



Intraspecific variability drives functional changes in lichen epiphytic communities across Europe

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Abstract. Traditional approaches in trait-based community ecology typically expect that trait filtering across broad environmental gradients is largely due to replacement of species, rather than intraspecific trait adjustments. Recently, the role of intraspecific trait variability has been largely highlighted as an important contributor mediating the ability of communities to persist under changing conditions and determining the community-level trait variation, particularly across limited environmental gradients. Unfortunately, few studies quantify the relative importance of species turnover versus intraspecific variability mediating the response of communities different from vascular plants. Here, we studied the functional changes in epiphytic lichen communities within 23 beech forests across large latitudinal (ca. 3,000 km) and environmental gradients in Europe to quantify the relative contribution of species turnover and intraspecific variability and the role of climate controlling community-level trait changes. For 58 lichen species, we focused on a set of 10 quantitative functional traits potentially affected by climatic conditions and related to photosynthetic performance ($n = 1,184$ thalli), water use strategy ($n = 1,018$ thalli), and nutrient uptake ($n = 1,179$ thalli). Our results showed that intraspecific trait variability explained most of the functional changes in lichen communities in response to the latitudinal gradient. Further, such functional changes were determined by the covariation between intraspecific trait variability and species turnover, which varied in sign depending on the trait considered. Finally, different climatic predictors explained functional variation due to both intraspecific trait variability and species turnover. We propose that lichen communities cope with contrasting climatic conditions by adjusting the functional trait values of the most abundant species within the communities rather than by the replacement of the species. Consequently, intraspecific variability should be explicitly incorporated to understand the effect of environmental changes on lichen communities, even over large environmental variations, better. Our results challenge the universality of the hypothesis that species turnover chiefly drives functional trait changes across large environmental gradients and call for a wider test of such important assumptions in trait ecology in different organism types and ecosystems.

Key words: beech forests; climate; community ecology; functional ecology; functional trait variation; intraspecific variability; latitudinal gradient; lichen; species turnover.

INTRODUCTION

Understanding the patterns of biodiversity across environmental gradients is a primary goal to predict the potential response of communities in a global change context (Díaz and Cabido 2001, de Bello et al. 2013). In this way, functional traits provide a link for assessing not only the performance of communities under different environmental scenarios (Webb et al. 2010), but also

the impact of such contrasting conditions on ecosystem functioning (de Bello 2010). Most studies so far have assessed changes in community trait structure, neglecting the possible effect of intraspecific trait adjustments. Particularly, over broad environmental gradients it is expected that the effect of intraspecific trait adjustments should be negligible (Auger and Shipley 2013).

Overall, the functional variation in natural communities across different gradients can be determined by three components: species turnover, intraspecific trait variability, and their covariation (Lepš et al. 2011). Species turnover reflects the effect of replacement of functionally different species in terms of species identity and

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abundance. Intraspecific trait variability reflects the effect of trait adjustments within species due, for example, to plasticity or genetic variability. Although existing tests of the importance of these sources of trait variation are rather limited, it should be noticed that the relative contribution of these drivers may differ across sites and biological groups. A great number of studies relied on the assumption that differences in functional traits are larger among than within species (Garnier et al. 2001), which justifies why intraspecific trait variability has been largely ignored (Shiple et al. 2016). In the case of vascular plants, intraspecific trait variability does contribute to functional variation (Albert et al. 2010), but species turnover tends to have a considerably greater relative contribution (Kichenin et al. 2013, Siefert 2015). Moreover, the general expectation is that across broader environmental gradients, species turnover should have a more marked role than intraspecific trait adjustments (Auger and Shipley 2013).

Until now, these hypotheses and the importance of intraspecific trait variability has been largely explored in plant communities, but less is known about the sources and extent of functional variability in different organisms such as animals (Moretti 2017) or lichens (Asplund and Wardle 2014, Roos et al. 2019). For instance, Garcia-Raventós et al. (2017) found that species turnover explained most of the functional trait variation in freshwater macroinvertebrate communities. Conversely, intraspecific variability emerged as the main contributor to functional trait variation in response to environmental changes in lichen communities (Asplund and Wardle 2014), especially for chemical traits (i.e., tissue nitrogen [N] and phosphorous [P] content, N:P ratio, and pH; Roos et al. 2019), which supports the importance of intraspecific variation as acclimation to changing environmental conditions (Björklund et al. 2009). This finding challenges the generalization of the trends found in vascular plants and suggests that the inclusion of intraspecific variability might better predict the response of communities by better explaining the ability of species to cope with environmental changes (Kichenin et al. 2013). However, as far as we know, only Asplund and Wardle (2014) and Roos et al. (2019) have sought to acknowledge the importance of species turnover and intraspecific variability in lichen communities, and more studies, including a wider suite of traits and environmental conditions, are needed to elucidate general patterns.

Lichens are amongst the most sensitive organisms to environmental changes (Matos et al. 2015), and represent a valuable ecological system to anticipate and model the response of other less-sensitive organisms present in the ecosystems (Pinho et al. 2011). They are poikilohydric organisms particularly sensitive to precipitation and temperature factors, because their physiology is tightly linked to cycles of hydration and dehydration and they lack mechanisms to control their water and nutrient content (Prentice et al. 1992). Therefore, they have been largely recognized as meaningful ecological indicators of

environmental factors such as climate and nutrient deposition (Giordani et al. 2012, Matos et al. 2015). The lichen functional traits typically used as response traits (*sensu* Bellau and Shipley 2018) are growth form, photobiont type, and reproductive strategy, which are easily recognizable morphoanatomical attributes that inform about different aspects related with the physiology and activity of lichens. On the other hand, and as in vascular plants, a different set of functional traits inform about specific functions of these organisms (Cornelissen et al. 2007), such as photosynthetic performance (i.e., chlorophyll content), water use strategy (i.e., specific thallus mass [STM] and water-holding capacity [WHC]) and resource uptake and retention (i.e., thallus nutrient concentration and isotopic ratios; Gauslaa 2014). These continuous traits capture precise functions and impact ecosystem processes (e.g., water and nutrient cycling, litter decomposition, and food webs; Ellis 2012), but most studies have used categorical traits extracted from databases (such as growth form, photobiont type, reproductive strategy), rather than measuring continuous traits. Meanwhile, the leaf mass per area (LMA) has been largely used and probed to reflect distinct responses to environmental gradients in vascular plants successfully (Wright 2004, Poorter et al. 2009), but very few studies have characterized the variation of the analogous lichen functional trait (i.e., STM) occurring in natural communities across wide environmental gradients (Gauslaa and Coxson 2011, Asplund and Wardle 2014).

In the present study, we assess the universality of the hypothesis that species turnover chiefly drives functional trait changes across broad environmental gradients. To that end, we quantify the relative contribution of species turnover and intraspecific variability determining the functional variation in epiphytic lichen communities across a broad environmental gradient. We also assess the role of climate in controlling the species turnover, intraspecific variability, and total variation in the community-level trait measures. To address these questions, we studied lichen epiphytic communities in 23 beech forests across a latitudinal gradient in Europe comprising the entire distribution range of *Fagus sylvatica* L. We focused on a set of 10 quantitative functional traits potentially affected by climatic conditions and related to photosynthetic performance, water use strategy, and nutrient uptake. Based on previous studies on lichens (Asplund and Wardle 2014) and contrary to the findings for vascular plants (Kichenin et al. 2013, Siefert et al. 2015), we hypothesize that functional changes at community level across the broad latitudinal gradient will be influenced by both species turnover and intraspecific variability, with a higher relative contribution of the latter. Furthermore, we expect that climatic factors will be the main drivers behind both species turnover and intraspecific variability in lichen communities given the inherent limitations of these poikilohydric organisms to actively buffer contrasting climatic conditions.

METHODS

Sampling design

The study was carried out across a latitudinal gradient in Europe comprising more than 3,000 km from southern Sweden to southern Italy. The latitudinal gradient reflects a climatic gradient in temperature and precipitation, both in annual values and seasonality, with differences of 7°C in mean annual temperature and 1,080 mm in total annual precipitation (Appendix S1: Table S1). We surveyed lichen epiphytic communities in 23 mature and well-conserved monospecific stands of European beech with a tree cover >65% and without tree cutting during the last 50 yr (Fig. 1). In all cases, we surveyed mature epiphytic communities with presence of *Lobaria pulmonaria* (L.) Hoffm.

We followed the protocol of Aragón et al. (2012) to record the composition of the lichen community, accounting for more than a 90% of the species present in each stand. Within each stand, we randomly selected five 25 × 25 m plots, 100 m apart from forest edge and 500 m among plots. Within each plot, we selected 10 beech trees (diameter at breast height [dbh] > 25 cm) and placed four 20 × 30 cm grids on each trunk, in the north and south faces and at breast height and tree base. Within each grid, we estimated the cover (%) of all lichen species found following Smith et al. (2009) and Clauzade and Roux (1985) for species identification. In total, we surveyed 1,150 trees and collected data from 4,600 sample units (grids).

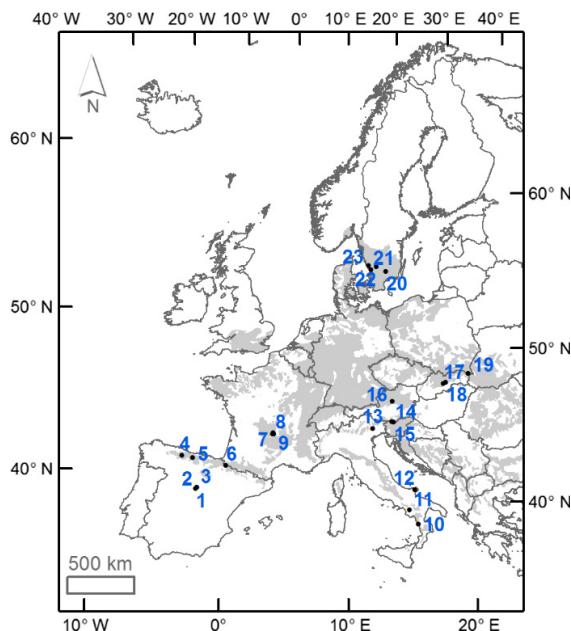


FIG. 1. Map of the study area showing the distribution area of *Fagus sylvatica* (light gray) and the 23 sampling sites (black dots). Full name of the sampling sites in Appendix S1: Table S1.

In addition, we randomly collected four thalli in each forest, whenever possible, of all macrolichen species found across the gradient for the measurement of quantitative functional traits in the laboratory. Mature thalli with similar size of each lichen species were collected in different trees. All the samples were collected after spring leaf budding, between June and September 2015 and 2016. After collection, samples were air-dried and frozen (−20°C), as is recommended for later physiological studies in lichens (Honegger 2003).

Trait data

A total of 58 species of macrolichens were found across the latitudinal gradient (Appendix S1: Table S2). For these species we measured 10 quantitative functional traits related to photosynthetic performance ($n = 1,184$ thalli), water use strategy ($n = 1,018$ thalli), and nutrient uptake ($n = 1,179$ thalli; mean values in Appendix S1: Table S3). Regarding photosynthetic performance, we quantified chlorophyll a content (Chla, in mg/g), chlorophyll b content (Chlb, in mg/g), and normalized phaeophytinization index (NPQI, unitless) following Barnes et al. (1992) and equations given by Wellburn (1994). In relation to water use strategy, we measured specific thallus mass (STM, in mg dry mass/cm²) and water-holding capacity (WHC, in mg water/cm²) according to Merinero et al. (2014). We hydrated the thalli (c. 120 mg) until full saturation with deionized water, scanned them, and analyzed the images (Adobe Photoshop CS6 Extended, Adobe Systems, San Jose, California, USA) to record the thallus area (A). Immediately after, we fully hydrated and gently blotted the thalli with filter paper to measure wet mass (WM) to the nearest mg. Then, we oven-dried (60°C for 72 h) the thalli to quantify dry mass (DM) in milligrams. $STM (mg DM/cm^2) = DM/A$ and $WHC (mg H_2O/cm^2) = (WM - DM)/A$. For the nutrient uptake, we quantified thallus carbon content (%C), thallus nitrogen content (%N), carbon–nitrogen ratio (C/N), carbon isotopic ratio ($\delta^{13}C$, in ‰) and nitrogen isotopic ratio ($\delta^{15}N$, in ‰) using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at UC Davis Stable Isotope Facility.

Climatic predictors

We used a selection of 4 climatic variables, out of an original set of 19 available variables, in order to assess the contribution of these climatic conditions to explain the species turnover and intraspecific trait variability of the lichen communities across the gradient (SeeData analysis section). Climatic information at forest level was retrieved from the high-resolution climate data set CHELSA (Karger et al. 2017) including variables related

to annual values and seasonal ranges of temperature and precipitation (Appendix S1: Table S1).

Data analysis

We first quantified the amount of species turnover across the 23 beech forests studied across Europe. We did this by quantifying the variation of the species composition of macrolichen communities across the latitudinal gradient (i.e., beta diversity), accounting for both the species turnover and the nestedness of assemblages without the influence of richness gradients. Species turnover informs about the replacement of some species by others reflecting environmental, spatial, and historical constraints, and nestedness reflects a nonrandom process of species loss (Baselga 2010). We used the *beta.multi* function implemented in the *betapart* package (Baselga and Orme 2012) to compute the value of species turnover measured as Simpson dissimilarity (*beta.sim*), the value of nestedness measured as the nestedness-resultant fraction of Sørensen dissimilarity (*beta.sne*), and the value of the overall beta diversity measured as Sørensen dissimilarity (*beta.sor*). *Beta.sim*, *beta.sne*, and *beta.sor* range from 0 to 1, representing the highest similarity and lowest similarity, respectively.

We then performed a variance partitioning analysis to unveil the drivers behind the variability of the quantitative traits measured in macrolichen communities across the gradient, and their response to the climatic conditions. We did this by testing whether the variation of community-weighted mean indices (CWM) across the gradient for the 10 quantitative traits studied was caused by species turnover, intraspecific trait variability, or their covariation, following the method proposed by Lepš et al. (2011). This method allows the assessment of trait variation across gradients and the quantification of the relative effect caused by species turnover, intraspecific trait variability or their covariation (Siefert et al. 2015). The method can be applied for each trait to test, overall, how much the changes in CWM values across sampling units depend on species turnover, intraspecific trait variability, or their covariation, irrespective of any potential environmental predictor. Further, it can allow the test of given environmental predictors on species turnover, intraspecific trait variability, and total variation. For each quantitative trait the approach is based on computing three community parameters, the specific CWM (CWMs using the traits measured in a given stand), the fixed CWM (CWMf using species trait averages across all stands), and the difference between them. We calculated the first two with the *functcomp* function implemented in the *FD* package (Laliberté and Legendre 2010). The CWMs were computed with the trait values measured in each of the 23 forest stands (i.e., mean value at a given site for each species). Variations across the forests in CWMs inform about the total functional variation across forest stands, which can be due to both species turnover and intraspecific trait variability. The

CWMf was computed using the same trait value for a given species, irrespective of where it is growing (i.e., the overall mean value across all sites). The CWMf informs about the species turnover only, as it uses the same trait value for species in all sites. The difference between CWMs and CWMf accounts exclusively for the effect of intraspecific trait variability. Thus, CWMs, CWMf, and their difference were used to quantify the amount of variability explained by species turnover or intraspecific trait variability by using a sum-of-squares decomposition procedure through the R function *trait.flex.anova* (see Lepš et al. 2011 for further details about the procedure). The method also provides a measure of covariation between turnover and intraspecific effects. When the covariation is positive (i.e., the sum of variance due to turnover and intraspecific trait variability is below 100%), it indicates that turnover and intraspecific trait variability have a similar effect; for example, in those conditions where smaller species are favored, smaller individuals within those species are favored. When the sum of the variance explained by species turnover and intraspecific trait variability is larger than 100%, there is a negative covariation between them. A negative covariation suggests that the variations in traits across the studied gradient due to species turnover counterbalance each other (i.e., the changes in trait values have a different sign, for example, in those conditions where smaller species are favored, bigger individuals within those species are favored).

To apply the method by Lepš et al. (2011) we determined the relative contribution of species turnover and intraspecific trait variability on the total variation of CWM values across the 23 forest stands in Europe, irrespective of any environmental predictor. We also investigated the direct influence of a selection of climatic predictors in such variation. Among the set of all environmental variables, we selected four main climatic predictors, two related to temperature (isothermality and mean temperature of driest quarter), and two related to precipitation (precipitation of driest quarter and precipitation of coldest quarter). These predictors were not significantly correlated (Spearman's $\rho < 0.7$, $P > 0.05$) and showed high load in the axes of the principal components analysis performed with an original selection of 19 climatic variables available (Appendix S1: Fig. S1). We repeated the procedure explained above for each of the 10 quantitative traits (dependent variable) using, in a single model, isothermality, mean temperature of driest quarter, precipitation of driest quarter, and precipitation of coldest quarter as explanatory variables. We used a slight modification of the function *trait.flex.anova*, to include analysis of variance (ANOVA) type II for quantifying the relative contribution of each predictor explaining the trait variability and the significance of the testable effects. We used ANOVA type II to assess the variance explained by each explanatory variable, which was not confounded with variance explained by the other explanatory variables. Finally, we evaluated the

direction of the changes in community-level trait measures due to species turnover, intraspecific trait variability, and total variation in response to the different climatic predictors (Appendix S1: Fig. S2). All statistical analyses were performed using R version 3.5.0 (R Development Core Team 2018).

RESULTS

We found a large amount of variation of the species composition of macrolichen communities across the latitudinal gradient (i.e., beta diversity), with β_{sor} close to 1 (i.e., $\beta_{sor} = 0.9$). Particularly, 91% of the overall beta diversity was due to species turnover ($\beta_{sim} = 0.8$), and nestedness performed a much lower contribution explaining around 9% of overall beta diversity ($\beta_{sne} = 0.1$).

Decomposition of total variability indicated that the relative contribution of intraspecific trait variability and species turnover differed in the 10 quantitative traits studied (Fig. 2). In six of the measured traits (Chla, NPQI, STM, WHC, %C, and $\delta^{13}C$), the intraspecific trait variability was the main determinant of functional trait variations, meaning that changes in dominant trait values across the gradient derived from the variation of the trait values within given species, rather than changes in the species pool conforming the macrolichen communities in each forest stand. In contrast, only in %N did the species turnover show the highest contribution to the total variability. Another suite of three traits (Chlb, C/N, and $\delta^{15}N$) had a similar relative contribution of intraspecific trait variability and species turnover, being always higher for the former. The covariation found between intraspecific trait variability and species turnover was positive in the case of four traits (Chlb, %C, C/N, and $\delta^{15}N$), but negative in six traits (Chla, NPQI, STM, WHC, %N and $\delta^{13}C$; Fig. 2).

Changes in community-weighted trait values due to species turnover and intraspecific trait variability responded to climatic predictors, with mean temperature of driest quarter being the only predictor affecting all the studied traits (Table 1). Both total trait variation and variation due to intraspecific trait variability were better explained by climatic predictors than functional variation due to species turnover. Overall, temperature variables showed a greater contribution than precipitation variables explaining the observed functional variation. In particular, functional variation linked to species turnover was only explained by temperature variables: Isothermality (temperature fluctuations within a month relative to the year) and mean temperature of driest quarter were the main drivers determining changes in Chla, Chlb, and STM due to species turnover, whereas WHC, $\delta^{13}C$, and $\delta^{15}N$ were only affected by mean temperature of driest quarter (Table 1). Both isothermality and mean temperature of driest quarter influenced Chla, Chlb, and STM negatively, as well as Chlb and $\delta^{13}C$, respectively; meanwhile the latter variable affected Chla,

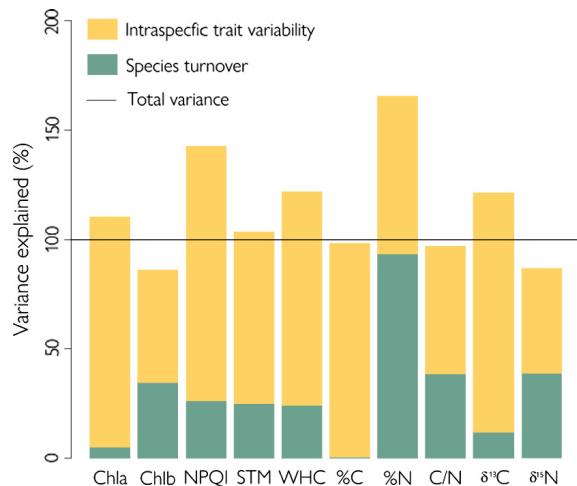


FIG. 2. Relative contribution of intraspecific trait variability and species turnover to community trait variation across the gradient. Yellow and dark green denote the contribution of intraspecific trait variability and species turnover, respectively. The covariation between intraspecific trait variability and species turnover is represented through the space between the column and the black line (total variance). When the column goes beyond the black line there is a negative covariation, and when the column does not reach the black line, the covariation is positive. Abbreviations: Chla, chlorophyll a content; Chlb, chlorophyll b content; NPQI, normalized phaeophytinization index; STM, specific thallus mass; WHC, water-holding capacity; %C, thallus carbon content; %N, thallus nitrogen content; C/N, carbon/nitrogen ratio; $\delta^{13}C$, carbon isotopic ratio; $\delta^{15}N$, nitrogen isotopic ratio.

STM, WHC, and $\delta^{15}N$ (Table 1; Appendix S1: Fig. S2) positively. Regarding functional variation linked to intraspecific trait variability, both temperature and precipitation variables contributed: We observed a reduction in Chla, NPQI, STM, WHC, %C, and %N, and an increase in $\delta^{13}C$ due to a higher mean temperature of the driest quarter and an opposite pattern in relation with precipitation of the coldest quarter (higher NPQI, STM, %C, %N, and lower $\delta^{13}C$ with increasing precipitation); isothermality affected STM and WHC (Table 1; Appendix S1: Fig. S2) positively. Finally, the total variation at community level in four of the studied traits (Chlb, NPQI, %C, and C/N), was explained by mean temperature of the driest quarter, positively for Chlb and negatively for NPQI, %C, and C/N (Table 1; Appendix S1: Fig. S2). NPQI also responded positively to isothermality and precipitation of the coldest quarter, as well as STM and %C due to the latter variable (Table 1; Appendix S1: Fig. S2).

DISCUSSION

Our results showed that intraspecific trait variability explained most of the functional changes in lichen communities in response to a wide latitudinal gradient (Fig. 3), in contrast to the broad trends typically expected in plant communities (Auger and Shipley 2013,

TABLE 1. Relative contribution (%) of climatic factors to species turnover, intraspecific trait variability (ITV), and total variation of functional traits across the gradient.

	Isothermality			Mean temperature of driest quarter			Precipitation of driest quarter			Precipitation of coldest quarter		
	Turnover	ITV	Total	Turnover	ITV	Total	Turnover	ITV	Total	Turnover	ITV	Total
Chla	1.54 ↓	–	–	1.89 ↑	23.88 ↓	–	–	–	–	–	–	–
Chlb	4.55 ↓	–	–	8.21 ↓	–	22.05 ↓	–	–	–	–	–	–
NPQI	–	–	15.24 ↑	–	30.64 ↓	15.18 ↑	–	–	–	–	17.97 ↑	11.92 ↑
STM	4.36 ↓	15.55 ↑	–	4.57 ↑	–	–	–	–	–	–	12.15 ↑	18.38 ↑
WHC	–	23.63 ↑	–	5.61 ↑	17.28 ↓	–	–	–	–	–	–	–
%C	–	–	–	–	21.06 ↓	19.66 ↓	–	–	–	–	14.34 ↑	15.64 ↑
%N	–	–	–	–	22.09 ↓	–	–	–	–	–	12.08 ↑	–
C/N	–	–	–	–	–	17.06 ↓	–	–	–	–	–	–
$\delta^{13}\text{C}$	–	–	–	3.40 ↓	28.25 ↑	–	–	–	–	–	14.24 ↓	–
$\delta^{15}\text{N}$	–	–	–	9.22 ↑	–	–	–	–	–	–	–	–

Notes: Only significant relationships are shown. Arrows represent the direction of the changes, \uparrow = positive effect, \downarrow = negative effect (see Appendix S1; Fig. S2 for further details). %C, thallus carbon content; %N, thallus nitrogen content; C/N, carbon/nitrogen ratio; Chla, chlorophyll a content; Chlb, chlorophyll b content; NPQI, normalized phaeophytinization index; STM, specific thallus mass; WHC, water-holding capacity; $\delta^{13}\text{C}$, carbon isotopic ratio; $\delta^{15}\text{N}$, nitrogen isotopic ratio.

Siefert et al. 2015). Such functional changes at community level were also determined by the covariation between intraspecific trait variability and species turnover, which varied in sign depending on the trait considered. Most studies centered on plants (e.g., Lepš et al. 2011, Kumordzi et al. 2014, Carvajal et al. 2018, Liu et al. 2019) and the few including lichens (Roos et al. 2019), found that species turnover and intraspecific trait variability tend to positively covary, whereas here we found the opposite; that is, twice as many cases showed a negative covariation (Fig. 2). Functional variation due to both intraspecific trait variability and species turnover was explained by different climatic predictors. Whereas intraspecific trait variability and total trait variation in lichen communities across the gradient responded to temperature and precipitation variables, species turnover was only explained by temperature. Climatic predictors affected traits differently. We discuss these three main findings.

Overall, intraspecific variability was the highest contributor explaining the variation of lichen functional traits at the community level, even though there is considerable turnover in species across the gradient considered. This finding differs with respect to vascular plants (e.g., Lepš et al. 2011, Kirchein et al. 2013) and non-chemical traits in lichens (Roos et al. 2019) in which interspecific variation tends to be larger than intraspecific variation at community level, especially across broad environmental gradients (Auger and Shipley 2013), but see Siefert et al. (2015) regarding chemical traits.

Similarly to Asplund and Wardle (2014), we identified a much greater contribution of intraspecific variability than species turnover in six of the functional traits studied (Chla, NPQI, STM, WHC, %C, and $\delta^{13}\text{C}$), meaning that the responses of lichen communities to environmental changes are influenced by the high phenotypic plasticity, or selection of heritable genetic variation, of the most abundant species across the communities considered (Fridley and Grime 2010), and not by the replacement of the species alone. These results are also in accordance with those focused on single species, which found an important within-species variation for traits as WHC or STM (Gauslaa and Coxson 2011, Merinero et al. 2014), supporting the plasticity of lichen species to cope with the environmental variation along wide climatic gradients. Therefore, traits related to photosynthetic performance, water use strategy, and carbon allocation showed large differences within lichen species and were highly sensitive to the environmental conditions, pointing out that intraspecific variation of this suite of traits cannot be safely ignored across the studied latitudinal gradient (Shipley et al. 2016). However, Chlb and traits related to nitrogen allocation showed a different pattern, with an increasing relative importance of species turnover. Exclusively for the thallus nitrogen content, species turnover was considerably more important than intraspecific trait variability, which is in disagreement with results from Roos et al. (2019). Interestingly, those traits with an important contribution to species turnover were related to the main

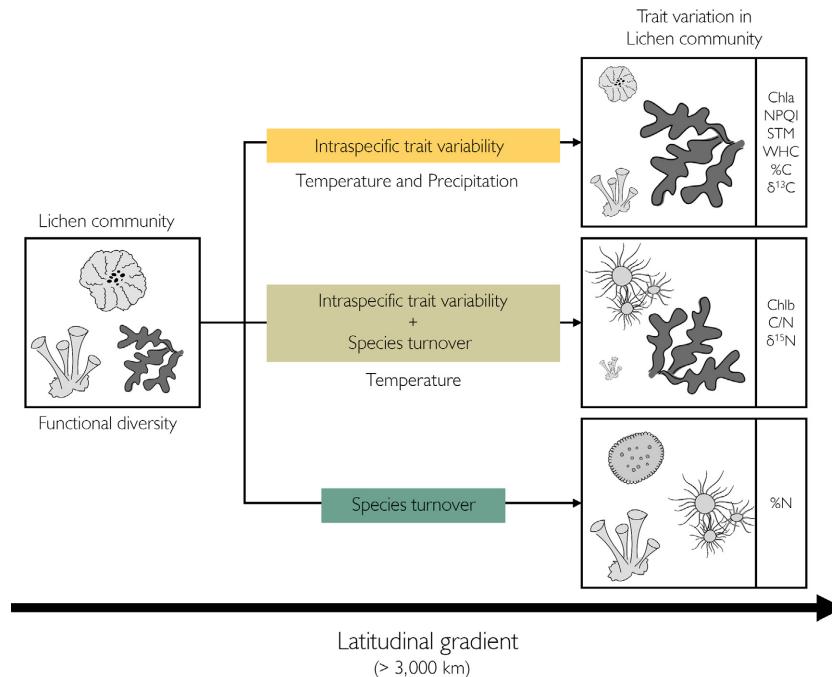


FIG. 3. Summary of the main results. Intraspecific trait variability explained most of the functional changes in lichen communities in response to a latitudinal gradient across Europe. We identified three patterns of functional variation at community level with: (1) intraspecific trait variability performing the highest relative contribution in Chla, NPQI, STM, WHC, %C, and $\delta^{13}\text{C}$; (2) intraspecific trait variability having the highest relative contribution, but species turnover showing a similar relative contribution in Chlb, C/N, and $\delta^{15}\text{N}$; and (3) species turnover showing the highest contribution to variability in %N. Functional variation due to intraspecific trait variability responded to temperature and precipitation variables in Chla, NPQI, STM, WHC, %C, and $\delta^{13}\text{C}$, whereas functional variation linked to intraspecific trait variability and species turnover in Chlb, C/N, and $\delta^{15}\text{N}$ was only explained by temperature. Abbreviations as in Fig. 2.

photosynthetic partner in the lichen symbiosis (i.e., green algae or cyanobacteria), supporting its genetic constraint mostly at the interspecific level (Marks 2007). As such, cyanolichens are known to lack Chlb (Palmqvist and Sundberg 2000) and to possess the ability for fixing atmospheric nitrogen (Palmqvist et al. 1998). Thus, changes in the abundance of chloro- and cyanolichens within the communities across the gradient seem to underpin the high contribution of species turnover to the trait variation (see Appendix S1: Fig. S3).

For all studied traits, species turnover and intraspecific trait variability covaried, but the direction of such covariation differed. We found a positive covariation in those traits with a similar relative contribution of species turnover and intraspecific variability, meaning that the same drivers would favor both the abundance of species with higher values of Chlb, C/N, and $\delta^{15}\text{N}$, and the increase of these trait values within individuals of the same species. In turn, we found the opposite result when species turnover or intraspecific trait variability displayed a much larger relative contribution than the other. As such, the same factor would reduce the relative abundance of species with high levels of Chla, NPQI, STM, WHC, %N, and $\delta^{13}\text{C}$, but increase the intraspecific trait values in response to the gradient. Surprisingly, even for the same biological group (i.e., lichens) our

results are not completely consistent with those obtained by Asplund and Wardle (2014) and Roos et al. (2019), who observed positive covariation in the studied traits across a soil fertility and elevational gradient in northern Sweden and southern Norway, respectively. This contrasting result emphasizes the importance of the precise environmental gradient considered (Kichenin et al. 2013) and prevents establishing general patterns of covariation between intraspecific variability and species turnover. One possible reason explaining why a high thallus nitrogen content within species is not accompanied by an increase of the abundance of species with higher %N could be related to differences in the factors determining both determinants of functional variation. Nutrient content in lichens is tightly determined by the amount of atmospheric nutrients (Johansson et al. 2010) and, consequently, high levels of atmospheric nitrogen may increase the %N in lichens without nitrogen-fixing photobionts. However, the increase of the relative abundance of cyanolichens, with nitrogen fixation ability and higher %N, may depend on the availability of compatible photobionts (Cardós et al. 2019) rather than on the atmospheric nutrient deposition.

Regarding the role of climate, we observed contrasting climatic factors affecting the species turnover and intraspecific trait variability of lichen communities

across the latitudinal gradient. The amount of variation explaining the species turnover was relatively low (i.e., 1.5–9.2%), which may be partially explained by the low relative contribution of species turnover to the community-level variation in most studied traits. Furthermore, those traits with a relatively high contribution of species turnover are related with the type of photobiont and the atmospheric nutrient availability, suggesting that factors such as the availability of compatible photobionts, the level of specificity for the photobiont, and nitrogen deposition affect species turnover more than climate does (Cardós et al. 2019, Johansson et al. 2010). The species turnover may also respond to biotic interactions of competition and facilitation, and to the dispersal capacity and effective local establishment of different species due to their reproductive strategy (Prieto et al. 2017). Nonetheless, isothermality and temperature of the driest quarter were the main climatic factors driving species turnover in lichen communities, suggesting that temperature rather than precipitation was the constraining factor determining changes in community composition across the latitudinal gradient. Conversely, community-level responses linked to intraspecific trait variability responded to both temperature and precipitation factors, with different directions in some cases, reflecting the combined sensitivity of these poikilohydric organisms (Matos et al. 2015), which are physiologically active according to cycles of hydration and dehydration. In particular, isothermality, temperature of the driest quarter, and precipitation of the coldest quarter mediated intraspecific changes in traits related to photosynthetic performance, water use strategy and nutrient allocation, pinpointing that precise physiological changes at individual level may shape lichen community responses to climate. Thus, mean temperature of the driest quarter, which negatively influences the Chla, NPQI, WHC, %C, and %N, would indicate a resource-conservative strategy for lichens under stressful conditions (Palmqvist and Sundberg 2000). Surprisingly, when considering the total trait variation, five traits were not affected by climate (Chla, WHC, %N, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$) and, for the other five traits (Chlb, NPQI, STM, %C, and C/N) the climatic factors accounted for less than ca. 40% of the total variation. This relatively low impact of the large-scale climatic drivers considered suggests that those traits linked to precise physiological functions could play an important role in understanding the effects of environmental changes on ecosystem functioning rather than as ecological indicators. However, species may respond to finer-scale changes in environmental variables that cannot be predicted using climatic averages (Kimball et al. 2010).

CONCLUSIONS

Disentangling the relative contribution of inter- and intraspecific trait variability and the environmental drivers underpinning the responses of natural communities

is critical in community ecology for gaining insight into the effects of the ongoing global change. Indeed, unveiling whether the replacement of species or the adjustment of trait values as the climate changes mediate the response of communities across environmental gradients will improve our understanding on the ability of the communities to persist under changing conditions. We found that the relative contribution of intraspecific trait variability and species turnover mediating the response of lichen communities to a broad latitudinal gradient differed among traits, but intraspecific trait variability was larger than species turnover. Therefore, intraspecific variability likely has significant ecological consequences and should be explicitly incorporated to understand the effect of environmental changes on lichen communities better. In particular, traits related to photosynthetic performance, water use strategy, and carbon allocation varied mostly at the intraspecific level, showing their high sensitivity to the environment and suggesting that this suite of traits allow lichen species to respond to environmental changes. Conversely, traits tightly linked to the main photosynthetic partner in the lichen symbiosis were more conserved and varied mostly at the interspecific level. More generally, our findings highlight the need to avoid average values for the studied traits and the measurement of trait values at the level of individuals for assessing community-level responses. In plant communities, integrating intraspecific trait variability in community ecology may strengthen understanding of processes operating at community and ecosystem levels (Siefert et al. 2015), but in lichen communities, this is essential, because intraspecific trait variability performed the highest contribution mediating these community-level functional responses in a wide set of traits. Therefore, our results challenge the universality of the hypothesis that species turnover chiefly drives functional trait changes across large environmental gradients and call for a wider test of such important assumptions in trait ecology in different organism types and ecosystems.

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