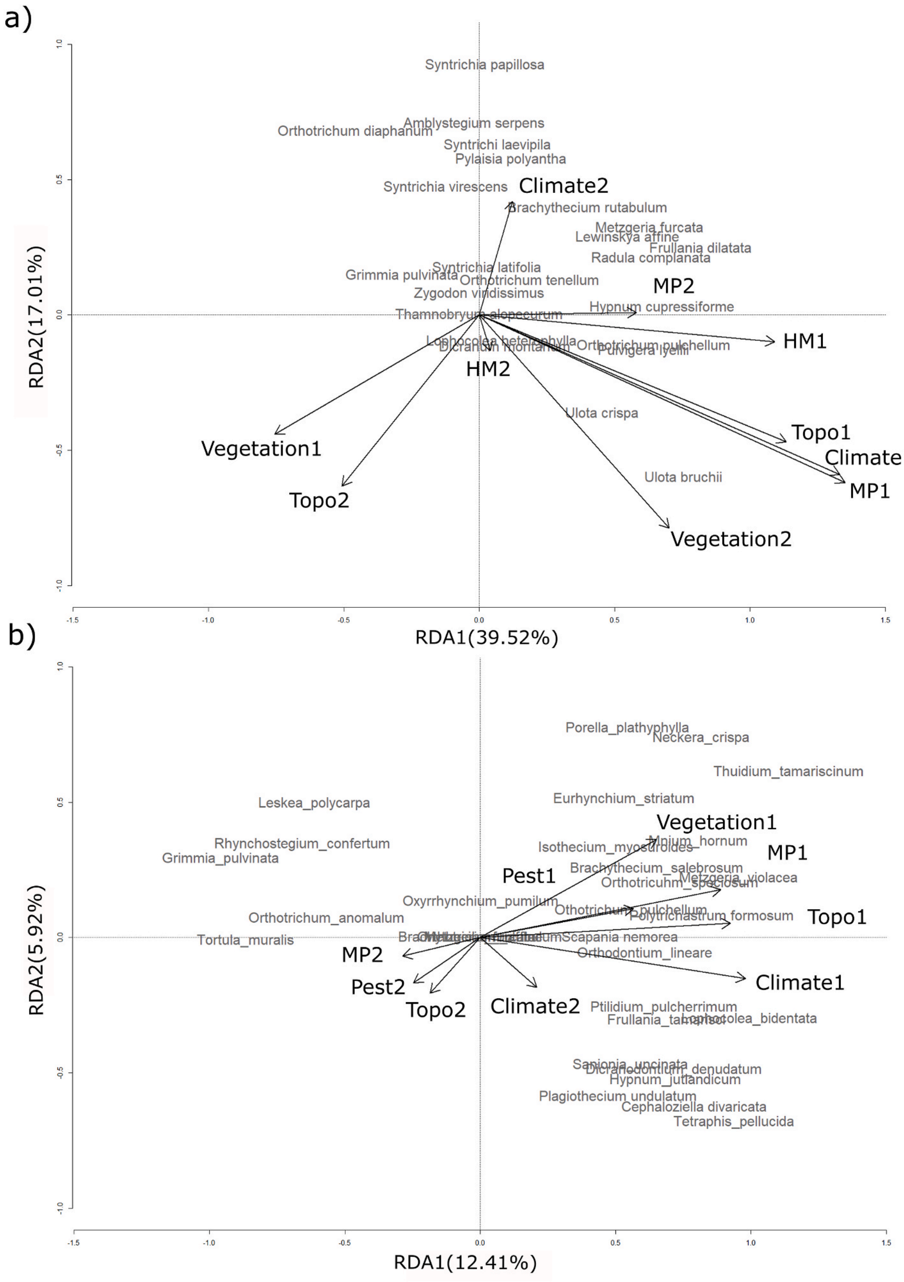

Fig. 2. Redundancy analysis of the variation in epiphytic bryophyte community composition, background environmental variables and air pollutant concentrations. (a). Species frequencies at 20 trees surrounding the measuring stations vs measured concentrations of heavy metals (arsenic, cadmium, chromium, nickel, lead, zinc, summarized by the first two axes of their principal component analysis, labelled as HM1 and 2), other major pollutants (SO_2, NO_2, O_3, Particle Matters, summarized by the first two axes of their principal component analysis, labelled as MP1 and 2), and background environmental factors (topography, climate, vegetation). (b). Species presence/absence at 43 pixels of 16 km^2 vs interpolated measured concentrations of pollutants of agricultural origin (NH_3, herbicides, insecticides, fungicides, all pesticides, summarized by the first two axes of their principal component analysis, labelled as PEST1 and 2), other major pollutants (see above), and background environmental factors (see above).

nitrogen preference along the main pollution gradient retrieved here is, at first sight, consistent with similar trends reported in epiphytic lichens (Ellis and Coppins, 2009) and non-epiphytic bryophytes (Pakeman et al., 2022), and with previous reports on the impact of NO_2 on the composition of epiphytic communities in urban environments (Sebald et al., 2022). In the context of decreasing NO_2 pollution, and given that shifts in species composition also involved a shift towards species with high pH preferences, whereas NO_2 deposition leads to substrate acidification however, this trend is puzzling. One interpretation is that other N sources than NO_2 have played a role in the increase of nitrophilous species.

In particular, NH_3 was the fifth most important variable and second pollutant in terms of predictor of species occurrences. Experimental evidence in fens exposed to different sources of N pollution suggests that NH_y impacts bryophyte vegetation much more severely than NO_x. NH_y may have both direct and indirect effects on epiphytic bryophytes communities. Indirect effects would result from increased competition due to eutrophication. On trees, fast spreading, thick mats of colonies formed by dendroid growth forms, such as *Isothecium*, whose creeping primary shoots rapidly spread over the surface and whose erect secondary shoots overarch underlying species, are considered as typical examples of effective competitors (Peck & Frelich, 2008). The role of

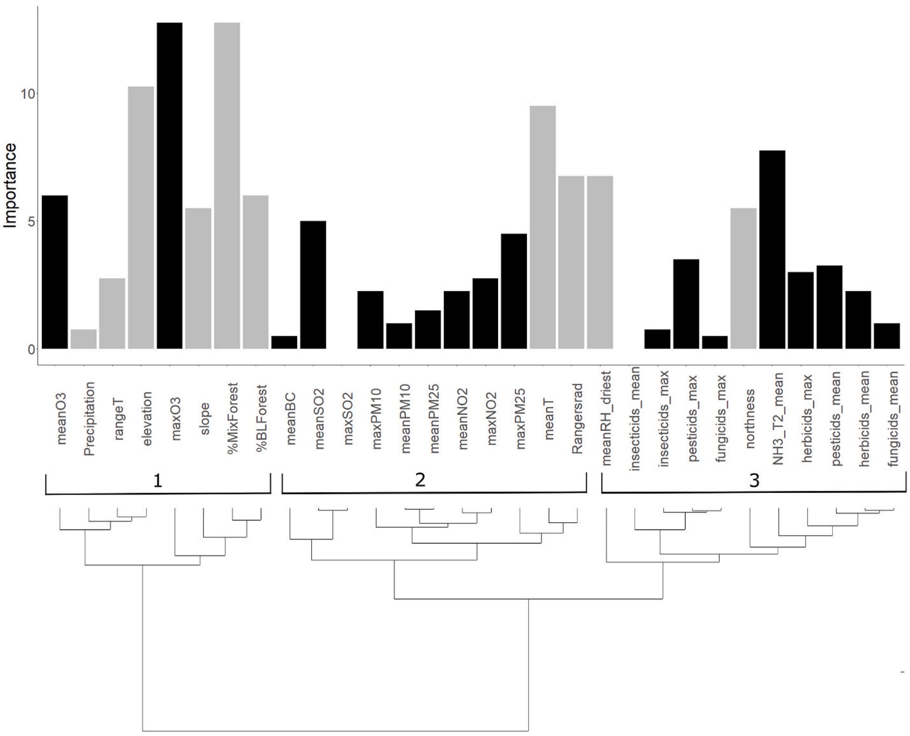

Fig. 3. Importance of environmental variables (black: air pollutants, grey: background environmental conditions, see Table 1 for abbreviations) accounting for the distribution of strictly epiphytic bryophytes in southern Belgium. The importance of each variable is a function of the proportion of models, in which it was included by the embedded covariate selection (keeping the four best variables per model), weighted by its ranking during the selection procedure. The panel below is a dendrogram of similarity among variables based on the correlation coefficient among variables and using Ward's algorithm as the clustering criterion.

competition among bryophyte communities has, however, remained an area of controversy (Ma et al., 2024, and references therein). Co-occurrence analyses suggested that, in epiphytic communities, biotic interactions play a secondary role as compared to environmental filtering, with a very marginal contribution of competitive exclusion (Shen et al., 2023), supporting the notion that competitive exclusion is a rare process in bryophyte communities (Steel et al., 2004; Mälson & Rydin, 2009; Udd et al., 2016).

Direct effects of NH_y, which accumulates beyond the N-saturation point, include impacts on photosynthesis performance and C fixation, uptake regulation of P and K, and oxidative stress, causing the production of reactive oxygen species whose accumulation can lead to the deterioration of cell membranes (Izquieta-Rojano et al., 2018). In addition, NH_3 deposition results in an increase of bark pH (Van Herk, 2001; Manninen et al., 2023), resulting in a decrease of the acidophilous lichen flora (Manninen et al., 2023; Gauslaa, 2024) and most likely contributing to the decrease of the acidophilous bryophyte flora over the past decades (Hutsemekers et al., 2023). The increase of bark pH due to NH_3 pollution could explain the observed trend for calciphilous epixylic species typically found on concrete (e.g., *Grimmia pulvinata*, *Orthotrichum anomalum*, *Tortula muralis*) to grow as epiphytes in the most polluted areas. Altogether, bark pH and NH_y concentration in the stemflow therefore appeared as the main drivers of the regional variation in epiphytic bryophyte and lichen communities (Mitchell et al., 2005).

Among pollutants whose variations in concentrations best correlated with epiphytic bryophyte species distributions, ozone was identified as the most important one. O_3 is a strong oxidizing agent that damages plant internal structure and impacts physiological functions, accelerating senescence and decreasing photosynthesis (see Zhao et al., 2020 and references therein). The substantial increase of O_3 since the pre-industrial era (Young et al., 2013) is such, that current concentrations are sufficiently high to impact crop yield (Du et al., 2024) and forest primary production (Sorrentino et al., 2025). In bryophytes, reduction of photosynthetic activity and membrane leakage was reported in four of 22 species exposed to concentrations of 300 $\mu g/m^3$ (Lee et al., 1998), which are almost in the range of the maxima currently recorded in southern Belgium. The ecophysiological impact of O_3 on cryptogamic epiphytes remains, however, poorly known (Ellis and Coppins, 2009). In addition, the fact that O_3 concentrations are higher in rural than urban areas (Paoletti et al., 2014), together with the fact that O_3 concentrations were highly correlated with background environmental factors, and not with other pollutants, suggests that O_3 may actually be interpreted as a land-use marker rather than a factor actually impacting epiphytic floras. This suggests that the contribution of air pollutants as compared to that of other background environmental factors reported above may even be overestimated.

Among other investigated pollutants, pesticides, whose impact on ecosystems has been widely acknowledged (Albaseer et al., 2025 and references therein), marginally contributed to explain variations in epiphytic bryophyte community composition and species distributions. Several herbicides, some of which are specifically used against bryophyte development in, e.g., golf courses (Fausey, 2003; Post et al., 2016), have an effective impact on bryophyte ecophysiology. For example, bryophytes suffer detrimental effects after exposure to widely used herbicides such as asulam at concentrations similar to those that affect bracken, against which they are implemented (Rowntree et al., 2003). The response of bryophytes varies, however, among species and depending on the kind of pesticide implemented (Newmaster et al., 1999; Rowntree et al., 2003; Rowntree et al., 2005; Fuselier and Carreiro, 2022). Detailed information on pesticide concentrations in the air, as well as further experimental investigations on the impact of pesticides on bryophyte ecophysiology, would be necessary for a more accurate evaluation of the impact of pesticides on bryophyte floras.

Another family of pollutants whose impact was not revealed by the present analyses are heavy metals. In contrast to angiosperms, which tend to develop specialized tolerant strains, bryophytes tend to exhibit 'all-purpose' genotypes and a high intrinsic tolerance to metals through physiological acclimatization (Shaw, 2000). Mounting evidence points to the role played by metabolites such as allantoin, which have increasingly been identified as important mediators of stress, enhancing tolerance to metals (Dresler et al., 2023). Nevertheless, the limited amount of data on heavy metals in the present study due to the impossibility to accurately interpolate their concentrations in the air, calls for further research on their potential impact.

Substantial variations in species composition along a gradient of air pollution were retrieved here, with a shift from oligotrophic assemblages comprised of true epiphytes to eutrophic assemblages comprised of generalist species. This supports the notion that individual species exhibit different ‘indicator values’ for air pollution, underlaying the implementation of air-monitoring schemes that have been widely developed to survey spatio-temporal changes in air quality (Zechmeister et al., 2007; Sérgio et al., 2016; Jiang et al., 2020). There was, however, a substantial drop of the percent explained variance of the bryophyte community when using species presence absence vs relative frequencies. Beyond actual species composition, species relative frequencies thus yield important information regarding environmental factors and should be recorded in epiphytic vegetation surveys.

In conclusion, our survey reveals that background environmental factors prevail over air pollution to explain extant epiphytic bryophyte community composition and species distributions at a regional scale. Major pollutants such as SO_x and NO_x, which prevailed a few decades ago, now seem to marginally impact epiphyte floras. The impact of pesticides, which was, to our knowledge, not previously assessed on epiphyte floras, was marginal as well. Since pesticides were experimentally shown to impact bryophyte ecophysiology, we suggest that further empirical studies on the role of pesticides in the wild would be necessary to assess whether our findings are generalizable. Ultimately, while bryophytes have been efficiently used as biomonitors of atmospheric deposition of heavy metals (Harmens et al., 2010), they have not been the focus of long-term community composition survey programmes as vascular plants, which revealed substantial shifts in species distributions associated with global environmental change in general and N deposition in particular (Sanczuk et al., 2024). Given the extreme sensitivity of bryophytes to air pollution and climate change, we suggest that a network of permanent plots surveyed on a regular basis, following standardized protocols across a range of geographical regions and habitats (e.g., Cateau et al., 2024), should be implemented to disentangle the role played by individual drivers of global change on biodiversity patterns.

CRediT authorship contribution statement

Lea Mouton: Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Virginie Hutsemekers:** Validation, Data curation. **Flavien Collart:** Methodology, Formal analysis. **Alain Vanderpoorten:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.126495.

Data availability

The link to the data is shared in the manuscript

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Supplementary material for Chapter II

Table 1. Datasets of epiphytic bryophyte species, air pollutant concentrations, and environmental background (climate, topography, vegetation) in southern Belgium

	Dataset 1	Dataset 2	Dataset 3
Sampling units	Air pollution measuring stations (n=20)	16km2 pixels (n=43)	16km2 pixels (n=633)
Floristic data	frequency of all epiphytic bryophyte species recorded on 20 phorophytes surrounding the station (n=74)	presence/absence of all epiphytic bryophyte species per pixel (n=140)	occurrence of only strict epiphytic bryophyte species per pixel (n=51)
Topographic data	Elevation, northness, slope	Elevation, northness, slope, averaged across each 16km^2 pixel	idem
Climatic data	Annual mean air temperature (mean T), annual range of air temperature (range T), annual total precipitation, mean relative humidity of the driest month, (meanRH_driest), annual range of radiations at 1km2 resolution (Rangersrad)	Idem, averaged across each 16km^2 pixel	idem
Pollutants	Measured concentrations at n=20 stations: - Heavy metals (arsenic, cadmium, chromium, nickel, lead, zinc) - Other major pollutants (NO_2, O_3, SO_2, black carbon (BC), fine particles with an aerodynamic diameter <10 µm (Particle Matter, PM10) and 2.5µm (PM2.5)	Interpolated concentrations - Pollutants of agricultural origin (NH3 and modelled loads of herbicides, insecticides, fungicides, all pesticides) - Other major pollutants (NO_2, O_3, SO_2, black carbon (BC), fine particles with an aerodynamic diameter <10 µm (Particle Matter, PM10) and 2.5µm (PM2.5)	idem
Forest	Frequency of host tree species, % broadleaf forest cover (%BLForest), % mixed forest cover (%MixForest) in a 50m (1ha) and 200m (10ha) radius around the station	% broadleaf forest cover (%BLForest), % mixed forest cover (%MixForest) per pixel	idem

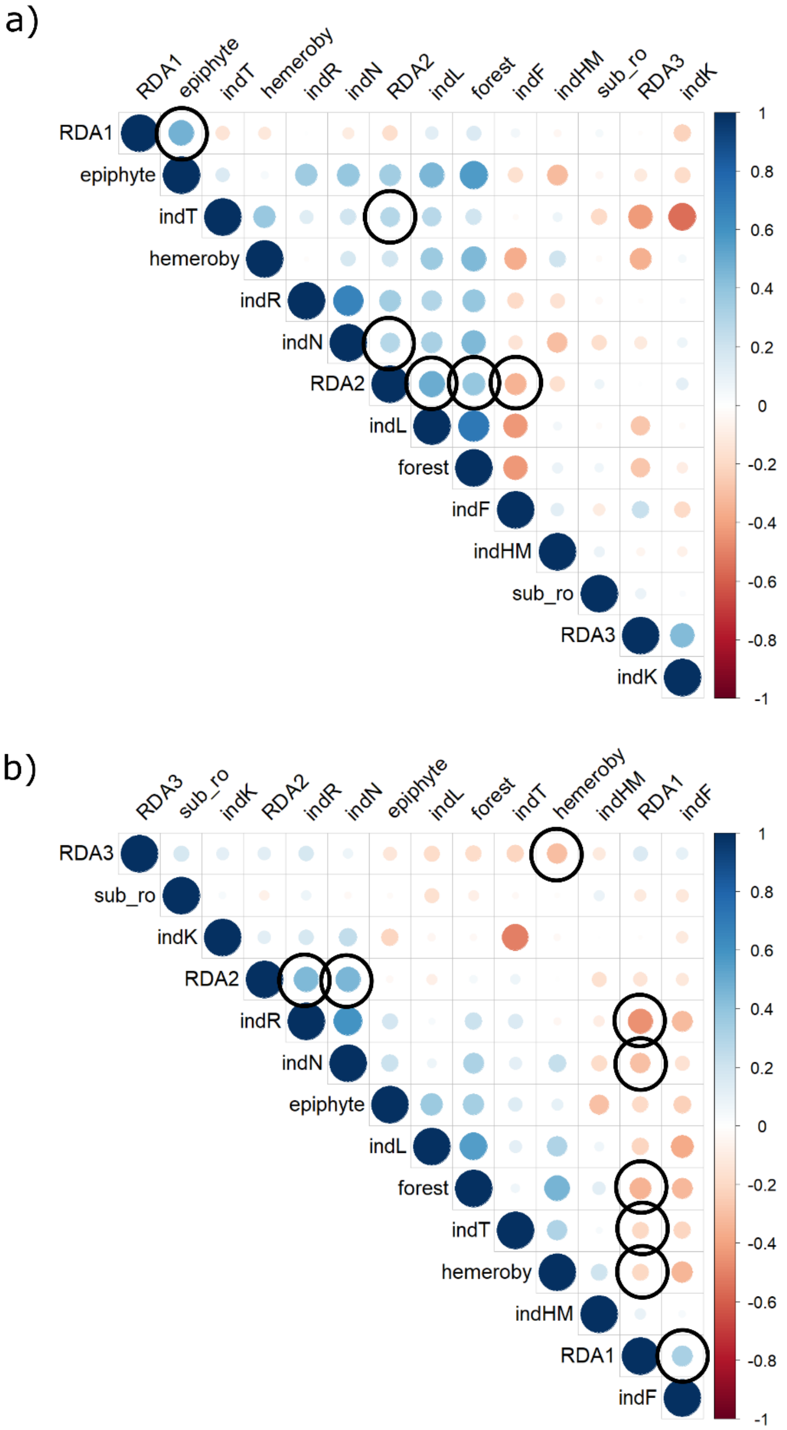


Fig. S1. Correlation coefficient between the species scores of the redundancy analysis (first two axes RDA1 and 2) and species ecological traits. a) RDA based on species frequencies 20 trees surrounding each of 20 air quality measuring stations (Fig. 2a). b) RDA based on species presence/absence in 43 16km2 pixels (Fig. 2b). indL, indT, indF, indR, indN, indHM: Ellenberg indicator value for light, temperature, moisture, acidity, nutrient availability, and heavy metals, respectively. Epiphyte, sub_ro: species classified as epiphytes and epixylix (on rocks), respectively. Hemeroby: species preference for man-made habitats. The significant correlations are circled in black.

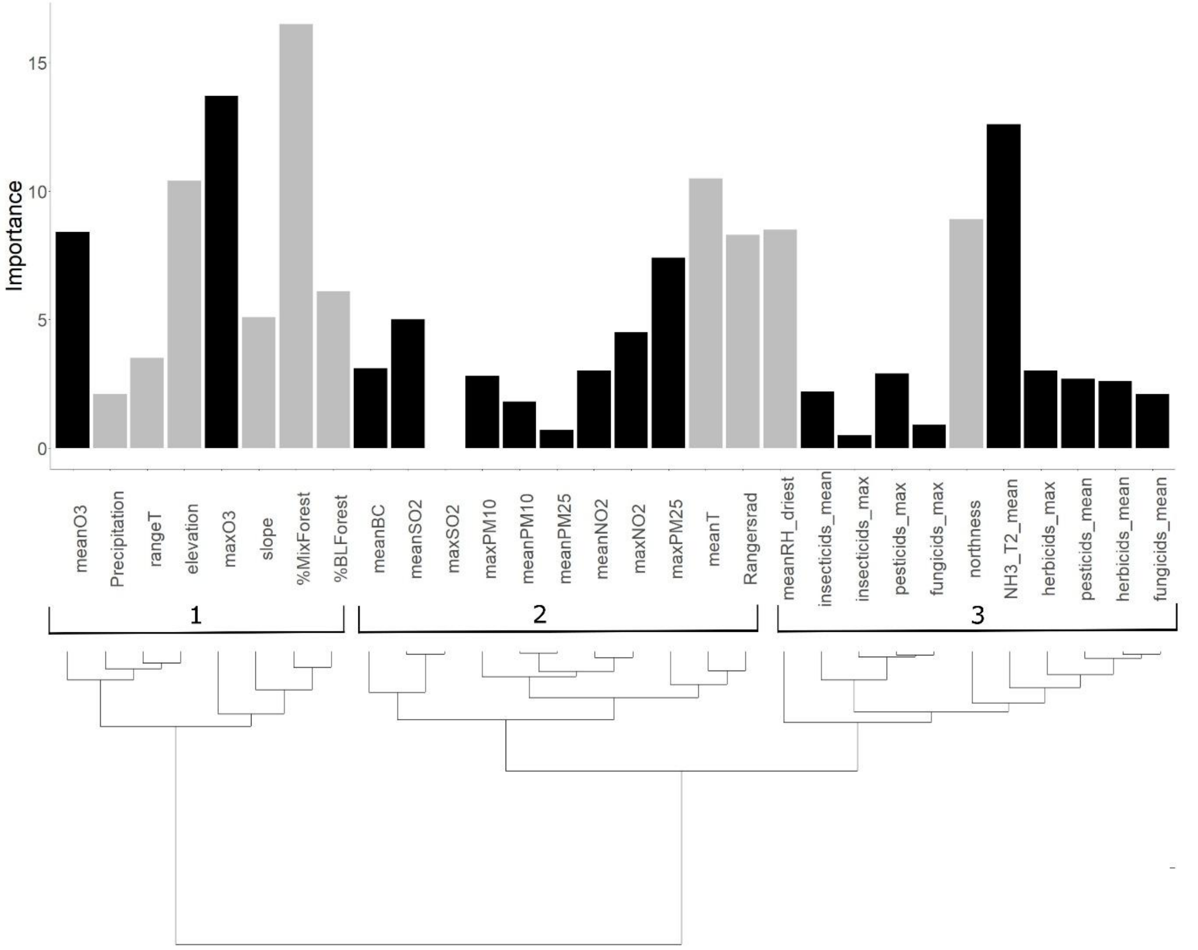


Fig. S2. Importance of environmental variables (black: air pollutants, grey: background environmental conditions, see Table 1 for abbreviations) accounting for the distribution of strictly epiphytic bryophytes in southern Belgium. The importance of each variable is as a function of the proportion of models, in which it was included by the embedded covariate selection (keeping the variables consistently selected by the three algorithms per model), weighted by its ranking during the selection procedure. The panel below is a dendrogram of similarity among variables based on the correlation coefficient among variables and using Ward's algorithm as the clustering criterion.

4. Chapter III

In search of forest microrefugia: an analysis of the microclimatic effect

cover picture:A datalogger in its shelter on a tree, Mormont (Wallonia), picture from Lea Mouton, April 2024

Chapter III – In search of forest microrefugia: an analysis of the microclimatic effect

The authors' contribution to the article.

Lea Mouton: flora identification, dataloggers’ records and analysis, statistical analysis, lead writing

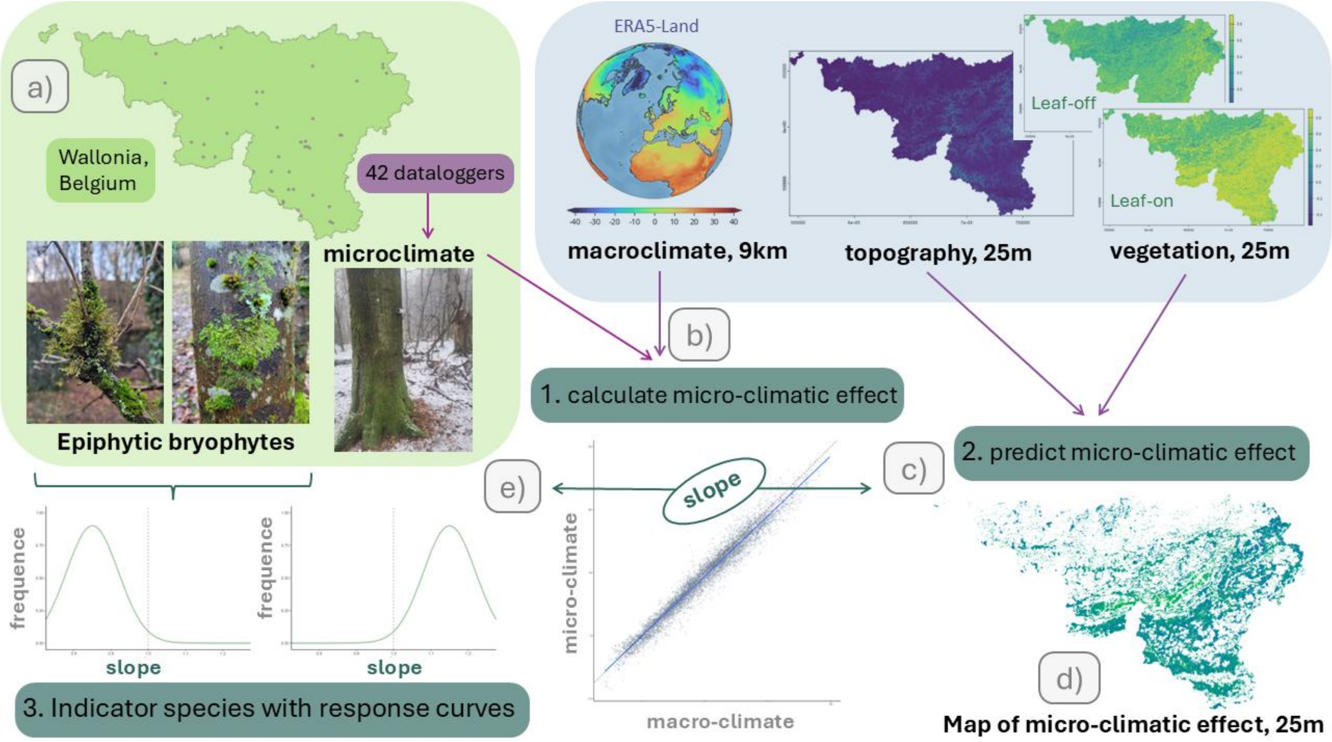
Virginie Hutsemékers: field support, flora identification

François Jonard: remote sensing of vegetation structure data

Flavien Collart: macroclimatic data, statistical analysis support

Alain Vanderpoorten: conception, supervision, Funding and writing

The following article is *in prep.*



Methodological overview

In search of forest microrefugia: an analysis of the microclimatic effect

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Alain Vanderpoorten: conception, supervision, Funding and writing

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Abstract

Forests best exemplify the microclimatic effect, i.e., the difference between macro (free-air) and microclimatic (measured *in-situ*) conditions in the understorey due to the joint factors of topography and canopy structure. In the context of climate change, quantifying the microclimatic effect has substantial applications, such as the identification of highly buffered forests called to play a crucial role as microrefugia and adapt forest management practices to promote forest resilience and preserve threatened species against climate change. Here, we investigated the response of highly sensitive organisms, namely epiphytic bryophytes, to the microclimatic effect. *In-situ* temperature and relative humidity were recorded for one year at 42 locations in *Quercus-Fagus* stands in southern Belgium. The microclimatic effect was characterized at the level of each sensor through the "slope and equilibrium" approach and its variation across sensors was modelled using spatially explicit variables of topography and vegetation structure obtained by satellite imagery. Species response curves to microclimatic effects were analysed using GLMs. The results show excellent linear relationships between macro and microclimate, leading to an accurate estimation of local microclimatic effects. Models aiming at predicting the spatial variation of the latter exhibited, however, a low accuracy, calling for the deployment of additional sensors to better capture the spatial heterogeneity of broadleaf forests and the implementation of additional spatial predictors, such as ones derived from LiDAR. The analysis of the response curves identified species with an optimum under buffered conditions of temperature (e.g., *Thuidium tamariscinum* and *Zygodon conoideus*) and relative humidity (e.g., *Leucodon sciuroides*, *Frullania* spp. and *Metzgeria* spp.). Overall, this preliminary study explores promising techniques for quantifying forest microclimates and identifying potential microrefugia in order to adapt forest management practices.

Key words

Indicator species – bryophytes – microclimate – forest - continuity

Introduction

Trees generate specific environmental conditions in forest understoreys. Under their canopy, light intensity reaching the floor is reduced, creating a shady environment. Shade prevents the exposure of understorey species to direct solar radiation and extreme temperatures. Tree density also reduces the wind that penetrates the forest, and, therefore, protects understorey species from drought. The difference between the microclimate experienced by organisms inside the forest and the macroclimate that prevails in open conditions is termed the "microclimatic effect". Forest understories typically experience buffered microclimates (De Frenne *et al.*, 2021; Gril *et al.*, 2023a), characterized by more stable conditions inside than outside the forest. On average, maximum temperatures are 4.1 °C lower while minimum temperatures are on average 1 °C warmer inside than outside forests (De Frenne *et al.*, 2019 and 2021). This is called the "buffering effect" (De Frenne *et al.*, 2021; Gril *et al.*, 2023a). In forest openings or forest edges (Ewers and Banks-Leite, 2013), conversely, the microclimate can be exacerbated (Carnicer *et al.*, 2019; Gril *et al.*, 2023a; Vandewiele *et al.*, 2023), meaning that microclimate variations have a larger amplitude than macroclimate variations outside the forest. For example, in forests clearings where surrounding trees prevent efficient air mixing, maximum temperatures can be higher and minimum temperatures lower than outside the forest. This is called the "amplifying effect" (De Frenne *et al.*, 2021; Gril *et al.*, 2023a).

The intensity of the microclimatic effect varies depending on a series of factors, including canopy shape, vegetation structure and forest environmental conditions (Frey *et al.*, 2016a; Gril *et al.*, 2023a; Kovács *et al.*, 2017), and is thus directly impacted by forest management. Frey et al. (2016b) showed a difference of maximum spring monthly temperatures up to 2.5 °C between plantation forests and old-growth forests, which represents the predicted increase of global temperature for the next 50 years due to differences in canopy structure and biomass (Frey *et al.*, 2016b; Schreiber *et al.*, 2025). In a context of fast global changes, forest environments, and in particular, areas experiencing the most buffered conditions due to specific conditions of topography and forest structure, could play a crucial role as microrefugia (Kemppinen *et al.*, 2024; Greiser *et al.*, 2020). Consequently, adapting forest management to enhance forest resilience to climate change, prevent forest species loss by identifying the most suitable forest areas in terms of microclimatic refugia while continuing relying on forest production has become a true contemporary challenge.

Understorey species exhibit contrasting responses to microclimatic effects (Gril *et al.*, 2025). Forest generalist species, which can be found in forest environments, although not restricted to them, exhibit broad microclimatic preferences. On the opposite, forest specialists are restricted to forest understoreys due to their strict reliance on buffered climatic conditions. They developed adaptations to the specific conditions found under dense canopies with low light intensities, by either completing their life cycle before the leaf-on season or by developing strategies to capture maximum light intensity, such as minimizing self-shading or increasing chlorophyll content (Chelli et al., 2021; Decocq and Hermy, 2003). Developing such adaptations and growing in a stable environment, forest specialists invest less in terms of reproductive effort than generalist species (Pellissier *et al.*, 2013; Verheyen and Hermy, 2001). Furthermore, they exhibit lower dispersal capacities, limiting their ability to migrate from one suitable area to another (Hermy *et al.*, 1999),), making them good indicators of mature forests.

Located at the interface between trees and atmosphere, epiphytic bryophytes are one of the most sensitive organisms to microclimatic variations (Vanderpoorten and Goffinet, 2009). Because they lack roots and a vascular system, and because they live unconnected to the ground, epiphytic bryophytes rely exclusively on precipitation for their water and nutrient uptakes (Bates, 2000). Epiphytic bryophytes are therefore sensitive to drought and global warming (Tuba *et al.*, 2011; Glime, 2007) and rely on the stability of their surrounding microclimate. Specialized epiphytic bryophytes are therefore found in forests with high buffering capacity, i.e., forests with complex vegetation structure characterizing mature forests. In addition, epiphytic bryophytes, particularly species associated with mature forests, have limited dispersal capacities (Patino *et al.*, 2018). Dispersal counter-selection occurs through the production of large spores, short setae or reduced peristomes. Consequently, epiphytic bryophytes appear as good candidates to characterize the intensity of the microclimatic effect (Gril *et al.*, 2025) and well-structured mature forests.

This study aims to (1) characterize microclimates and predict microclimatic effects using spatially-explicit predictors of topography and remote-sensing in temperate broadleaf forests (2) identify indicators species of the most buffered conditions, analysing response curves of epiphytic bryophytes species.

Materials and methods

Epiphytic bryophyte frequency was analysed over 42 forests in Wallonia, south Belgium. Microclimate temperature and relative humidity were recorded in-situ and linked to macroclimate using the « Slope and the equilibrium » method of Gril *et al.* (2023a). A model predicting the spatial variation of microclimatic effects based on the correlation between the microclimatic effect and predictors, such as topography and vegetation structure variables (Gril *et al.*, 2023a; Gril *et al.*, 2023b), was generated to map microclimatic effects in the study area. In order to identify indicator species, response curves were modelled to predict the occurrence of each species according to the microclimatic effect (Gril *et al.*, 2025) (Figure 1).

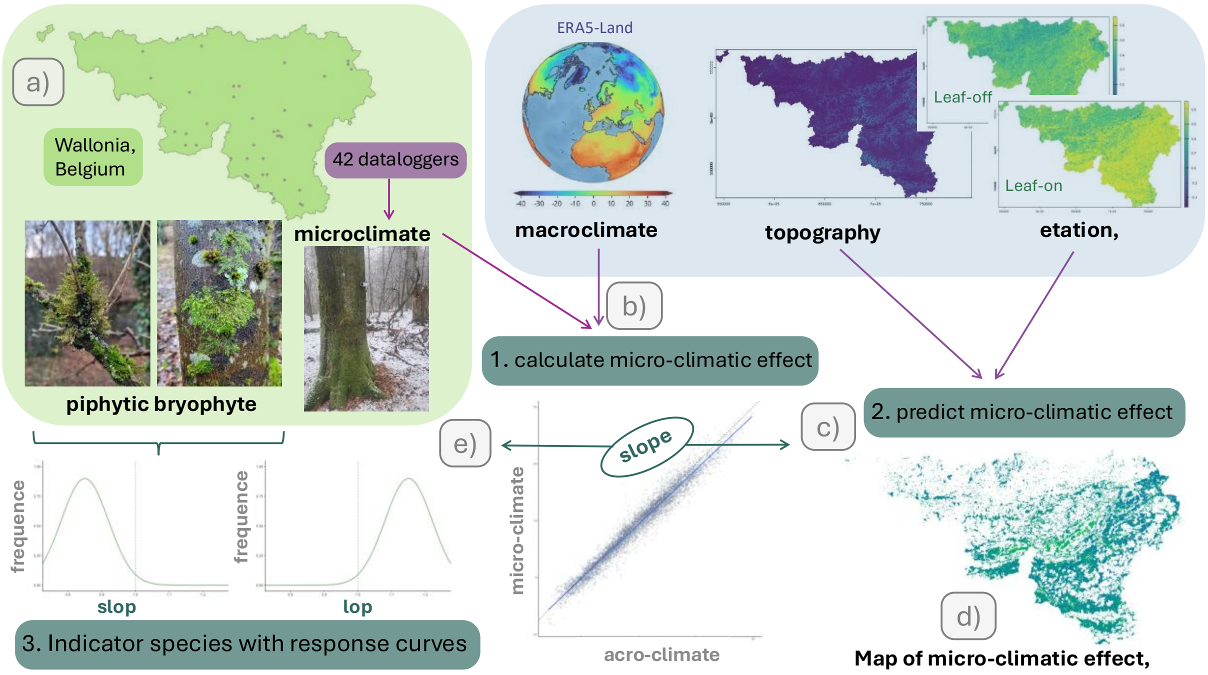


Figure 1. Methodological overview. (a) The frequency of epiphytic bryophyte species and microclimatic temperature and relative humidity were recorded hourly for one year at 42 forest plots of 25 m in diameter in Wallonia, south Belgium. (b) The recorded microclimatic data of each logger were combined with hourly macroclimatic data for the same period. The slope of this relationship quantifies the microclimatic effect, with slopes >1 and <1 characterizing buffered and amplified microclimatic conditions, respectively (Gril et al. 2023a). (c) Slope variation across sensors was modelled using spatially-explicit variables of topography and remote sensing (NDVI) reflecting vegetation conditions as predictors. (d) The model was projected across Wallonia at 25m resolution to produce a map of forest microclimatic effects. (e) The slope of the macro/microclimatic relationship was used to model species response curves to the microclimatic effect.

Study area

Wallonia is, with 32 % of forest cover over 901.4 km^2 (SPW, 2022), the most densely wooded region of Belgium, including more than 75 % of Belgian forests (Alderweireld et al., 2016). Almost 60 % of Walloon forests are broad-leaf forests (SPW, 2023a), the climax stage of the landscape in the region. Elevation ranges from ±100 to 700 meters above sea level (SPW, 2018). This study focuses on 42 beech-oak groves dominated by sessile oak (*Quercus petraea*), pedunculate oak (*Quercus robur*) and beech (*Fagus sylvatica*). They were selected to reflect the variation across bioclimatic regions (Figure 2) and management regimes, with 21 forests identified as natural, 14 as degraded and 7 as planted (De Coene *et al.*, 2012; Dufrêne *et al.*, 2006). In each forest, one circular plot with a radius of 25 m was defined as the sample unit based upon previous monitoring programmes (Dufrêne *et al.*, 2006).

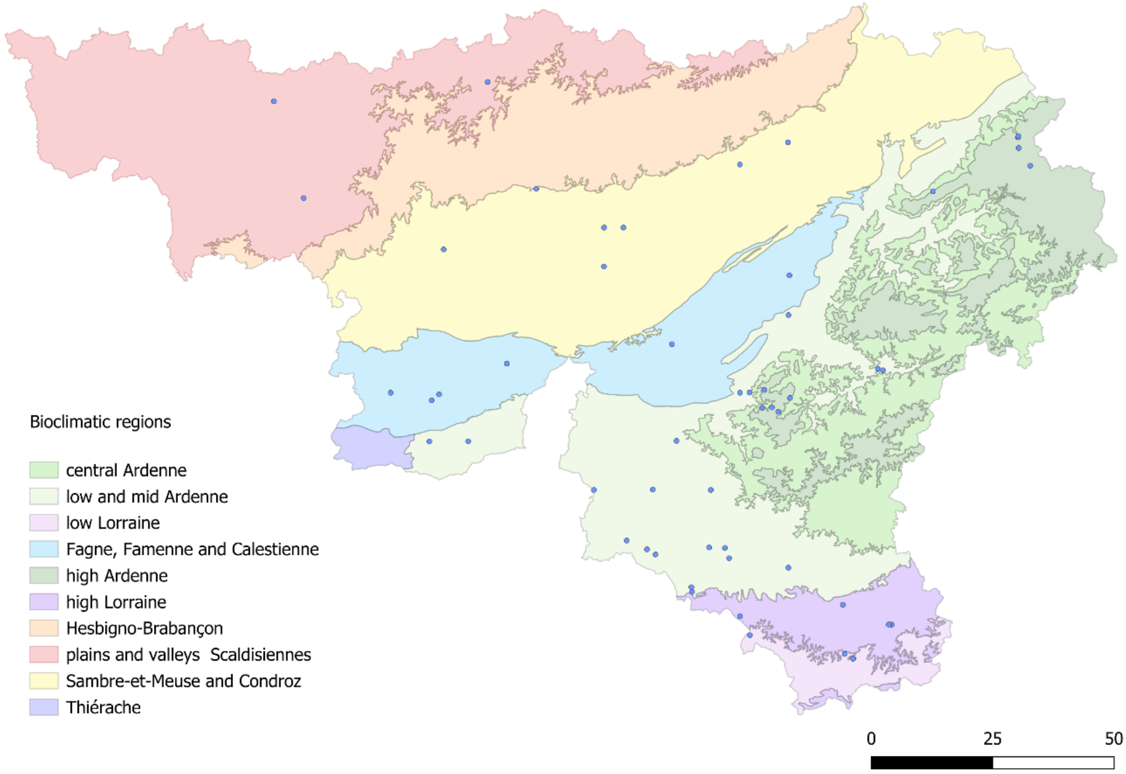


Figure 2. Map of Wallonia, South Belgium, with the 10 bioclimatic regions and the location of 42 forest plots of 25 m radius.

Climatic data

Microclimatic temperature and relative humidity were recorded hourly in each of the 42 forest plots using HOBO® ProV2 and Lascar® (El-USB2) sensors. HOBO® loggers have a manufacturer-reported accuracy of 0.2° C for temperature and 2.5 % for relative humidity (ONSET, 2020) while Lascar® sensors have an accuracy of 0.45° C for temperature and 3 % for relative humidity (EasyLog, 2024). Loggers were attached on the north face of the central tree at 1.5 m height. Each logger was protected from direct solar radiation (Gril *et al.*,2023 ;Zellweger *et al.*, 2019) by a light grey PVC pipe of 15 cm length. The loggers stayed on site over 1 year, from March 2024 to March 2025.

Macroclimatic temperature and relative humidity were extracted hourly from ERA5-land, for the same period of time, from March 2024 to March 2025, at a resolution of 9 km (Muñoz Sabater, 2019).

Floristic data

The epiphytic bryophyte survey was carried out from August 2023 to Novembre 2024. In each of the 42 forest plots, presence-absence of epiphytic bryophyte species from the base up to 2.5 m above ground was recorded on each living tree with a DBH (diameter at breast height) >15 cm. Although epiphytes may occur towards tree crown, epiphytic bryophyte communities are progressively depauperating upwards. In temperate forests, the bole above 2.5m and the crown contribute to only 4 % of the total epiphytic diversity of a trees (Boch *et al.*, 2013). Surveys restricted to the first 2.5 m capture the vast majority of epiphytic communities (Boch *et al.*, 2013).

Environmental variables

Topographic data

Topographic variables, such as slope, aspect, eastness, east slope mean, altitude, northness, north slope mean, roughness, TWI (topographic wetness index), TPI (topographic position index) and TRI (terrain ruggedness index) were extracted from a Digital Elevation Model (DEM) using the 'terra' (Hijmans, 2025) package in R version 4.5.1 (R Core Team 2025), with the exception of TWI, which was calculated with SAGA version 9.5.1 (Böhner and Selige, 2002). The DEM was initially at a resolution of 1 m (2021-2022) and aggregated by taking the average values at 25 m resolution. Because forest plots do not exactly match with the topographic pixel, a weighted mean based on the surface area of the 25 m pixels present in the 25 m circle was calculated for each of the 42 plots.

Vegetation data

Optical vegetation indices, such as the Normalized Difference Vegetation Index (NDVI; derived from red and near-infrared surface reflectance, sensitive to chlorophyll absorption and canopy structure), Enhanced Vegetation Index (EVI; designed to minimize atmospheric and soil background influences and to reduce saturation in dense vegetation canopy compared to NDVI), Normalized Difference Moisture Index (NDMI; exploits the contrast between near-infrared and shortwave infrared reflectance to provide an indicator of vegetation moisture status) and Chlorophyll Index - Green (Cg; exploits the contrast between green and near-infrared reflectance and is used as a proxy for leaf chlorophyll concentration) were obtained from satellite imageries taken by the Sentinel-2 (A and B) satellites. The multispectral instrument on Sentinel-2 samples 13 spectral bands: in the visible and near infrared range at 10 m, in the red edge and short-wave infrared range at 20 m, and atmospheric bands at a spatial resolution between 10 and 60 m. To obtain a raster map of Wallonia for each variable and each month at a resolution of 25m, Google Earth Engine (GEE) was used to gather all satellite images over the last 5 years, applying a maximum cloud probability threshold of 10% to minimize cloud contamination. GEE extracted the values of each remote sensing variable from satellite imagery and generated monthly raster maps for each variable, using the mean of all satellite imageries acquired during the same month. 48 (12 months x 4 variables) maps were then obtained. Although Sentinel-2 (A and B) satellites acquire imagery over Wallonia approximately every 2–3 days, the number of usable cloud-free observations is often limited to only a few scenes per month due to persistent cloud cover. Even taking 5-year periods, no data for some 25 m pixels were available. The gaps were filled with the SAGA program using the stepwise resampling method. As vegetation structure changes seasonally, 2 raster maps for each variable were built this way, one with the mean of the months for leaf-on period and one for leaf-off period.

Statistical analysis

All analyses were conducted in R version 4.5.1 (R Core Team 2025). The packages used were the 'tidyr' (Wickham and Henry, 2024) to handle microclimatic data, 'terra' (Hijmans, 2025) to handle the climatic, topography and vegetation maps, and 'randomForest' (Liaw and Wiener, 2002) to predict microclimatic effect.

Quantifying microclimatic effect

To quantify the microclimatic effect of the forest canopy, a linear regression between the hourly measurements of micro- and macro-temperatures was computed for each sensor (Gril *et al.*, 2023a; Gril *et al.*, 2023b). The slope of the regression between micro and macroclimate characterizes the "microclimatic effect". A slope >1 represents an amplification effect and a slope <1 a buffering effect. Because strong changes occur in forests' buffering effect over the year according to the loss of leaves (Gril *et al.*, 2023a), two periods were defined: leaf-on from May to November and leaf-off from December to April (Gril *et al.*, 2023a). The slope was computed for each of the 42 sensors under leaf-on and under leaf-off periods separately. This approach, initially developed for temperature (Gril *et al.*, 2023a), was further implemented here for relative humidity.

Mapping the microclimatic effect

To predict the forest microclimatic effect, the variation of the slope of the macro/microclimate relationship across the 42 sensors was modelled by Random Forest (RF). To avoid multicollinearity, a correlation coefficient was computed for each pair of variables, and one variable in each pair with r >0.7 was removed until no pair of variables with r >0.7 remained. The RF model to predict the thermo-microclimatic effect was based on 11 out of 16 topographic and vegetation variables (TWI, TPI, NDMI, Cg, EVI, aspect, TRI, Northness_slope, East_slope, altitude and leaf period) and the random forest model to predict the hygro-microclimatic effect was based on 11/16 topographic and vegetation variables (TPI, TWI, NDMI, EVI, CgI, Eastness, Northness_slope, slope_w, East_slope, altitude and leaf period). Each model was tested by realizing 100 cross validations using 70 % of data to calibrate the model and 30 % to evaluate it.

The model was subsequently projected over the entire forest area to generate a preliminary map of the forest microclimatic buffering effect. A 25*25 m pixel was considered as forested if more than 50 % of 2 m pixels constituting it were scored as forest. Four different maps were then generated, one for the microclimatic effect based on temperature for leaf-on period, another one for leaf-off period, the third one for the microclimatic effect based on relative humidity for leaf-on period and the last one for leaf-off period.

Identifying indicator species

To identify potential indicator species of the forest microclimatic effect, 102 species response curves to the microclimatic effect for temperature and relative humidity, respectively, were generated. Species with more than 5 occurrences or less than 5 absences were discarded. Species response curves were modelled using GLM with a binomial function and a prevalence set to 0.5 equally weighting presence and absence data. Predictors included the slope of the macro/microclimatic relationship for temperature and relative humidity, respectively, and their quadratic terms. We determined whether significant models were obtained using analysis of variance. When significant models were obtained, we visually determined whether response curves were monotonic or unimodal (Figure 3).

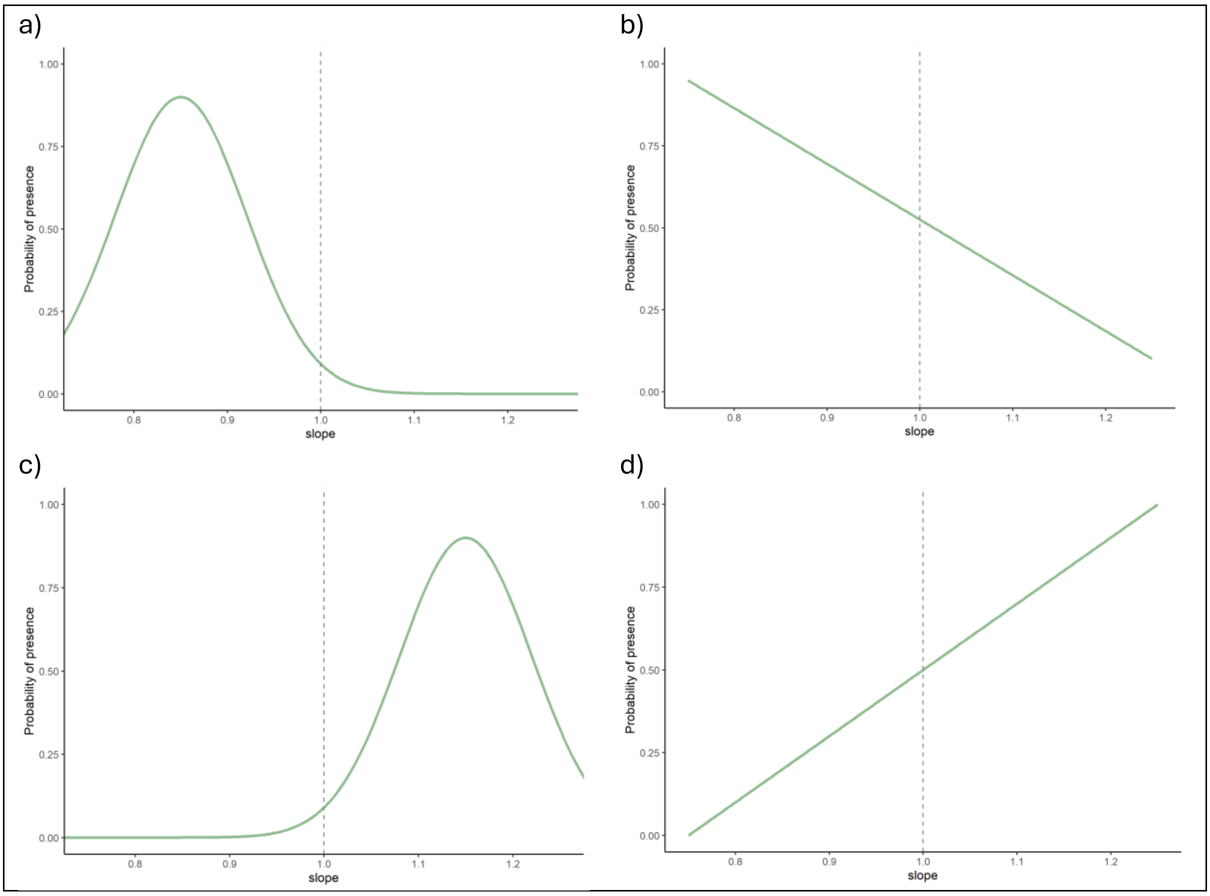


Figure 3. Modelling epiphytic bryophyte species responses to microclimatic effects. Microclimatic effects characterize the difference between macro and microclimate conditions. They are quantified through the slope of the hourly variation between micro- and macroclimate conditions. When the slope of the macro/microclimate >1, microclimatic conditions are amplified as compared to macroclimatic conditions. When the slope of the macro/microclimate <1, microclimatic conditions are buffered as compared to macroclimatic conditions. (a) and (c) represent unimodal responses to microclimatic effects, with an optimum for buffered (a) and amplified (c) microclimates, respectively. (b) and (d) represent monotonic responses, a decrease (b) or an increase (d) of the probability of occurrence under increasingly amplified microclimatic conditions (increasing values for the slope of the macro/microclimate).

Results

Microclimatic temperatures varied between -13.0 and 43.09 °C, with a mean of 9.79±6.66 °C, while macroclimatic temperatures varied between -13.64 and 32.68 °C, with a mean of 10.25±6.75 °C. The mean of the difference of macro- and micro-temperatures was 1.19±1.04, with a minimum difference of 9.8 10^{-6} and a maximum difference of 15.70 °C. The macro and microclimatic relative humidity means were 87.37±12.04 and 83.78±12.17, respectively. The minimum and maximum values are 21.88 % and100 % for micro-relative humidity and 34.80 % and 100 % for macro-relative humidity, respectively (Figure 4).

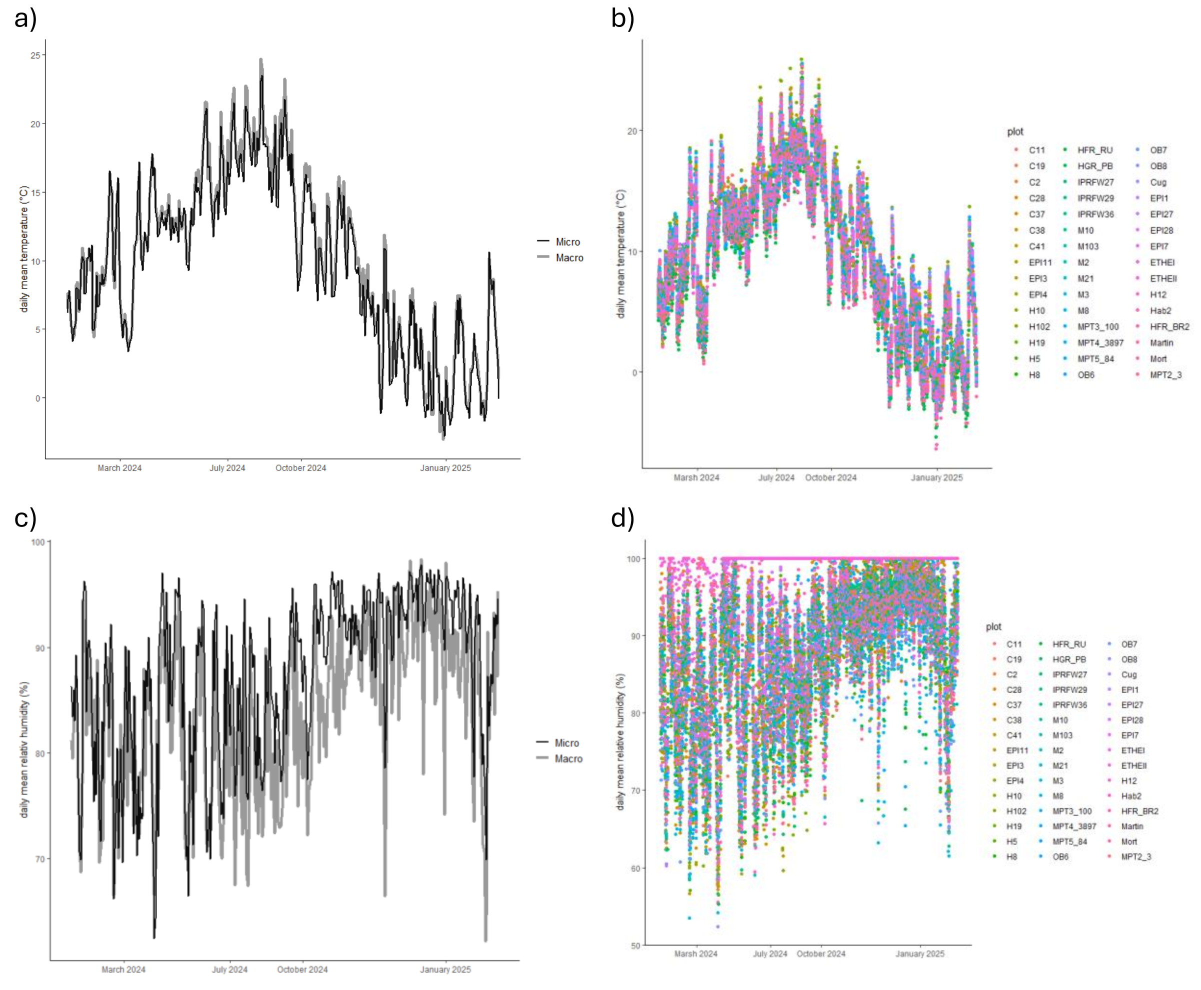


Figure 4. Daily mean of micro and macroclimatic conditions of 42 forest plots in Wallonia between Marsh 2024 and March 2025. (a) Mean daily micro- and macro-temperature of all plots, (b) mean daily micro-temperature for each plot, (c) mean daily micro and macro-relative humidity of all plots, (d) mean daily micro-relative humidity for each plot.

Concerning the microclimatic temperature effect of the 42 studied plots, 6 slopes were higher than 1 (max=1.15) under leaf-on period for temperature, and 12 under leaf-off (max=1.05). The minimum slope values were 0.79 and 0.87 for temperature under leaf-on and leaf-off periods, respectively. No slope higher than 1 for relative humidity was observed, except one under the leaf-off period. The minimum slope for the microclimatic effect for relative humidity was 0.02 and 0.06, and the maximum was 0.96 and 1.01 under leaf-on and leaf-off periods, respectively. The linear relationship between the hourly variation of micro- and macro-temperature had a mean r^2 of 0.93±0.022 under leaf-on and a mean $r^2$of 0.93±0.02 under leaf-off period. The slope of the linear relationship between the hourly variation of micro- and macro-relative humidity had a mean 0.64±0.08 under leaf-on and 0.69±0.06 under leaf-off.

The RF model used to predict the temperature and relative humidity microclimatic effects explained 23.5 % and 37.3 % of the variance, , respectively. The explained variance dropped to 15 % and 20 %, respectively, after cross validation.

The predicted maps of microclimatic effect based on temperatures during leaf-on (Figure 5.I. a) and leaf-off periods (Figure 5.I.b) show that almost all Walloon forests produce a thermo-buffering effect (Appendix Figure S1.I). During the leaf-on period, this thermo-buffering effect is lower for the forests in the Calestienne and the Lorraine regions and stronger in the Ardenne region (Figure 5.I.a). During the leaf-off period, all forest loses thermo-buffering power strength except some forests in the Ardenne (Figure 5.I.b). The predicted maps of microclimatic effect based on relative humidity during leaf-on and leaf-off period show that almost all Walloon forests produce a hygro-buffering effect (Appendix Figure S1.II), with no clear difference between leaf-on and leaf-off. Concerning the intensity of this hygro-buffering effect, forests in the Calestienne region show the lowest hygro-buffering effect during leaf-on period (Figure 5.I.a). During leaf-off period, all forests lose their hygro-buffering power, except some forests in the Ardenne (Figure 5.I.b).

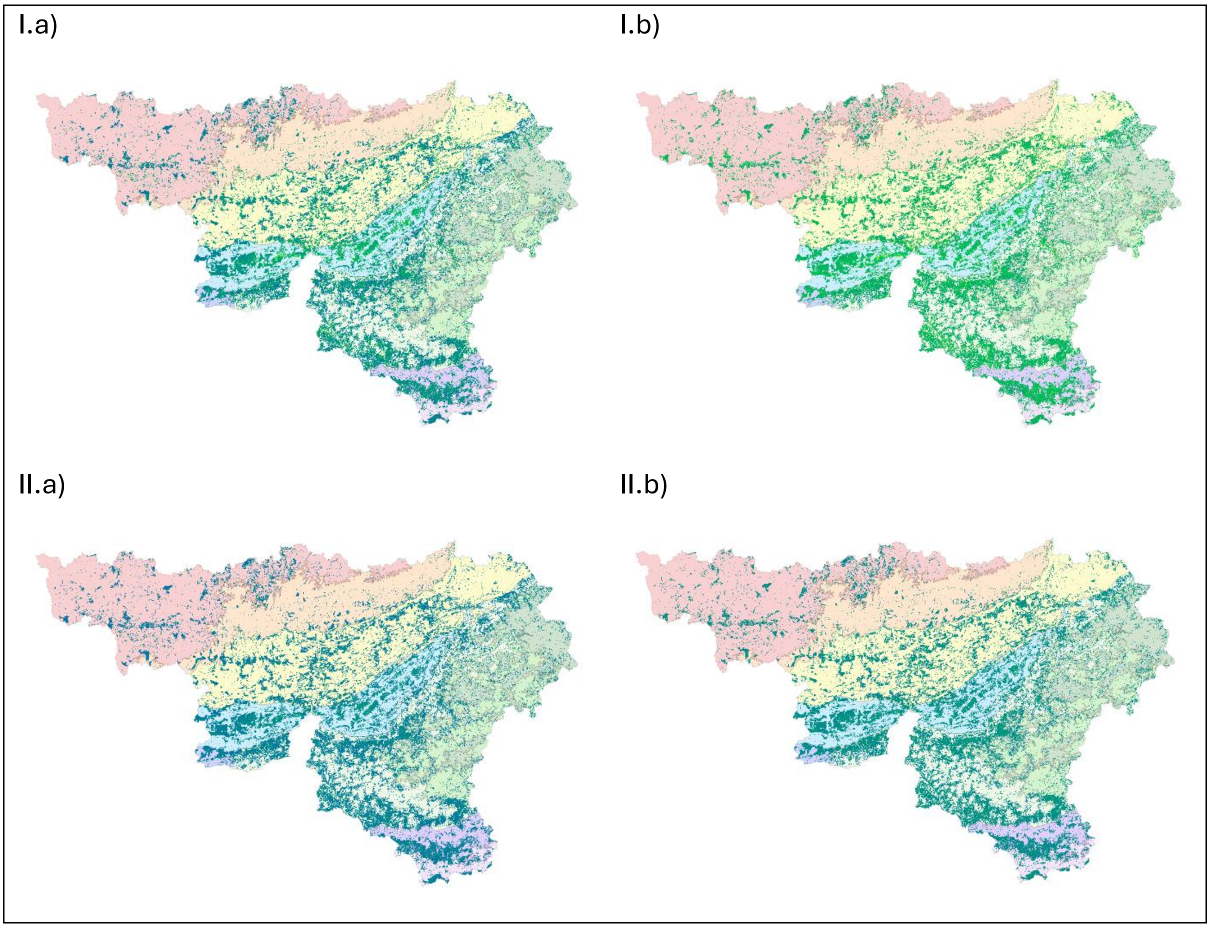
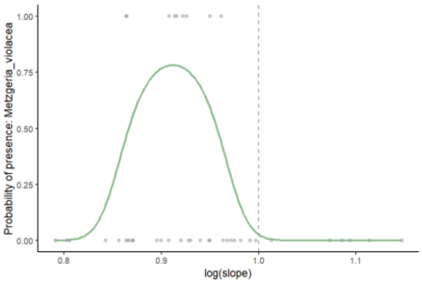
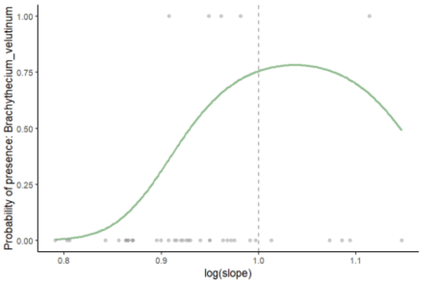
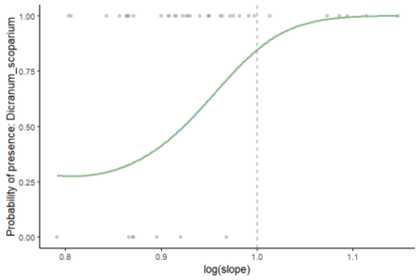


Figure 5. Maps of the predicted microclimatic effect for temperature and relative humidity of forests in Wallonia. Yellow-red colours reflect an amplifying effect and blue-green colours a buffering effect. Microclimatic effect is the slope of the linear regression between I) the micro- and the macro-temperatures and, II) between the micro- and the macro-relative humidity, of a) leave-on period (May to November) and b) leaf-off period (December to April). The slope for all 25m pixel of forest was predicted thanks to its correlation with topographic variables and vegetation structure variables.

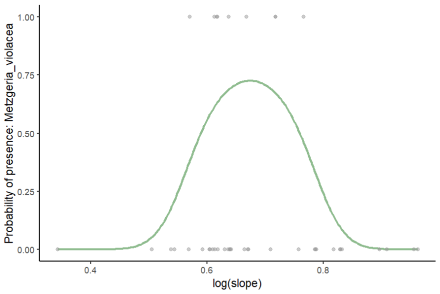
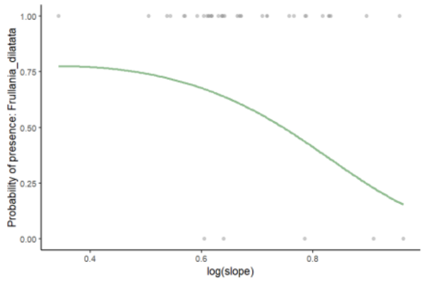
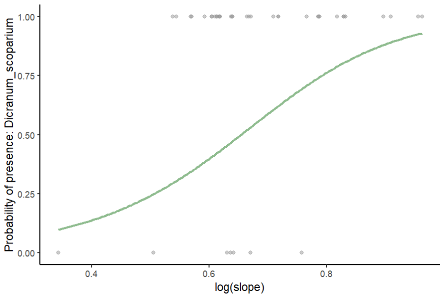
Among the 50 species investigated for the relationship with microclimatic effects, a significant model for the variation of species presence with regard to microclimatic temperature is obtained for 8 of them. For the temperature microclimatic effect (Table 1), three main types of response curves were observed: (i) species with a broad optimum for buffered conditions (*Frullania dilatata, Kindbergia praelonga, Leucodon sciuroides, Metzgeria violacea* and *Syntrichia papillosa*), (ii) species with a broad optimum for non-buffered conditions (*Brachythecium velutinum*), and (iii) species with increasing probabilities of occurrence with decreasing buffering effects (*Dicranum scoparium* and *Plagiothecium denticulatum*).

Table 1. Three different response curve types of indicator species for temperature microclimatic effects, with associated p-value and pseudo-R^2.

Response curv	Indicator specie	u	udo-R^2
i. Gaussian curve with an optimum at slope value <1 	*Frullania dilatata*	< 0.001	0.144
	Kindbergia praelonga	0.019	0.090
	Leucodon sciuroides	< 0.001	0.135
	Metzgeria violacea	< 0.001	0,266
	Syntrichia papillosa	< 0.001	0.252
ii. Gaussian curve with an optimum at slope value >1 	*Brachythecium velutinum*	< 0.001	0.164
iii. Linear increase 	*Dicranum scoparium*	< 0.001	0.151
	Plagiothecium denticulatum	0.043	0.073

Three other main types of response curves were observed for the hygro-microclimatic effect (Table 2): (i) species with a broad optimum for buffered conditions (*Metzgeria furcata, Orthotrichum striatum, Synthrichia papillosa and Zygodon conodeus*), (ii) species with decreasing probabilities of occurrence with decreasing buffering effects (*Frullania dilatata, Thuidium tamariscinum and Zygodon rupestris)*, and (iii) species with increasing probabilities of occurrence with decreasing buffering effects (*Dicranum scoparium*).

Table 2. Three different response curve types of indicator species for relative humidity microclimatic effects, with associated p-value and pseudo-R^2.

Response curv	**Indicator specie**	**u**	**udo-R^2**
i. Gaussian curve with an optimum at slope value <1  	*Metzgeria furcata*	< 0.001	0.192
	Orthotrichum striatum	0.014	0.086
	Synthrichia papillosa	0.006	0.103
	Zygodon conoideus	0.019	0.118
ii. Linear decrease  	*Frullania dilatata*	0.003	0.115
	Thuidium tamariscinum	0.025	0.078
	Zygodon rupestris	0.016	0.112
iii. Linear increase  	*Dicranum scoparium*	0.002	0.130

Discussion

Predicting forest microclimatic effects

With a mean r^2 reaching 0.95 for temperature and higher than 0.6 for relative humidity, the relations of macro/microclimate provide reliable quantification of microclimatic effects. The microclimatic effect varied from a buffering to an amplifying effect for temperature but only a buffering effect was detected for relative humidity. Significant models based on topographic and vegetation structure predictors were obtained to describe the spatial variation of the microclimatic effect and this study is, to our knowledge, the first to obtain a significant model to predict microclimatic effects for relative humidity. The r^2 of these models are, however, relatively low. For instance, they are twice lower compared to previous studies implementing a similar approach (Gril *et al*., 2025; Gril *et al*., 2023a). In addition to the deployment of additional dataloggers to better capture the heterogeneity of forests in the study area, another way of improving the models is to add other predictors, such as variables derived from LiDAR (Gril *et al*., 2025). For example, the maximum height, the Plant Area Index and the Vertical Complexity Index are typical vegetation structure variables that can be derived from LiDAR. These vegetation structure variables have already shown an increase of model performance when added as predictors of microclimatic effects (Gril *et al*., 2025).

Indicator species of the microclimatic effect

Among the species indicating a microclimatic effect, 6 had an optimum of occurrence under buffered conditions for temperature (e.g., *Frullania dilatata, Thuidium tamariscinum and Zygodon rupestris)* and 7 for relative humidity (e.g., *Metzgeria furcata, Orthotrichum striatum, Synthrichia papillos and Zygodon conoideus)*. The results are consistent with the ecological traits database from van Zuijlen *et al*. (2023). *Thuidium tamariscinum* is a hygrophilous pleurocarpous moss and *Zygodon conoideus* is an indicator for forests.

Concerning the microclimatic effect for temperature, the 6 species indicating a buffering effect are *Frullania dilatata, Kindbergia praelonga, Leucodon sciuroides, Metzgeria violacea* and *Syntrichia papillosa*. These species have an ecological indicator value for temperature between 3 and 6, with a value of 1 indicating a preference for cold conditions and 9 for extremely warm conditions (van Zuijlen *et al*.,2023). *Kindbergia praelonga* is common in shady areas (BBS, 2021), where temperatures are reduced compared to the macroclimate. As *Leucodon sciuroides* grows preferentially on old trees (BBS, 2021), it has already been identified as an indicator for old-growth forests (Marino *et al*., 2025 ;Blasi *et al*., 2010), characterized by high vegetation structure and complexity. The selection of *Syntrichia papillosa* is, conversely, surprising, because it has become common nowadays, including in open landscapes and urban areas, due to the reduction of SO_2 air pollution and the general trend for eutrophication.

Dicranum scoparium shows a reverse pattern: the probability of its occurrence increases with the decrease of the buffering intensity, for both temperature and humidity. This response curve pattern should point to species specialised to amplifying environments. In this case, however, it reflects the capacity of this species to tolerate less buffered environments. In fact, *Dicranum scoparium* is a species that is not restricted to forests but can also be found in open and relatively dry environments, such as heathland and acidic grasslands (BBS, 2021). It has therefore a score of 4 on a scale of 9 for moisture (van Zuijlen *et al*.,2023). *Dicranum scoparium* and *Plagiothecium denticulatum* show similar pattern of response curve of microclimatic effect for temperature, which is in line with their indifference for temperature, as scored by indicator values (BryForTrait Düll 1991 (Ellenberg & Leuschner 2010).

The selection of *Frullania dilatata* and *Metzgeria furcata* as indicators for buffering effect based on relative humidity is also unexpected because, among liverworts, these two are relatively tolerant to drought. It is also reflected in the ecological indicator values for moisture as they score 4, on a scale of 1 for extreme dryness and 9 for wet-site indicator.

In fact, Ellenberg indicator values and the present optima for microclimatic effects presented here may be decoupled. As compared to Ellenberg indicator values, the response curves for microclimatic effects exhibit several advantages. First, they are based on a quantitative, data-based assessment of species optima instead of representing a qualitative assessment of species preferences based on expert knowledge. Second, the slope of the macro/microclimatic relationship is an integrative variable for ecological preference of species to microclimatic conditions, but also for the conditions of topography and canopy structure that generate specific microclimatic conditions (Gril *et al.*, 2024). Finally, the slope of the macro/microclimatic relationship as derived in the present study characterizes microclimatic conditions actually experienced by organisms. In contrast, Ellenberg indicator values were shown to be decoupled from microclimatic variation (Gril *et al*, 2024). As a result, the microclimatic species optima such as those derived in the present study can serve to assess variations in microclimatic conditions at fine resolution, whereas Ellenberg indicator values reflect species macroclimatic preferences characterizing climatic differences at much larger scales (Gril *et al*, 2024).

Conclusions and perspectives

The microclimatic effect was accurately characterised in this study by the slope of the relationship between macro and microclimate. This study also represents a first attempt at modelling microclimatic conditions for relative humidity. Our preliminary model for inferring the spatial variation of the microclimatic effect, although significant, exhibited a weak accuracy and needs to be further developed, using additional dataloggers to better capture forest heterogeneity in the study area and implementing additional predictors, and in particular, variables of canopy structure derived from LiDAR data. The LiDAR data available in southern Belgium at a resolution of 25 m are unfortunately useless to derive vegetation structure variables because they were generated in winter (SPW, 2023b). Nevertheless, LiDAR data for leaf-on period and additional microclimatic sensors are presently recorded in two newly designed National Parcs in southern Belgium, namely Semois valley and Entre-Sambre-et-Meuse.

Ultimately, improved models will be spatialised to generate maps of microclimatic effects. One of the primary applications of such a map is the identification of microrefugia under specific conditions of topography and canopy structure, and in particular, in old-growth forests characterized by the highest level of heterogeneity of the canopy (Kemppinen *et al.*, 2024; Greiser *et al.*, 2020). In the context of climatic changes, these microrefugia could play a crucial role providing shelter to specific forest species and stepping stone or corridors for more mobile species (Rull, 2009). Identifying these microrefugia and preserve them is therefore essential to assist species coping with climate change (Finocchiaro *et al.*, 2023; Zellweger *et al.*, 2020; Lenoir *et al.*, 2017). In addition, forests with high buffering power are also more resilient to climate change (Carnicer *et al.*, 2021). Identifying the buffering power of each forest could further help forest managers to adapt forest management practices and exploitation to promote forest resilience and production in the future.

To conclude, this preliminary study lays the groundwork for assessing the microclimatic effect in temperate forests in two ways: (i) by measuring the microclimate and predicting the microclimatic effect using spatially-explicit predictors of topography and remote-sensing to eventually generate a map of the microclimatic effect in forests at a regional scale and (ii) by selecting indicator species for buffered conditions in forests. Identifying and quantifying microclimatic effect is crucial to provide guidelines for conservationists and forest managers in order to promote forest resilience to climatic change and to provide microrefugia to species in a warming context.

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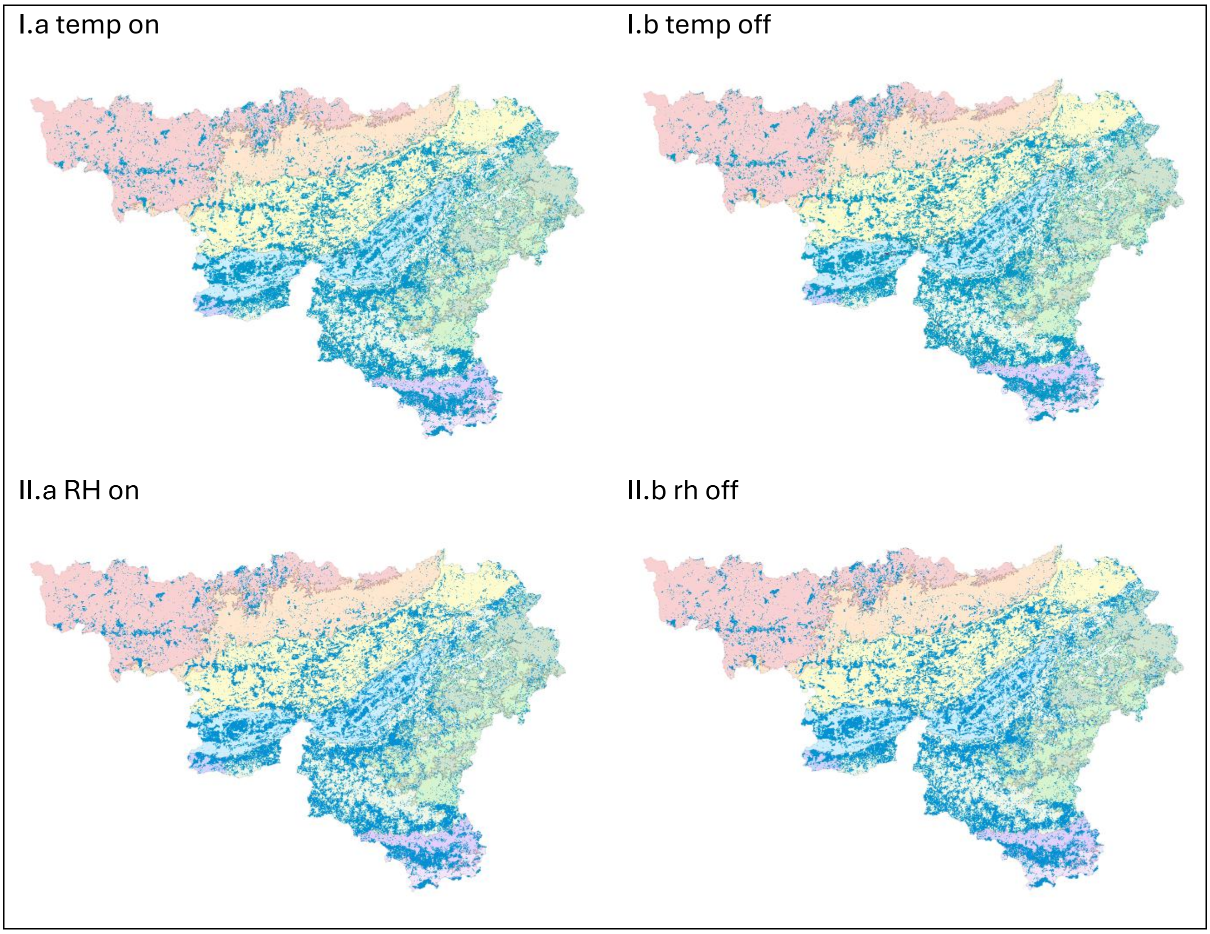
Appendix

Appendix S1 :buffering or amplification effect.



I.a temp on

I.b temp off

II.a RH on

II.b rh off

Figure S1. Maps of the predicted microclimatic effect for temperature and relative humidity of forests in Wallonia. Blue for buffering effect (slope<1) and red amplificatory effect (slope>1). Microclimatic effect is the slope of the linear regression between I) the micro- and the macro-temperatures and, II) between the micro- and the macro-relative humidity, of a) leave-on period (May to November) and b) leaf-off period (December to April). The slope for all 25m pixel of forest was predicted thanks to its correlation with topographic variables and vegetation structure variables.

5. General discussion

cover picture:epiphytic bryophytes (*Cryphaea heteromalla*) on a branch, Châtelet (Wallonia), picture from Lea Mouton, Decembre 2025

General discussion

In the context of global changes characterising the Anthropocene, this thesis aims to document the impacts of climate change and modifications in air quality on biodiversity through the analysis of epiphytic bryophyte communities over space and time. The thesis addresses three main questions. The first objective is to disentangle the effect of climate change and of the decrease in major pollutants that prevailed in the 1960s-1980s (SO_2 and NO_2) on the spectacular expansion of epiphytic bryophytes in Europe (Figure 8). Second, the thesis investigates whether epiphytic bryophyte communities are still impacted by these industrial pollutants and whether new or overlooked pollutants affect epiphytic bryophytes at the present time. Finally, this thesis revisits the climate change issue through analyses of microclimate, investigating how species respond to microclimatic variations under the canopy, which can largely depart from free-air, macroclimatic conditions.

Signs of a spectacular expansion of epiphytic bryophytes

A re-survey of the epiphytic bryophyte flora across 81 grid-cells of 16 km^2 that had been initially monitored from 1980 to 2015 (Sotiaux and Vanderpoorten, 2015) revealed that, on average, species frequencies increased (Figure 8), contributing to a growing body of evidence attesting to massive recolonization by epiphytes since the end of the 20th century (Bates *et al.*, 1997; Duckett and Pressel, 2010; Pescott *et al.*, 2015; Purvis *et al.*, 2010; Sérgio *et al.*, 2016; Stebel and Fojcik, 2016). The increase in species frequencies is paralleled by an increase of species richness through time, with the emergence of "newcomers" in the flora (such as *Myriocoleopsis minutissima*, and *Plenogemma phyllantha*) since the late 1990s and the beginning of the 21st century (Sotiaux and Vanderpoorten, 2015; De Zuttere, 1992; Hoffmann, 1985).

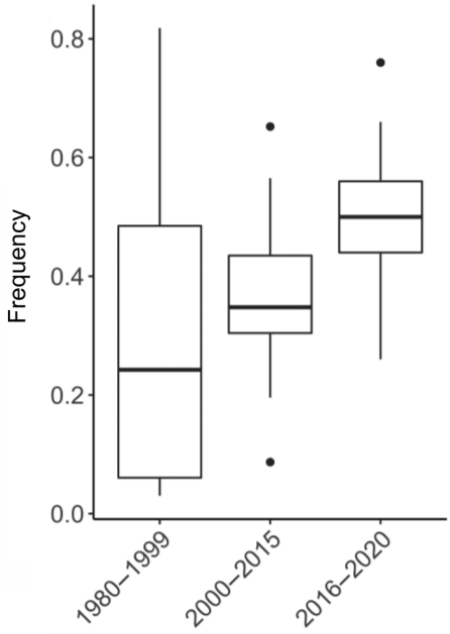


Figure 8. Temporal variation of mean frequencies of epiphytic bryophytes species in southern Belgium, derived from a diachronic analysis across 81 grid-cells of 16km2 between 1980 and 2020.

The temporal difference of species composition of the same site at two different periods of time (temporal beta diversity) is twice higher than the difference of species composition between sites at the present time (spatial beta diversity). This trend is illustrated by the MDS ordination of the grid-cells recorded at different periods of time, wherein species community composition sequentially varies along a temporal gradient (Figure 9).

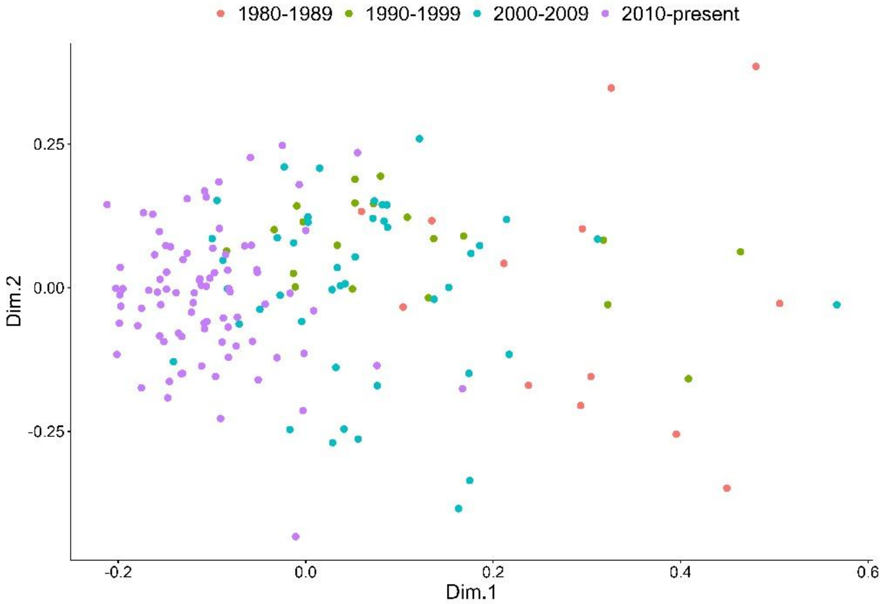


Figure 9. MDS ordination of 81 16 km2 pixels in southern Belgium based on a Sorensen distance matrix of their composition in epiphytic bryophytes in 1980–1990, 1991–2000, 2001–2010, and 2016–2020. Each dot represents an individual pixel, with a colour reflecting the time (decade) of record.

Response of the epiphytic bryophyte flora to changes in air pollutant concentrations over the past decades

The variation partitioning analysis (Figure 10) showed that temporal changes in air pollution loads account for 38 % of the observed temporal differences in community composition within the same pixel at different time periods (βsne temp). Among pollutants, NO_2 and SO_2 together explain 25 % of the variance of βsne temp against 9 % for O_3 alone.

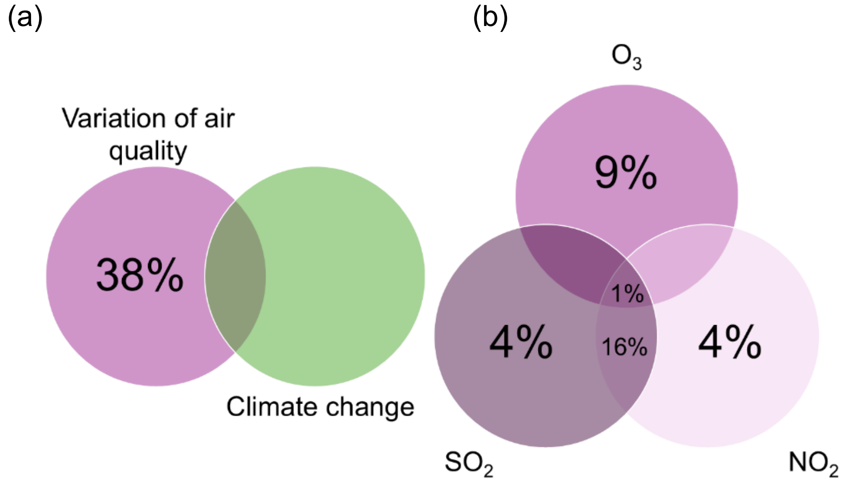


Figure 10. Venn diagram representing the contribution (in % variance explained) of (a) the temporal variation in air pollutant concentrations (SO2, NO2, O3) and climate change (precipitation and temperature) and (b) of each pollutant individually, to temporal differences of epiphytic bryophyte community composition within the same site at two time periods (expressed as βsne·temp) since 1990 in southern Belgium.

High loads of SO_2 and NO_2 in the air led to substantial acidification of ecosystems during the pollution peaks that prevailed in the 1970s-1980s (Lee *et al.*, 1998; Bates *et al.*, 1996). The drastic decrease of SO_2 concentrations associated to coal burning and of NO_2 concentrations thanks to the efficiency of the regulations on vehicle engines, has been identified as the main change that has enabled the spectacular recolonization of epiphytic bryophytes in formerly polluted areas. These changes in the concentrations of major air pollutants have been paralleled by the decline of acidophilous species (such as *Dicranoweisia cirrata*, *Dicranum* spp.), as reported elsewhere in Europe (Manninen *et al.*, 2023; Gauslaa, 2024) and the expansion of acid-sensitive species (such as *Pylaisia polyantha*), as evidenced by an increase of Ellenberg's index for pH averaged over communities from the 1980s onwards (Figure 11).

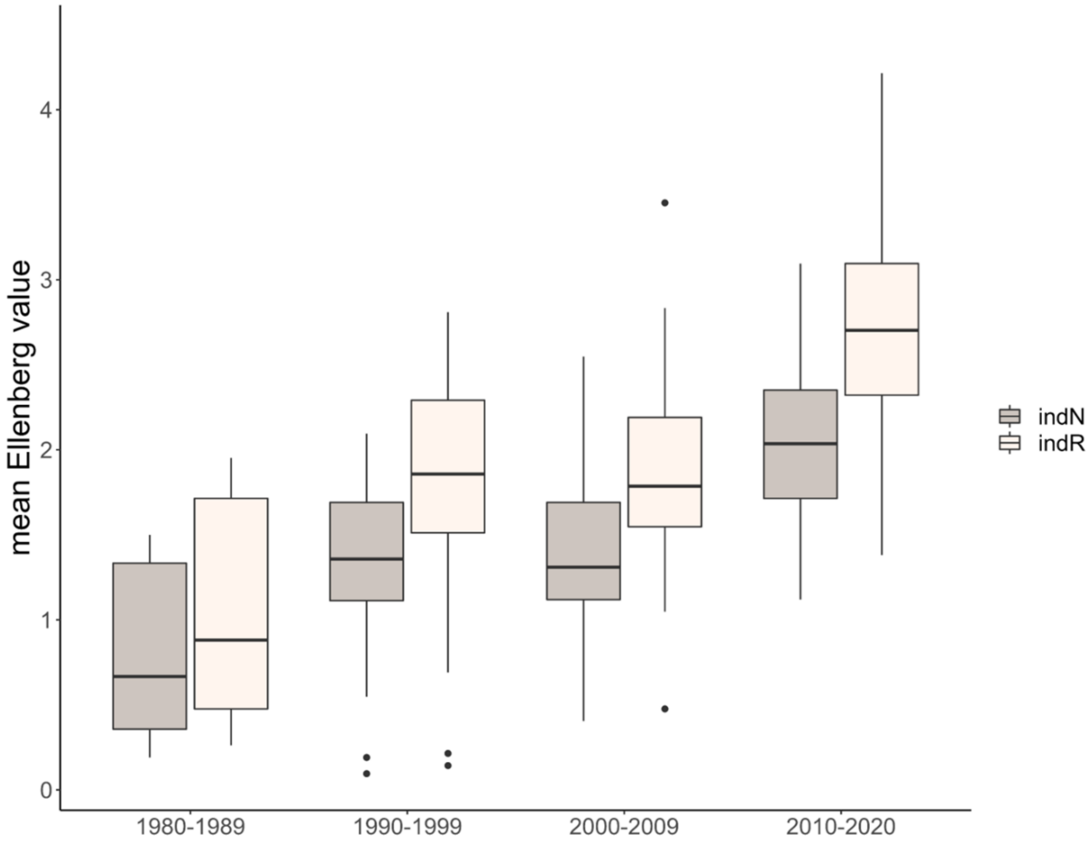


Figure 11. Temporal variation of Ellenberg indicator values for pH (indR, ranging from 3 for acid to 7 for strongly basic) and for N (indN, ranging from 2 for infertile to 8 for richly fertile substrates) of epiphytic bryophytes community in southern Belgium.

Current variations of epiphytic floras are better explained by the spatial variation of climatic conditions than by air pollution loads (Figure 12). This suggests that, as a result of air quality improvement in southern Belgium, the regional variation in climatic conditions mainly driven by an elevation gradient (from < 100-700 m) is now the main driver of the composition of epiphytic communities.

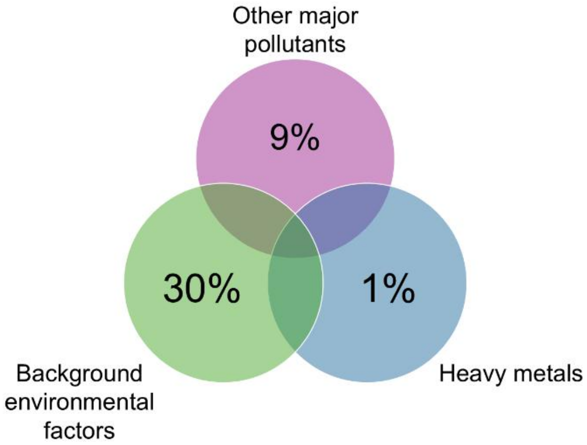


Figure 12. Venn diagrams of the variation partitioning analysis between epiphytic bryophyte species composition, air pollutant concentrations and background environmental factors. Species frequencies at 20 trees surrounding the measuring stations vs measured concentrations of heavy metals (X1), other major pollutants (X2, including SO_2, NO_2, O_3, PMs), and background environmental factors (X3, including topography, climate, vegetation).

The fact, that SO_2 and NO_2 were not selected as the main drivers for current species distributions, further suggests that these pollutants, unlike in some cities (Rocha *et al.*, 2022; Sebald *et al.*, 2022) or countries of central Europe (Procházková *et al.*, 2025), have reached in southern Belgium concentrations that no longer affect epiphytic bryophyte communities.

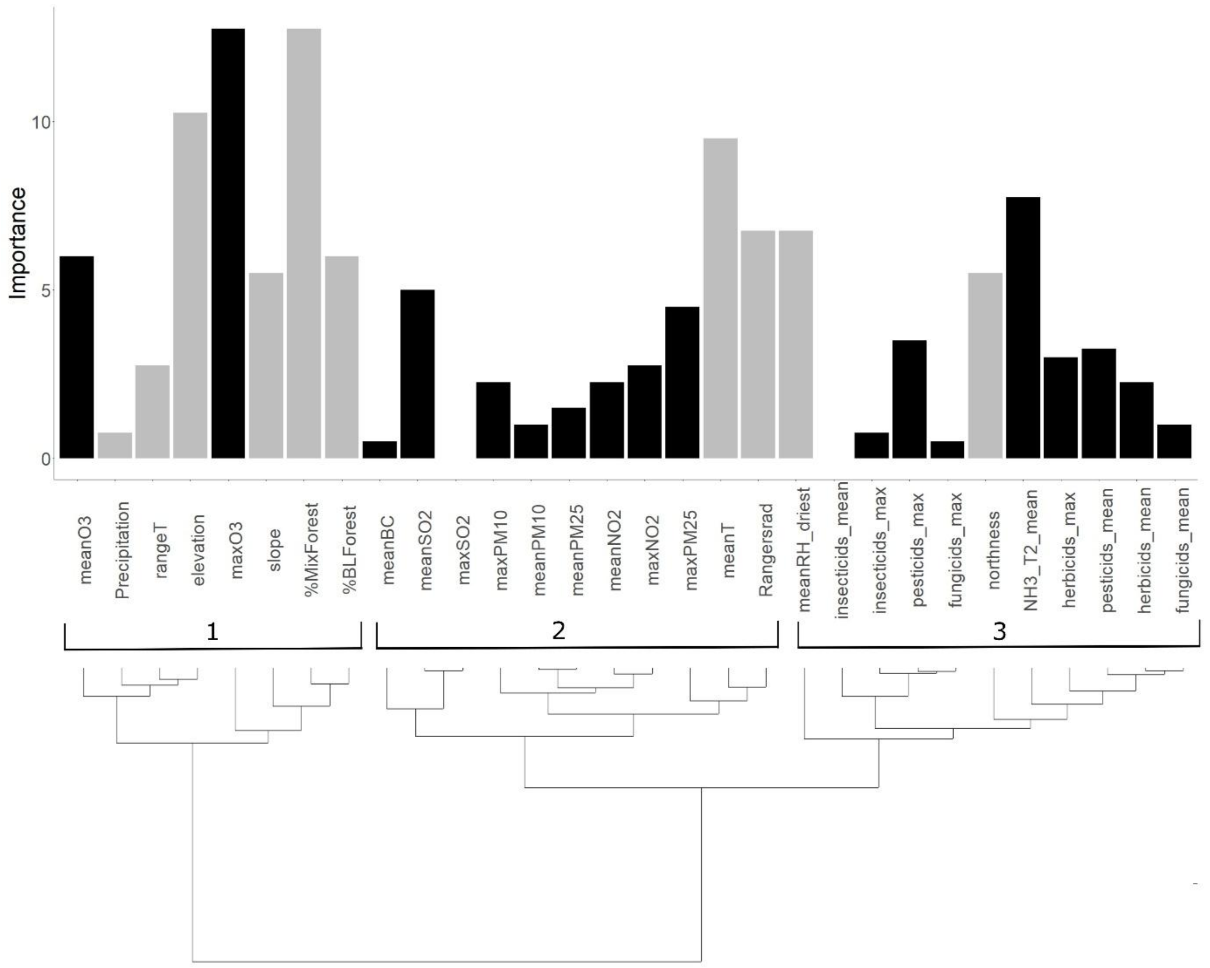


Figure 13. Importance of environmental variables (black: air pollutants, grey: background environmental conditions, see Table 1 from Chapter II for abbreviations) accounting for the distribution of strictly epiphytic bryophytes in southern Belgium. The importance of each variable is a function of the proportion of models, in which it was included by the embedded covariate selection (keeping the four best variables per model), weighted by its ranking during the selection procedure. The panel below is a dendrogram of similarity among variables based on the correlation coefficient among variables and using Ward's algorithm as the clustering criterion.

Other pollutants may have replaced SO_2 and NO_2 in explaining current epiphytic bryophyte species distribution patterns. In particular, O_3 is identified as the most frequently selected variable to explain species distributions in southern Belgium (Figure 13). O_3 reduces photosynthesis in bryophytes exposed to concentrations of 300 µg/m³ (Lee *et al.*, 1998), which represents current maxima of the range in Belgium. The variation of the concentrations in O_3 is, however, highly correlated with variations in a series of environmental factors, including precipitations, temperatures, elevation, slope and forest cover. Because its concentration decreases at night in urban areas due to reaction with NO_2, O_3 is, indeed, interpreted as a land-use marker (Paoletti *et al.*, 2014). If variations in O_3 concentrations can be interpreted as the signature of an urban to rural gradient, then the major drivers of current epiphytic bryophytes distribution in southern Belgium identified in Chapter II reflect natural abiotic variations of climate and topography at a landscape scale, suggesting that pollutants themselves might no longer impact epiphytic floras.

Nevertheless, although the most recent decline of highly nitrophilous species, such as *Orthotrichum diaphanum* (Simmel *et al*., 2021), could be the signature of a forthcoming global decline of nitrophilous species as global N deposition continues to decrease, we demonstrated in Chapter II that NH_3 is one of the major drivers of current epiphytic bryophyte species distribution patterns in southern Belgium (Figure 13). Large concentrations of N can affect species directly and indirectly.

Along a gradient of eutrophication, N may indirectly affect bryophytes through competition. Competition among bryophytes is, however, controversial (Ma *et al*., 2024, and references therein). In temperate forests, dendroid pleurocarps, such as *Isothecium*, whose creeping primary shoots rapidly spread over the surface and whose erect secondary shoots overarch underlying species, are considered as typical examples of effective competitors (Peck and Frelich, 2008). In a co-occurrence analysis of epiphytic bryophyte species in a tropical rainforest, we showed that biotic interactions play a secondary role as compared to environmental filtering, with a very marginal contribution of competitive exclusion (Figure 14) (Shen et al., 2023, see Appendix III). Increasing N concentrations are unlikely to lead to increased competition levels among bryophyte communities, supporting the notion that competitive exclusion is a rare process in bryophyte communities (Udd *et al*., 2016; Mälson and Rydin, 2009; Steel *et al*., 2004).

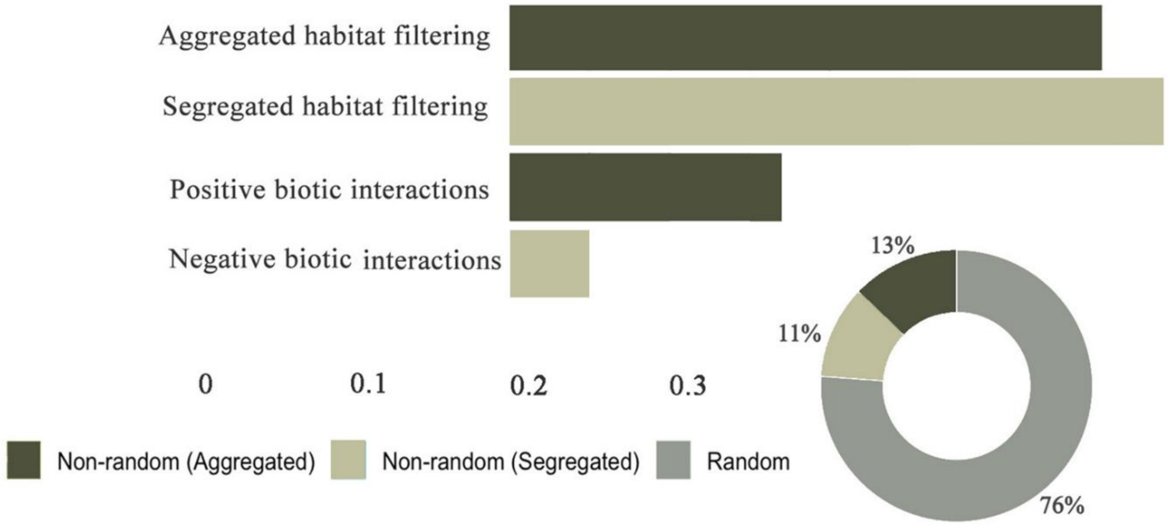


*Figure 14. Disentangling habitat filtering from biotic interactions in epiphytic bryophyte communities through an analysis of co-occurrence patterns controlling for environmental variation (adapted from Shen et al., 2023, article presented in Appendix III). The figure represents the proportion of randomly and non-randomly associated species pairs among epiphytic bryophytes in a tropical rainforest (Yunnan, China) and the contribution (%) of the factors accounting for non-random species associations using a combination of environmentally constrained null models (D'Amen et al. 2018). **Aggregated habitat filtering**: proportion of significantly aggregated species pairs whose co-occurrence can be explained by the sharing of the same abiotic niche; **Segregated habitat filtering**: proportion of significant non-coincidences between two species that can be explained by abiotic differences; **Positive biotic interaction**: proportion of significantly aggregated species pairs that cannot be explained by the sharing of the same abiotic niche and are interpreted in terms of facilitation; **Negative biotic interaction**: proportion of significant non-coincidences between two species in similar abiotic conditions, which cannot be explained in terms of habitat differences and are interpreted in terms of competition.*

Direct effects of NH_3, which accumulates beyond the N-saturation point, include impacts on photosynthesis performance and C fixation, uptake regulation of P and K, and oxidative stress, causing the production of reactive oxygen species whose accumulation can lead to the deterioration of cell membranes (Izquieta-Rojano *et al*., 2018). In addition, NH_3 deposition leads to an increase of bark pH (Van Herk, 2001; Manninen *et al*., 2023), resulting in a decrease of the acidophilous lichen flora (Manninen *et al*., 2023; Gauslaa, 2024) and most likely contributing to the decrease of the acidophilous bryophyte flora over the past decades. The increase of bark pH due to NH_3 pollution could explain the observed trend for calciphilous epixylic species typically found on concrete (e.g., *Grimmia pulvinata*, *Orthotrichum anomalum*, *Tortula muralis*) to grow as epiphytes in the most polluted areas. This would at least partly invalidate the notion that the decline of acidophilous species mirrors an improvement of air quality, while in fact, an increase of NH_3 may be the main cause (Van Herk, 2001).

Among other investigated pollutants, pesticides, whose impact on ecosystems has been widely acknowledged (Albaseer *et al*., 2025 and references therein), marginally contribute to explain variations in epiphytic bryophyte community composition and species distributions. Several herbicides, some of which are specifically used against bryophyte development in, e.g., golf courses (Post et al., 2016; Fausey, 2003), have an effective impact on bryophyte ecophysiology. For example, bryophytes suffer detrimental effects after exposure to widely used herbicides such as asulam at concentrations similar to those that affect bracken, against which they are implemented (Rowntree *et al*., 2003). The response of bryophytes varies, however, among species and depending on the kind of pesticide implemented (Fuselier and Carreiro, 2022; Rowntree *et al*., 2003; Rowntree *et al*., 2005; Newmaster *et al*., 1999).

The failure of our analyses to detect an impact of pesticides may potentially reflect the lack of accurate data on pesticide concentrations in the air. In fact, the present analyses were based on the only information available to date, that is, an extrapolated estimation of the amount of pesticides implemented on average per crop field (Habran *et al*., 2022). Detailed information on pesticide concentrations in the air, as well as further experimental investigations on the impact of pesticides on bryophyte ecophysiology, would be necessary for a more accurate evaluation of the impact of pesticides on bryophyte floras.

Response of the epiphytic bryophyte flora to climate change

Analyses presented in Chapter I did not identify changes in climatic conditions over the past four decades in southern Belgium as a driver of changes in community composition. The contribution of climate change alone to the observed temporal shifts in community composition ranged between 0 and 7 %, depending on the analysis performed. This suggests that the drastic compositional shifts in epiphytic bryophyte communities observed in the study area, and recurrently reported across Europe, cannot be attributed to climate change.

The lack of climatic impact on epiphytic floras reported here does, however, not suggest that such floras are resilient to climate change. While, in line with the inconclusive trends reported across central Europe (Gudmundsson and Seneviratne, 2016), no temporal trends in the precipitation regime were observed in the course of the past four decades in southern Belgium, anthropogenic climate change has already increased drought risk and number of dry days in southern and northern Europe, respectively (Gudmundsson and Seneviratne, 2016). Furthermore, Europe has been experiencing its worst drought in at least 500 years in the course of the past couple of years (European Drought Observatory, 2025). Bryophytes, which do not have a vascular system, are sensitive to drought but also to heat, which increases evaporation. As bryophyte species of temperate forests do not survive more than a few days at temperatures over 30°C when moisturized (Furness and Grime, 1982), the tolerance limit for many, even common bryophytes, could be reached soon, if not already. This, together with record-breaking temperatures in Europe during the 2022 summer (Witze, 2023), suggests that climate change impacts on bryophytes will start to become apparent during the next decades. Predictions based on species distribution modelling in *Dicranum viride*, an epiphytic moss relying on high air humidity and temperature stability, suggest that the species will have to shift its distribution range from the lowlands to mountain regions to track areas with suitable climatic conditions (Wierzcholska et al., 2020).

The impact of climatic conditions in the analyses presented here, and more generally, in all studies employing climate data at 1 km^2 or coarser resolution, may, however, have been poorly estimated. In fact, such climatic data reflect macroclimatic conditions interpolated from measurements made at meteorological stations, which are typically located in open areas. Epiphytes, the bulk of which occur on the lower trunk and in areas exposed to high air humidity (Ellis and Eaton, 2021; Ellis, 2020), experience microclimatic conditions under the canopy that are substantially buffered as compared to the ones prevailing in open areas (see De Frenne *et al.*, 2021 for review) calling for a paradigm shift from macro-to micro-scales (Lembrechts and Lenoir, 2020).

The need to shift from macro-to micro-scales for microspecies

Forests best exemplify the microclimatic effect, i.e., the difference between macro (free-air) and microclimatic (measured *in-situ*) conditions in the understorey due to factors of topography and canopy structure. In Chapter III, we quantified the microclimatic effect based on *in-situ* measurements of temperature and relative humidity recorded for one year at 42 locations in *Quercus-Fagus* stands in southern Belgium. The mean of the difference of macro- and micro-temperatures was 1.2 °C, but hourly differences reached up to no less than 15.7 °C. The microclimatic effect was characterized at the level of each sensor through the ‘slope and equilibrium’ approach (Gril *et al.*, 2023a). Microclimatic effects were accurately described, with a mean r^2 reaching 0.95 for temperature and higher than 0.6 for relative humidity. The microclimatic effect varied from a buffering to an amplifying effect for temperature (Figure 15), but only a buffering effect was detected for relative humidity.

Significant models were obtained to describe the spatial variation of the microclimatic effect across sensors using spatially explicit variables of topography and vegetation structure obtained by satellite imagery. This study is, to our knowledge, the first to obtain significant models to predict microclimatic effects for relative humidity. The r^2 of these models are, however, relatively low. For instance, they are twice lower compared to previous studies implementing a similar approach (Gril *et al.*, 2025 ;Gril *et al.*, 2023a). In addition to the deployment of additional dataloggers to better capture the heterogeneity of forests in the study area, one other way of improving the models is to add other predictors, such as variables derived from LiDAR (Gril *et al.*, 2025).

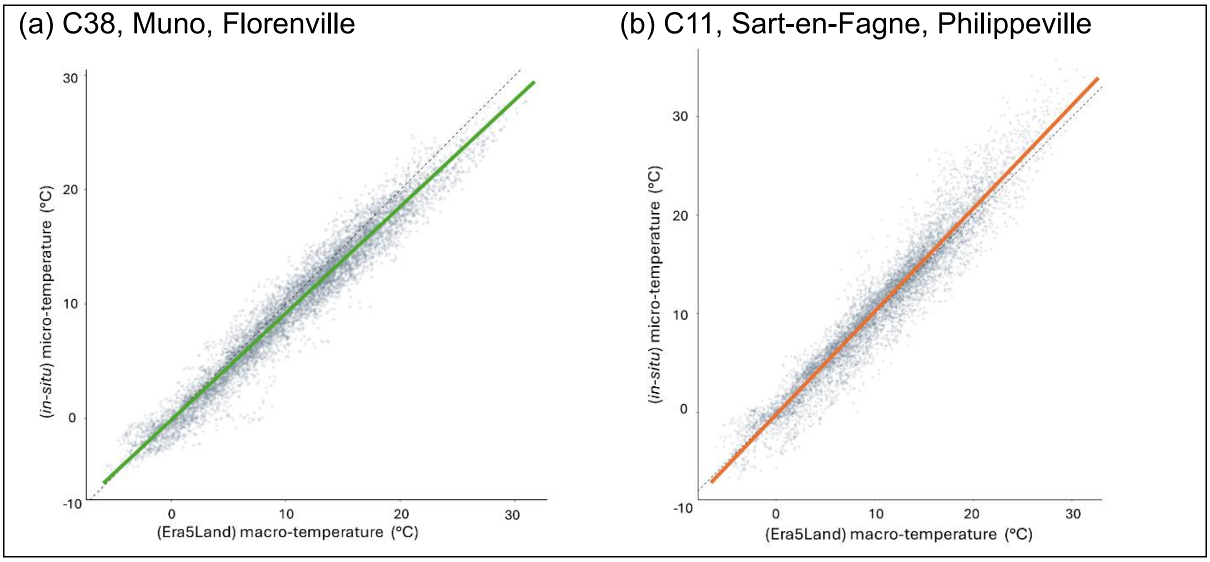


Figure 15. The microclimatic effect under forest canopies. The graph shows the hourly variation of micro-temperature, recorded in-situ for 7 months at two locations in southern Belgium during leaf-on period (May to November) and macro-temperature provided by ERA5Land for the same period of time. In (a), the slope of the macro-microclimate is <1, evidencing that microclimate temperature variation is buffered compared to macroclimate temperature variation. In (b), conversely, the slope of the macro-microclimate is >1, evidencing that microclimate temperature variation is amplified compared to macroclimate temperature variation.

The microclimatic effect can also be detected though indicator species. Chapter III identified, according to the shape of the species response curve to microclimatic effect, 6 and 7 species with an optimum probability of occurrence under buffered conditions of temperature and relative humidity, respectively. The optimum of the response curve to microclimatic effects provides information on species microclimatic preferences. Contrary to Ellenberg indicator values, this optimum is based on a quantitative assessment of species response curves and characterizes microclimatic conditions actually experienced by organisms. In addition, the slope of the macro/microclimatic relationship is an integrative variable for ecological preference of species to microclimatic conditions including topography and canopy structure that generate these microclimatic conditions (Gril *et al*., 2024). This integrative variable can also be used, to a certain extent, as an indicator of ancient forests typically characterized by complex and heterogenous canopy structures leading to the most buffered microclimatic conditions (Frey *et al*., 2016).

6. Conclusion and perspectives

cover picture:epiphytic bryophytes on a branch, Mormont (Wallonia), picture from Lea Mouton, April 2024

Conclusion

Epiphytic bryophytes communities experienced a massive increase in frequencies and richness since the 1980s. The increase in species frequencies was paralleled by an increase of species richness through time, with the emergence of “newcomers” in the flora (*Habrodon perpusillus*, *Lewinskya shawii*, *Myriocoleopsis minutissima*, *Orthotrichum consimile*, *O. rogeri*, *O. scanicum*, *Plenogemma phyllantha*, *Ulota rehmannii*) since the late 1990s and the beginning of the 21th century that represent 15% of the current epiphytic flora. The concomitant global warming and shifts in air pollution have long blurred the interpretation of these temporal changes in epiphytic floras (Bates and Preston, 2011). Analyses from Chapter I and Chapter II revealed that the sharp reduction of pollutants in the air, in particular SO_2 and NO_2, allowed the massive (back) colonisation of epiphytic bryophytes. The increase of frequency and richness of highly sensitive organisms, such as epiphytic bryophytes, is seen as the positive consequence of the efficiency of policy measures to improve air quality over the last decades.

Nevertheless, current epiphytic floras may still not have reached their levels of diversity dating back to the pre-industrial era. In fact, Dittrich et al. (2016) reported that the bryophyte floras in the early 20th century were richer and more abundant than today. Dittrich et al. (2016) further evidenced a significant increase in the mean Ellenberg value for temperature and nutrients from 1900 and 2013, pointing to a general eutrophication and a global warming. This underlines the necessity to correctly identifying the starting point for comparison. Jacobs (2025) and Monsarrat *et al*. (2019) speak about “shifting baselines” to describe the loss of biodiversity benchmarks from one human generation to the next, blurring a global loss of biodiversity. Jacobs (2025), therefore, calls for establishing comparison baselines further back in time.

The massive compositional shifts in species observed in southern Belgium from the 1980s, and recurrently reported across Europe, cannot be attributed to climate change. The lack of evidence for the climatic impact on the temporal change in epiphytic communities, however, does not suggest that such floras are resilient to climate change. Moving from macro to microclimatic provides a more accurate understanding of the impact of climate change (Lembrechts *et al*. 2019; Zellweger *et al*. 2019; Greiser *et al*. 2018; Slavich *et al*. 2014), especially in the case of small species such as bryophytes. Through the study of microclimate, this thesis laid the groundwork for quantifying and modelling the microclimatic effect in broadleaf forests of southern Belgium. By calculating the slope of the relationship of micro/macro-temperature and relative humidity, the microclimatic effect can be quantified and then spatially projected using predictors such as topography and vegetation structure. These microclimatic effect maps can be used in numerous applications in biodiversity conservation, such as the identification of microrefugia, the selection of integral nature reserves and recommendations for forest management under climate change. The integration of microclimatic effect in nature conservation programs and forest management is essential to preserve biodiversity and promote forest resilience to climate change.

Perspectives

Considering microclimate in biodiversity conservation programs under climate change

Finding microclimate refugia

Macroclimatic changes induce species to move towards suitable climatic conditions to survive. Nevertheless, species may be preserved locally in microrefugia, characterised with thermal and hygric insulation (Zhou *et al*., 2024). Forest structure and the density of the canopy play a central role in in this context by mitigating the effect of climate change (Zhou *et al*., 2024). Quantifying the microclimatic effect of forests is therefore essential to identify and preserve microrefugia, where species are expected to undergo less pressure of climatic change, at least in the short term (De Frenne *et al*., 2021; De Frenne *et al*. 2019; Lenoir *et al*. 2017; Ewers and Banks-Leite, 2013; von Arx *et al*. 2013). Given their poor transposability, the microclimatic models presented here will need to be optimized through the implementation of a dense network of microclimatic sensors capturing the heterogeneity of topography and forest structure. If LiDAR data across the study area at 25 m resolution during the leaf-on period become available, other predictors of vegetation structure could be added to the model to increase performance of the prediction of the microclimatic effect. Once accurate and transposable, this model will allow us to generate a map at 25 m resolution of the microclimatic effect and the microclimate in broadleaf forests in southern Belgium, with numerous applications.

Target areas of high conservation relevance

High-resolution maps of forest microclimatic effects and microclimates would be of tremendous interest to guide the selection of protected areas. This application is extremely timely in the context of two newly designed national parcs in southern Belgium, namely Semois and Entre-Sambre-et-Meuse (SPW, 2026). One of the objectives of these National Parks is to increase the surface of integral protected areas. The management committees of these parks are currently designing a network of integral nature reserves, which they must select *de novo*. Maps of forests microclimatic effect will enable to identify forests with strong buffering effects, typically characterizing old-growth forests with complex canopy structure (Blumroeder *et al*., 2024; Máliš *et al*., 2023; Lindenmayer *et al*., 2022). Old-growth and mature forests should be protected as a priority because they are the result of long forest historical continuity (Spies, 1996) and host species of high conservation relevance, characterized by narrow niche requirements and poor dispersal capacities. In bryophytes, this is the case for a series of species characteristic of the most buffered conditions effect (Man *et al*., 2022; Ódor *et al*., 2013) and sensitive to forest fragmentation and edge effect (Oishi, 2009), but also of connectivity (Wierzcholska *et al*., 2020; Fritz *et al*., 2008) integrity and naturalness (Czerepko *et al*., 2021).

Softwares, such as the *prioritizr R* package (Hanson *et al*., 2025), are increasingly used to identify priority areas for conservation according to different environmental, biodiversity and socio-economic constraints. Integrating microclimatic maps to epiphytic bryophyte species distributions into such programs would ensure that the best combination of sites, capturing a maximum of biodiversity but also characterized by the most buffered conditions, typical of the best-preserved forest stands, are selected

Adapting forest management to climate change

Forests characterized with a strong buffering capacity, due to a complex canopy structure, show better resilience to climatic change (Kramer *et al.*, 2020; Thompson, 2009) Understanding how forest structure impacts microclimatic effect may help guiding forest management in a climate change context (Menge *et al.*, 2023).

The use of indicator species

In a context of global changes, policy-makers are relying increasingly on the use of various bioindicators methods (i.e., biomonitoring, ecological indicator value, indicator species, …), to take decisions about nature conservation (Caro, 2010) and ecosystem management (Siddig *et al.*, 2016). Identifying and monitoring bioindicator species is helpful to measure the efficiency of environmental policies, chose areas to protect and adapt landscape management practices. Due to their specific eco-physiological features rendering them extremely sensitive to environmental conditions, epiphytic bryophytes appear as extremely relevant bioindicators.

Bryophytes can be used as bioindicators notably through their Ecological Indicator Values (EIVs). Calculating, for example, the mean Ellenberg index of the community enable to quantify environmental characteristics (Delgado and Ederra, 2013). When repeated surveys are performed, this approach makes it possible to infer temporal environmental shifts, such as eutrophication (Pakeman, 2019). Chapter III showed that indicator values for microclimatic preferences can be derived from species response curve to microclimatic effects based on species occurrence data at fine resolution and microclimatic data. Such newly derived indicator values would open the door to the indirect characterization of microclimates and the identification of the forest stands most relevant for conservation. More widely, with the optimum of the species' response curve to a given environmental factor, it would be possible to update the EIVs in a quantitative manner (Gril *et al.*, 2024). The use of optimum response curves to revisit Ellenberg index is promising, as it has the advantage of being based on a quantitative assessment of species optima and characterizes ecological conditions actually experienced by organisms.

Future research on air quality

Bryophytes have long been used for monitoring spatio-temporal changes in pollution loads (Baczewska-Dąbrowska *et al.*, 2023; Chaudhuri and Roy, 2023; Dołęgowska *et al.*, 2021; Godzik, 2020; Kapusta and Godzik, 2020). As demonstrated for microclimatic effects in Chapter III, response curves to pollution could similarly be used to identify reliable air-quality indicator species. A promising development to monitor air quality, while at the same time increasing public awareness about environmental issues, would be to encourage citizens to record the presence and monitor frequency shifts in selected, easily recognizable species with high indicator values, such as *Orthotrichum diaphanum*, within their immediate environment. It would eventually be possible to generate a map of air quality based on the indicator species present and recorded through citizen participation.

A curiosity arose during fieldwork and remains unresolved this day. We noticed that in some forests, young trees are sometimes richer in epiphytic species, or at least host more rare species, than older trees. This raises the question of whether older trees that lived during the 1950s-1980s, the period of massive pollutant emissions, have a form of pollution memory. The remnants of past pollution trapped in tree tissues would lead to delay epiphyte colonisation on older tree. The delay of colonisation is therefore not noticed at the scale of the forest if younger trees, hosting the large epiphytic diversity, are recorded in the study. Measuring the pollutants concentration in the bark of trees would then eventually allow to determine whether the epiphytic flora has indeed recovered or whether remnants of industrial pollution in the air still impact on the distribution of epiphytic bryophytes.

To conclude, the human being caused a change in the geological area entering the Anthropocene and threatening living conditions on Earth. Through its industrial activities, the human being is responsible for a large amount of pollutants released in the atmosphere, causing unprecedented changes in climate and irreversible pollution on Earth. This thesis, among many other scientific works, is just one more piece of evidence that there is sufficient knowledge to change the course of human impacts on our own planet. Sources of pollution and greenhouse gases are known, indicator species are identified, microclimate maps are almost available, and forestry guidelines are adjusted. It is also recognised that strong restrictions, such as those imposed by the European Union to reduce air pollutants since 1980s, are effective, even in the short term. What is missing is the collective motivation and sense of responsibility to restore decent living conditions for all the inhabitants, Human and non-Human, of the Earth.

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8. Appendices

cover picture:epiphytic bryophytes on a tree, Liège (Wallonia), picture from Lea Mouton, January 2026

Appendix I

IV. Ting Shen, Richard Corlett, Flavien Collart, Thibault Kasprzyk, Xin-Lei Guo, Jairo Patiño, Yang Su, Olivier Hardy, Wen-Zhang Ma, Jian Wang, Yu-Mei Wei, **Lea Mouton**, Yuan Li, Liang Song, and Alain Vanderpoorten, 2022. Microclimatic variations in tropical canopies: a glimpse into the processes of community assembly in epiphytic bryophyte communities. Journal of Ecology. 10, 3023–3038. https://doi.org/10.1111/1365-2745.14011

cover picture: trees full of epiphytes in Ailao Mountains (China) in October 2019, from Lea Mouton

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RESEARCH ARTICLE

Microclimatic variation in tropical canopies: A glimpse into the processes of community assembly in epiphytic bryophyte communities

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Abstract

1. Epiphytic communities offer an original framework to disentangle the contributions of environmental filters, biotic interactions and dispersal limitations to community structure at fine spatial scales. We determine here whether variations in light, microclimatic conditions and host tree size affect the variation in species composition and phylogenetic structure of epiphytic bryophyte communities, and hence, assess the contribution of environmental filtering, phylogenetic constraints and competition to community assembly.
2. A canopy crane giving access to 1.1 ha of tropical rainforest in Yunnan (China) was employed to record hourly light and microclimatic conditions from 54 dataloggers and epiphytic bryophyte communities from 408 plots. Generalized Dissimilarity Modelling was implemented to analyse the relationship between taxonomic and phylogenetic turnover among epiphytic communities, host-tree characteristics and microclimatic variation.
3. Within-tree vertical turnover of bryophyte communities was significantly about 30% higher than horizontal turnover among-trees. Thus, the sharp vertical variations in microclimatic conditions from tree base to canopy are more important than differences in age, reflecting the likelihood of colonization, area, and

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habitat conditions between young and old trees, in shaping the composition of epiphytic bryophyte communities.

4. Our models, to which microclimatic factors contributed most (83–98%), accounted for 33% and 18% of the variation in vertical turnover in mosses and liverworts, respectively. Phylogenetic turnover shifted from significantly negative or non-significant within communities to significantly positive among communities, and was slightly, but significantly, correlated with microclimatic variation. These patterns highlight the crucial role of microclimates in determining the composition and phylogenetic structure of epiphytic communities.
5. *Synthesis*. The mostly non-significant phylogenetic turnover observed within communities does not support the idea that competition plays an important role in epiphytic bryophytes. Instead, microclimatic variation is the main driver of community composition and phylogenetic structure, evidencing the role of phylogenetic niche conservatism in community assembly.

KEYWORDS
beta diversity, biotic interactions, environmental filters, epiphytic bryophytes, forest canopy, microclimates, niche conservatism, phylogenetic constraints

1 | INTRODUCTION

The relative influence of community assembly mechanisms varies depending on spatial and temporal scales (Kneitel & Chase, 2004; Kraft & Ackerly, 2014). In Grime's competitive, stress-tolerant, ruderal (CSR) theory of plant ecological strategies (Grime, 1977), community composition is controlled by selection for traits depending on levels of competition, stress and disturbance. Along a gradient of decreasing habitat filtering, community composition is expected to shift from a dominance of stress-tolerant species to competitive and ruderal species (Escobedo et al., 2021). At larger spatial scales, and hence, as variation in environmental conditions increases, community composition is conversely increasingly driven by environmental filtering (Powell et al., 2015). The contribution of environmental and biotic filters to community assembly is, however, often confounded (Cadotte & Tucker, 2017), especially at small spatial scales, at which both processes may occur (Xu et al., 2021).

In this context, phylogenetic turnover, which characterizes the phylogenetic structure of communities, offers an appealing framework to disentangle the processes involved in community assembly (Graham & Fine, 2008). While taxonomic turnover measures the extent to which some species are replaced by others along environmental gradients, phylogenetic turnover measures the extent to which species replacement is phylogenetically constrained, so that species within a community are more or less phylogenetically related to each other than expected by chance.

Positive phylogenetic turnover occurs when species in a community are more closely related to each other than species from different communities. A clumped phylogenetic distribution of taxa (phylogenetic clustering) indicates that habitat-use is a conserved trait within the pool of species in the community, and hence, evidences phylogenetic niche conservatism (Webb et al., 2002). The application of the phylogenetic niche conservatism hypothesis has substantial ecological and evolutionary implications because it makes it possible to determine whether niche preferences are evolutionarily labile or, to the reverse, are phylogenetically constrained, potentially hampering the chances of species to respond to climate change.

Negative phylogenetic turnover (phylogenetic overdispersion) occurs when species from the same community are more phylogenetically distant than species from different communities. While phylogenetic overdispersion points to non-random species assemblages, its interpretation has been controversial. In line with Darwin's competition-relatedness hypothesis, which posits that closely related species compete more strongly than distantly related ones (Cahill et al., 2008), phylogenetic overdispersion has primarily been interpreted in terms of competition among related species sharing limited resources within the same niche (Anacker & Strauss, 2014; Wiens & Graham, 2005). Phylogenetic overdispersion may, however, also result from niche convergence (Cavender-Bares et al., 2004) or facilitation (Valiente-Banuet & Verdu, 2007) among phylogenetically unrelated species.

Epiphytes appear as an interesting model to address the question of the factors shaping community structure at small spatial scales (Adams et al., 2017, 2019; Méndez-Castro et al., 2020). For epiphytes, host-trees typically function as habitat islands, exhibiting, like oceanic islands but at much smaller spatial scales and shorter time frames, sharp spatio-temporal variations in their abiotic environment (Adams et al., 2017; Hidasi-Neto et al., 2019; Itescu, 2019; Taylor & Burns, 2015).

The ecological conditions that prevail along a vertical gradient, from the base to the uppermost canopy, typically vary in terms of extrinsic (e.g. air humidity, light intensity, temperature) and intrinsic (physical properties of the substrate, such as bark texture and physico-chemistry, branch orientation and diameter) features (Cornelissen & ter Steege, 1989). In the outer canopy, the high light intensity and extremely low humidity, high wind exposure and daily variation in temperature and relative humidity, exert strong selection pressure for traits similar to those seen in desert habitats, such as leaf succulence, small stature, slow growth rate, water and nutrient storage capabilities and UV protection (Spicer & Woods, 2022). Progressing towards the tree base, physical stability of the support and relative humidity (RH) increases, while light, temperature and the daily variation in microclimatic conditions decrease, resulting in a more stable environment inhabited by species that are less tolerant of drought and high light intensity (Cornelissen & ter Steege, 1989; Freiberg, 1996; Watkins et al., 2007; Woods et al., 2015).

These conditions further vary along horizontal gradients not only due to differences among host-tree species in terms of branching architecture, bark texture and physico-chemistry (Hidasi-Neto et al., 2019) but also due to age differences among host trees. As the likelihood of colonization increases with time, old trees typically exhibit a higher epiphytic species richness than young ones (Taylor & Burns, 2015). Old trees also have a larger area for colonization and a higher diversity of micro-habitats than young ones (Paillet et al., 2019).

In this context, Grime's CSR theory of plant ecological strategies (Grime, 1977) allows us to make predictions on the importance of interactions among epiphytes depending on the ontogenetic stage of their host-tree and the habitat they occupy (Spicer & Woods, 2022). On a tree, competition is expected to increase from the canopy, characterized by large variations in light and microclimatic conditions, to tree base, with more buffered environmental variations. Competition is also expected to increase from young to old host-trees, as pioneer species progressively accumulate before entering competition with specialized competitors (Ellis & Ellis, 2013).

Despite these expectations, where and when competition and facilitation dominate, if at all, remains relatively unexplored in epiphyte ecology (Francisco et al., 2018; Spicer & Woods, 2022). Furthermore, while the vertical structures of epiphyte communities (Gehrig-Downie et al., 2013; Mota de Oliveira et al., 2009; Mota de Oliveira & ter Steege, 2015; Zotz, 2016) and, to a lesser extent, associated variations in microclimatic conditions (Murakami et al., 2022; Stuntz et al., 2002; Toivonen et al., 2017), have long been documented, no analysis has, to our knowledge, examined the relationship between microclimatic variation and species composition in a spatially explicit framework. In fact, although forest canopy science has been an active discipline since the 19th century, its progress has been slow, partly due to the limited accessibility of canopies (Nakamura et al., 2017) and the limited availability of fine-scale microclimatic data (De Frenne et al., 2021), a critical issue for canopy epiphytes (Murakami et al., 2022).

Bryophytes represent an important component of epiphytic floras, to which they contribute up to 75% of the biomass, and hence, play a key role in nutrient and water cycles (Gradstein et al., 2010). Bryophytes are poikilohydric and rely on rainfall or moisture in the atmosphere for water uptake. They are hence ideal models to investigate the impact of microclimatic variation on community composition, which strikingly varies from the base to the canopy (Mota de Oliveira & ter Steege, 2015; Sporn et al., 2010). Although mounting evidence points to the relevance of climatic niche conservatism for the assembly of bryophyte floras over large spatial and evolutionary time scales (Collart et al., 2021; Piatkowski & Shaw, 2019; Wilson & Coleman, 2022), whether shifts in community composition along vertical microclimatic gradients and along horizontal gradients in host-tree size are structured phylogenetically, that is, whether niche conservatism could operate at such micro-scales, remains to be tested.

Furthermore, it has been suggested that bryophytes may not compose communities similar to those of vascular plants, but instead, that the distributions of individual species would be driven by niche preferences and dispersal capacities, regardless of other species (Wilson et al., 1995). The unbounded relationship between epiphytic species richness and tree age has been interpreted in terms of the unrestricted increase in species richness in the absence of competition in unsaturated communities (Boudreault et al., 2000; Fritz, Brunet, & Caldiz, 2009). In line with this hypothesis, a significantly lower evenness, which could reflect weaker competition, was reported in bryophyte communities compared with those formed by vascular plants (Steel et al., 2004). Results from common garden experiments conversely revealed that competition is more important than temperature for the performance of bryophyte species (Greiser et al., 2021). The role of competition in bryophyte communities has, thus, long been questioned (Rydin, 2009). Wilson et al. (1995) concluded that there is community structure among bryophytes, in that species exclude each other to the same degree as higher plants do in their communities. They failed, however, to identify groups of species within a community that are mutually exclusive because of similarity in resource use, leading them to conclude that bryophyte species all form one guild.

Taking advantage of one of the world's 22 canopy cranes, the goal of the present study is to determine whether variations in light, microclimatic conditions and host tree size affect the variation in species composition and phylogenetic structure of epiphytic bryophyte communities, and hence, assess the contribution of environmental filtering, phylogenetic constraints and competition to community assembly. More precisely, we address the following questions: Is variation in species composition among epiphytic communities more important vertically, reflecting within-tree changes in microhabitat and microclimatic conditions, or horizontally, reflecting differences in age, and hence size and microhabitat diversity, among trees (Q1)? To what extent are these changes in community composition phylogenetically constrained (Q2)? Among communities, we test the hypothesis of an increasingly positive phylogenetic turnover along microclimatic gradients, pointing to phylogenetic niche conservatism (H1). Within communities, we test the hypotheses that species exhibit increasingly competitive interactions, and hence, increasing phylogenetic overdispersion, from the canopy to the base, and from young to old trees (H2).

2 | MATERIALS AND METHODS

2.1 | Study site and sampling design

This study took place in a 1.44 ha square plot in a lowland (643–700 m) seasonal rain forest (101°34′59.1″E, 21°37′2.6″N) in Mengla, one of the five subdistricts that together form Xishuangbanna National Natural Reserve (Yunnan, SW China). Mean monthly RH and mean monthly temperature recorded by dataloggers from 12 trees at 2 m during 2017–2019 were 95.3% and 20.8°C, respectively, with the coldest month in January (15.8°C) and the warmest month in June (25.2°C). This site was selected because it is equipped with an 81 m-high canopy crane (TCT7015-10E, Zoomlion Heavy Industry, Changsha, China) whose 60 m-long arm provides access to the canopy within a 1.1 ha circular area (Figure 1c).

Xishuangbanna National Natural Reserve covers an area of 242,510 ha that comprises the largest tropical forest area in China. The region experiences a typical monsoon climate with a 6-month dry season from November to April and a rainy season from May to October. In a 20-ha plot of tropical seasonal rainforest of Xishuangbanna, 468 tree species in 213 genera and 70 families were recorded. The tallest trees attain 70 m, and there are 4791.70 stems and a total basal area of 42.34 m^2 per hectare (Lan et al., 2012). Within the 1-ha plot investigated, the canopy layer (height > 30 m) is dominated by *Parashorea chinensis* (Dipterocarpaceae), which contributes 19.5% of the trees with a diameter at breast height (DBH) ≥ 5 cm and most of the tallest trees. It is accompanied by *Canarium album*, *Pometia tomentosa*, *Sloanea tomentosa* and *Semecarpus reticulata*. The sub-canopy layer (16–30 m) is dominated by *Ficus langkokensis*, *Litsea dilleniifolia*, *Barringtonia fusicarpa*, *Diospyros atrotricha* and *Pseuduvaria indochinensis*, and the understorey layer (6–16 m) by *Pittosporopsis kerrii*, *Baccaurea ramiflora*, *Diospyros xishuangbannaensis*, *Cleidion brevipetiolatum* and *Mitrephora maingayi*.

Epiphytic bryophytes were recorded only on the dominant host-tree species, *Parashorea chinensis*, to control for host specificity (González-Mancebo et al., 2003; Guan et al., 2017; Schmitt & Slack, 1990). *Parashorea chinensis* is an evergreen species, characterized by large buttresses, and hosts abundant epiphytic bryophytes (Shen et al., 2018). Although our analyses were restricted to the communities found on *P. chinensis*, these are representative of the entire epiphytic bryophyte community of the area. 102 epiphytic bryophyte species we found on 42 tree individuals, that is, slightly more than the 90 species reported from 69 individual trees belonging to 14 different tree species in the same plot in a previous investigation (Shen et al., 2018).

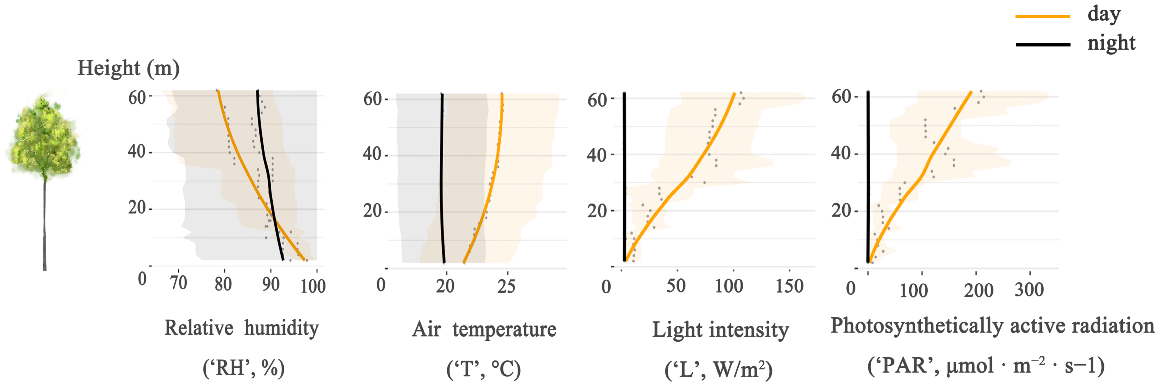
Trees with a DBH < 5 cm or covered by vines and lianas were discarded, resulting in a total of 42 with a DBH ranging from 5.4 to 135 cm. Each tree was divided into six height zones based on a slightly modified version of Johansson's (1974) zonation scheme (see e.g. Figure 7.11 in Zotz, 2016), which is not based on absolute height, but on tree architecture, as follows: tree base (zone 1), <2 m and corresponding to the buttresses; lower trunk (zone 2), between zone 1 and middle height of the trunk; upper trunk (zone 3), between the middle height of the trunk and the first ramifications of the canopy; inner, middle, and outer canopy (zones 4–6), corresponding to the lowest, middle and upper thirds of the canopy.

For each height zone, two plots were haphazardly located vertically. From the 504 initial plots, 96 had no bryophyte species, leading us to focus on 408 plots (see Shen (2021a) for individual plot coordinates) with at least one species, suitable for analyses of beta diversity (see below). Although orientation typically plays a limited role in explaining variation in epiphytic community composition in tropical cloud forests (Song et al., 2011), we controlled for this factor by sampling, for each plot, four sub-plots of 20 × 20 cm (as measured with a tape) on the trunk or branches (zones 1–4). These four sub-plots were organized in pairs, with the two plots of a pair being diametrically opposed and the pairs being perpendicular to each other. At zone 5, branches may be narrower than 20 cm, and we recorded epiphytes within a shape of 80 × 5 cm. At zone 6, we recorded an area of *c.* 400 cm^2 of twigs. This led to a total of 1632 sub-plots, 1156 of which had bryophytes.

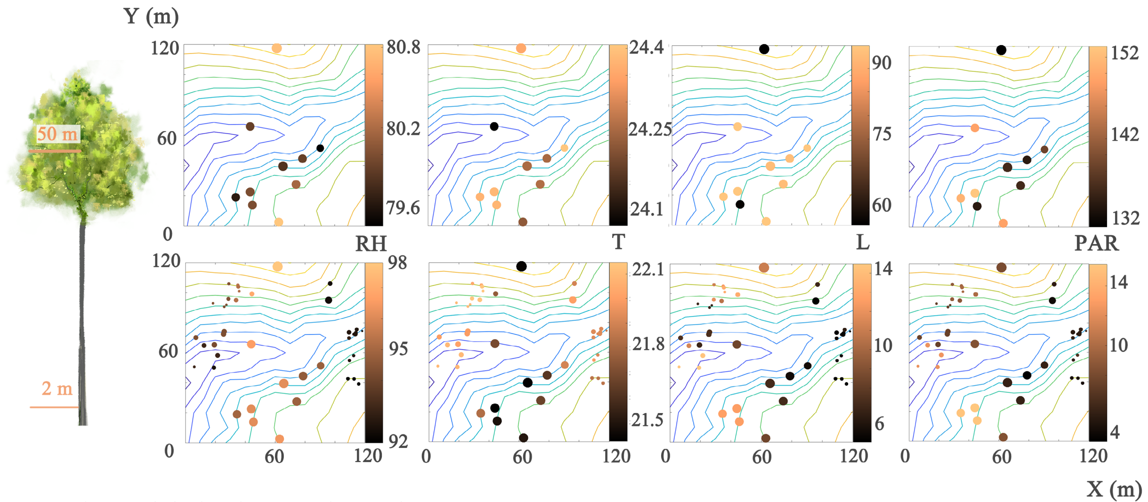
Within each sub-plot, a complete species inventory was conducted. Representative specimens of each species were sampled in each sub-plot, resulting in 1156 collections that were subsequently analysed in the laboratory using relevant microscopic techniques and monographs (Shen et al., 2018). In some instances, the material available was too scanty to allow for an identification at the species level, and sometimes, even at the genus level. This was the case for six moss taxa, labelled as sp1–6, respectively (Table S4). Voucher specimens of each of the species included in the 1156 collections are kept at the Herbarium of the University of Liège (LG). The observations performed at the level of each sub-plot were then merged to produce presence-absence data for each of the moss and liverwort communities at the level of each plot (data available at https://doi.org/10.6084/m9.figshare.17057615.v8).

FIGURE 1 Experimental design and 3D microclimatic modelling of temperature (T), relative humidity (RH), photosynthetically active radiation (PAR) and light intensity (L) in a 1.44 ha tropical canopy crane facility, Yunnan, SW China. (a) Vertical profile of day (orange line) and night (black line) monthly averages (and standard deviation, grey ribbon) of T, RH, PAR and L modelled at the level of Tree #1; (b) topographic map of the study area representing the position of the 42 sampled trees in a x-y space and the modelled horizontal variation in monthly average of day T, RH, PAR, and L at 2 m ($n = 50$) and at 50 m ($n = 10$) height, respectively; (c) experimental design. Circle diameters in (b) are proportional to tree DBH.

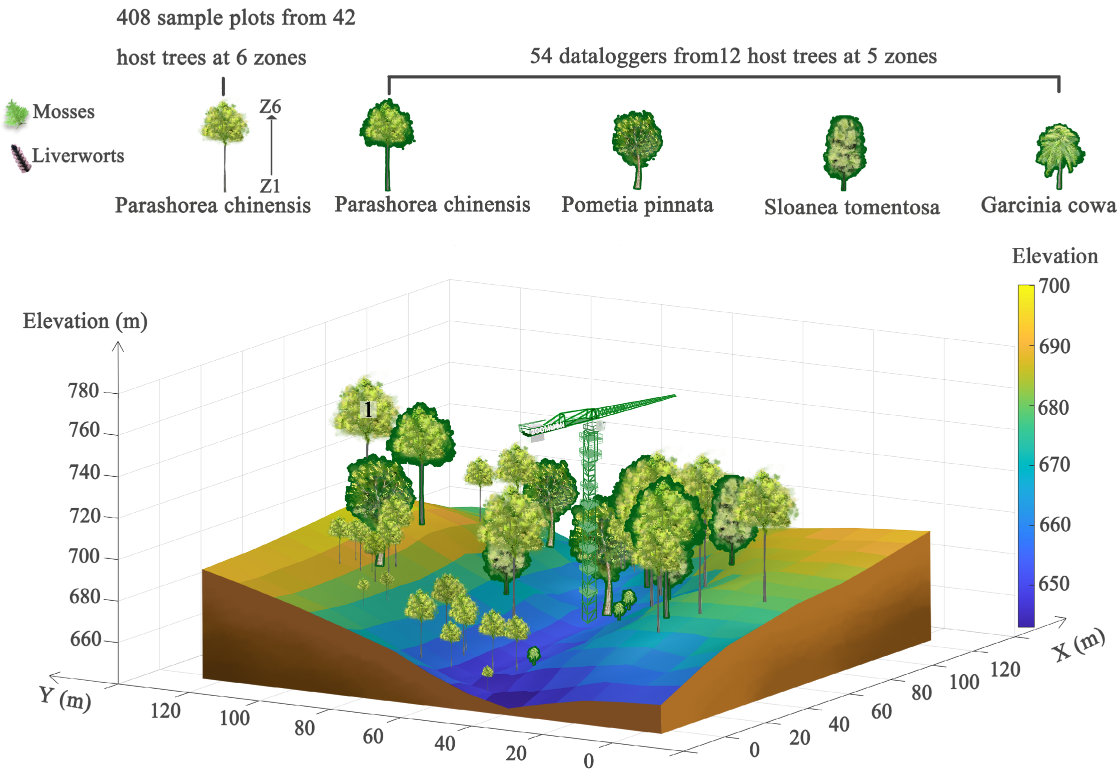
(a) Vertical microclimate #1



(b) Horizontal microclimate



(c) Experimental design in a 3D fine scale



2.2 | Geographic and environmental variables

Nine ecological and geographic variables were recorded at each plot and were used to derive differences in ecological and geographic conditions among plots for subsequent analyses (Figure 2a).

The *X*–*Y* coordinates of each tree (Shen, 2021a) were used to compute the horizontal distance (hereafter, 'GeoDist', ranging between 0 and 114.82 m) among tree bases and the relative position between two treesin the *x*–*y* space (distance to a reference point, 'TreePos'). Tree height and plot height on the tree (*Z* coordinate) were measured with a tape from the hanging basket of the canopy crane. DBH of each tree was measured at 1.3 m above ground. The difference in DBH (hereafter, 'ΔDBH') was computed among all pairs of trees. We measured the elevation at 10 m intervals via the autopilot vehicle (LiAIR VUX-1350) equipped with VUX-1UAV Laser (RIEGL Laser Measurement Systems GmbH) and generated a 10 m resolution map with the measurements as pixel centroids using the RASTER package (Hijmans, 2021). The difference

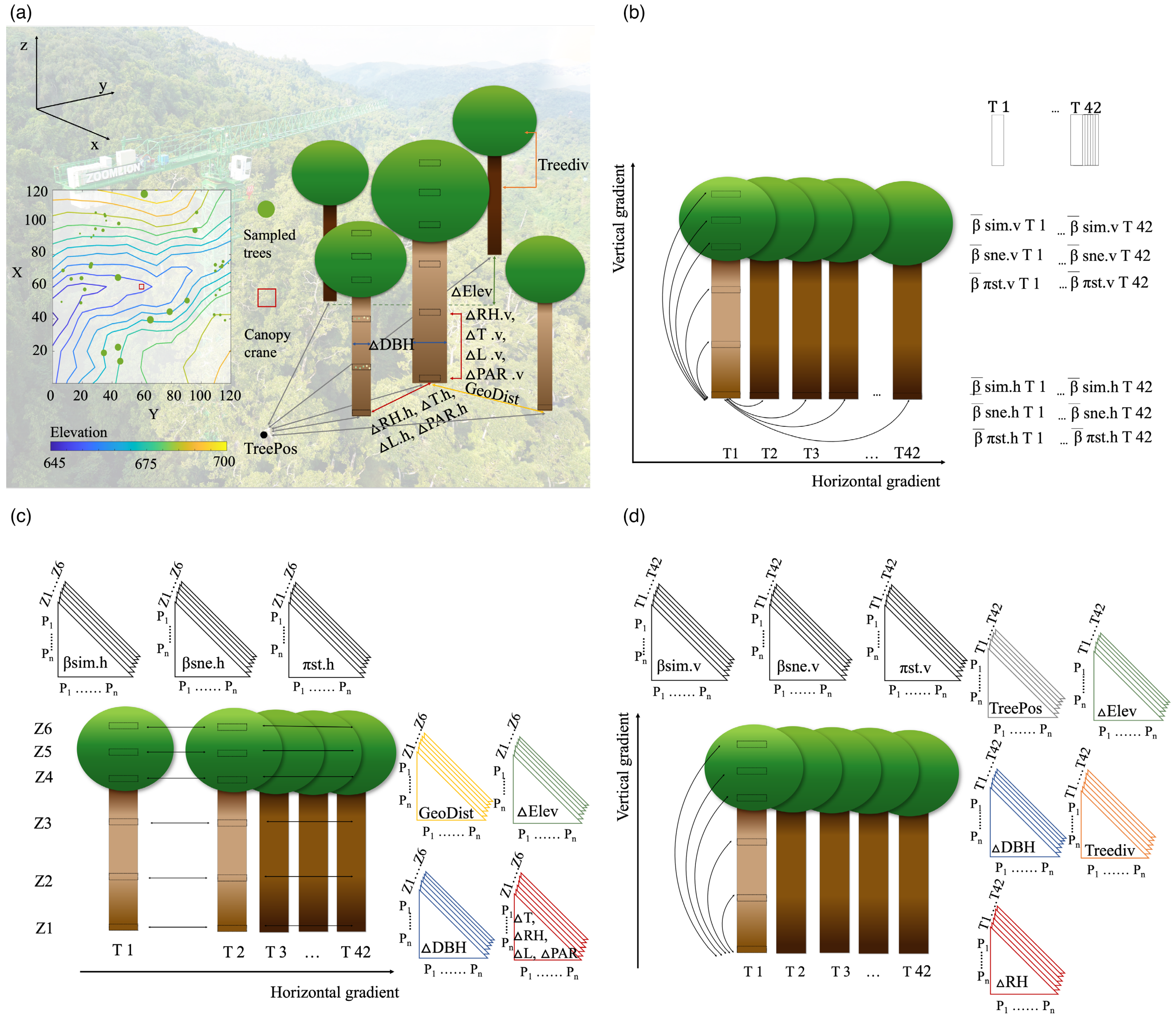


FIGURE 2 Statistical design implemented for the analysis of the turnover (βsim) and nestedness (βsne) components of beta diversity and the phylogenetic turnover (πst) among epiphytic moss and liverwort communities. (a) Factors used in the analyses. Ecological and geographic distances among plots used as predictors include the horizontal (.h) and vertical (.v) differences in relative humidity (ΔRH), temperature (ΔT), light (ΔL), photosynthetically active radiation (ΔPAR), microtopography (relative difference in elevation among trees), ΔElev, derived from a topographic map of the area (insert), a variable accounting for within vs among trunk/canopy comparisons (Treediv), a variable reporting the relative position between two trees in the *x*–*y* space (distance to a reference point, TreePos), difference in DBH among trees (ΔDBH), the geographic distance among trees (GeoDist); (b) comparisons of the vertical (.v) and horizontal (.h) variation in βsim, βsne and πst; (c) horizontal variation in βsim, βsne and πst within each of the six height zones (Z1–Z6) as a function of differences in ΔRH, ΔT, ΔL, ΔPAR, ΔDBH and GeoDist; (d) vertical variation in βsim, βsne and πst among height zones within trees as a function of ΔRH, ΔDBH, ΔElev, TreePos and Treediv.

in elevation ('ΔElev') was then computed among all pairs of trees. To characterize the main habitat difference between the trunk and the canopy, a binary variable ('Treediv') was used to describe whether plots were both located on the trunk or in the canopy. In pairwise plot comparisons, two plots located on the trunk or the canopy had a 'Treediv' of 0, whereas pairs of plots including one plot from the trunk and the other plot from the canopy had a 'Treediv' of 1. Finally, we generated a binary variable indicating whether pairs of plots being compared are located on the same tree (0) or on different trees.

Air temperature ('T', °C), relative humidity ('RH', %), photosynthetically active radiation ('PAR', μmol m^{-2} s^{-1}) and light intensity ('L', W m^{-2}) were used to characterize light and microclimatic environmental conditions. T and RH were measured by HYS15 air temperature and relative Moisture Sensors, Unism, China. PAR and L were measured by LI-190R Quantum Sensor, LI-COR Biosciences (Figure S1). These variables were recorded by 54 dataloggers every hour during 30 months from July, 2017 to December, 2019, to document the spatio-temporal variation of microclimates and calibrate microclimatic models (see below). To cover the range of vertical and horizontal microclimatic variation within the 1.44 ha plot, these dataloggers were located at regular height intervals on 12 trees scattered across the study area (Table S1). Because of datalogger failures, for instance during storm events, data could not be collected by all the dataloggers over this entire time period. To avoid missing data, we therefore averaged the values recorded for the same hour and month across years (data available at https://doi.org/10.6084/m9.figshare.17057624).

2.3 | Spatial microclimate modelling

To predict the light and microclimatic conditions at each of the 408 plots from the data collected by the 54 dataloggers, we modelled hourly variation in T, L, RH and PAR in an *X–Y–Z* space (thus including tree height and elevation) using Random Forest (Liaw & Wiener, 2002) as implemented by the RANDOMFOREST package in R v4.0.4 (R Development Core Team, 2021). Random forest is an efficient technique to model complex interactions among predictor variables (Cutler et al., 2007) and non-linear responses (Arulmozhi et al., 2021), which has increasingly been used in climatic modelling (Arulmozhi et al., 2021; Ellis & Eaton, 2021; Su et al., 2021). 80% and 20% of the data were used to train and assess the models, respectively. The models were tuned by searching the best hyperparameter values after 10-fold cross-validation (see Figure S2 for a flow chart of the protocol used). Model predictions were used to compute the Euclidian distance (ΔT, ΔRH, ΔL, ΔPAR) of the hourly difference in predicted microclimatic conditions between each pair of plots.

2.4 | Taxonomic and phylogenetic beta diversity

Taxonomic beta diversity was partitioned into nestedness (βsne) and turnover (here represented by Simpson's dissimilarity index, βsim) with the BETAPART package (Baselga & Orme, 2012). Nestedness occurs when species found at the poorest plots represent a subset of the species pool found in the richest plots (Baselga, 2010), reflecting, for example, the accumulation of species on trees with time. Species turnover, in turn, reflects the shift in species composition that typically occurs along ecological gradients, and is expected here among communities from the base to the canopy.

Phylogenetic turnover was quantified through the πst statistics, which is a measure of the average phylogenetic distance among species within versus among plots (Hardy, 2008; Hardy et al., 2012). To determine whether there was a significant phylogenetic overdispersion (πst < 0) or clustering (πst > 0) of epiphytic communities (Q2), we computed an average πst from all pairwise comparisons of plots, both within and among height zones and DBH classes. We determined whether πst was significantly lower or higher than expected by chance by comparing the distribution of observed πst values with that obtained with 100 randomized phylogenies among the tips to build the distribution of the null hypothesis. For each of the 100 randomly resolved phylogenetic trees, we re-computed the pairwise πst values among plots, which served to generate the distribution of 100 average πst among plots that would be expected if phylogenetic relationships among species were random. An observed average πst was significantly lower or higher than expected by chance if it was lower or higher than 95% of the values obtained after phylogeny permutations.

Phylogenetic distances among species pairs were computed from the moss and liverwort chronograms produced by Laenen et al. (2014). These chronograms resulted from large-scale analyses using genera as sampling units and including a single species per genus. The liverwort phylogeny was derived from the analysis of eight genes from all genomic compartments and includes 303 genera, representing 84% of the total extant generic diversity. The moss phylogeny was based on the analysis of one nuclear, one mitochondrial and one chloroplast gene and includes genera representing 64% of the total extant generic diversity of mosses. Phylogenetic trees were pruned to only keep the tips corresponding to observed species to generate suitable distributions of the null hypothesis (Hardy & Senterre, 2007). Twelve genera, which were not sampled in the phylogenies, were assigned to their closest genus based on phylogenetic evidence (Table S2). Since the phylogenies included a single species per genus, all congeneric species included in the present dataset were grafted onto the genus-level phylogeny, ensuring that phylogenetic relationships and branch lengths within genera were random and that the ages of genus crown nodes ranged between time present and the age of their stem node. In total, 100 trees with randomly resolved relationships among congeneric species were generated and separately analysed to take phylogenetic uncertainty into account. Taxa which could not be identified at the genus level were omitted from the analysis.

2.5 | Statistical analyses

Comparing vertical and horizontal patterns in βsim, βsne and πst (Q1) involves the inclusion of the same plot multiple times, violating the assumption that the observations are independent from each other. We therefore computed, for each tree, the average βsim, βsne and πst among plots on the same tree, avoiding comparisons among plots located within the same height zone. This generated a distribution of 42 average vertical βsim, βsne and πst (Figure 2b). We then computed, again for each tree, the average βsim, βsne and πst among plots between the focal tree and all other trees, making sure to restrict the comparisons among plots from the same height zone and to trees that belong to the same DBH class to avoid non-homologous comparisons (e.g. plots from the canopy of a 9 m and 70 m tree). The categories considered were small trees (DBH of 5.4–19.6 cm, *n* = 16), medium trees (DBH of 20.3–39.6 cm, *n* = 15), and large trees (DBH of 66.9–135 cm, *n* = 11). This generated 42 average horizontal βsim, βsne and πst distributions (Figure 2b). The vertical and horizontal distributions of average βsim, βsne and πst significantly departed from normality (Kolmogorov–Smirnov test, $p < 0.001$) and homoscedasticity (Bartlett's test, $p < 0.001$) for both mosses and liverworts. We therefore applied a paired Wilcoxon rank test to test the hypothesis that, on average, vertical βsim, βsne and πst are larger than horizontal βsim, βsne and πst values.

To disentangle the contribution of the factors affecting βsim and βsne along horizontal and vertical gradients, we implemented Generalized Dissimilarity Modelling (GDM; Ferrier et al., 2007). Because the GDM program needs values in the biological dissimilarity matrix ranging between 0 and 1, πst values were rescaled accordingly in these analyses. For horizontal gradients, we generated six matrices (Figure 2c), each of which encompassed all pairwise comparisons among plots located within the same height zone within and among trees. Predictors included GeoDist, ΔDBH, ΔElev, ΔT, ΔRH, ΔL and ΔPAR among each pair of plots. For the vertical patterns, we focused on pairs of plots located on the same tree and generated a matrix including all pairwise plot comparisons within the 42 trees (Figure 2d). Predictors included ΔT, ΔRH, ΔL, ΔPAR and Treediv. To inform the model of the structure in the data, wherein only within-tree comparisons were allowed, we added the variable TreePos.

To circumvent collinearity among predictors, we computed, for each analysis, the correlation between environmental predictors as well as the variation inflation factor (VIF). If any of the predictors exhibited a VIF > 5, the predictor with the highest VIF was removed. The VIF of the remaining variables was re-computed, and so on until all predictors had a VIF < 5 (Akinwande et al., 2015). We then performed variable significance testing with 50 permutations per step until only significant ($p < 0.05$) variables remained in the model. We finally estimated the contribution of each variable to the model using the gdm.varImp function (Fitzpatrick et al., 2021).

To determine how πst varies along environmental gradients, we performed analyses at the level of average πst within and among communities and pairwise πst among plots. We visualized the variation of average πst per DBH class and height zone as a function of an ordinal ecological distance, computed as the number of height zone difference between communities. The significance and strength of this relationship was assessed with a Mantel test (VEGAN package, Oksanen et al., 2020). We then performed a second series of analyses using pairwise plot comparisons using the GDM framework described above.

3 | RESULTS

3.1 | Microclimatic modelling

Microclimatic conditions exhibited substantial vertical variations (Figure 1a). Between 2 and 62 m above ground, day (8 am–7 pm) RH ranged between 53.6% and 99.9% (monthly average 79.7–93.5%), day temperature between 12.0 and 31.7°C (monthly average 17.8–27.8°C), light intensity between 2.3 $\mathrm{W\,m^{-2}}$ and 208.0 $\mathrm{W\,m^{-2}}$ (monthly average 27. 8–51.0 $\mathrm{W\,m^{-2}}$), and PAR between 0.0 $\mu\mathrm{mol\,m^{-2}\,s^{-1}}$ and 407.6 $\mu\mathrm{mol\,m^{-2}\,s^{-1}}$ (monthly average 41.4–94.0 $\mu\mathrm{mol\,m^{-2}\,s^{-1}}$). Average variations ± standard deviation (SD) in the day between 2 m and 50 m were of 2.4 ± 1.6°C for temperature, −16.7 ± 10.2% for RH, 85.0 ± 47.3 $\mathrm{W\,m^{-2}}$ for light intensity, and 128.0 ± 77.1 $\mu\mathrm{mol\,m^{-2}\,s^{-1}}$ for PAR. Horizontal variation was more subtle (Figure 1b), with average maximum variations (differences between maximum and minimum) in the day at 2 m and at 50 m reaching, respectively, 0.9 ± 0.5°C and 0.5 ± 0.3°C for temperature, 4.4 ± 1.9% at and 3.3 ± 2.1% for RH, 13.1 ± 13.5 $\mathrm{W\,m^{-2}}$ and 24.2 ± 18.0 $\mathrm{W\,m^{-2}}$ for light, 12.7 ± 12.4 $\mu\mathrm{mol\,m^{-2}\,s^{-1}}$ and 48.9 ± 40.4 $\mu\mathrm{mol\,m^{-2}\,s^{-1}}$ for PAR. This variation was captured by Random Forest models, with R^2 ranging from 0.96 for PAR to 0.99 for temperature (Figure S3; Table S3).

3.2 | Species richness and composition

Totals of 50 moss and 52 liverwort species were recorded (Table S4). The base (zone 1) was dominated by *Circulifolium microdendron*, *Caduciella mariei* and *Claopodium prionophyllum*. Along the trunk (zones 2–3), *Plagiochila parviramifera* and *Plagiochila fordiana* prevailed on small trees, and *Frullania monocera*, *Mastigolejeunea repleta* and *Caduciella mariei* on large trees. The most representative species were *Erythrodontium julaceum* and *Groutiella tomentosa* in the inner canopy of large trees, *Lejeunea flava*, *Cheilolejeunea eximia* and *Groutiella tomentosa* in the inner canopy of medium and large trees, and *Frullania ericoides*, *Acrolejeunea recurvata* and *Sematophyllum subhumile* in the outer canopy of large trees. The most frequent epiphylls were *Caudalejeunea reniloba*, *Cololejeunea planissima* and *Leptolejeunea subacuta*.

In mosses, species richness decreased from the base, with an average ± (SD) of 4.5 ± 1.5 species per plot (14.5 ± 3.3 species per DBH class) to 2.6 ± 1.0 species per plot (4.5 ± 2.5 species per DBH class) in the outer canopy of large trees. In liverworts in contrast, species richness increased from the base (small trees), with 1.4 ± 0.5 species per plot (7.6 ± 2.7 species per DBH class), to the outer canopy, with 2.9 ± 1.3 species per plot (12.3 ± 6.9 species per DBH class) on large trees (Figure 3).

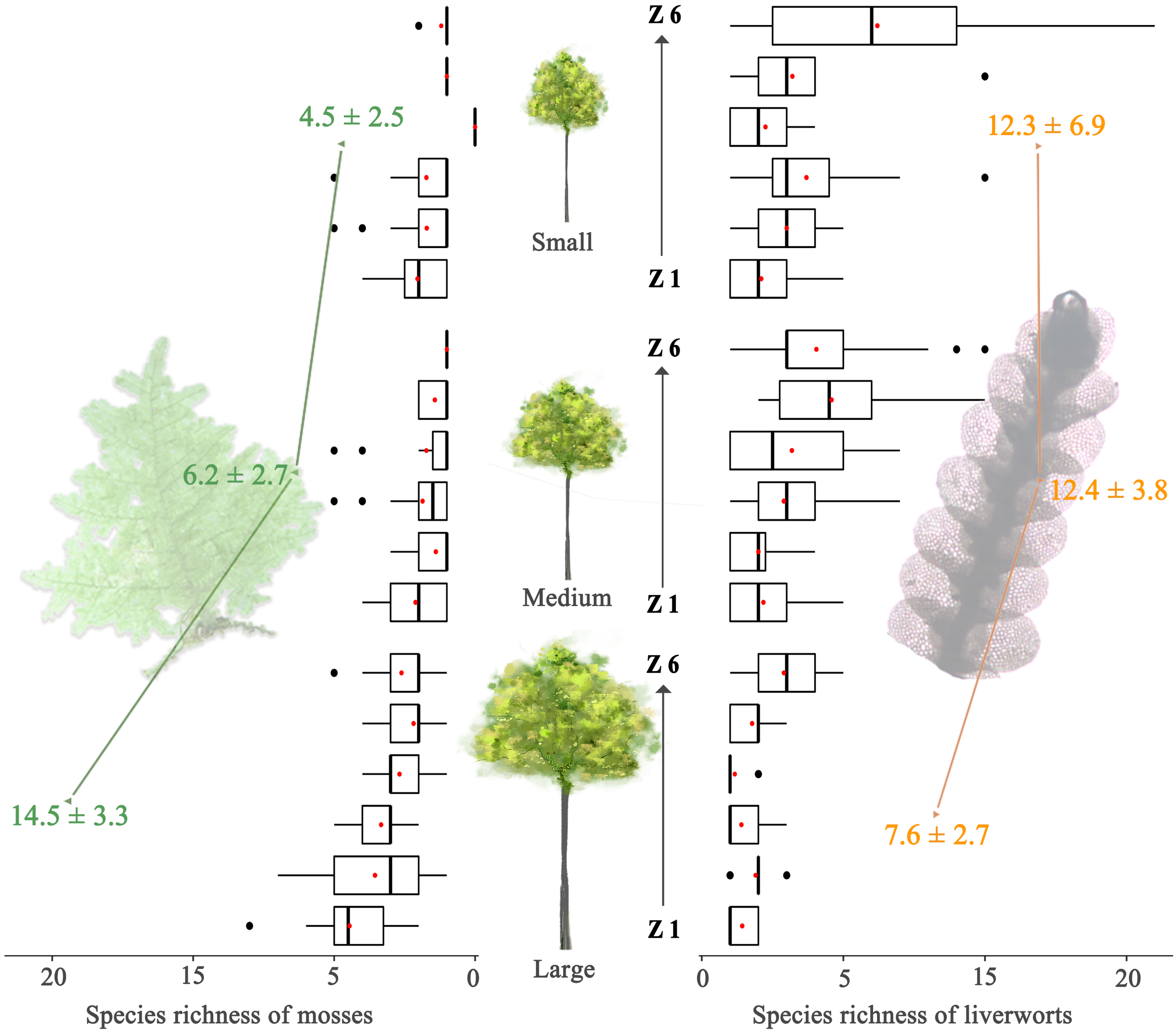


FIGURE 3 Vertical variation in species richness of epiphytic mosses (left) and liverworts (right) in a 1.44 ha tropical canopy crane facility, Yunnan, SW China. The box-plots [showing the first and third quartiles (upper and lower bounds), second quartile (center), average (red dots), 1.5* interquartile range (whiskers) and minima–maxima beyond the whiskers] represent species richness per height zone on small (DBH of 5.4–19.6 cm, $n = 16$), medium (DBH of 20.3–39.6 cm, $n = 15$) and large (DBH of 66.9–135.0 cm, $n = 11$) *Parashorea chinensis* individuals. The line represents the average (mean ± SD) of moss (green triangles) and liverwort (orange triangles) species richness per DBH class.

3.3 | Taxonomic beta diversity

Turnover contributed about three- to fivefold more to taxonomic beta diversity than nestedness (Figure 4). Vertical turnover of moss and liverwort communities was significantly higher than horizontal turnover (average ± SD of vertical $\beta sim = 0.59 \pm 0.25$ and 0.66 ± 0.24, average horizontal $\beta sim = 0.42 \pm 0.20$ and 0.50 ± 0.16 in mosses and liverworts, respectively, $p < 0.001$ for the differences between vertical and horizontal βsim in both mosses and liverworts). Nestedness exhibited the reverse pattern (average horizontal $\beta sne = 0.15 \pm 0.07$ and 0.14 ± 0.06, average vertical $\beta sne = 0.11 \pm 0.09$ and 0.14 ± 0.06 in mosses and liverworts, respectively, $p < 0.001$ for the differences between vertical and horizontal βsne in both mosses and liverworts).

In GDM analyses focusing on the horizontal variation in taxonomic turnover within the same height zone, which accounted, on average, for 19.3 ± 21.8% (mosses) and 11.1 ± 2.6% (liverworts) of the explained deviance across height zones, the difference in DBH among trees was the best predictor, with a relative contribution ranging between 64.7% and 99.8% across height zones (Table S5). For horizontal nestedness, no model was significant except for liverworts in height zone 2 (Table S5).

In analyses focusing on the vertical variation in beta diversity (Figure S4), models contributed to 33.3% and 17.8% of the total deviance of species turnover in mosses and liverworts, respectively. Difference in RH among plots was the most important variable in the model, contributing to 98.8% and 83.0% of the deviance in species turnover of mosses and liverworts, respectively, while Treediv

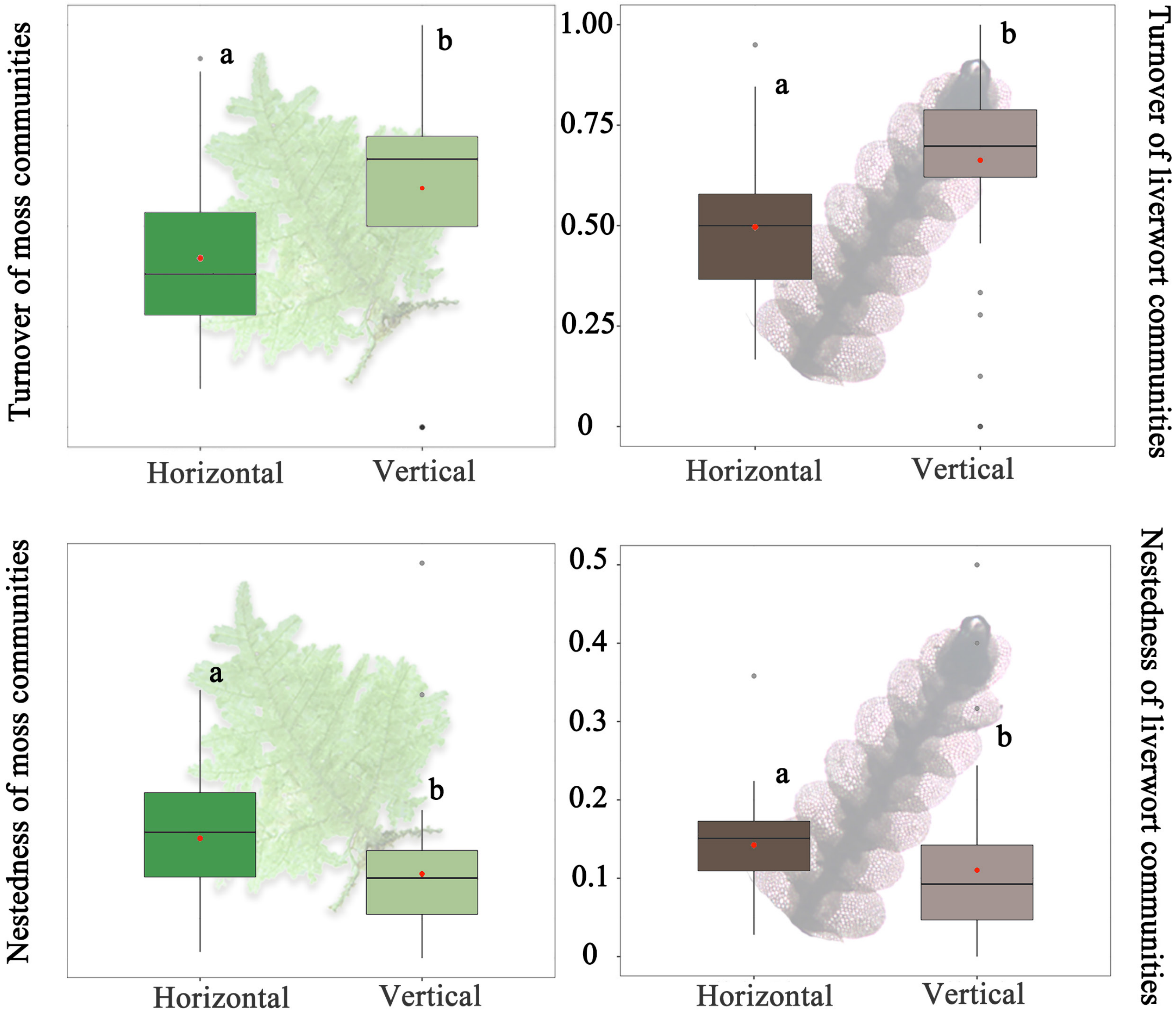


FIGURE 4 Vertical and horizontal patterns of turnover and nestedness in epiphytic mosses and liverworts on *Parashorea chinensis* in a 1.44 ha tropical canopy crane facility, Yunnan, SW China. Box-plots [showing the first and third quartiles (upper and lower bounds), second quartile (center), average (red dots), 1.5* interquartile range (whiskers) and minima-maxima beyond the whiskers] represent the vertical turnover, nestedness for pairs of plots on the same tree and horizontal turnover, nestedness within the same height zone and among trees belonging to the same class of diameter at breast height (DBH; small trees, DBH of 5.4–19.6 cm, medium trees, DBH of 20.3–39.6 cm, and large trees, DBH of 66.9–135 cm) of moss and liverwort epiphytic communities. Letters above each box-plot indicate which comparisons significantly differ.

contributed to less than 1% and 15%. No model was significant for the vertical variation in nestedness.

3.4 | Phylogenetic turnover

Average πst per height zone and DBH class significantly increased ($r = 0.25$, $p < 0.001$ for liverworts and $r = 0.09$, $p < 0.001$ in mosses) and shifted from mostly non-significant or rarely significantly negative (at the base of small trees in both mosses and liverworts and in the outer canopy of large trees in mosses, Table S6) to consistently significantly positive along a gradient of height zone differences (Figure 5).

In pairwise plot comparisons, horizontal phylogenetic turnover could not or could marginally be predicted from horizontal variation in microclimatic conditions and differences in DBH among trees (Table S5). For vertical phylogenetic turnover (Figure S5), the GDM accounted, on average across the 100 phylogenetic trees randomly resolved among congeneric species, for 6.6 ± 0.3% and 11.5 ± 0.7% of the total deviance in mosses and liverworts, respectively. In mosses, the best predictor was RH, which contributed to more than 99% of the explained deviance. A different pattern was observed in liverworts, where the variable accounting for within versus among trunk/canopy comparisons accounted for 65.6 ± 3.3% of the explained deviance, while the position of each individual tree in the

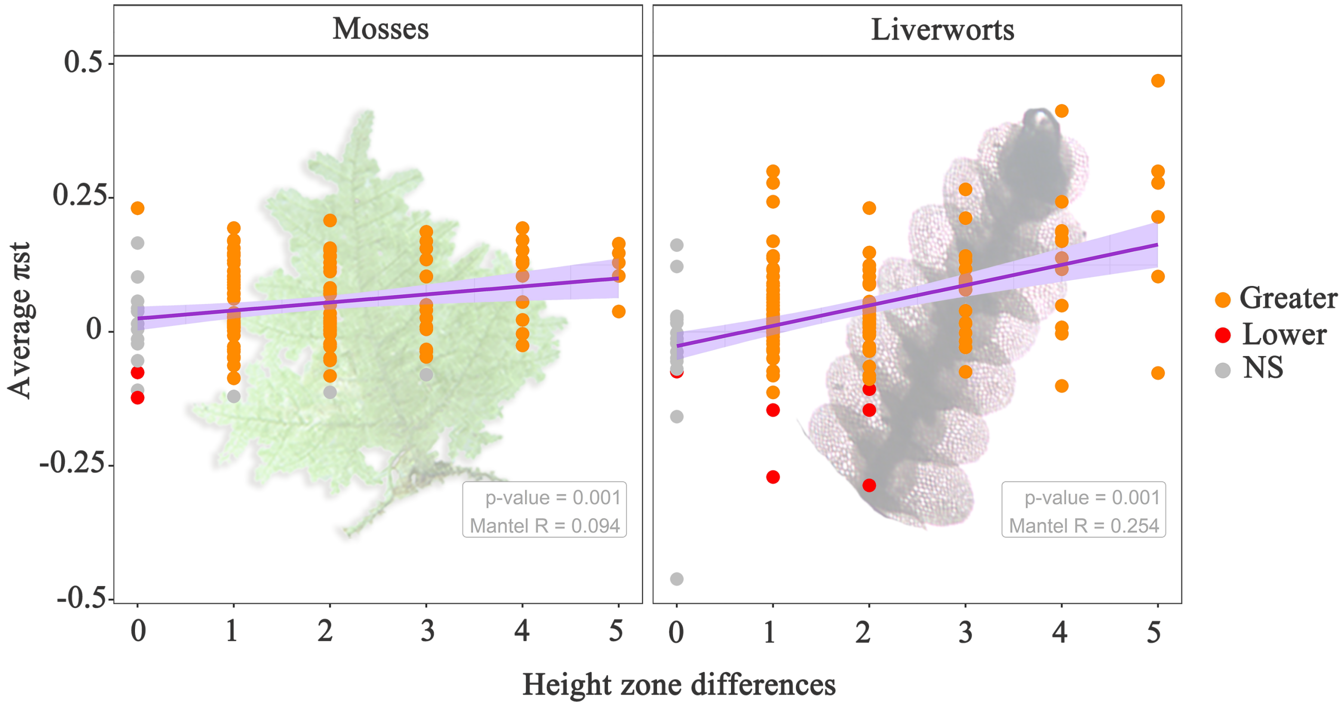


FIGURE 5 Variation of average phylogenetic turnover within epiphytic moss and liverwort communities from the same height zone and on trees from the same DBH class in a lowland dipterocarp forest (Xishuangbanna, Yunnan, SW China) along a gradient of height zone difference. Average πst for a given environmental distance class that were significantly greater, significantly lower, and non-significantly (NS) different than expected by chance, are represented by orange, red and grey dots, respectively.

x-y space and RH each contributed to less than 20% of the explained deviance.

4 | DISCUSSION

The microclimatic data reported here and their three-dimension modelling help to fill a gap in our knowledge of the spatial variation of forest microclimates and to address the question of how microclimatic variation within and below tree canopies impacts community species richness and composition (De Frenne et al., 2019; Ellis & Eaton, 2021; Murakami et al., 2022). In line with previous assessments of microclimatic variation within canopies (see Murakami et al., 2022 and references therein), variations in temperature and RH were progressively buffered from the canopy to the tree base, with a substantially higher day/night difference in temperature and RH in the canopy. Monthly average temperatures of 28.9°C were recorded in the upper canopy and 26.1°C at the base, in line with previous reports of a mean difference of 4°C between forest understory and open ground due to the absorption of solar radiation by the canopy and increased evapotranspirative cooling (De Frenne et al., 2019).

The much wider range of vertical versus horizontal variation in microclimatic conditions explains why vertical species turnover is significantly higher than horizontal species turnover, despite the large differences in habitat conditions between young and old trees in terms of bark texture and chemistry (Fritz & Heilmann-Clausen, 2010; Fritz, Niklasson, & Churski, 2009; Wagner et al., 2015) as well as the effect of time, reflected by age differences among host trees, which impacts on the likelihood of colonization (Hidasi-Neto et al., 2019). Due to the prevalence of vertical microclimatic gradients, the contribution of nestedness to vertical beta diversity is negligible because specialist species segregate among height zones. In Amazonian rainforests for instance, more than half of the epiphyte species are height-zone specialists (Mota de Oliveira et al., 2009). Consequently, communities from the canopy share almost no species with communities from the tree base, preventing any nested pattern from emerging.

Our models accounted for 33% and 18% of the variation in vertical turnover in mosses and liverworts, respectively, and the predominant contributions of microclimatic factors (83–98%) to this pattern evidences their crucial role in determining the composition of epiphytic communities. The similar explanatory power of microclimatic conditions for moss and liverwort species turnover hides, however, opposite patterns of species richness in the two groups, with moss richness decreasing and liverwort richness increasing from the base to the canopy. Horizontal turnover in the two groups was similarly explained by the same factor, that is, tree size, but moss species richness peaked on large trees, while liverwort species richness peaked on small trees. Epiphytic moss and liverwort community composition thus responded in an opposite way to the same gradients, highlighting substantial differences in niche preferences between them. In vascular epiphytes, large, old trees tend to host a higher epiphytic richness than young ones due to the larger amount of time for colonization, larger space availability and greater diversity of microhabitats (Mayumi Francisco et al., 2021; Patiño et al., 2018; Zotz & Schultz, 2008; Zotz & Vollrath, 2003), and have therefore been a major focus for conservation (Adhikari et al., 2021; Wang et al., 2017). Our results suggest that epiphytic bryophyte diversity assessments in tropical forests must also include small, understorey trees (Sporn et al., 2010), which should also be considered for conservation.

Despite a comprehensive set of environmental variables, our models accounted for only 1/5–1/3 of the variation in species turnover, within the range of similar analyses for vascular epiphytes (0.10–0.57, Zotz & Schultz, 2008; Woods et al., 2015). Although additional variables characterizing microhabitat conditions, such as bark texture and chemistry, branch diameter, or percentage cover of canopy humus (Woods et al., 2015), would certainly increase model accuracy, we interpret the large proportion of unexplained variance in terms of stochasticity associated with dispersal limitations. Although epiphytes need to track patches of suitable trees (or leaves in the case of epiphylls) in a dynamic landscape for persistence (Snäll et al., 2005), mounting evidence suggests that dispersal capacity is counter-selected in epiphytic bryophytes. Epiphytic bryophytes typically exhibit spatially clustered distributions (Löbel et al., 2006; Snäll et al., 2003; Wagner et al., 2015) and their fine-scale patterns of genetic variation are strongly spatially structured (Ledent et al., 2020; Vanderpoorten et al., 2019), pointing to important effects of isolation-by-distance. These patterns are paralleled by morphological adaptations counter-favouring dispersal. For instance, the peristome, a ring of hygroscopic teeth that enhance spore dispersal in mosses, and the seta, which elevates the capsule above the substratum, are typically reduced in epiphytic species (Hedenäs, 2012). Peristome reduction is itself significantly associated with hygrochasy, i.e., the release of spores under wet conditions (Zanatta et al., 2018), further decreasing chances of long-distance dispersal but enhancing rates of establishment (Johansson et al., 2016).

The shift between negative or non-significant average phylogenetic turnover to consistently significant clustering that was observed along a gradient of height zone differences suggests that phylogenetic constraints further contribute to shaping the assembly of epiphytic bryophyte communities. The slight, but significant correlation between this trend for an increasing phylogenetic clustering with variation in microclimatic conditions adds to emerging evidence for the role of phylogenetic niche conservatism in community assembly through time (Saladin et al., 2019; Segovia et al., 2020), including at the much smaller spatial scales of epiphytic communities. Phylogenetic niche conservatism in epiphytic bryophyte communities, along with mounting evidence for niche conservatism in vascular epiphytes (Müller et al., 2017), shows that the specialization for vertical niches and their associated microclimatic conditions is phylogenetically inheritable, and hence, that species may be limited in their ability to shift among niches. The deep phylogenetic level (genus-level phylogeny), at which the analysis was conducted, further points to deeply nested phylogenetic constraints, which may have evolved during the burst of diversification of epiphytic lineages triggered by the development of large, humid, megathermal angiosperm forests (Feldberg et al., 2014).

The fact that there was no significant horizontal phylogenetic clustering of liverworts communities, and that the horizontal phylogenetic clustering observed in moss communities was not explained by differences in DBH among trees, conversely suggests that the succession of communities on a tree depending on its age is not phylogenetically constrained. Typically, early pioneers are short-lived organisms with a high reproductive effort, whereas late-colonizers have a longer lifespan and are characterized by limited reproductive investment (During, 1992). Although restricted to a set of 42 trees in a specific 1.44 ha plot, our results thus suggest that these life-history strategies arose multiple times during the evolutionary history of epiphytes.

The negative πst observed at tree base in mosses and liverworts is consistent with the expectation that competition in epiphytic communities should occur at levels characterized by lower variation of daily and seasonal microclimatic conditions rather than high-up in the canopy (Spicer & Woods, 2022). Although even strong competition levels can leave no trace in community phylogenetic structure (Bennett et al., 2013), the non-significant phylogenetic turnover that mostly characterized communities from the same height zone and trees of the same DBH class does not support the idea that competition plays an important role in shaping epiphytic bryophyte communities. In vascular epiphytes of lowland rain forests, which use only a small proportion of the available bark surface, the importance of competition has been similarly questioned (Zotz, 2016; Zotz & Vollrath, 2003). Competition could, however, be more important in montane forests, where epiphytes are typically much more abundant (Burns & Zotz, 2010). Instances of niche displacement were already reported among epiphytic bryophytes (Wiklund & Rydin, 2004), raising a series of questions on how species may shift niche to avoid competition.

5 | CONCLUSIONS

We provide here, through the spatially-explicit modelling of microclimatic conditions in a tropical forest, explicit support for the long-held notion that vertical variation in light, temperature and humidity conditions are the main driver of epiphytic species turnover along a tree. Epiphytic bryophyte communities were phylogenetically clustered, and the low, but significant correlation between phylogenetic turnover among communities and vertical microclimatic variation evidences fine-scale phylogenetic niche conservatism. Despite the comprehensive description of the host-tree environment, our analyses captured, however, only 1/5–1/3 of the floristic variation among communities, calling for further improvements and opening the door to new research perspectives. First, our analyses did not allow us to assess the potential role of positive interactions. In vascular epiphytes, positive co-occurrence patterns suggest potential facilitation (Burns & Zotz, 2010; Ceballos et al., 2016), as a dense clumping of epiphytes could enhance temperature and drought stress, which is expected to increase towards the outer canopy (Spicer & Woods, 2022). Second, our analyses failed to consider bryophyte-vascular epiphyte interactions, whose role in the structuring of bryophyte communities should be further investigated as vascular and bryophytic epiphytes significantly co-occur (Lu et al., 2020; Zotz & Vollrath, 2003).

The tight link between community composition and microclimatic conditions, as well as evidence for niche conservatism, raise questions about the ability of epiphytic bryophyte communities to move down along the trunk to track the shift of their niche in the context of climate change. How macroclimatic changes will impact the changes within canopies remains, however, uncertain. While the statistical modelling of microclimatic conditions as we implemented here may successfully capture the spatial variation of microclimatic conditions, the potential of such approaches to forecast novel conditions is somewhat questionable, calling for the development of mechanistic models based on first-principles physics (Maclean & Klinges, 2021).

AUTHOR CONTRIBUTIONS

A.V., T.S., L.S., R.T.C. conceived the study. T.S., L.S., W.-Z.M., J.W., Y.-M.W., L.M. and Y.L. collected the data. T.S., F.C., T.K., Y.S. and L.M. performed the analyses. F.C., X.L.G., J.P. and O.H. provided suggestions throughout the process. A.V., T.S., R.T.C. and L.S. wrote the paper with the assistance from all co-authors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The raw information on each individual tree and epiphytic species distribution data are available at https://doi.org/10.6084/m9.figshare.17057615.v8 (Shen, 2021a). The microclimatic data are available at https://doi.org/10.6084/m9.figshare.17057624 (Shen, 2021b).

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SUPPORTING INFORMATION

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Appendix II

V. **Lea Mouton**, Jairo Patiño, Marc Carine, Fred Rumsey, Miguel Menezes de Sequeira, Juana María González-Mancebo, Rosalina Maria de Almeida Gabriel, Olivier J. Hardy, Manuela Sim-Sim, J. Alfredo Reyes-Betancort, Flavien Collart, and Alain Vanderpoorten. (2023). Patterns and drivers of beta diversity across geographic scales and lineages in the Macaronesian flora. Journal of Biogeography. 50, 858–869. https://doi.org/10.1111/jbi.14580

cover picture: *Euphorbia canariensis*; Tenerife in July 2022, from Lea Mouton

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RESEARCH ARTICLE

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Patterns and drivers of beta diversity across geographic scales and lineages in the Macaronesian flora

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Abstract

Aim: How spatial, historical and ecological processes drive diversity patterns remains one of the main foci of island biogeography. We determined how beta diversity varies across spatial scales and among organisms, disentangled the drivers of this variation, and examined how, consequently, biogeographic affinities within and among archipelagos vary among land plants.

Location: Macaronesia.

Taxon: Bryophytes, pteridophytes, spermatophytes.

Methods: Species turnover and nestedness were compared within and among archipelagos across taxonomic groups. The relationship between species turnover and nestedness, climatic, geological and geographic factors was analysed using generalized dissimilarity models.

Results: Species turnover, but not nestedness, increased with the geographic scale. This increment decreased from spermatophytes, pteridophytes and bryophytes, wherein the median turnover was less than half that in spermatophytes. Bryophytes exhibited a significantly higher nestedness and lower turnover than spermatophytes. Extant climatic conditions and island age contributed the most to all models but the importance of island age for bryophyte and pteridophyte turnover was marginal. Spermatophyte floras clustered by archipelago, whereas the clustering patterns in pteridophyte and bryophyte floras reflected macroclimatic conditions.

Main Conclusions: The lower increment of species turnover with spatial scale and the higher nestedness in bryophytes and pteridophytes than in spermatophytes reflect the variation in dispersal capacities and distribution ranges among land plants. Accordingly, extant climatic conditions contributed more to explain turnover in bryophytes and pteridophytes than in spermatophytes, whereas factors associated with dispersal limitations, including island age, geographic distance and archipelago structure, exhibited the reverse trend. The differences in beta diversity patterns, caused by different responses of Macaronesian land plant lineages to the main factors shaping their community composition, explain their different biogeographic affinities. These differences reflect a distinct origin and different mechanisms of speciation among Macaronesian land plant lineages and archipelagos.

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1 | INTRODUCTION

How spatial, historical and ecological processes drive diversity patterns, and how area, elevation and isolation influence community composition, are two of the main foci of island biogeography (Patiño et al., 2017). Beta diversity, the variation of biological communities through space and time, measures changes in community composition along environmental, spatial and temporal gradients (Soininen et al., 2018). This metric can be partitioned into two process-related components: species replacement (turnover) and richness difference (nestedness) (Baselga, 2010). Species turnover characterizes changes in species composition along gradients of geographical and/or ecological distance (Qian et al., 2020), whereas nestedness occurs when a set of species at one site is a subset of the species at a richer site, which has been interpreted in terms of ordered extinction events (Baselga, 2010).

Beta diversity varies depending on both extrinsic (environmental) and intrinsic (biological) factors related to species niche breadth and dispersal capacities. For instance, species turnover is expected to be inversely proportional to species dispersal capacities (Soininen et al., 2018; Varzinczak et al., 2019). In land plants, this hypothesis is in line with the steeper slope of the species-area relationship reported in spermatophytes than in pteridophytes and bryophytes explained by the production of smaller, wind-dispersed diaspores in the two latter groups (Patiño, Weigelt, et al., 2014). Furthermore, since nestedness can only arise for areas that share a common source pool, a high nestedness at large scales is expected in organisms with high dispersal capacities (Greve et al., 2005).

Beta diversity also varies depending on geographic scale (Soininen et al., 2018). If an increase in species turnover with geographic scale due to dispersal limitations and stronger environmental filtering over larger environmental gradients has been recurrently documented (Gusmao et al., 2020; Qian et al., 2020; Soininen et al., 2018), the relationship between nestedness and geographic scale has been more controversial. Nestedness is expected to peak at a small scale if local variations of habitat quality and availability lead to variation in species richness within habitats among sites (Gusmao et al., 2020). In contrast, high nestedness at large scales is expected when extinctions vary depending on major geographic gradients (Soininen et al., 2018). In particular, nestedness is expected to increase with latitude (Batista et al., 2021; Soininen et al., 2018).

Oceanic islands offer an ideal framework to investigate the variation of species turnover and nestedness across geographic scales and to determine the relative contribution of contemporary climatic factors and dispersal limitations associated with geographic isolation. Oceanic islands are readily geographically circumscribed (Whittaker & Fernández-Palacios, 2007), offering naturally isolated and often replicated operational geographic units (OGUs). Furthermore, because they were colonized de novo, oceanic islands accumulate species from continental or alternative insular sources at rates depending on connectivity and in situ speciation (Whittaker & Fernández-Palacios, 2007) that vary with geographic isolation, environmental complexity, island age and species dispersal capacities (Heaney, 2000).

In particular, the three northern archipelagos of the Macaronesian region (the Azores, Madeira and Canary Islands) have long been identified as an excellent model for hypothesis testing in biogeography (Florencio et al., 2021). In fact, these archipelagos vary in terms of geographic isolation and macroclimatic conditions, so that their floras exhibit sharply different distribution patterns. In the Canarian spermatophyte flora, single-island endemics (SIEs) are much more frequent than multiple-island endemics (MIEs), whereas the reverse pattern prevails in the Azores (Carine & Schaefer, 2010). This, together with the sharper ecological gradients in the Canaries (and to some extent in Madeira), has led to the idea that speciation in the Canaries is primarily driven by ecological radiations, whereas allopatric speciation prevails in the Azores among islands that are more distant among each other and from continental sources than in the Canaries (Carine & Schaefer, 2010; Price et al., 2018). Spore-producing plants exhibit a lower proportion of SIEs and a higher proportion of Macaronesian regional endemics (i.e. taxa that are endemic to two or more Macaronesian archipelagos) than spermatophytes (Vanderpoorten et al., 2011), owing to their higher dispersal capacities, allopatric speciation modes and ecological affinities (Patiño, Carine, et al., 2014). Therefore, biogeographic relationships across Macaronesia vary among major land plant groups. The grouping of spermatophyte floras reflects the structure of archipelagos (de Nicolás et al., 1989), whereas floristic analyses at the archipelago level revealed conflicting relationships among spore-producing floras (Vanderpoorten et al., 2007).

Building on previous analyses on the drivers of the spatial variation of plant species richness (Aranda et al., 2014; Hobohm, 2000) on the one hand, and on the partitioning of beta diversity in Macaronesian vascular floras (Chiarucci et al., 2010) on the other, we examine how the components of beta diversity vary across spatial scales among the four main lineages of land plants, namely liverworts, mosses, pteridophytes and spermatophytes, and identify what are the drivers of this variation. In this framework, we address the following questions and test the following hypotheses:

- How does beta diversity vary across taxa and archipelagos? We expect that species turnover increases from intra- to inter-archipelago comparisons (H1a). We expect this increase to be significantly lower for spore-producing plants than for spermatophytes (H1b).
- Does nestedness decrease with increasing spatial scale and do the patterns differ for different groups? We test the hypothesis that nestedness decreases from intra to inter-archipelago comparisons (H2a). We expect that this decrease is stronger from spermatophytes to ferns, and then, bryophytes (H2b), due to the presumed higher dispersal capacities and, hence, higher proportion of shared species across archipelagos in spore-producing plants.
- What are the drivers of beta diversity, and how do they vary among taxa? We expect that climatic variation is more important than geographical isolation and island age in explaining species turnover for bryophytes and pteridophytes, and that geographical isolation does not correlate with bryophyte and pteridophyte nestedness, whereas we expect the reverse patterns for spermatophytes (H3).
- How do biogeographic affinities within and among archipelagos vary among land plants? Following de Nicolás et al., (1989) and del Arco Aguilar and Rodríguez Delgado (2018), we expect that islands cluster primarily by archipelago in spermatophytes, but not in spore-producing plants, wherein islands are expected to cluster depending on climatic similarity, irrespective of the archipelago in which they are found (H4).

2 | MATERIALS AND METHODS

2.1 | Study area

Macaronesia (Figure 1) is a biogeographic region located in the Atlantic Ocean between 15.8 and 40.8°N (Florencio et al., 2021). We focused here on the Azores, Madeira (including Madeira, Porto Santo, and the Desertas islands) and the Canary Island archipelagos. Within the latter, we tested the impact of the inclusion of the islets of Isla de Lobos, Montaña Clara, Alegranza and Graciosa, whose size and elevation are, respectively, more than 95 and six times lower than that of the main Canarian islands. The Desertas include Deserta Grande, Chão and Bugio. Due to their very small size, close proximity to each other, connection during the Last Glacial Maximum and very low species richness, these islands are treated as a single unit in available species lists, a position that we also adopted here. We excluded Cabo Verde, whose cryptogamic flora clearly belongs to sub-Saharan Africa (Vanderpoorten et al., 2007), and the Selvagens, whose very limited flora and number of islands did not warrant inclusion in the present analyses.

2.2 | Data collection and matrices

Lists of moss, liverwort, pteridophyte and spermatophyte species per island were retrieved from a review of the literature and personal unpublished observations of the authors (Appendix S1). Sub-species, whose circumscription is typically associated with a restricted geographic range (Mallet, 2013), and are hence potentially informative in spatial analyses of taxonomic composition among OGUs, were included. Due to the very low number of hornwort species (6), the latter were included within liverworts, to which they are the most similar in terms of morpho-anatomy and life-history traits. Nomenclature was standardized following Hodgetts et al. (2020) for mosses and liverworts, Hassler (2018) for pteridophytes and Euro+Med (2006) for spermatophytes. In bryophytes, *Isothecium interludens*, *Racomitrium affine*, *Lophocolea coadunata* and *Frullania tamarisci* agg., which have recently been raised at species level, were not distinguished pending for a critical re-assessment of their distributions. *Bryoxiphium madeirense* was considered as a synonym of *B. norvegicum* based on phylogenetic evidence (Patiño et al., 2016).

Introduced species were excluded from the analysis as they lead, at the geographic scale of entire islands, to a substantial human-induced homogenization of their floras (Otto et al., 2020). Assessing the native status of a taxon is challenging, and this status may further vary for the same taxon among archipelagos (e.g. *Clethra arborea*, Madeiran endemic invasive in the Azores), or even among islands from the same archipelago (e.g. *Echium nervosum* introduced from Madeira to Porto Santo), preventing us from defining a global list of introduced species across Macaronesia. We therefore relied on the status defined for each taxon by the most recent databases (Appendix S1) for each archipelago. Many widespread Mediterranean species considered as native in the Canary Islands and Madeira, such as *Helminthotheca echioides*, *Hypochaeris radicata* and *Trifolium* spp., are, however, considered as introduced in the Azores, whereas they thrive in the same kinds of habitats across archipelagos. We thus performed a second set of analyses, wherein all species considered as introduced in the Azores, and for which unambiguous evidence of a native status was missing in the Canary Islands and Madeira is missing (Table S16), were excluded. Species distribution matrices were employed to compute, for each of the four lineages considered, species turnover, expressed here as Simpson dissimilarity index (βsim) and nestedness-resultant dissimilarity (βsne) among all possible pairs of islands using the package 'betapart' (Baselga et al., 2021) in R 4.2.1. (R Core Team, 2020).

Environmental variables included climatic conditions (annual mean temperature, minimum annual temperature range, annual precipitation and minimum coefficient of variation in monthly precipitation), geographic distance among islands, area, elevation, distance to the closest mainland, and age of each island. The first nine variables were recorded from Weigelt et al. (2013) while island age was obtained from Torre et al. (2019).

2.3 | Data analysis

Comparing turnover (βsim) and nestedness (βsne) among islands within and among archipelagos (H1a, H2a, Figure 1) involves the inclusion of the same observation multiple times (the same

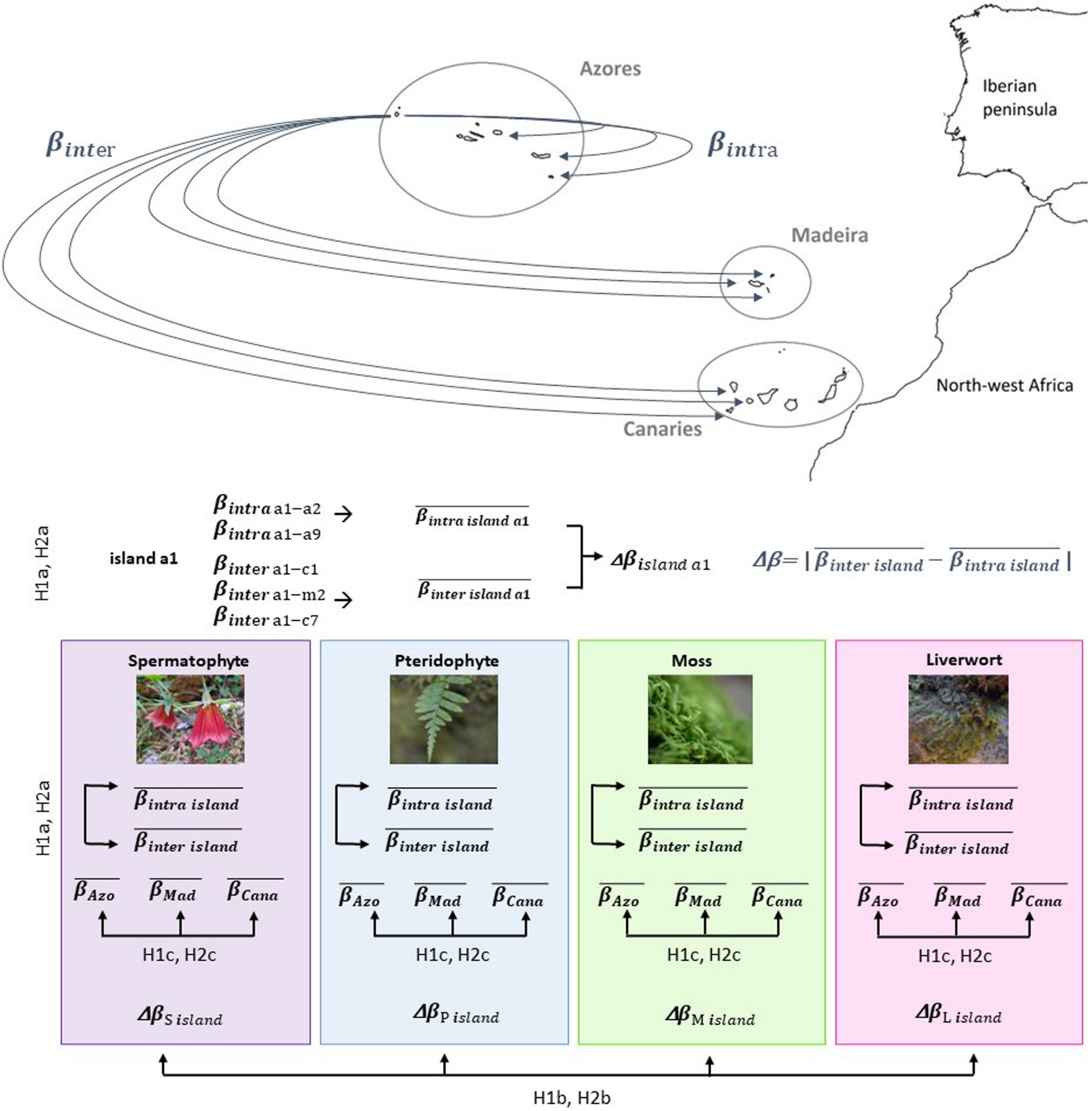

FIGURE 1 Study area and statistical framework to calculate beta diversity (β) and its two components, turnover (βsim) and nestedness (βsne) between islands within (intra) and between (inter) archipelagos (Azores: Azo, 9 islands labelled as a1-a9, Canary Islands: Cana, 7 islands labelled as c1-c7, and Madeira: Mad, 3 islands labelled as m1-m3) among land plant lineages (spermatophytes: S; pteridophytes: P; mosses: M; and liverworts: L).

occurrence of a species on an island serving to compute βsim and βsne both within and among archipelagos), violating the assumption that the observations are independent from each other. We therefore computed, for each island, the average βsim and βsne values with all the other islands from the same archipelago (βintra, Figure 1). Then, we computed, for each island, the average βsim and βsne values with each island from the other archipelagos (βinter, Figure 1). The average β values within archipelagos were finally compared to those among archipelagos. Although the data were homoscedastic (Fisher test = 1 for all lineages), departure from normality for the turnover of spermatophytes and for the nestedness of all lineages (Shapiro test, $p < 0.01$) led us to apply paired Wilcoxon rank tests.

To assess the variation of β from intra- to inter-archipelago comparisons (H1b and H2b, Figure 1), we computed, for each island, the difference ($\Delta\beta$) of the average β between that island and all other islands from the same archipelago (βintra) and the average beta between that island and all other islands from different archipelagos

(βinter) (Figure 1). $\Delta\beta$ values were not normally distributed in the case of turnover for spermatophytes, and in the case of nestedness for mosses, ferns, and spermatophytes. Therefore, non-parametric Friedman's and post-hoc Nemenyi tests, applying Bonferroni correction on the *p*-value, were implemented with the package 'PMCMRplus' (Pohlert, 2021) to search for significant differences of $\Delta\beta$ values per island among lineages. In order to assess, for each lineage, differences of nestedness and turnover among archipelagos, we implemented Kruskal–Wallis and post-hoc Dunn tests, applying Bonferroni correction for multiple comparisons, with the package 'PMCMRplus' (Pohlert, 2021).

To determine how beta diversity varies depending on geographic distance (both among islands and between islands and nearest continents) and variation in climatic conditions, age, area, and elevation across taxa and archipelagos (H3), Generalized Dissimilarity Model (GDM, Ferrier et al., 2007) was employed using the 'gdm' R package (Fitzpatrick et al., 2021) for each of the four lineages independently. To control for the archipelago structure of the data, we assigned each pair of islands from the same archipelago a distance of 0, and each pair of islands from different archipelagos a distance of 1, and then treated this binary distance measure in the same manner as geographical distance (Ferrier et al., 2007). To avoid multicollinearity, we computed Pearson correlation coefficients among each pair of predictors and kept one predictor among any pair with a correlation coefficient >0.75. As annual mean temperature, annual precipitation, distance to the closest mainland, distance among islands and minimum coefficient of variation in monthly precipitation were strongly correlated, we kept only the latter, hereafter referred to as 'precipitation'. This approach allowed us to identify six predictors, including area, elevation, minimum annual temperature range, island age, precipitation and archipelago. We implemented stepwise backward variable elimination as implemented in the gdm.varImp function of the package 'gdm' (Fitzpatrick et al., 2021) until all variables had a $p < 0.05$.

To address hypothesis H4, a cluster analysis of islands as a function of their taxonomic composition was performed using Ward's algorithm based on a total beta diversity matrix derived from Sørensen distances. The optimal number of clusters for each lineage was determined with the 'NbClust' package (Charrad et al., 2014) using 30 indexes (i.e. all indices except GAP, Gamma, Gplus and Tau). To help visualizing the groupings, a classical (Metric) multidimensional scaling (MDS) based on the Sørensen distance matrix was performed.

All the analyses were repeated without the four Canarian islets to examine the impact of the latter on the explanatory power of the models and the clustering patterns, and without 172 spermatophyte species considered as introduced in the Azores, and for which unambiguous evidence of a native status in the Canary Islands and Madeira is missing.

3 | RESULTS

Our species distribution matrices (10.6084/m9.figshare.17099840) included 226 liverwort & hornwort species, 516 moss species, 83 pteridophytes species and 1810 spermatophyte species and subspecies. The proportions of endemics per lineage and archipelago are displayed in Table 1.

For all lineages, paired Wilcoxon tests showed a significant increase of turnover among islands from intra to inter-archipelago comparisons (Figure 2). No significant variation from intra- to inter-archipelago comparisons was observed for nestedness (Table S1). The difference in turnover between intra and inter-archipelago comparisons ($\Delta\beta$sim) was significantly higher in spermatophytes (0.57 ± 0.12) than in bryophytes and pteridophytes (Friedman test, $p < 0.01$). $\Delta\beta$sim in pteridophytes (0.34 ± 0.08) was significantly higher than in mosses (0.25 ± 0.08) but not than in liverworts (0.28 ± 0.07). Among bryophytes, $\Delta\beta$sim did not differ significantly.

Turnover among islands within archipelagos did not significantly vary among archipelagos for mosses and liverworts (Figure 3a). Turnover was significantly higher in the Canary Islands than in the Azores and Madeira in pteridophytes. For spermatophytes, turnover in the Canary Islands was significantly higher than in the Azores. Nestedness among islands within archipelagos was consistently higher in Madeira than in the Azores and the Canary Islands across lineages (Figure 3b). Probably due to the low statistical power associated with the low number of islands in Madeira (3), the difference of turnover between Madeira and the Canaries, and of nestedness between Madeira and the Azores were, however, not significant for spermatophytes (Tables S3 and S4).

The variation in species turnover and nestedness within archipelagos among lineages is illustrated in Figure 4. Turnover was significantly higher in mosses and spermatophytes than in pteridophytes and liverworts. Mosses, liverworts and pteridophytes exhibited a significantly higher nestedness than spermatophytes.

The GDM explained 67%, 62%, 79% and 92% of the deviance of liverwort, moss, pteridophyte and spermatophyte turnover, respectively. Precipitation and island age were the variables most contributing to all models, but with different relative contributions across lineages (Table S5). Thus, the contribution of island age was about half that of precipitation in spermatophytes, but only about 1/10–1/20 in bryophytes and pteridophytes. The factor 'archipelago' was selected only in spermatophytes.

The GDM explained less than 50% of nestedness in liverworts, mosses and spermatophytes and 68% in pteridophytes. Elevation and temperature were selected in all models, but the contribution of the former was about three times higher than that of the latter. Area was selected for spermatophytes while island age was selected for all the spore-producing plants (Table S6).

The groups resulting from the clustering analyses of islands as a function of their species composition in each lineage are shown on the first plane of the NMDS of islands depending on the floristic distance among them (Figure 5). The stress values, a goodness-of-fit statistic that is minimized in MDS and characterizes the extent to which the actual floristic dissimilarities among islands are well represented on the plot, were 0.032 for liverworts, 0.030 for mosses, 0.031 for pteridophytes and 0.042 for spermatophytes, indicating good to excellent fit. In spermatophytes, the

TABLE 1 Numbers of native and endemic species and subspecies of liverworts, mosses, pteridophytes and spermatophytes in the Azores, Canary Islands, Madeira archipelago and Macaronesia (Macaronesian endemics corresponding to species or subspecies restricted to Macaronesia but distributed across at least two archipelagos).

	Azores	Madeira	Canaries	Macaronesia
Liverworts				
Endemic/total/%	3/156/2	4/182/2	1/148/0.7	12/226/5
SIE/% SIE	0/0	2/1	0/0	—
MIE/% MIE	3/2	2/1	1/0.7	12/4
Mosses				
Endemic/total/%	7/295/2	8/359/2	6/359/1.7	16/516/3
SIE/% SIE	3/1	7/1.95	2/0.7	—
MIE/% MIE	4/1	1/0.5	4/1	16/1
Pteridophytes				
Endemic/total/%	4/53/8	5/59/8	3/58/5	7/83/8
SIE/% SIE	0/0	05 August	0/0	—
MIE/% MIE	4/8	0/0	3/5	7/8
Spermatophytes				
Endemic/total/%	69/167/41	141/683/21	653/1398/47	57/1810/3
SIE/% SIE	8/5	97/14	416/30	—
MIE/% MIE	61/36	44/6	237/17	57/3

Abbreviations: MIE, %MIE: number and proportion of multiple islands endemic species and subspecies; SIE, %SIE: number and proportion of single island endemic species and subspecies.

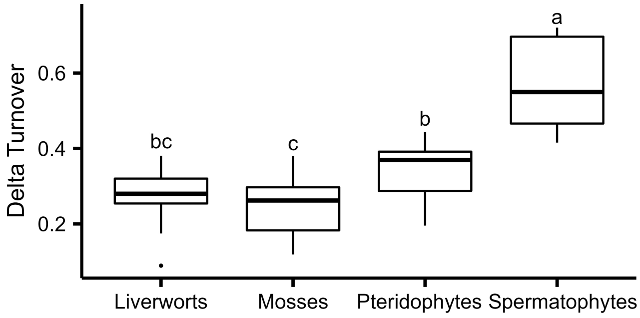

FIGURE 2 Box-plots (showing the first and third quartiles (upper and lower bounds), second quartile (centre), 1.5×interquartile range (whiskers) and minima-maxima beyond the whiskers) of the difference of turnover ($\Delta\beta$sim) of liverwort, moss, pteridophyte and spermatophyte communities among islands within (intra) and among (inter) archipelagos in Macaronesia (see Table S1 for actual values). Letters above each box-plot indicate, which comparisons significantly differ (see Table S2 for the *p*-values of the post-hoc Friedman Nemenyi tests), identical letters being used for lineages whose turnover does not significantly differ from each other.

three clusters identified correspond to the Azores, the Canary Islands and Madeira. In liverworts, the Azorean islands clustered together, the Canarian islands of Fuerteventura and Lanzarote, and the Desertas of Madeira archipelago, formed a second cluster, while Madeira clustered with Porto Santo and the remaining Canary Islands. The grouping observed with the moss floras was almost identical, except that Porto Santo clustered with Fuerteventura and Lanzarote and the Desertas. In pteridophytes, Madeira clustered with the Azores while Porto Santo and the Desertas (Madeira archipelago), Lanzarote and Fuerteventura (Canary Islands) formed a second cluster, and the western and central Canary Islands formed a third final cluster.

The results of the analyses including Isla de Lobos, Montaña Clara, La Graciosa and Alegranza in the Canary archipelago are described in S7-S15. The most important differences with the analyses on the main islands include (i) an increase of the average nestedness among the Canarian islands in spermatophytes and mosses from 0.116 to 0.236 and from 0.233 to 0.564 (Tables S4 and Table S10); (ii) a decrease of the deviance explained by the GDM for the turnover in mosses (33% vs 62%) and pteridophytes (30% vs 80%), while no significant model was obtained for liverworts nor spermatophytes (Table S13); and (iii) the clustering of the islets independently from the main Canarian islands (Figure S15).

Analyses excluding 172 species (listed in Table S16) considered as introduced in the Azores, and for which unambiguous evidence of a native status was missing in the Canary Islands and Madeira (S17–S24), yielded similar results to those of the complete dataset.

4 | DISCUSSION

Our analyses on the spatial patterns of variation in plant beta diversity components across Macaronesia revealed that turnover, but not nestedness, significantly increased from intra to inter-archipelago comparisons. The increase of turnover with the extent of the geographic scale is in line with our hypothesis H1a, based on theoretical and empirical evidence pointing to the role of stronger dispersal limitation and environmental filtering owing to stronger environmental gradients and larger geographic distances across larger spatial scales (Menegotto et al., 2019; Soininen et al., 2007, 2018). In contrast with our second hypothesis (H2), our analyses

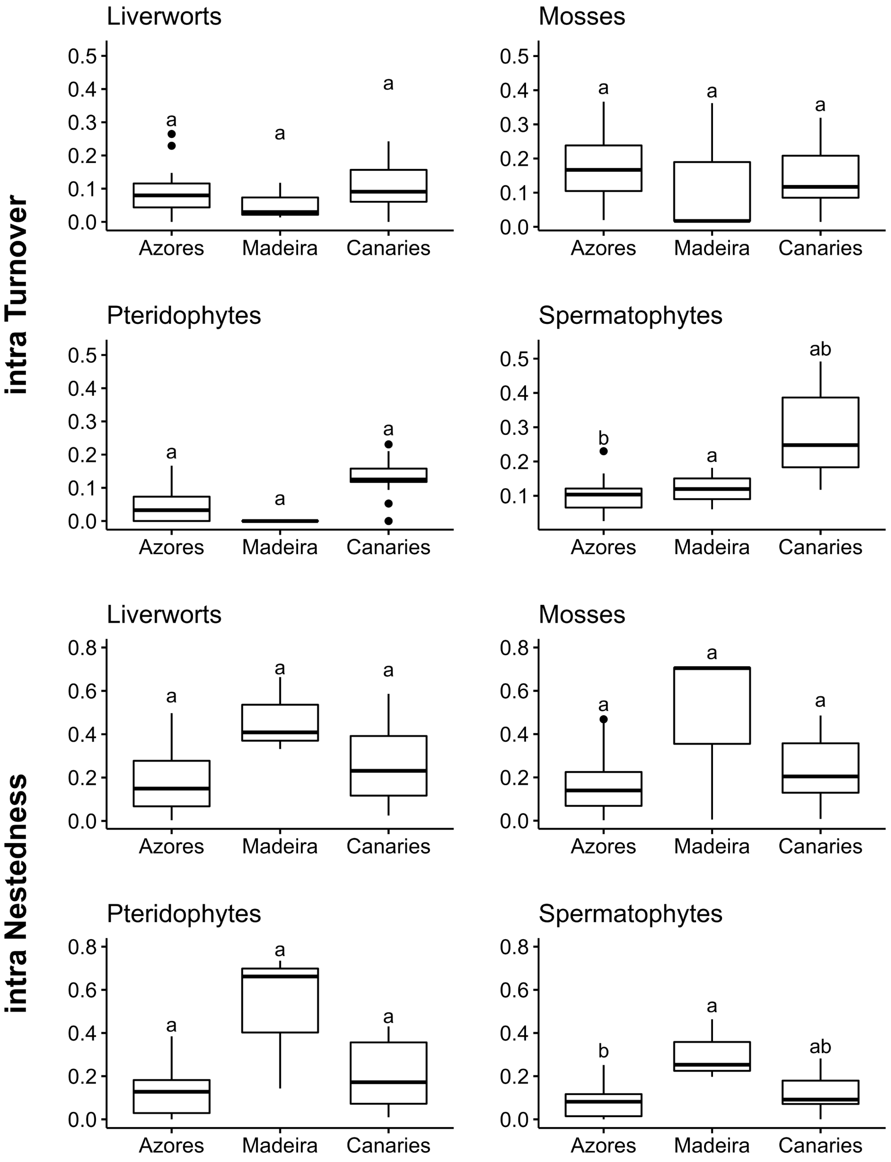

FIGURE 3 Box-plots (showing the first and third quartiles (upper and lower bounds), second quartile (centre), 1.5 × interquartile range (whiskers) and minima–maxima beyond the whiskers) of turnover and nestedness of liverwort, moss, pteridophyte and spermatophyte communities among islands from the same archipelago in Macaronesia. Letters indicate, for each lineage, the archipelagos among which turnover and nestedness significantly differs (see Tables S3 and S4 for *p*-values of the posthoc Kruskal–Wallis Dunn tests), a same letter indicating non-significantly different average values between the archipelagos considered. The *y*-axis represents the values of the species turnover (based on Simpson index of dissimilarity) and nestedness components of beta diversity among islands of the same archipelago.

showed no significant variation of nestedness with scale (Menegotto et al., 2019). Nestedness arises when species-poor sites represent subsets of the biota occurring in species-rich sites (Baselga, 2010; Cantor et al., 2017). In Macaronesian bryophytes and pteridophytes, species richness patterns are similar across archipelagos, so that no clear nested pattern is apparent. Differences in species richness among archipelagos are more evident in spermatophytes, with 1398, 683 and 167 native species and subspecies (out of 1810 in total in Macaronesia) in the Canarian, Madeiran and Azorean floras, which is reflected by the near-significance of the difference in nestedness for within—and among—archipelago comparisons.

In line with our expectations H1b and H2b, the extent to which species turnover increased at large spatial scales varied, however, among lineages, being larger in spermatophytes than in pteridophytes, and then, mosses and liverworts, wherein the median turnover was less than half that in spermatophytes. In turn, nestedness was significantly higher in mosses, liverworts and pteridophytes than in spermatophytes. The progressive decrease of turnover and increase of nestedness from spermatophytes to bryophytes reflects their differences in dispersal capacities, pteridophytes producing bigger spores (30–50 µm on average) than bryophytes (10–20 µm on average). It also reflects major differences of speciation modes between these groups, with some spectacular radiations in the spermatophyte flora, whereas island bryophytes and ferns typically fail to radiate (Patiño, Carine, et al., 2014). These differences of dispersal capacities and speciation modes are themselves reflected in differences of distribution patterns and especially, patterns of endemism. For instance, the 0.7%, 1.7% and 5% of Canarian endemic liverwort, moss and pteridophyte species, 0, 33.3% and 0 of which are SIEs, pale by comparison with the 47%, Canarian endemic spermatophyte species species and subspecies that include 64% of SIEs.

Patterns of turnover also varied among archipelagos in pteridophytes and spermatophytes, but not in bryophytes. The higher turnover observed in the Canaries for spermatophytes and pteridophytes, despite the shorter mean distance between islands than in the Azores, reflects the steeper altitudinal floristic gradients, as well

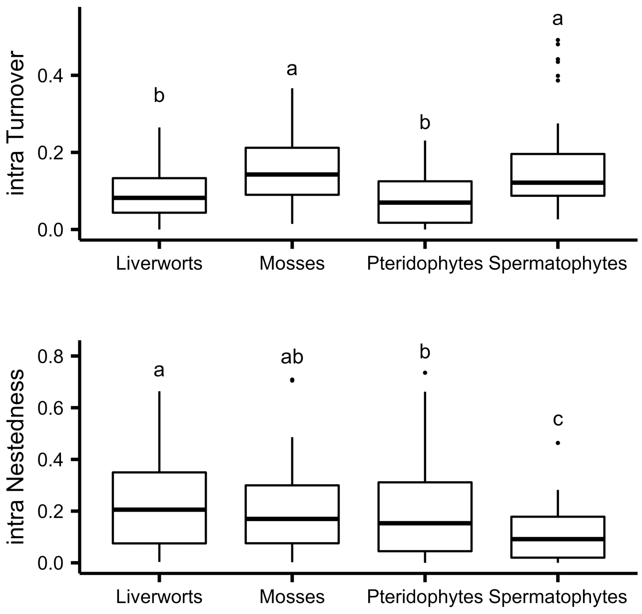

FIGURE 4 Box-plots (showing the first and third quartiles (upper and lower bounds), second quartile (centre), 1.5 × interquartile range (whiskers) and minima-maxima beyond the whiskers) of the turnover and nestedness of liverwort, moss, pteridophyte and spermatophyte communities among islands within archipelagos in Macaronesia. Letters indicate the lineages among which turnover significantly differs, a same letter indicating non-significantly different average values between the lineages considered. The *y*-axis represents the values of the species turnover (based on Simpson index of dissimilarity) and nestedness components of beta diversity among islands of the same archipelago.

as, globally, greater heterogeneity in climate, islands age and habitat types between the Canarian islands than between the Azorean islands (del Arco Aguilar & Rodríguez Delgado, 2018; Triantis et al., 2012). These differences are well reflected in the distribution of endemism among archipelagos, with the bulk of Canarian endemics being SIEs (64% of Canarian endemic spermatophytes), whereas Azorean endemics tend to be MIEs (88% of Azorean endemic spermatophytes), often widespread across the archipelago (Carine & Schaefer, 2010; Schaefer et al., 2011). The similarity of turnover among archipelagos in bryophyte floras is, at first sight, more striking. Indeed, large differences in bryophyte species composition would have been expected, due to poikilohydric condition of the group, between islands as different from each other as the Canary Islands. In reality, the specialized xerophytic floras of the eastern Canary Islands, characterized by low elevation and dry climates and dominated by thalloid liverworts (*Riccia* spp.) and annual mosses (especially of the family Funariaceae and Pottiaceae), can also be found at low elevation in the western Canary Islands. As a result, Canarian bryophyte communities are more nested than Canarian spermatophyte communities.

Differences of nestedness among archipelagos revealed a recurrent pattern across lineages, according to which nestedness in Madeira was higher than in other archipelagos. The archipelago of Madeira was represented in our analyses by Madeira, Porto Santo and the Desertas. The latter two exhibit much lower elevation, and much drier climates than Madeira, so that their species richness is comprised of the most drought-tolerant elements of the Madeiran flora, resulting in a strong nested pattern that correlates with variation in climatic conditions.

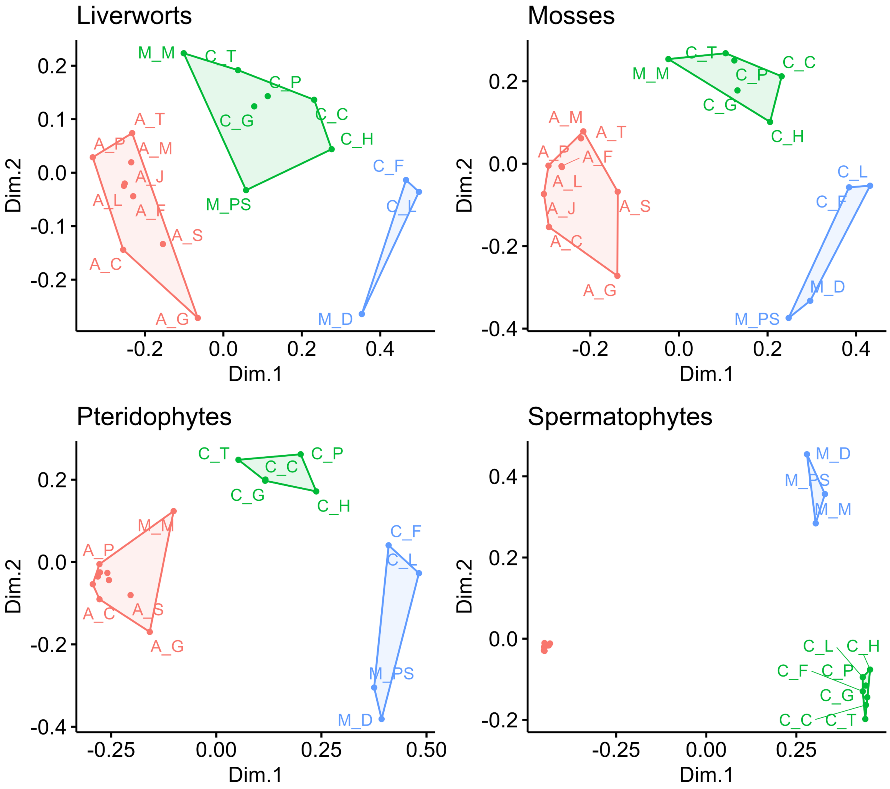

FIGURE 5 MDS ordination of the Macaronesian islands depending on their floristic composition (spermatophytes, pteridophytes, mosses and liverworts), based on Sørensen dissimilarity. Colours represent the clusters identified using Ward's clustering algorithm and correspond to the optimal number of clusters obtained by the consensus of 30 indexes.

Variation in turnover and nestedness among land plant lineages depending on their dispersal capacities was paralleled by differences in their drivers. In agreement with the idea that spore-producing plants exhibit higher dispersal capacities than spermatophytes, and hence, that their distributions are better explained by extant environmental conditions than by historical factors associated with dispersal limitations (H3), extant climatic factors accounted more to bryophyte and pteridophyte turnover than to spermatophyte turnover. In contrast, island age contributed more to explain variation of turnover among islands in spermatophytes than in bryophytes and pteridophytes. Island age can be interpreted as a composite variable that accounts for time per se, but also, and most importantly, for the environmental heterogeneity during the life-cycle of oceanic islands, and especially, elevation and topographic complexity. Hence, island age does not necessarily reflect the amount of time available for colonization as in fact, the extant composition of the Macaronesian flora largely reflects dynamic interchanges with continental sources in both spore-producing lineages (Vanderpoorten et al., 2007) and spermatophytes (García-Verdugo et al., 2019; Hooft van Huysduynen et al., 2021). Instead, island age characterizes opportunities for speciation depending on habitat heterogeneity and availability, which peak as islands reach their highest elevation (Patiño et al., 2013; Whittaker et al., 2008). In this context, speciation plays a much more important role in the extant diversity of spermatophyte than of bryophyte and pteridophyte species, as evidenced by the striking difference in endemism rates per archipelago among lineages, of <8% in bryophytes and pteridophytes and >45% in spermatophytes. In bryophytes and pteridophytes in fact, endemic species are typically 'isolated' in their genus ("anagenesis", Stuessy et al., 2006), whereas in Macaronesian spermatophytes, the ratio between the number of genera including endemic species and the number of endemic species ranges between 1.5 and 3 (Patiño, Carine, et al., 2014; Patiño, Weigelt, et al., 2014).

Differences in dispersal capacities, and hence, endemism patterns among the mainland plant lineages, were further evidenced by the selection of the factor 'archipelago structure' in the model for species turnover in spermatophytes, but not in bryophytes and pteridophytes. The relevance of archipelago structure in spermatophytes, but not spore-producing plants, again mirrors patterns of endemism among those groups, with 0.7%–2%, 1.7%–2%, 5%–8% and 21%–47% of archipelago endemics in liverworts, mosses, pteridophytes, and spermatophytes, respectively.

In turn, area, one of the main drivers of species richness on islands due to decreased extinction rates, but most importantly, increased chances of colonization by airborne propagules (target area effect, Whittaker & Fernández-Palacios, 2007) and of speciation (Kissel & Barraclough, 2010), was never selected as a significant driver of species turnover. This was, at first sight, surprising because taxa with high dispersal capacities are expected to require larger areas to speciate than taxa with low dispersal capacities (Kissel & Barraclough, 2010), which should be reflected in higher levels of endemism on large islands, and hence, larger differences in species turnover among islands of different sizes. One of the main reasons why area did not play a role in the observed patterns of turnover is that all Macaronesian islands are much larger than the minimal area, <10 km^2, required for neutral genetic differentiation in spermatophytes (Kissel & Barraclough, 2010). In addition, Macaronesian bryophytes typically failed to speciate in situ (Patiño, Carine, et al., 2014; Vanderpoorten et al., 2011). Even in genera that include several Macaronesian endemics, endemic species do not form a monophyletic group and each speciation event follows a long-distance dispersal event from continental sources (Patiño & Vanderpoorten, 2015). Unlike sympatric speciation, allopatric speciation depends on geographic isolation from sources, and not island size, contributing to the lack of relationship between island area and beta diversity patterns in spore-producing plants. Pteridophytes failed to radiate in Macaronesia as well. In fact, most Macaronesian ferns genera include only one endemic species that evolved by allopolyploidisation (Vanderpoorten et al., 2011), a mechanism that is typically independent from area (Kissel & Barraclough, 2010).

It is worth noting that, when the four Canarian islets were included in the analysis, no significant model was obtained for liverwort and spermatophyte turnover and the percentage of explained deviance dropped of 32 and 50% in mosses and pteridophytes, respectively. Such an impact of small islands is reminiscent of the small-island effect, an anomalous pattern of the species–area relationship that predicts the existence of a threshold area, below which species richness varies independently of island area. The small-island effect is mostly driven by the decoupling of area and environmental heterogeneity on small islands (Chen et al., 2020; Matthews et al., 2020). The drop of explained deviance in our models similarly suggests that, beyond a certain threshold area, the factors affecting species composition change. As for the link between species richness and area (Yu et al., 2020), this effect varies among taxonomic groups, being most obvious in bryophytes and pteridophytes, whose floras are extremely depauperate on the Canarian islets.

In line with differences in beta diversity patterns among land plant lineages, caused by different responses of turnover to variation of climatic conditions and geographic distance, and with our hypothesis H4, spermatophyte floras clustered by archipelago, whereas pteridophyte and bryophyte floras did not. Fuerteventura, Lanzarote, Desertas and Porto Santo host similar cryptogrammic floras of low-elevation, dry islands, whereas the western Canary Islands and Madeira, which share typical laurel forest floras, formed another cluster. In spermatophytes, this signal is erased by the predominance of the endemic element at the archipelago level (see above), so that the turnover between islands from different archipelagos is substantially higher in spermatophytes (0.74 ± 0.07) than in pteridophytes (0.41 ± 0.08), mosses (0.40 ± 0.10) and liverworts (0.37 ± 0.10). The inclusion of the Canarian islets slightly changed the pattern, as, for all the taxonomic lineages and as previously reported (Torre et al., 2019), these islets clustered together, independently of their archipelagic adscription.

The differences of beta diversity patterns among Macaronesian land plant lineages illustrate two major evolutionary differences among those groups depending on their dispersal capacities. First, spermatophytes speciate in situ at much faster rates than spore-producing plants, wherein allopatric speciation following long-distance dispersal is the rule (Patiño, Carine, et al., 2014; Patiño, Weigelt, et al., 2014). Second, the higher dispersal capacities of spore-producing plants explains the striking tropical affinities of Macaronesian endemic spore-producing plants (Vanderpoorten et al., 2011), which contrasts with the predominantly Mediterranean origin of Macaronesian endemic spermatophytes (Carine et al., 2004). Combined with the differences in beta diversity reported here, these observations point to different assemblage mechanisms in terms of origin, timing and mode of colonization among Macaronesian land plant lineages. These differences in the mechanisms of assembly among land plant in Macaronesia call for a comparative analysis of the geographic origin of these floras in an explicit time-frame.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The matrices of species distributions for liverworts, mosses and spermatophytes are available at https://figshare.com. doi: 10.6084/m9.figshare.17099840.

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BIOSKETCH

Lea Mouton is a PhD student at the University of Liège. Her research focuses on the variation of beta diversity with geographic scale.

Author contributions: AV and LM conceived the project; LM, MC, FR, JP, MMS, JMGM, RMAG, MSS, JARB, AV collected the data; LM, FC and OJH analysed the data; and LM and AV led the writing with assistance from all co-authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix III

VI. Ting Shen, Liang Song, Richard Corlett, Antoine Guisan, Jian Wang, Wen-Zhang Ma, **Lea Mouton**, Alain Vanderpoorten and Flavien Collart, 2023. Disentangling the roles of chance, abiotic factors and biotic interactions among epiphytic bryophyte communities in a tropical rainforest (Yunnan, China). Plant Biol. 25(6):880-891. https://doi.org/10.1111/plb.13570

cover picture: View from the canopy-crane, Ailao Mountains (China) in October 2019, from Lea Mouton

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RESEARCH ARTICLE

Disentangling the roles of chance, abiotic factors and biotic interactions among epiphytic bryophyte communities in a tropical rainforest (Yunnan, China)

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Keywords

Competition; epiphytes; facilitation; life form; liverworts; mosses; niche preference; stress-gradient hypothesis.

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ABSTRACT

- Epiphytes offer an appealing framework to disentangle the contributions of chance, biotic and abiotic drivers of species distributions. In the context of the stress-gradient theory, we test the hypotheses that (i) deterministic (*i.e.*, non-random) factors play an increasing role in communities from young to old trees, (ii) negative biotic interactions increase on older trees and towards the tree base, and (iii) positive interactions show the reverse pattern.
- Bryophyte species distributions and abiotic conditions were recorded on a 1.1 ha tropical rainforest canopy crane site. We analysed co-occurrence patterns in a niche modelling framework to disentangle the roles of chance, abiotic factors and putative biotic interactions among species pairs.
- 76% of species pairs resulted from chance. Abiotic factors explained 78% of non-randomly associated species pairs, and co-occurrences prevailed over non-coincidences in the remaining species pairs. Positive and negative interactions mostly involved species pairs from the same *versus* different communities (mosses *versus* liverworts) and life forms, respectively. There was an increase in randomly associated pairs from large to small trees. No increase in negative interactions from young to old trees or from the canopy to the base was observed.
- Our results suggest that epiphytic bryophyte community composition is primarily driven by environmental filtering, whose importance increases with niche complexity and diversity. Biotic interactions play a secondary role, with a very marginal contribution of competitive exclusion. Biotic interactions vary among communities (mosses *versus* liverworts) and life forms, facilitation prevailing among species from the same community and life form, and competition among species from different communities and life forms.

INTRODUCTION

Trees are striking examples of habitat islands, characterized by substantial spatiotemporal variations in their characteristics as hosts, such as the area available for colonization and habitat conditions in terms of branching architecture, bark texture and chemistry, from the base to the canopy, and from young to old trees (Taylor & Burns 2015). In this context, epiphytes offer an appealing framework to disentangle the contributions of chance, biotic and abiotic drivers of species distributions, because specific hypotheses regarding the contributions of these factors to variations in species co-occurrence patterns depending on their position on the tree and tree age can be formulated (Spicer & Woods 2022).

During early colonization stages, species are expected to progressively accumulate, with high levels of stochasticity associated with dispersal chance (Taylor & Burns 2015). Reproductive traits determine the order of appearance of species, driving the segregation of primary and late successional species assemblages, as evidenced in epiphyllous communities (Sierra *et al.* 2019). In line with the idea that, during community succession, niche-based processes (niche preference and biotic interactions) progressively prevail over neutral processes associated with stochastic dispersal events. As Mežaka *et al.* (2022) hypothesized in epiphyllous communities, the contribution of random factors to community composition can be expected to decrease on ageing trees. Following an optimal stage of ontogenetic development of the host tree, during which both primary and late successional species can be found, competition is expected to play an increasing role, as suggested by the decrease in species richness following an optimum reported in epiphytic lichens (Ellis & Ellis 2013).

The importance of biotic interactions in epiphytic community composition is also expected to vary vertically on the same tree because of variations in light intensity, relative humidity, wind exposure, temperature, and nutrient availability. The stress gradient hypothesis, primarily developed in terrestrial ecosystems (Bertness & Callaway 1994; Qi *et al.* 2018), has most recently been applied to epiphytic communities (Mežaka *et al.* 2022; Spicer & Woods 2022) and provides a framework for predicting when and where interactions among epiphytes may be important. For epiphytes, the outer canopy is a high stress environment characterized by high exposure to UV radiation and large daily temperature and relative humidity fluctuations, wherein facilitation is expected to prevail (Soliveres *et al.* 2015). For instance, the likelihood of vascular epiphyte colonization increases with the extent of bryophyte cover thanks to the high water-holding capacities, and hence, the humid microhabitats provided by the latter (Acevedo *et al.* 2020). The relevance of facilitation by bryophytes for vascular epiphytes is such that bryophyte cover contributed more to models of vascular epiphyte richness than host tree size, which has been identified as the major predictor of epiphyte richness (Zotz & Vollrath 2003; Zotz & Schultz 2008; Francisco *et al.* 2021). As light exposure and daily variations in temperature and relative humidity decrease towards the tree base, interactions are expected to become more competitive (see Spicer & Woods 2022 for review), especially among epiphytes from different communities, as reported in moss, liverwort, and epiphyllous lichen communities (Mežaka *et al.* 2022).

Here, we focused on epiphytic bryophyte communities, which are a key component in tropical forest ecosystems for water and nutrient cycles, enhancing seed anchorage and germination, and providing shelter for diverse and abundant fauna (Holz & Gradstein 2005, and references therein), and wherein biotic interactions are thought to play an important role. As a comparison, facilitation and competition were, respectively, the focus of 22% and 35% of the non-vascular epiphyte literature, but only 8% and 5% of the vascular epiphyte literature (Spicer & Woods 2022).

Bryophyte species may compete with each other by producing allelopathic substances that prevent the growth of protonema (Whitehead *et al.* 2018), or by having a life form and growth strategy promoting the development of dense colonies that quickly invade available space. For example, fast spreading, thick mats of colonies formed by dendroid growth forms, such as *Isothecium* in temperate rainforests, whose creeping primary shoots rapidly spread over the surface and where erect secondary shoots overarch underlying species, potentially entering into competition for light with the latter, are considered as typical examples of effective competitors (Peck & Frelich 2008; Woods *et al.* 2019). The role of competition among bryophyte communities has, however, remained an area of controversy (Frego & Carleton 1995; McAlister 1995; Økland & Økland 1996; Rydin 1997; Bates 1998; Økland 2000; Bergamini *et al.* 2001; Mälson & Rydin 2009; Udd *et al.* 2016). In Grime's (1977) description of competitive, ruderal and stress-tolerant strategies in plants, for instance, bryophytes are not included in the competitors (Grime *et al.* 1990). Bryophytes are traditionally viewed as pioneer organisms that are efficiently wind-dispersed through small spores. This would, at first sight, suggest that they disperse to newly available suitable habitats instead of competing locally for persistence. In such groups with high dispersal capacities, the notion that trees represent habitat islands has been challenged (Cook *et al.* 2004; Belinchón *et al.* 2009). Several lines of evidence suggest, however, that epiphytic bryophytes experience severe dispersal limitation, making them suitable models in an island habitat context. In fact, epiphytic mosses are typically characterized by reduction of the peristome (Hedenäs 2012), whose teeth movements enhance spore dispersal. Peristome reduction is correlated with hygrochasy, *i.e.*, the release of spores under wet conditions, which decreases their chances of long-distance dispersal, and might be seen as a safe-site strategy, forcing spores to land in appropriate, nearby patches where their survival is favoured (Medina *et al.* 2014). Aggregated distribution patterns are frequently reported in epiphytic bryophytes (*e.g.*, Snäll *et al.* 2003, 2004; Löbel *et al.* 2006, Wagner *et al.* 2015), and strong spatial genetic structures at fine spatial scales were consistently reported in the (few) epiphytic bryophyte species investigated so far (Snäll *et al.* 2004; Vanderpoorten *et al.* 2019; Ledent *et al.*).

Here, we take advantage of a canopy crane in a tropical rainforest to test the following hypotheses: (i) the proportion of randomly associated species pairs decreases on old trees due to the increasing role of deterministic (*i.e.*, non-random) factors (H1); (ii) non-coincident species distribution patterns, potentially associated with negative biotic interactions, play an increasing role as trees age (H2) and from the canopy to the tree base (H3); (iii) species co-occurrences, potentially associated with positive interactions, show the reverse pattern. We further hypothesize that competition prevails among, rather than within, moss and liverwort communities (Mežaka *et al.* 2022) (H4a). Within moss and liverwort communities, we expect competition to prevail among species of different life forms because of the higher competitive ability of certain life forms over others. We also expect facilitation to prevail among species sharing the same life form to maximize shoot density within colonies, and hence, water retention thorugh capillarity (H4b).

MATERIAL AND METHODS

Study area and data collection

This study took place in a 1.44 ha square plot in a lowland (643–700 m a.s.l.) seasonal rain forest (101°34′59.1″ E, 21°37′2.6″ N) in Xishuangbanna National Natural Reserve (Yunnan, SW China). This site was chosen because of the availability of comprehensive, fine-scale information on environmental conditions and epiphyte species distributions (Shen *et al.* 2018, 2022a,b), which could be collected thanks to an 81-m-tall canopy crane (TCT7015-10E, Zoomlion Heavy Industry, Changsha, China), whose 60-m-long arm provides access to the canopy within a 1.1-ha circle.

Sampling protocols and environmental data collection were described in Shen *et al.* (2022a,b) and are briefly summarized below. Bryophyte species composition was determined for 408 plots of 1600 cm^2 on 42 *Parashorea chinensis* host trees using a stratified sampling across three height zones: lower trunk (from tree base to the middle height of the trunk), upper trunk (from the middle height of the trunk to the lowest third of the canopy), and canopy top (from to the middle third of the canopy to the upper third of the canopy). Since tree age was not available for each individual tree investigated, we used diameter at breast height (DBH) as a proxy for tree age based on very accurate allometric equations ($r^2 = 0.99$) linking the age of individual *P. chinensis* with their DBH in Xishuangbanna (Tang *et al.* 2008). Three DBH classes were considered: small (DBH ≤20 cm, n = 16), medium (≥20 & < 40 cm, n = 15), and large (≥40 cm, n = 11), corresponding to trees <60 years, 60–100 years, and >100 years, respectively.

Forty-six environmental variables were recorded or derived (Table 1), including microclimate (relative humidity and temperature; HYS15 air temperature and relative Moisture Sensors, Unism) and light (intensity and photosynthetically active radiation; LI-190R Quantum Sensor, LI-COR Biosciences) recorded at 1-h intervals from 12 trees (one in the lower trunk, two in the upper trunk, and two in the canopy) from July 2017 to December 2019 and predicted in an x-y-z space (Shen *et al.* 2022a) using Random Forest (Liaw & Wiener 2002).

Community modelling

We disentangled the contribution of chance, abiotic environmental drivers, and biotic interactions to explain species associations using a community modelling framework in three steps (Fig. 1, based on a slightly modified version of D'Amen *et al.* 2018). This approach first involves the identification of species pairs that can be explained by abiotic environmental conditions (species co-occurring due to shared niche preferences – *i.e.*, environmental filtering – or avoiding each other – *i.e.*, limiting similarity – because of niche divergence) through species distribution modelling (see below). Focusing on species pairs whose co-occurrence or non-coincident patterns cannot be explained in terms of abiotic environmental conditions, the second step involves the identification of non-randomly associated species pairs (*i.e.*, species that tend to co-occur or exclude each other more than expected by chance). The third step involves an analysis of species distribution ranges to identify, among non-coinciding distributions, which of these concern species with non-overlapping ranges. Allopatric ranges were interpreted by D'Amen *et al.* (2018) in terms of dispersal limitations, but at the very fine spatial scale investigated here, non-overlapping distributions in the x-y-z space, rather characterize differences in habitat conditions that would not have been considered. Finally, non-randomly associated species pairs with overlapping ranges, which cannot be explained in terms of abiotic environmental conditions, are interpreted in terms of facilitation (co-occurrences) or competition (non-coincidences).

During the first step, which involves environmentally constrained null models to determine whether two species tend to co-occur or to be spatially segregated more than expected given their niche preferences, the strength of association between each pair of species is measured by the C-score index (Stone & Roberts 1990). The C-score index is based on the number of records for the first species, for the second species, and for the number of plots where both species co-occur and are rescaled between 0 and 1. A value of 0 means that the species pair is maximally aggregated, and 1 is where a species pair is maximally segregated. Observed C-scores are then compared to a

Table 1. Abiotic environmental variables used to describe the niche of epiphytic bryophyte species at the Xishuangbanna canopy crane facility (Yunnan, SW China).

environmental variable	definition
DBH	Diameter at breast height (m) at 1.3 m above ground
Z	Vertical height from tree base to sampling location
W	Degree of regularity of the spatial distribution of the four trees nearest to the focal tree based on the classification of the angles between these four neighbours (Zhang *et al.* 2018)
U	Dominance index (relationship between size of the focal tree and its four nearest neighbours, describing whether a focal tree is larger or smaller than its neighbours) (Zhang *et al.* 2018)
meanDist	Average distance (m) between the focal tree and its four nearest-neighbour trees
Elevation	Elevation (m)
TPI	Topographic position index (relative topographic position of a focal tree as the difference between its elevation and the mean elevation of all other trees)
Slope	Slope (°) of the ground at the level of each focal tree
Eastwest	East/west orientation of the ground at the level of each focal tree; value from −1 (West) to 1 (East)
Northsouth	North/south orientation of the ground at the level of each focal tree; value from −1 (South) to 1 (North)
Bio1RH	Annual mean relative humidity
Bio2RH	Average monthly relative humidity ranges
Bio3RH	(Bio2RH/Bio7RH) (×100)
Bio4RH	Relative humidity seasonality (standard deviation ×100)
Bio5RH	Maximum relative humidity (%)
Bio6RH	Minimum relative humidity (%)
Bio7RH	Relative humidity annual range (%, Bio5RH − Bio6RH)
Bio8RH	Mean relative humidity of wet season
Bio9RH	Mean relative humidity of dry season
Bio1T	Annual mean temperature
Bio2T	Average monthly temperature ranges
Bio3T	Isothermality (Bio2T/Bio7T) (×100)
Bio4T	Temperature seasonality (standard deviation)
Bio5T	Maximum temperature (°C)
Bio6T	Minimum temperature (°C)
Bio7T	Temperature annual range (°C, Bio5T − Bio6T)
Bio8T	Mean temperature of wet season
Bio9T	Mean temperature of dry season
Bio1PAR	Annual mean photosynthetic active radiation
Bio2PAR	Average monthly photosynthetic active radiation ranges
Bio3PAR	(Bio2RAR/Bio7RAR) (×100)
Bio4PAR	Photosynthetic active radiation seasonality (standard deviation)
Bio5PAR	Maximum photosynthetic active radiation (μmol·m^{-2}·s^{-1})
Bio6PAR	Minimum photosynthetic active radiation (μmol·m^{-2}·s^{-1})
Bio7PAR	Photosynthetic active radiation annual range (W/m^2; Bio5RAR − Bio6RAR)
Bio8PAR	Mean photosynthetic active radiation of wet season
Bio9PAR	Mean photosynthetic active radiation of dry season
Bio1Light	Annual mean light intensity
Bio2Light	Average monthly light intensity ranges
Bio3Light	(Bio2Light/Bio7Light) (×100)
Bio4Light	Light intensity seasonality (standard deviation)
Bio5Light	Maximum light intensity (W·m^{-2})

Table 1. (Continued)

environmental variable	definition
Bio6Light	Minimum light intensity (W·m^{-2})
Bio7Light	Light intensity annual range (W·m^{-2}; Bio5Light − Bio6Light)
Bio8Light	Mean light intensity of wet season
Bio9Light	Mean light intensity of dry season

distribution of C-scores from a set of 10,000 null communities. The null communities were simulated by shuffling species occurrences among plots, according to their habitat suitability values resulting from SDMs (see below), as if niche preferences were the only driver of species distributions, regardless of biotic interactions. To control for false positives, a Bayesian approach, assuming independence of probabilities, was employed. This approach consists of comparing the C-score of each species pair with those resulting from the same species pair in the null matrices (Gotelli & Ulrich 2010).

The second step involves removing randomly associated species pairs. A total of 10,000 null communities were generated by reshuffling species among plots while preserving species occurrence frequencies. Observed C-scores were then compared to C-scores expected by chance, as described above.

The third step involves determining whether two species have overlapping or non-coincident ranges. For this, we compared the minimum convex hulls encompassing all of their occurrence points in an x-y space (ICUN 1994). If these ranges overlapped, we then determined whether their vertical range (z-axis, which includes height of the plots on the tree and the elevation) overlapped. If the ranges in the x-y space and along the z-axis both overlapped, a multivariate ANOVA (MANOVA) of coordinates in the x-y space depending on the factor species was implemented. A significant MANOVA test was interpreted as partial overlap, while a non-significant difference of species coordinates in the x-y space was interpreted as complete overlap.

The above analyses were performed at the level of the entire dataset to test H1; of lower trunks, upper trunks and canopy tops, respectively, to test H2; and of small, medium and large trees, respectively, to test H3.

To determine whether the observed association patterns were associated with life-forms (H4), we assigned all species to one of the following functional groups, as defined by Mägdefrau (1982) (Table S1): smooth mat (shoots creeping over the substrate with rhizoids adhering to substratum, with flat branches); rough mat (idem, with many erect lateral

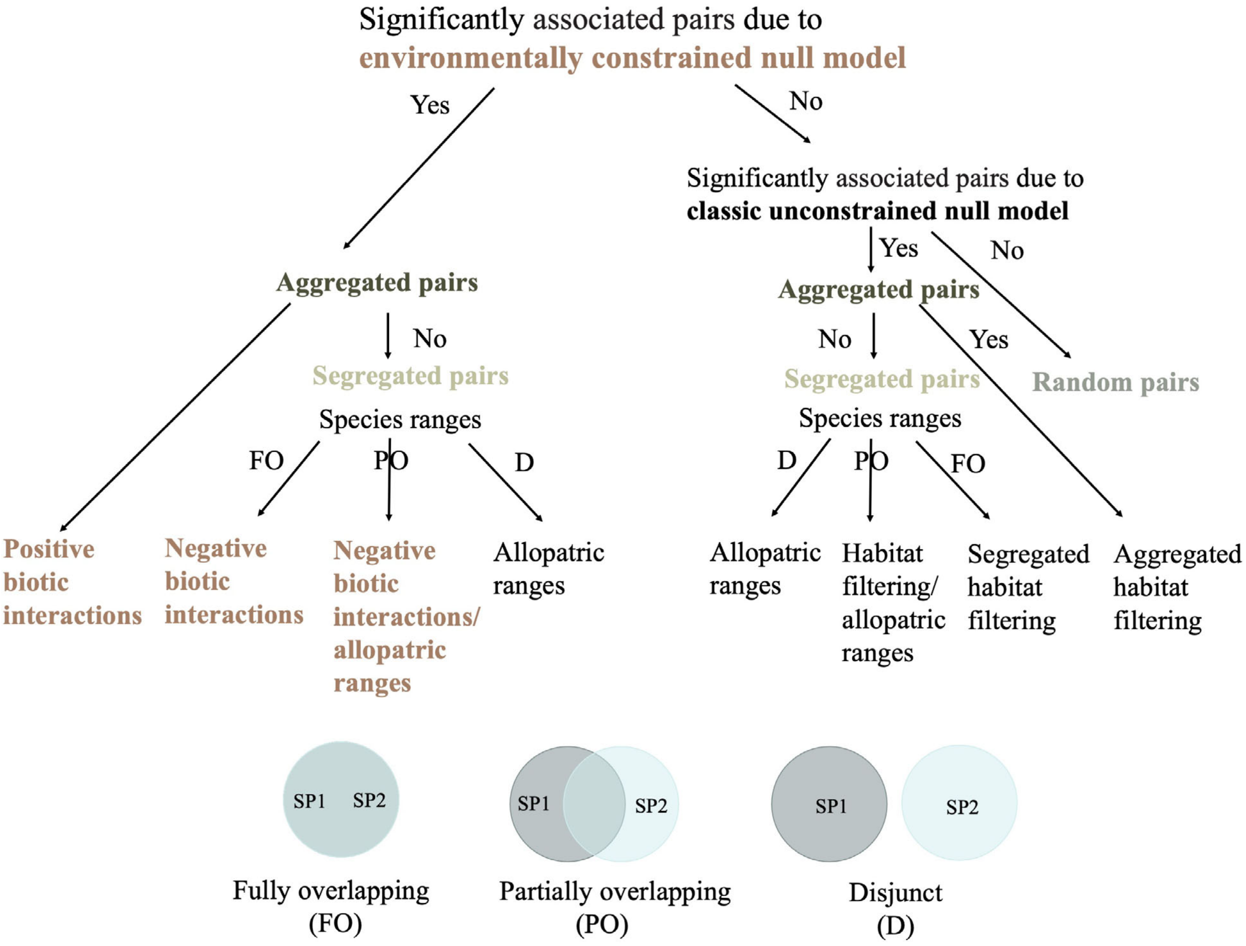

Fig. 1. Decision tree to disentangle community drivers (adapted from D'Amen *et al.* 2018) using a combination of environmentally constrained null models and classic unconstrained null models.

branches); fan (creeping on vertical substrate, with branches in one plane and leaves usually flat); dendroid (main shoots creeping, secondary shoots erect); pendant (hanging shoots); weft (shoots growing erect and horizontally, with multiple branching stems distributed throughout the main stem); turf (stems erect, parallel and close together, often covering extensive areas). As suggested by Lett *et al.* (2022), we further considered more broadly defined functional groups resulting from the collation of individual groups. In particular, since the Lett *et al.* classification includes leafy liverworts as a group, we added a 13th category, mosses, in the analyses to determine whether specific traits of moss and liverwort could help interpret the observed species association patterns.

Species distribution models

From an initial number of 102 species, we retained 40 with at least ten occurrences, which was defined as the lowest limit for model evaluation based on AUC from presence–absence data (Jiménez-Valverde 2020). To decrease the number of predictors and avoid multicollinearity, the variation among these predictors was reduced using Principal Components Analysis. The first two axes, which explained 73% of the total variance, were used as predictors.

To take model uncertainty into account, we used an ensemble modelling approach (Araújo & New 2007). We generated SDMs with the R package biomod2 (Thuiller *et al.* 2021) using five algorithms: Generalized Linear Models (GLM), Generalized Additive Models (GAM), Gradient Boosting Machine (GBM), Random Forest (RF), and Maximum Entropy (MaxEnt) using the default parameters in biomod2 (Thuiller *et al.* 2021).

Models were evaluated using the area under the ROC curve (AUC), the maximum value of the true skill statistic (MaxTSS) and the Boyce Index (Hirzel *et al.* 2006; Guisan *et al.* 2017). For model evaluation, 20 replicates were run, each comprising 70% of the data to calibrate the models (training set) and 30% to evaluate them (test set). Given the small size of each individual test set, we refrained from computing AUC, MaxTSS and the Boyce Index from each individual test set, and then averaging them across replicates, as in Breiner *et al.* (2015, 2018). Instead, following Collart *et al.* (2021), we pooled the suitability values from the 20 test sets. As the same presence point is likely to be sampled in multiple replicates of the test set, the suitability values for each presence point were averaged across replicates.

This generated a series of suitability values independent from the data used to calibrate the models, but with a much larger size than the individual test sets, roughly equal to the initial occurrence dataset (as some occurrence points may not have been sampled in any of the 20 replicates). This recomposed evaluation dataset allowed direct computing of the Boyce index, and by combining it with the absence points, the AUC and maxTSS, using ecospat ersion 3.4 (Broennimann *et al.* 2022). We finally generated ensemble models, wherein each individual model contributed proportionally to its Somer's D (Somer's D = 2AUC − 1) value.

RESULTS

Ensemble species distribution models exhibited AUC, SomersD, Boyce, and MaxTSS values ranging between 0.78 ± 0.08, 0.56 ± 0.17, 0.74 ± 0.16 and 0.51 ± 0.15 across species, respectively (Table S2).

Globally, the observed C-scores of 76% of species pairs were not significantly different than random C-scores (Fig. 2). Among the 24% of remaining non-random pairs, 78% could be explained by abiotic environmental

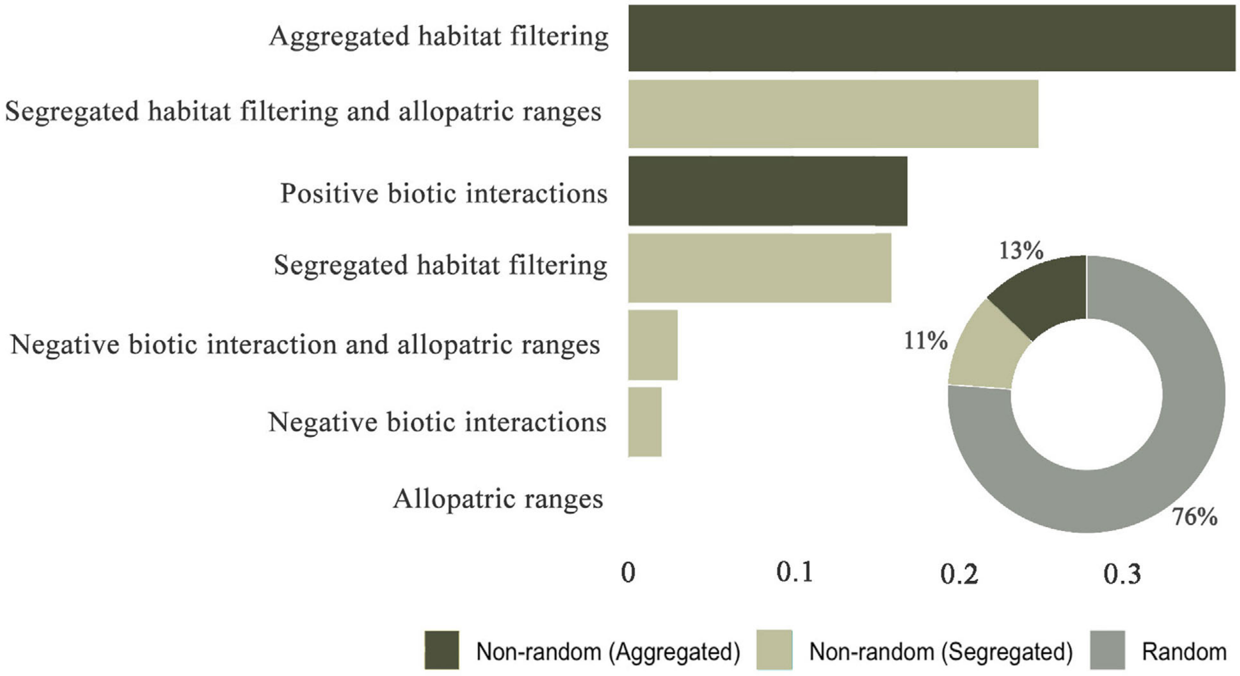

Fig. 2. Proportion of randomly and non-randomly associated species pairs among epiphytic bryophytes in a tropical rainforest (Yunnan, China) and contribution (%) of the factors accounting for non-random species associations.

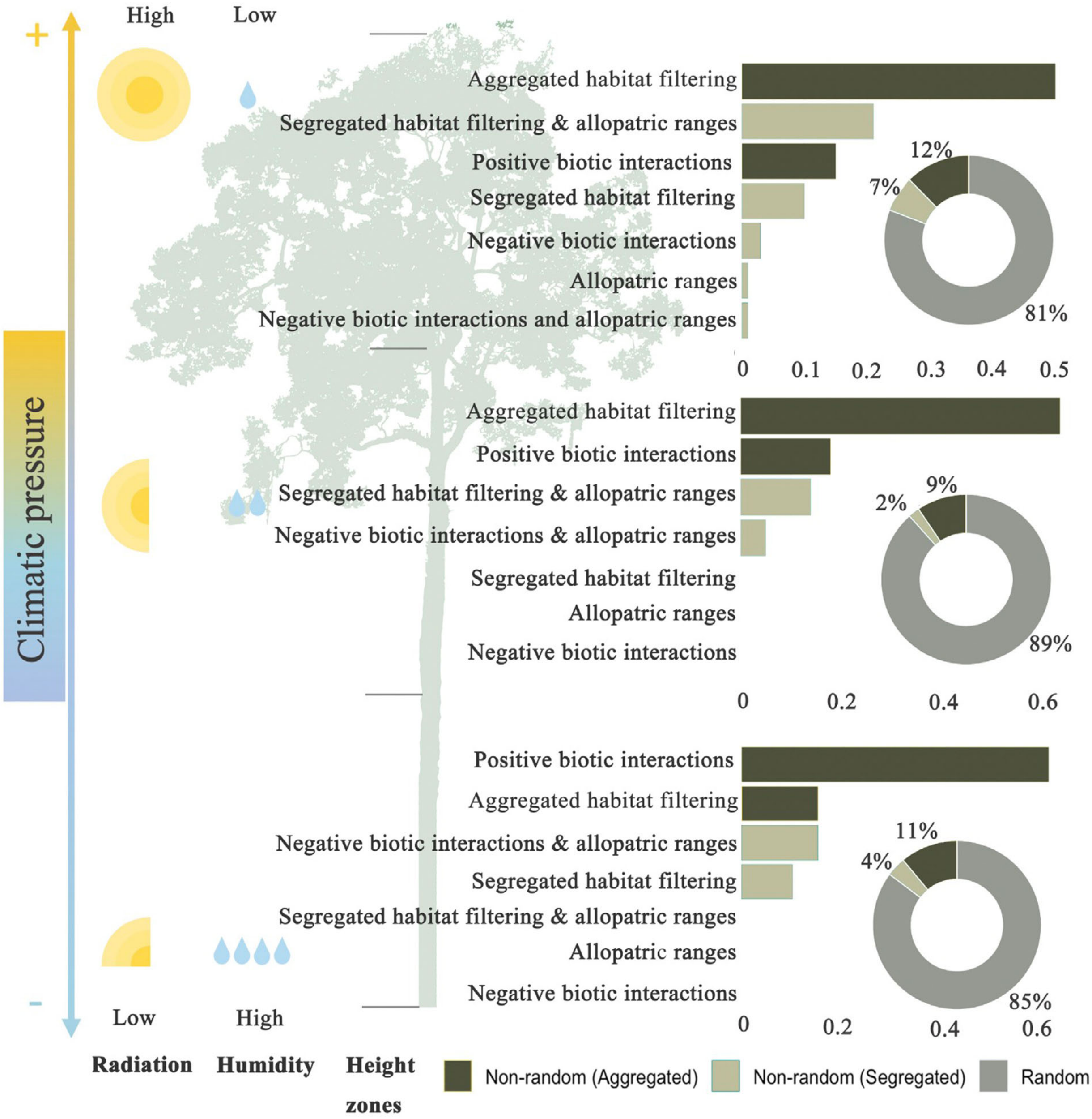

Fig. 3. Proportion of randomly and non-randomly associated species pairs among epiphytic bryophyte communities of the lower trunk, the upper trunk, and the canopy in a tropical rainforest (Yunnan, China) and contribution (%) of the factors accounting for non-random species associations.

conditions, 17% by positive interactions, and 2% by negative interactions. Abiotic factor was the most important factor among communities from the canopy and the upper trunk, but not from the tree base, wherein positive interactions prevailed (Fig. 3). The proportion of randomly associated species pairs increased from large (77%) to small (88%) trees (Fig. 4). Among non-randomly associated species pairs, positive biotic interactions were almost as important (36%) as abiotic environmental conditions on small trees but explained only 21% and 7% of non-randomly associated species pairs on medium and large trees, respectively (Fig. 4).

The taxonomic identity of the species involved in either positive or negative biotic interactions on small, medium, and large-sized trees and at the lower trunk, upper trunk and in the canopy are listed in Tables S3 and S4, respectively. Species from the same (moss–moss pairs or liverwort–liverwort pairs) and from different communities (mosses *versus* liverworts) tended to be involved in positive and negative interactions, respectively (Fig. 5a). Species sharing the same life form tended to be involved in positive associations, whereas species of different life forms tended to be involved in negative associations, with the exception of the lower trunk, where the reverse pattern prevailed (Fig. 5b).

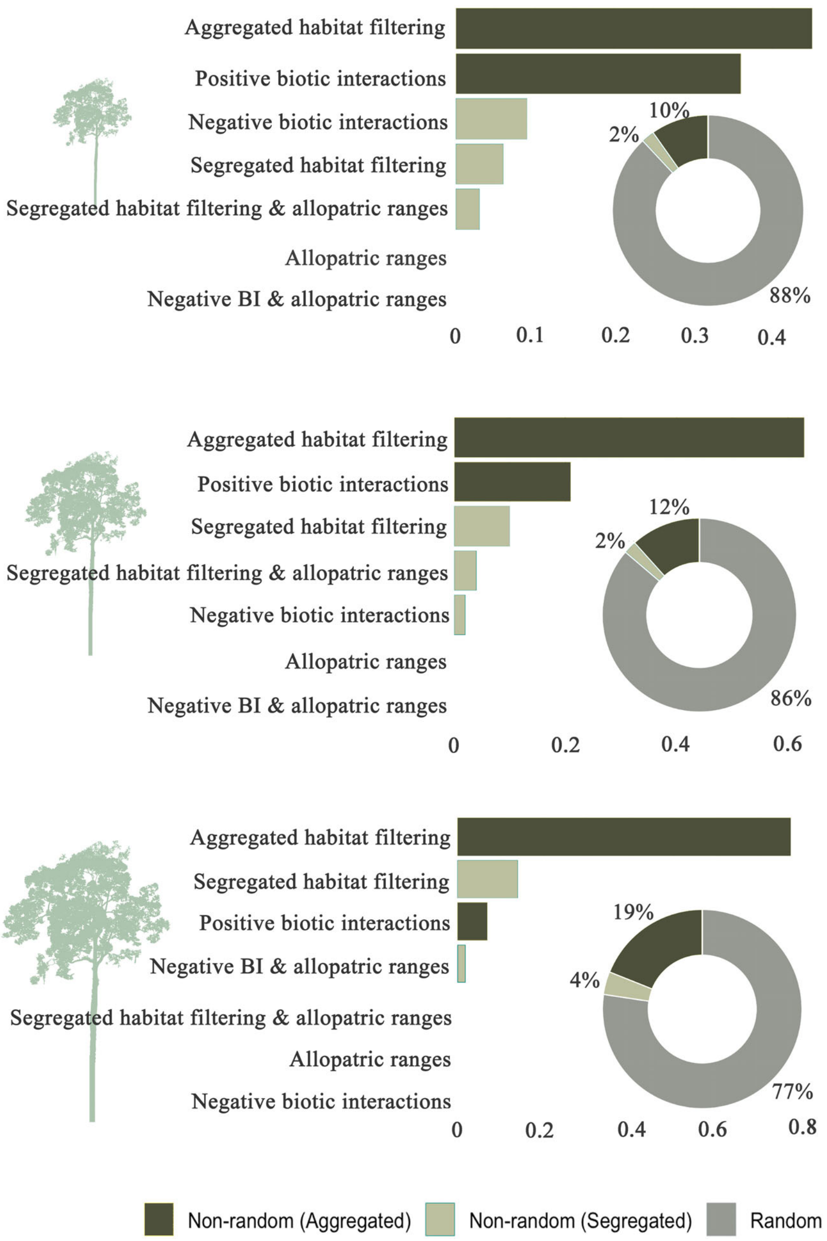

Fig. 4. Proportion of randomly and non-randomly associated species pairs among epiphytic bryophyte communities on small, medium-sized and large trees in a tropical rainforest (Yunnan, China) and contribution (%) of the factors accounting for non-random species associations.

DISCUSSION

A large majority (76%) of species pairs formed by epiphytic bryophytes was not significantly higher than expected by chance, in line with previous assessments on the role of chance in plant communities (D'Amen *et al.* 2018; Scherrer *et al.* 2019). Among non-randomly associated species pairs, abiotic environmental conditions explained more than 78% of the remaining variation. Biotic interactions, although widely accepted as an important driver of plant species distributions (Grant *et al.* 2014; Gavini *et al.* 2019), especially at the fine spatial scale investigated here (Götzenberger *et al.* 2012; Mod *et al.* 2020; Rossignaud *et al.* 2022, and references therein), contributed less than 20% of the observed co-occurrences. This is in line with the general perception that assembly mechanisms in bryophyte communities, especially epiphytic ones (Mota de Oliveira *et al.* 2009; Mota de Oliveira & ter Steege 2015), are primarily driven by niche preferences at this scale, with biotic interactions playing a secondary role (Slack 1990). The contribution of biotic interactions may even be overestimated here. We implemented a method (D'Amen *et al.* 2018) which assumes that non-randomly associated species pairs that cannot be explained in terms of abiotic environmental conditions or dispersal limitations result from biotic interactions. Although we attempted to describe species niches from a wide range of abiotic environmental conditions, it cannot be excluded that residual associations arise from missing environmental factors (Blanchet *et al.* 2020), such as bark texture and

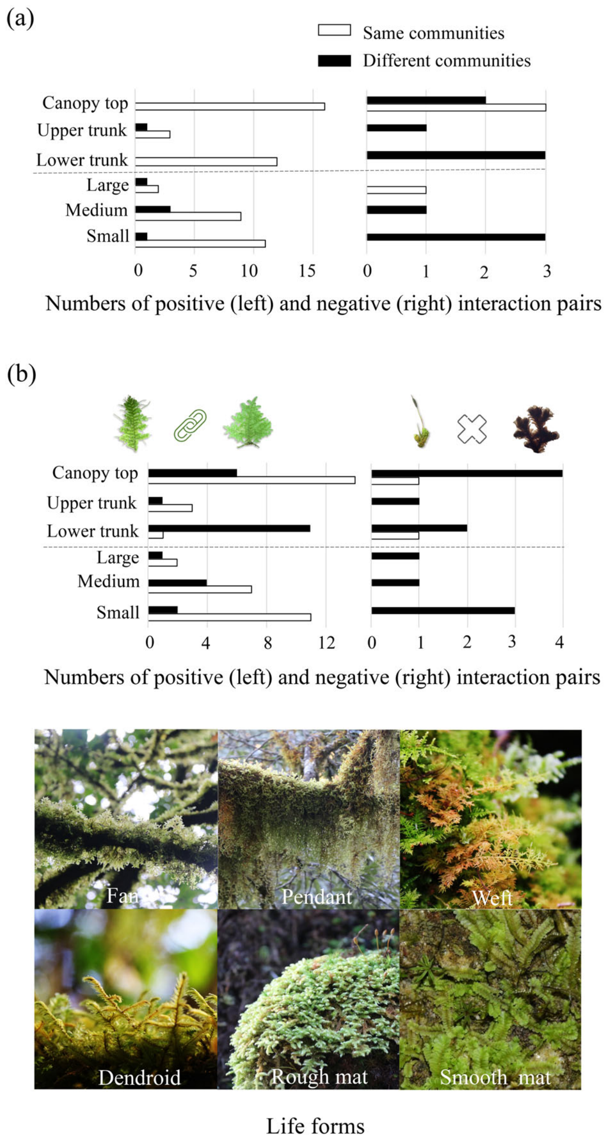

Fig. 5. (a) Numbers of positive and negative interactions among epiphytic bryophyte species in a tropical rainforest (Yunnan, China) involving pairs of species from the same (moss–moss pairs or liverwort–liverwort pairs) *versus* different communities (mosses *versus* liverworts). (b) Numbers of positive and negative interactions among epiphytic bryophyte species involving pairs of species from the same *versus* different life forms.

chemistry, branch diameter, or percentage cover of canopy humus (Woods *et al.* 2015).

Among the non-random species pairs that could not be explained by ecological preferences, co-occurrences prevailed over non-coincidences, involving 17% and 2% of non-randomly associated species pairs, respectively. A similar prevalence of co-occurrences over non-coincidences was previously reported among boral forest bryophyte communities (Økland 1994). The clustering of epiphyte species on the same tree may reflect a succession of species sharing similar ecological and/or dispersal traits. Early colonizers are characterized by higher rates of expression of traits involved in sexual and asexual reproduction (Sierra *et al.* 2019). As trees develop, characteristics such as bark texture and chemistry change, leading to a turnover of specialized communities that segregate on the tree as a function of its characteristics as a host. Furthermore, early colonizers initiate the process of soil formation, subsequently allowing late-succession species that depend on canopy soil to establish themselves (Victoriano-Romero *et al.* 2020), leading to 'facilitation cascades' (Zotz 2016). Together, these processes could account for the observed non-random species co-occurrences. We observed, however, similar proportions of co-occurring species pairs among communities growing at the same level of the tree and on trees within the same DBH class, thus sharing similar characteristics associated with their age. The fact, that observed co-occurrences cannot be explained by community succession, suggests that actual facilitation occurs, as proposed for plants in harsh environments (D'Amen *et al.* 2018; Scherrer *et al.* 2019). Epiphytes rely on rainfall for water, and species occurring in the canopy are exposed to extremely harsh conditions in terms of light and wind exposure, temperature range, and periods of drought. One strategy to mitigate the impact of such harsh conditions is to grow in dense colonies to enhance facilitation, explaining why vascular epiphyte distributions are typically aggregated and density-dependent and their communities are nested (see Spicer & Woods 2022 for review). This is especially true for bryophytes due to their poikilohydric nature. Moderately dense stands are dehydrated less rapidly than loose stands or isolated shoots because a dense packing of shoots may reduce water loss by effectively reducing the diameter of capillary spaces among close neighbours. Bryophytes growing in dense communities are therefore able to remain physiologically active for a larger part of the growing season, resulting in higher biomass (Bates 1998). Hence, shoot size, biomass production, and species diversity are often positively related to shoot density (Økland & Økland 1996; Økland 2000; Bergamini *et al.* 2001).

In agreement with our first hypothesis (H1), we observed a decrease in randomly associated species pairs from small to large trees, supporting the idea that deterministic processes play an increasingly important role in explaining species composition as habitat complexity and diversity increase (Måren *et al.* 2018). In contrast, in epiphyllous communities, Mežaka *et al.* (2022) found similar random and aggregated species distribution patterns across young and old leaves. These differences may be explained by the fact that epiphylls occupy a very temporary substrate that, as opposed to an entire tree, offers similar habitat conditions through time. Although early colonizers were shown to exhibit higher probabilities of sexual and specialized asexual expression than late colonizers (Sierra *et al.* 2019), all epiphylls must exhibit high dispersal capacities, and changes in species composition on leaves with time reflect a random process of progressive accumulation of species due to increased chances of colonization with time, rather than species turnover (Mežaka *et al.* 2019). In epiphytic bryophyte communities, in contrast, species turnover contributes substantially more to changes in species composition over time than nestedness, illustrating the suite of specialized communities that succeed on a tree through time in response to the increasing number of niches as the tree develops (Patiño *et al.* 2018).

In turn, the marginal contribution of negative interactions reported here supports the notion that competitive exclusion is a rare process in bryophyte communities (Steel *et al.* 2004; Mälson & Rydin 2009; Udd *et al.* 2016). The weak role of competition on old trees reported here challenges the idea that competition would be important enough to have a negative impact on the accumulation of species richness on trees following an optimum on middle-aged trees, when both pioneers and competitors co-exist (Ellis & Ellis 2013). Observations of co-occurrence patterns may, however, fail to fully capture negative interactions as they already represent the outcome of their effect (Poggiato *et al.* 2021). To avoid competition, species may, in fact, disperse or shift niche (Stroud *et al.* 2019, and references therein). Although its importance would require further research, niche displacement has, however, very rarely been reported in bryophytes (Wiklund & Rydin 2004).

In contrast to our second and third hypotheses (H2 and H3), no increase in competition from young to old trees or from the canopy to the base was observed. To the reverse, positive interactions potentially associated with facilitation prevailed at the tree base. These results were unexpected because, in line with a number of studies supporting the application of the stress-gradient theory in plant communities (Casalini & Bisigato 2018; Kjær *et al.* 2018; Muscarella *et al.* 2018; but see-Maestre *et al.* 2006; Qi *et al.* 2018), positive and negative interactions are thought to prevail in communities exposed to harsh and mild environmental conditions, respectively. Although microclimatic conditions substantially vary in terms of daily variations in light exposure, temperature and relative humidity along a tree (Shen *et al.* 2022a), at first sight generating strong stress gradients from the base to the canopy, the stress-gradient hypothesis is not always supported, even along apparently strong stress gradients (Maestre *et al.* 2006; Qi *et al.* 2018). For instance, Scherrer *et al.* (2019) similarly failed to identify changes in biotic interaction patterns among plant communities along an elevation gradient, and no support for the stress-gradient hypothesis was reported among epiphyllous bryophyte communities from gap and closed forest sites (Mežaka *et al.* 2022). In line with Mežaka *et al.* (2022), we tentatively suggest that different stress factors may operate and affect species co-occurrence patterns. For example, while canopies are typically regarded as high-stress habitats exposed to severe drought, temperature and light conditions, tree bases are characterized by extreme shade conditions, acting as a major stress factor (Körner 2003). The similarly low levels of competition observed on trees from all DBH classes suggests that competition on old trees is counter-balanced by the increasing diversity of microhabitats.

In agreement with our hypothesis H4a, co-occurrences involved species pairs from the same community (moss–moss pairs or liverwort–liverwort pairs), whereas non-coincidences involved pairs from different communities (moss–liverwort pairs). Negative interactions leading to spatial segregation were similarly reported in epiphyll communities composed of various communities, but play a minor role among species from the same community (Mežaka *et al.* 2022). This suggests that specific traits of moss and liverwort, or potentially, lineage-specific allelopathic substances, shape their biotic interactions, promoting facilitation among species from the same community and competition among species from different life forms.

Following our hypothesis H4b, negative interactions primarily occurred among species from different life forms. Some life forms are indeed prone to exclude others by competition. In particular, dendroids typically form dense colonies of erect secondary shoots expanding outwards from their primary, creeping shoots that behave as 'runners'. They thus combine the two extreme strategies developed by competitive clonal species, *i.e.*, the 'guerilla' strategy, wherein shoots infiltrate surrounding vegetation, maximizing interspecific contact, and allowing for a rapid spread and the 'phalanx' strategy, involving tightly packed advancing fronts of shoots (Doust 1981). The dendroid life form has therefore been interpreted as the result of selection for high-competitive ability (Bates 1998). Here, we observed that dendroids (*Pinnatella ambigua*), but also fans (*Caduciella mariei*, *Circulifolium microdendron*, and *Plagiochila* spp.), were recurrently involved in competitive interactions. In fact, fans, like dendroids, possess primary shoots creeping along the substrate and erect secondary shoots, the only difference being that fans exhibit strongly complanate leaves.

In turn, the higher proportion of species from the same life form than of species of different life forms in positive associations suggests that facilitation involves species sharing similar architectures. Experimental investigations indeed revealed that the strongest mutually beneficial association occurred between structurally similar species (Michel *et al.* 2012), maximizing a dense packaging of shoots, necessary for maintaining a maximum amount of water among shoots by capillarity and, hence, longer periods of physiological activity.

A different pattern was, however, observed in the lower trunk, wherein co-occurrence patterns involved species of different life forms. We hypothesize that, while communities in the canopy are exposed to severe drought stress, leading to the observed clustering of species sharing the same life form to enhance water retention, communities from the tree base do not or to a much lower extent suffer from water limitation. In those conditions, wherein dense communities are not necessarily adaptive, combinations of species of different life forms may result in a better partitioning of available space and resources.

The findings presented here provide a first quantitative assessment of the contributions of environmental filtering and biotic interactions to epiphytic bryophyte species composition. The main benefit of the current approach over experimental investigations is that it allows for analyses at the level of the entire community. The interpretation of co-occurrence patterns in terms of biotic interactions has, however, been challenged, notably because undetected environmental factors could be erroneously attributed to competition or facilitation (Blanchet *et al.* 2020; Zurell *et al.* 2020; Poggiato *et al.* 2021). In particular, while our experimental setting allowed us to generate a 3D micro-climatic model, suitable to describe vertical and horizontal variations on trees at fine spatial scales, the microclimate that organisms actually experience depends on their size (Pincebourde & Woods 2020). On the surface of rugose trunks, up to 10 °C temperature differences have been reported between the bottom and edge of ridges (Nicolai 1986). Tall bryophyte species themselves may affect the climate conditions experienced by smaller species. Further characterizing the climate conditions actually experienced by epiphytic bryophytes would involve the shift from micro- to nano-climates, which would allow us to better characterize the niches of epiphytic bryophytes and, hence, improve our assessment of their biotic interactions based on the community modelling implemented here. Although this does not undermine our preliminary conclusion that competitive interactions may play a minimal role in the investigated epiphytic bryophyte communities, we propose that the species pairs interpreted here as positive or negative interactions could be targeted for subsequent experiments on the variation in their fitness when grown in pure stands or mixed colonies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

F. Collart, T. Shen, and A. Vanderpoorten conceived the project. T. Shen, L. Song, W.-Z. Ma, J. Wang, and L. Mouton collected the data. F. Collart and T. Shen performed the analyses. R. T. Corlett and A. Guisan provided suggestions on the manuscript. T. Shen and A. Vanderpoorten wrote the manuscript, with the assistance of all co-authors.

DATA AVAILABILITY STATEMENT

The dataset analysed in the current study is available in the Figshare repository, with the identifiers (1) https://figshare.com/s/c282472053224783bbf1; (2) https://doi.org/10.6084/m9.figshare.17057624 (Shen *et al.* 2022a).

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Life-forms of the dominant epiphytic bryophyte species recorded on *Parashorea chinensis* at the Xishuangbanna canopy crane site (Yunnan, China).

Table S2. Evaluations of the species distribution models (ensemble and individual algorithms: GAM, BBM and GLM) for dominant epiphytic bryophyte species recorded on *Parashorea chinensis* at the Xishuangbanna canopy crane site (Yunnan, China).

Table S3. Epiphytic bryophyte species pairs involved in positive and negative biotic interactions on small, medium-sized and large specimens of *Parashorea chinensis* at the Xishuangbanna canopy crane site (Yunnan, China).

Table S4. Epiphytic bryophyte species pairs involved in positive and negative biotic interactions on the lower trunk, upper trunk and canopy of *Parashorea chinensis* in a tropical rainforest (Yunnan, China).

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cover picture: base of a tree covered with *Isothecium*, Lompret (Wallonia) in August 2023, from Lea Mouton