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REPORT

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Carbon-concentrating mechanisms are a key trait in lichen ecology and distribution

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Abstract

Carbon-concentrating mechanisms (CCMs) are a widespread phenomenon in photosynthetic organisms. In vascular plants, the evolution of CCMs ([C44-carbon compound] and crassulacean acid metabolism [CAM]) is associated with significant shifts, most often to hot, dry and bright, or aquatic environments. If and how CCMs drive distributions of other terrestrial photosynthetic organisms, remains little studied. Lichens are ecologically important obligate symbioses between fungi and photosynthetic organisms. The primary photosynthetic partner in these symbioses can include CCM-presenting cyanobacteria (as carboxysomes), CCM-presenting green algae (as pyrenoids) or green algae lacking any CCM. We use an extensive dataset of lichen communities from eastern North America, spanning a wide climatic range, to test the importance of CCMs as predictors of lichen ecology and distribution. We show that the presence or absence of CCMs leads to opposite responses to temperature and precipitation in green algal lichens, and different responses in cyanobacterial lichens. These responses contrast with our understanding of lichen physiology, whereby CCMs mitigate carbon limitation by water saturation at the cost of efficient use of vapor hydration. This study demonstrates that CCM status is a key functional trait in obligate lichen symbioses, equivalent in importance to its role in vascular plants, and central for studying present and future climate responses.

KEYWORDS

carboxysomes, ecophysiology, green algae, photobiont, photosynthesis, pyrenoids

INTRODUCTION

Carbon-concentrating mechanisms (CCMs) are a widespread phenomenon in photosynthetic organisms, with their relative absence in land plants being a notable exception. These mechanisms have evolved to increase the ratio of carbon to oxygen within chloroplasts; examples include the C4 (4-carbon compound) and CAM (crassulacean acid metabolism) photosynthetic pathways in plants, pyrenoids in green algae and carboxysomes in cyanobacteria (Meyer & Griffiths, 2013).

In vascular plants, the evolution of CCMs (C4 and CAM) is considered a major innovation, associated with significant range shifts, most often to hot, dry and bright, or aquatic environments (Sage, 2017). The repeated evolution of CCMs in terrestrial plants (Sage, 2017) is mirrored by its repeated loss in algal lineages (Goudet et al., 2020; Meyer & Griffiths, 2013; Raven et al., 2011); in both contexts transitions in CCM status have occurred convergently in response to environmental shifts (Goudet et al., 2020; Sage, 2017). Carbon-concentrating mechanisms in single-celled algae and cyanobacteria often take the form of anatomical and/or biochemical specializations such as pyrenoids (Meyer & Griffiths, 2013). Unlike terrestrial vascular plants where CCMs are often associated with drought tolerance, CCMs in algae and cyanobacteria help overcome carbon limitation in water-saturated environments, where CO_2 diffusion is much slower than in air (Goudet et al., 2020; Raven et al., 2011).

In terrestrial environments algae and cyanobacteria (photobionts) can form lichen symbioses with fungi, which represent significant diversity, biomass and carbon fixation in many ecosystems (Asplund & Wardle, 2017). Most green algal photobionts and all cyanolichens have carbon-concentrating structures, such as pyrenoids and carboxysomes, however there are some genera that lack CCMs (Giordano et al., 2005). Recent studies of lichen communities suggest that photobiont types play a key role in shaping community responses to the environment (Hurtado et al., 2020; Koch et al., 2019; Matos et al., 2015). Traditionally photobionts have been classified according to coarse taxonomic categories: chlorococcoid green algal (e.g., *Trebouxia*), filamentous green algal (e.g., *Trentepohlia*) and cyanobacterial. However, these broad categories mask considerable variation in physiology, in particular within the chlorococcoid green algae. Indeed, recent work has found that finer scale, genus-level photobiont occurrence is associated with environmental changes during forest succession (Koch et al., 2022). These fine-scale responses align with the presence or absence of CCMs. This result, along with the striking physiological differences between lichens with and without CCMs (Palmqvist, 2000), led us to ask whether CCMs constitute a major trait in structuring lichen communities, as is the case in vascular plants.

The physiological impact of CCM presence can be expected to influence responses to the environment and therefore affect lichen distribution patterns. Lichenized algae obtain water from a range of sources, including liquid (e.g., precipitation) and vapor (Gauslaa, 2014). Saturation by liquid water easily leads to carbon limitation, hence we predicted a strong positive correlation between CCM presence and precipitation. In contrast, CCM-lacking photobionts benefit from consistent water vapor availability, which depends on the saturation vapor pressure (e_{sat}) hence we predicted a strong positive correlation between CCM absence and temperature.

To evaluate these hypotheses we use an extensive dataset of temperate eastern North American lichen communities. It includes extensive sampling from the southern Appalachian Mountains and Atlantic Coastal Plain, two globally recognized biodiversity hotspots for lichens (Allen & Lendemer, 2016a, 2016b; Lendemer et al., 2016; Tripp et al., 2019; Tripp & Lendemer, 2020). Both regions and the lichens they contain are also highly threatened (Allen & Lendemer, 2016a, 2016b; McMullin et al., 2019).

We hypothesize that photobiont identity varies across environmental gradients and that CCM presence or absence, scored using a genus-level photobiont classification, is a better predictor of environmental distribution than coarse-level photobiont groupings traditionally used in lichen studies. Specifically, based on the physiological roles of CCMs, we predict that:

1. Green algal lichens with pyrenoids will differ in the directionality and degree of their responses to environmental factors (e.g., temperature, precipitation, and moisture index) from green algal lichens lacking pyrenoids. Further, we predict the responses of green algal lichens with pyrenoids (CCMs present) may more closely resemble those of cyanobacterial lichens (CCMs present as carboxysomes) than that of green algal lichens without CCMs.
2. CCM presence will be positively correlated with mean annual precipitation (MAP). The absence of CCM will be unaffected or weakly negatively correlated to precipitation.
3. Temperature (mean annual temperature [MAT]), particularly at elevated climate moisture index, will be strongly positively correlated with CCM absence due to the increased availability of water vapor for hydration.

METHODS

Biodiversity dataset

To test whether the presence of CCMs, scored using a fine-scale genus-level photobiont classification (data available in Koch et al., 2023), affects lichen distributional patterns, we used a large-scale dataset of lichen communities from temperate eastern North America. The dataset comprised presence/absence species occurrence data from intensive expert-based inventories of 630 one-hectare plots (Appendix S1: Figure S1). The plots were sampled during three large-scale regional lichen biodiversity inventory projects led by JCL and colleagues: 208 plots in the southern Appalachian Mountains (see Tripp et al., 2019), 204 plots in the northern Appalachian Mountains of Pennsylvania (see Lendemer & Coyle, 2021), and 215 plots in the Mid-Atlantic Coastal Plain (see McMullin et al., 2019).

Methods of site delimitation and sampling are described fully in the publications cited for each project. In brief, each plot was delimited at one hectare, allowing for an irregular shape to ensure each consisted of a single vegetation type (e.g., swamp forest, spruce-fir forest). Within each plot an expert-based inventory of all lichens was carried out using a Floristic Habitat Sampling approach to more effectively detect total cryptogam biodiversity compared with randomized plot-based methods (e.g., Bowering et al., 2018). All lichens regardless of growth form and size (i.e., macrolichens and microlichens), occurring on all substrates (e.g., bark, leaves, soil, rock, wood), were included in the sampling and the final dataset included a total of 1208 species.

All vouchers were transferred to The New York Botanical Garden (NY) and the identifications were confirmed by one of us (JCL) to maintain consistent and standardized taxonomy, as well as minimize errors in the initial stages of field identification. All voucher data can be accessed in the NYBG institutional KEMu database (http://sweetgum.nybg.org/science/vh/; see Lendemer et al., 2019). The digital voucher data were exported from KEMu and used to build a presence/absence matrix of occurrences of each species at each site (data available in Koch et al., 2023).

Using the species occurrence matrix as a reference, photobionts were identified for each lichen species based on published literature (such as Muggia et al., 2018; Sanders & Masumoto, 2021). When possible, photobionts were reported at the level of algal or cyanobacterial genera. The small number of tripartite lichens (containing both green algal and cyanobacterial symbionts) were classified based on the primary (green) photobiont and analyzed separately. Where more than one primary photobiont genus has been reported for a lichen species, the most common association as determined from the published literature was used. In the rare cases when photobiont identity to genus could not be scored, records were not assigned a CCM status. Photobiont genera were further classified according to the reported presence or absence of CCM structures such as pyrenoids or carboxysomes (data available in Koch et al., 2023). In the cases where pyrenoid presence varies infragenerically (e.g., *Elliptochloris*), taxa were scored as pyrenoid-present, considering that the presence of this structure was possible. The complete dataset of species occurrences, photobiont taxonomy and photobiont traits is available in Koch et al. (2023) at https://doi.org/10.5061/dryad.5dv41ns8h.

Data analysis

To test our hypotheses, we used macroenvironmental variables drawn from the ClimateNA v6.40a software package (http://tinyurl.com/ClimateNA) based on methods described by Wang et al. (2016), extracting annual values for MAT, MAP and Hogg's climate moisture index (CMI). The CMI derives from the annual precipitation (P) minus the potential evapotranspiration (PET), considering a well vegetated landscape with adequate soil moisture ($\mathrm{CMI} = \mathrm{P} - \mathrm{PET}$, Hogg, 1997), so higher CMI indicates wet conditions and lower values, drought conditions. The environmental data are downscaled from 800×800 m resolution PRISM data and include annual averages for 1991–2020 (Wang et al., 2016). We based the analyses on two approaches (local and regional) and ran all analyses in R (R Core Team, 2020). First, we calculated the "local proportion" of species with a trait in the plot (normalized by the local number of species) (i.e., the proportion of species in a plot expressing a given trait state, e.g., $N_{A,i}/N_{\mathrm{total},i}$ where A is a given trait state [e.g., pyrenoid presence] and i is the plot number). This metric reflects the prevalence of a given trait in each plot. Second, we calculated the "regional occurrence" as the proportion of species with a trait compared with the total pool of plots sampled in temperate eastern North America (i.e., the number of species in a plot expressing a given trait state normalized by the number of species presenting that trait in the regional biota [the full dataset of species], e.g., $N_{A,i}/N_{A,\mathrm{total}}$). This metric reflects the regional distribution of diversity of species presenting a given trait. Traits were calculated using community weighted means (CWMs), the average of each trait value in a community, with the "functcomp" function of the *FD* package v.1.0-12 (Laliberté et al., 2014).

Generalized linear models (GLMs) were constructed using the "glm" function to test the relationships among traits (absence of CCMs; the presence of CCMs—pyrenoids; the presence of CCMs—carboxysomes, and commonly used photobiont categories: chlorococcoid lichens and green *Trentepohlia* separated, and combined as "all green," and cyanobacteria) using both the local proportion and regional occurrence metrics and the environmental variables, as well as their interactions. Prior to the analyses, we centered and scaled all variables to estimate comparable effect size (i.e., *z*-transformed: $(x - \mathrm{mean})/\mathrm{SD}$, using function "scale" built-in R). The full model was: trait ~ $(\mathrm{MAP+MAT+CMI})^2$. After analyzing the residual plots, we used Gaussian distributions in the three models and calculated the adjusted deviances (which takes into account the number of observations and predictors) with the function "Dsquared" of the package *modEvA* v. 2.0 (Barbosa et al., 2014). Kendall rank correlation tests were calculated between each genus and the macroenvironmental variables (MAP, MAT, CMI) with the "cor.test" function in the package *picante* (Kembel et al., 2010).

RESULTS

Carbon-concentrating mechanisms and environmental variables

The effects of environmental variables differed between types of photosynthetic pathways (Figure 1). Effects were strong with both local and regional approaches (Figure 1; Appendix S1: Tables S1 and S2). Lichen photobionts lacking CCMs were more frequent and diverse in areas with higher mean annual temperatures and with lower to intermediate MAP and CMI. Regional dominance of lichens with green algal CCMs (pyrenoids) increased strongly with MAP and CMI, while local occurrence was more responsive to MAT. Carboxysome-containing lichens responded strongly to MAT, MAP, and CMI (Figure 1a,b).

The three groups of photobiont physiologies differed mainly regarding their relationships with temperature and its interactions as ascertained by the analysis with the full model with all environmental variables (Figure 1c; Appendix S1: Figure S3, Table S1). The absence of CCMs was negatively correlated with climate moisture, and positively correlated with MAT and precipitation. Lichens with CCMs, both pyrenoids and carboxysomes, were positively correlated with higher levels of precipitation, but negatively with temperature and climate moisture (Figure 1c). Similar patterns were observed with the commonly used photobiont categories: models with green *Trentepohlia* were similar to the absence of CCMs, green coccoid, to the presence of CCMs—pyrenoids, and cyanobacteria similar to the presence of CCMs—carboxysomes, while models with all green algae combined ("green all") had completely different results and low adjusted Dsquared (Appendix S1: Figure S3, Table S1).

DISCUSSION

In this study, we found that lichen photobiont type (i.e., eukaryotic algae vs. cyanobacteria) and physiology/photosynthetic pathway (i.e., the presence or absence of carbon-concentrating mechanisms [CCMs]) was both important predictors of lichen distributional patterns considering a local and regional approach. While the first is well established in lichen ecology, the importance of the latter has been underappreciated, even though the presence of pyrenoids is well known to be physiologically important (Palmqvist et al., 1997, 2002). Additionally, multiple species-rich mycobiont clades in temperate eastern North America are known to be associated with diverse photobionts taxa that have different carbon-concentrating strategies (Appendix S1: Figure S4). The importance of CCMs in the interpretation of community composition is thus directly analogous to its well established importance in vascular plants (C3 vs. C4 and CAM). Carbon-concentrating mechanism presence and type (pyrenoid vs. carboxysome) each led to different environmental responses in distributions. As predicted from the physiological role of the CCM in tolerating saturating conditions, the presence of CCMs drove similar responses to precipitation in green algal and cyanobacterial lichens. In particular, the presence of CCMs in green algal lichens changed not just the degree but also the directionality of responses to temperature (MAT) and its interactions with precipitation (MAP) and moisture (CMI). As hypothesized, CCM presence was positively correlated with MAP, however CCM absence also showed a positive relationship with MAP. Although CCM absence was also positively correlated with MAT, it showed a strong negative correlation with CMI. These patterns were strong regardless of whether plot-level proportion ("local") or regionally scaled occurrence ("regional") were considered.

The importance of CCMs in lichen community ecology has most likely been overshadowed by the emphasis on the coarse-scale categorization of lichen photobionts into broadly defined groups rather than fine-grained taxonomic scales and key associated physiological differences. The differences between eukaryotic and prokaryotic photobionts are well established (Palmqvist, 2000). This approach likely owes its success to the outsized effect of the pyrenoid-containing genera *Trebouxia* and *Asterochloris*, especially among nontropical macrolichens, that has masked the physiological diversity of eukaryotic lichen photobionts. More recently, renewed attention has been given to the differences between *Trentepohlia* and chlorococcoid green algal lichen distributions (Käffer et al., 2021; Koch et al., 2022), and it is tempting to continue such an approach rather than using genus-level photobiont identification. However this simple clade-based distinction is still not mechanistic, overlooking the absence of CCMs in major chloroccoid genera such as *Symbiochloris*. This is a concern, as it may lead to incorrect predictions about future responses of green algal lichens whose physiology differs from the *Trebouxia*-dominated norm. The "classic" clade-based approach did not perform as well as a physiology-based classification, considering the fundamental role of CCMs in determining responses to environmental conditions (Palmqvist et al., 1997; Appendix S1: Table S2).

The absence of CCMs is polyphyletic across green photosynthetic eukaryotes and is thought to reflect repeated losses in response to environmental constraints (Goudet et al., 2020; Meyer & Griffiths, 2013). Much attention has been given to the adaptive importance of CCMs in reducing carbon limitation when diffusion is limited, either by liquid water (e.g., algae) or stomatal closure (i.e., C4 and

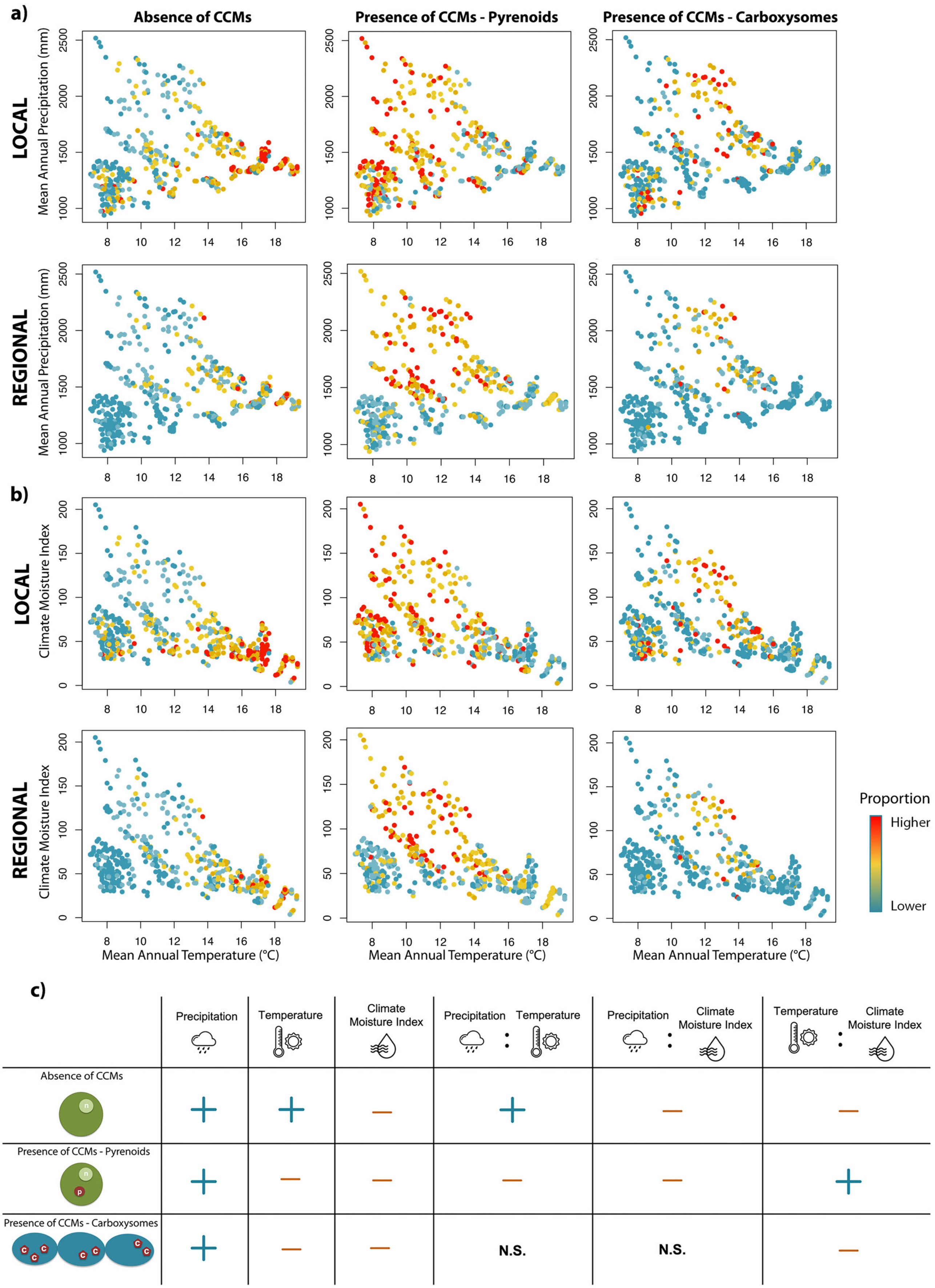

FIGURE 1 Legend on next page.

CAM plants; Borland et al., 2011; Sage, 2017). Less attention has been given to the advantages of the absence of CCMs (Maberly & Gontero, 2017). CCMs, whether unicellular (pyrenoids and carboxysomes) or multicellular (C4 and CAM), involve considerable energetic expense, as well as reductions in photosynthetic efficiency (Maberly & Gontero, 2017; Raven et al., 2014). The presence of CCMs is typically favored in high light, carbon-limited circumstances, whether as a result of drought (e.g., terrestrial and epiphytic vascular plants) or saturation (e.g., aquatic vascular plants and CCM-containing lichens). Because the microenvironments experienced by lichens and vascular plants differ, this shared the adaptive role of CCMs can translate into contrasting environmental distributions. While both vascular plant and lichen CCMs are more frequent in open habitats, CCMs are not necessarily associated with aridity in lichens. Carbon limitation in lichens is most likely to arise from water saturation of thalli, and therefore associated with direct wetting, such as from rainfall. This can be challenging to observe in lichen distributional patterns, because high precipitation biomes often contain humid but liquid-free and dark micro-habitats, such as bark surfaces in closed forests (Lakatos et al., 2006), as well as sunny and wind-exposed sites, where lichens could avoid suprasaturation and the adverse effects of slow CO_2 diffusion rates. Thus, the macroclimatic patterns found here are strong support for our results.

The role of pyrenoids and other CCMs in driving lichen distributions in eastern North America to likely apply much more broadly around the world. Recent studies have highlighted the differences between Trentepohlean lichen distributions and other green algal lichen taxa (Manzitto-Tripp et al., 2022), and our results suggest that those same studies might find similar patterns within the chlorococcoid chlorolichens that are currently masked by the predominance of a few CCM-containing genera (e.g., *Trebouxia*). A recent study in the Brazilian Atlantic Forest found decreasing presence of CCMs with canopy closure (Koch et al., 2022).

The incorporation of CCMs into studies of lichen communities still faces some limitations. Many chlorococcoid photobionts assigned to "*Trebouxia*" or other common genera have instead been found to belong to other clades of green algae (e.g., *Heveochlorella*; Sanders et al., 2016); this revision is still incomplete and older records of photobiont identity may be mistaken. Furthermore, on some occasions, photobiont associations can vary within lichenized fungal genera and even species (Ertz et al., 2018; Lindgren et al., 2020). For example, a recent re-examination of photobionts in the foliose genus *Sticta* found both CCM (*Chloroidium*, *Heveochlorella*) and non-CCM (*Symbiochloris*) algal associations, partitioned by geography (Lindgren et al., 2020). Last, even when the photobiont genus is correctly assigned, it may be insufficient to infer CCM presence; some algal genera are variable in the presence of pyrenoids (e.g., *Elliptochloris*, [Sanders & Masumoto, 2021]) and others show CCM characteristics in the absence of recognizable pyrenoids (Meyer & Griffiths, 2013). The above clearly illustrates an urgent need for further research into photobiont physiology, including the consistent and amplified use of tools such as anatomy, molecular biology, and stable isotopes to classify physiological modes.

The forecasting of responses of lichen communities to climate change requires attention to these physiological differences within traditional functional categories (e.g., chlorolichens). There is growing interest in the use of traits to describe lichen community responses to climate (Smith et al., 2020), as well as growing awareness of the limitations of some commonly used categorical traits (Ellis et al., 2021). Our findings here indicate that a widely used trait (photobiont type) can be converted into a physiologically informed predictor of lichen communities, but only if considered at the right scale.

The presence of CCMs is a key functional trait that helps to structure terrestrial autotroph communities. This has been well documented in vascular plants, but previously unreported in lichens. Using a large-scale dataset spanning a broad area of eastern North America, we found that the presence or absence of pyrenoids and similar CCM structures explained community variation along environmental gradients at subcontinental scales. These responses contrast with our mechanistic understanding of lichen physiology, whereby CCMs mitigate carbon limitation during periods of water saturation at the cost

FIGURE 1 Carbon-concentrating mechanisms (CCMs) along a gradient of (a) mean annual precipitation and mean annual temperature, and (b) climate moisture index and mean annual temperature in eastern North America. The points in the graphs represent the 630 plots sampled, and the color categories are based on the proportion of species with a trait in the plot normalized by the local number of species (LOCAL) and the proportion of species with a trait compared with the regional pool (REGIONAL). Higher values are shown in red, intermediate in yellow, and lower values are in blue. In (c) the graphical representation summarizing the different effects of mean annual precipitation, mean annual temperature, climate moisture index, and their interactions on the absence or presence of CCMs, namely pyrenoids (green algae) and carboxysomes (cyanobacteria) based on GLM. The results are based on models including the sum of all variables (mean annual precipitation, mean annual temperature, and climate moisture index) with their interactions (for details, see Appendix S1: Table S1). N.S., not significant.

of efficient use of a vapor hydration source. Further studies are needed to bridge this gap between thallus-scale dynamics and broad community patterns, as well as integrate CCMs with other lichen functional traits to try to identify strategies and trade-offs. Nonetheless, this study demonstrates that CCM status is a key functional trait in obligate lichen symbioses, equivalent in importance to its role in vascular plants, and central to the study of present and future climate responses.

AUTHOR CONTRIBUTIONS

Natália M. Koch and Daniel E. Stanton conceived the study. James C. Lendemer developed initial field protocols that were refined and expanded in collaboration with Erin A. Manzitto-Tripp and Christy McCain. James C. Lendemer led field sampling, verified voucher identifications, managed curation/digitization of data, and completed compilation of the species occurrence dataset. Erin A. Manzitto-Tripp co-led the field campaign and lichen sampling for approximately half of the southern Appalachian dataset, identified voucher specimens, and managed and curated resulting specimens and digital data at COLO. Natália M. Koch and James C. Lendemer scored the traits. Natália M. Koch and Daniel E. Stanton did the data analysis and led the manuscript writing, which had contributions and revisions from all the authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Koch et al., 2023) are available in Dryad at https://doi.org/10.5061/dryad.5dv41ns8h. All voucher data can be accessed in the NYBG institutional KEMu database (http://sweetgum.nybg.org/science/vh/; see Lendemer et al., 2019). Climate (Mean Annual Temperature [MAT], Mean Annual Precipitation [MAP] and Hogg's climate moisture index [CMI]) data has been generated with the ClimateNA v6.40b software package, available at http://tinyurl.com/ClimateNA, based on methodology described by Wang et al. (2016).

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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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