New Phytologist

Research

Herbarium specimens reveal that mycorrhizal type does not mediate declining temperate tree nitrogen status over a century of environmental change

Talia J. Michaud[1]*, Lauren C. Cline[2]*, Erik A. Hobbie[3], Jessica L. M. Gutknecht[4] and Peter G. Kennedy[1]

[1]Department of Plant and Microbial Biology, University of Minnesota, St Paul, MN 55108, USA; [2]Bayer Crop Sciences, St Louis, MO 63141, USA; [3]Earth Systems Research Center, University of New Hampshire, Durham, NH 03824, USA; [4]Department of Soil, Water, and Climate, University of Minnesota, St Paul, MN 55108, USA

Author for correspondence:
Talia J. Michaud
Email: micha938@umn.edu

Received: *6 October 2023*
Accepted: *27 October 2023*

New Phytologist (2023)
doi: 10.1111/nph.19452

Key words: herbarium, historical environmental change, mycorrhizal type, stable nitrogen isotopes.

Summary

- Rising atmospheric carbon dioxide concentrations (CO_2) and atmospheric nitrogen (N) deposition have contrasting effects on ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) symbioses, potentially mediating forest responses to environmental change.
- In this study, we evaluated the cumulative effects of historical environmental change on N concentrations and $\delta^{15}N$ values in AM plants, EM plants, EM fungi, and saprotrophic fungi using herbarium specimens collected in Minnesota, USA from 1871 to 2016. To better understand mycorrhizal mediation of foliar $\delta^{15}N$, we also analyzed a subset of previously published foliar $\delta^{15}N$ values from across the United States to parse the effects of N deposition and CO_2 rise.
- Over the last century in Minnesota, N concentrations declined among all groups except saprotrophic fungi. $\delta^{15}N$ also declined among all groups of plants and fungi; however, foliar $\delta^{15}N$ declined less in EM plants than in AM plants. In the analysis of previously published foliar $\delta^{15}N$ values, this slope difference between EM and AM plants was better explained by nitrogen deposition than by CO_2 rise.
- Mycorrhizal type did not explain trajectories of plant N concentrations. Instead, plants and EM fungi exhibited similar declines in N concentrations, consistent with declining forest N status despite moderate levels of N deposition.

Introduction

Mycorrhizal type is increasingly invoked to predict forest responses to environmental change (Terrer *et al.*, 2016; Averill *et al.*, 2018; Baldrian *et al.*, 2023). Most trees associate with either ectomycorrhizal (EM) or arbuscular mycorrhizal (AM) fungi (Steidinger *et al.*, 2019), which differ in their nutrient acquisition strategies (Smith & Read, 2010). Because EM fungi can obtain nutrients directly from organic matter (Lindahl & Tunlid, 2015; Shah *et al.*, 2016; Frey, 2019), EM plants are better adapted to conditions of low inorganic nutrient availability, while AM plants and fungi are better adapted to inorganic nutrient acquisition (Phillips *et al.*, 2013). As such, mycorrhizal type may mediate forest responses to environmental changes that alter soil nutrient availability or demand, such as atmospheric nitrogen (N) deposition or rising carbon dioxide (CO_2) concentrations (Mohan *et al.*, 2014).

Atmospheric N deposition increases inputs of bioavailable inorganic N to soils (Galloway *et al.*, 2004). Consistent with the idea that AM symbionts are better adapted to soils with high inorganic nutrient availability, Averill *et al.* (2018) found that AM tree recruitment increased and EM tree recruitment decreased with greater amounts of N deposition across the United States. Concurrently, some EM fungi appear sensitive to N deposition, evidenced by marked declines in sporocarp production followed by a shift in belowground community structure toward 'nitrotolerant' species (Arnolds, 1991; Lilleskov *et al.*, 2011). Indicators of decreased belowground EM fungal abundance have been documented with N addition (Högberg *et al.*, 2011), although this potential consequence is often overgeneralized (Lilleskov *et al.*, 2019; Karst *et al.*, 2021).

Rising atmospheric CO_2, however, may favor EM plants and fungi if increasing CO_2 concentrations stimulate plant N demand, requiring increased N acquisition from organic pools (Pellitier *et al.*, 2021). Historical declines in plant tissue $\delta^{15}N$ and [N] have been interpreted as evidence that rising CO_2 stimulates plant N demand, decreasing ecosystem N-loss pathways that discriminate against ^{15}N (McLauchlan *et al.*, 2010; Craine *et al.*, 2018). Furthermore, in a meta-analysis of free air CO_2 enrichment studies, elevated CO_2 enhanced the growth of EM

*These authors contributed equally to this work.

plants but not AM plants at low N availability sites (Terrer *et al.*, 2016). Alternatively, in highly N-limited environments, such as boreal forests (Näsholm *et al.*, 2013), EM fungi may instead limit N availability to plants under rising CO_2 by outcompeting plants for N (Alberton *et al.*, 2007; Dong *et al.*, 2018).

The analysis of herbarium collections offers a novel opportunity to evaluate the effects of rising atmospheric CO_2 and N deposition on plant N status over historical increases in both global change drivers. For instance, historical declines in foliar N concentrations and $\delta^{15}N$ reported across terrestrial ecosystems (Craine *et al.*, 2018) may not occur in an area with historical N deposition, particularly among AM plants better adapted to acquire inorganic N. By contrast, increased plant N demand under rising CO_2 may swamp N inputs from N deposition, resulting in declining foliar N concentrations and $\delta^{15}N$. However, these declines may be smaller or nonexistent among EM plants if their N supply can be supplemented by organic N. Alternatively, if N deposition mitigates organic N access among EM fungi and by extension, their ability to supplement plant N, while failing to directly satisfy increasing plant N demand, EM and AM plants may show similar declines in N concentrations under rising CO_2.

Since fungi are both subject to and mediators of soil N availability, sampling fungal herbarium collections in addition to plants allows for assessment of whether fungi have experienced concomitant declines in indices of fungal N status (Hobbie *et al.*, 2019; Kranabetter *et al.*, 2019) that have been observed in plant tissues (Craine *et al.*, 2018). The long-term trajectories of fungal N status may, however, differ by ecological guild (saprotrophic vs EM). For example, EM fungi with ready access to photosynthate may outcompete saprotrophic fungi for soil N (Fernandez *et al.*, 2019), potentially stabilizing N acquisition over environmental changes.

To evaluate the cumulative effects of historical environmental change on plant and fungal N status, we analyzed N concentrations and $\delta^{15}N$ among 493 plant and fungal collections made in Minnesota from 1871 to 2016. Over this period, atmospheric N deposition varied six-fold in Minnesota, peaking at 18 kg ha^{-1} yr^{-1} in the 1990s (Clark *et al.*, 2018) and atmospheric CO_2 increased by 40% (Belmecheri & Lavergne, 2020). This region was therefore well-suited for evaluating the cumulative effects of historical N deposition and CO_2 increase. Specifically, we tested whether (1) plant and fungal N concentrations and $\delta^{15}N$ declined in an area experiencing CO_2 rise and increasing N deposition and (2) trajectories of plant $\delta^{15}N$ and N concentrations diverged by mycorrhizal type. We also analyzed a subset of previously published foliar $\delta^{15}N$ values (Craine *et al.*, 2019) to parse the effects of CO_2 rise, N deposition, and mycorrhizal controls of $\delta^{15}N$. In this second dataset, we tested the extent to which (1) CO_2 rise or N deposition explains declines in foliar $\delta^{15}N$ and (2) $\delta^{15}N$ trajectories diverged by mycorrhizal type with CO_2 rise or N deposition.

Materials and Methods

Herbarium study: sample selection

Three hundred twenty-four plant and 232 fungal specimens were selected from the collections at the Bell Herbarium at the University of Minnesota from within a 130 km radius surrounding the University of Minnesota Saint Paul Campus, USA (44.9849°N, 93.1853°W) (Figs 1, 2a). This area is composed of temperate hardwood forests, with a mean annual temperature of 7.2°C and annual precipitation of *c.* 838 mm. Samples were selected from genera whose records spanned at least eight decades between 1880 and 2010. The 16 genera sampled belong to four ecological groups: EM plants, AM plants, EM fungi, and saprotrophic fungi (AM fungi do not typically produce macroscopic sporocarps, so could not be included). To eliminate the effects of plant life form, we selected only broadleaf deciduous genera. Plant mycorrhizal type was assigned using the FungalRoot database (Soudzilovskaia *et al.*, 2019). Because herbarium collections were made from mature *Populus* individuals, we considered *Populus* EM-associated (Teste *et al.*, 2020).

Fungal taxonomy was manually updated to reflect current knowledge; some collections, however, lacked species-level identification ($n = 16$). Out of 55 species represented in the collections, the median number of samples per species was two. Because we were interested in accounting for species-level variation, we excluded

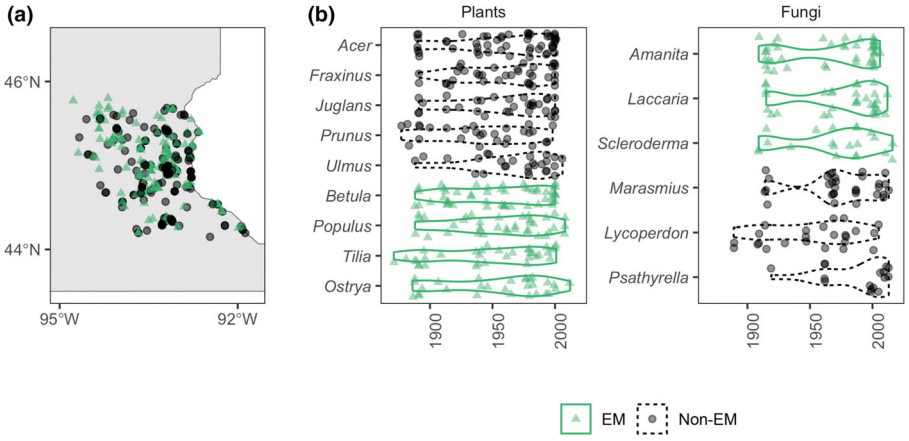

Fig. 1 Summary of foliar and sporocarp samples from herbarium collections made in Minnesota, USA, from 1871 to 2016. (a) Spatial distribution of ectomycorrhizal (EM) and non-EM collections with exact coordinate information. (b) Genus-level collections of arbuscular mycorrhizal (AM) and EM plants and EM and saprotrophic fungi across time. Black circles, dotted line = non-EM; green triangles, solid line = EM.

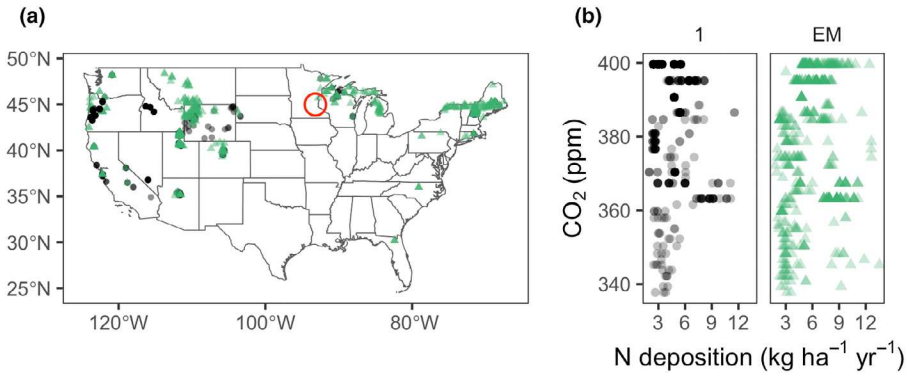

Fig. 2 Summary of foliar data compiled by Craine *et al*. (2018) from arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) plants analyzed (a) across the United States (red circle indicates the location of samples from Minnesota) and (b) with reference to contemporaneous atmospheric carbon dioxide (CO_2) concentrations and annual nitrogen (N) deposition rates. Black circles = AM plants; green triangles = EM plants.

samples without species identity and from species with fewer than three representatives ($n_{removed} = 63$, $n_{fungi\ retained} = 169$, $n_{all\ retained} = 493$). The EM fungal genus *Tricholoma* was excluded after this step because of sparse species-level collections. Summary information about the final dataset is in Supporting Information Table S1 and Fig. 1.

Herbarium study: elemental and isotopic analysis

Foliar and sporocarp tissues were homogenized before analysis via either an Elementar Vario Pyrocube (Hanau, Germany) interfaced to an Isoprime 100 isotope ratio mass spectrometer (Cheadle, UK) at the University of Minnesota or an Elementar Vario EL Cube interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK) at the University of California-Davis Stable Isotope Facility. See Methods S1 and Fig. S1 for quality control steps to ensure consistent data between the two facilities.

Herbarium study: statistical analyses

All statistical analyses were performed in R, v.4.3.0 (R Core Team, 2023). Models were fitted using the lmer function in the LME4 package (Bates *et al*., 2015), *P*-values produced using Satterthwaite's approximations of degrees of freedom with the LMERTEST package (Kuznetsova *et al*., 2017). Prediction intervals were produced using the predictInterval function in the MERTOOLS package (Knowles & Frederick, 2016).

Initial models included random effects to account for potential effects of species identity, genus, seasonality (month), and geographic location (county). To evaluate potential differences in baseline [N] and $\delta^{15}N$ and temporal trends between groups, the fixed effects of year, group, and their interaction were included. Year was a continuous variable (0–145 corresponding to years 1871–2016) and group was a categorical variable (EM plant, AM plant, EM fungus, and saprotrophic fungus). Backward stepwise selection using the function step in the LMERTEST package (Kuznetsova *et al*., 2017) was performed on the random effects to improve model parsimony and avoid overfitting. Final models included only the preserved random effects and original fixed effects.

Models of foliar and sporocarp [N] were run separately on plants and fungi, given the differences between leaf and mushroom stoichiometry. Natural log-transformed [N] values were used to satisfy the assumption of heteroscedasticity. Back-transformed statistics are reported in the text to aid interpretation. In the model of foliar [N], preserved random effects accounted for differences in seasonality (month), geography (county), and species and genus identity (species nested within genus). Backward stepwise selection on the model of sporocarp [N] eliminated the effects of seasonality, preserving species nested within genus and county.

Sporocarp and foliar $\delta^{15}N$ were modeled globally, treating $\delta^{15}N$ as a tracer comparable across leaves and mushrooms. Comparing trajectories of foliar $\delta^{15}N$ between EM and AM plants allows us to test potential mycorrhizal controls of $\delta^{15}N$. Specifically, the leaves of EM plants are typically ^{15}N-depleted relative to AM leaves, reflecting the preferential transfer of ^{15}N-depleted N compounds from EM fungi to their plant partners (Craine *et al*., 2009). ^{15}N depletion of transfer compounds is driven by both the fractionation that occurs during compound synthesis, and preferential retention of ^{15}N in EM fungal biomass (Hobbie & Högberg, 2012). EM foliar N may thus become less ^{15}N-depleted relative to AM foliar N when (1) EM plants source less N from EM fungi overall or (2) less ^{15}N is retained in EM fungal biomass (Hobbie & Colpaert, 2003). As in the [N] models, the full set of possible random effects was reduced using backward stepwise selection. The final set of random effects included species identity nested within genus. Fixed effects, as in the [N] model, included group, year, and their interaction to test for different temporal trends across groups.

Spatial foliar $\delta^{15}N$ analysis: data compilation

We extracted foliar $\delta^{15}N$ and [N] data from Craine *et al*. (2018) (Craine *et al*., 2019), in which each $\delta^{15}N$ and [N] value represents an average for a given species at a given site (defined by 0.1° latitude/longitude or *c*. 11 km) in 1 yr. We subsampled this data to only include EM and AM plants in the United States ($n = 2138$: $n_{EM} = 845$, $n_{AM} = 1293$, $n_{species} = 299$). While we were initially only interested in trees, data from AM trees were far less abundant than EM trees, resulting in an imbalanced dataset. We therefore included herbaceous plants as well, adding in random effects based on taxonomy in our model structure to account for the effects of life form, and excluded plants from AM-only ecosystems (explained later). These data cover the

period from 1980 to 2016. The original publication contains full details about data collection, mycorrhizal type assignment, and quality control procedures (Craine *et al.*, 2018).

We next compiled additional features at the same geographic scale that might capture variation in foliar $\delta^{15}N$, including climatic variables (mean annual temperature, aridity index, mean annual precipitation, and net primary productivity), edaphic features (soil pH, organic C content, total N, cation exchange capacity, clay, silt, sand fractions, and bulk density topsoil), land cover features (developed/cultivated land), and vegetation features (woody/herbaceous cover, EM/AM rootstocks). We also included the 182 level III ecoregions of North America (Bailey, 2014) to capture potential emergent effects of ecoregion. Annual atmospheric CO_2 concentrations were compiled from Belmecheri & Lavergne (2020). Gridded decadal estimates of annual N deposition at 12 × 12 km resolution were extracted for each site from Clark *et al.* (2018). Projected EM/AM rootstocks (Mg C ha^{-1}) were extracted from Barceló *et al.* (2023) as a proxy for potential EM/AM fungal abundance. The proportion of the colonized mycorrhizal rootstock belonging to EM plants was calculated for statistical analyses to represent EM fungal 'dominance'. Due to our interest in modelling EM $\delta^{15}N$ relative to AM $\delta^{15}N$ from comparable ecosystems, we excluded all sites with estimated EM rootstocks at 0 Mg C ha^{-1} ($n = 782$).

All data layers were aggregated to 10 km × 10 km to approximate the resolution of the averaged foliar $\delta^{15}N$ and [N] values from Craine *et al.* (2018). After combining all data layers and excluding missing values, the final dataset contained 1108 observations ($n_{EM} = 466$, $n_{AM} = 639$). Information about the frequency of species/genera included in the final dataset and their mycorrhizal type is presented in Table S2 and their distribution over space and relative to atmospheric CO_2 and estimated N deposition is summarized in Fig. 2. To avoid multicollinearity, all aforementioned predictors were included in a mixed model explaining foliar $\delta^{15}N$, with ecoregion and species nested within genus as random effects, after which VIF values for each predictor was computed using the vif function in the CAR package (Fox & Weisberg, 2018). Predictors with VIF above 3 were sequentially excluded to yield the following set of predictors: log-transformed foliar [N], plant mycorrhizal type, soil N content, soil pH, soil organic carbon, clay and silt fractions, decadal estimates of annual N deposition rates, annual atmospheric CO_2, herbaceous plant cover, developed land cover (cultivated land cover + built land cover), proportion of EM rootstocks, mean annual precipitation, mean annual temperature, latitude, longitude, net primary productivity, plant species, genus, and ecoregion. Source, initial resolution, and units of all original spatial layers are presented in Table S3.

Spatial foliar $\delta^{15}N$ analysis: statistical analyses

Species nested within genus and ecoregion were included as random effects to account for nonindependence arising from geographic and taxonomic clustering. These effects also captured differences in $\delta^{15}N$ arising from differences in plant life form. To test whether foliar $\delta^{15}N$ diverged by mycorrhizal type depending on variation in CO_2 concentrations or N deposition, we included two-way interactions between plant mycorrhizal type and CO_2 concentrations and plant mycorrhizal type and N deposition rates. Models were fitted using the lmer function in the LME4 package (Bates *et al.*, 2015), with *P*-values produced using Satterthwaite's approximations of degrees of freedom with the LMERTEST package (Kuznetsova *et al.*, 2017).

Results

Herbarium study: mixed models of historical trends in tissue $\delta^{15}N$ and [N] in Minnesota

Foliar N concentrations in EM and AM plants declined similarly over time in Minnesota ($slope_{year:AM}$: $-0.0019 \pm 0.0004\%$ yr^{-1}, $P_{AM} < 0.001$; $slope_{year:EM}$: $0.0011 \pm 0.0004\%$ yr^{-1}, $P_{EM} = 0.009$). Sporocarp [N] also declined in EM fungi ($slope_{year:EM}$: $0.0016 \pm 0.0007\%$ yr^{-1}, $P_{EM} = 0.019$), but remained stable among saprotrophic fungi ($P_{Sap} = 0.51$), although there was no significant difference between their slopes ($P = 0.25$) (Fig. 3). EM plants had lower foliar [N] in 1871 compared to AM plants (difference $= 0.145 \pm 0.073$, $P = 0.038$) and saprotrophic and EM fungi showed no significant differences in modeled [N] in 1871, although saprotrophic fungi trended higher ($P_{fungi} = 0.683$) (Fig. 3).

Tissue $\delta^{15}N$ declined significantly across all groups over time (Fig. 3). The decline was greatest among AM plants ($-0.043 \pm 0.005‰$ yr^{-1}, $P < 0.001$), followed by EM fungi ($-0.038 \pm 0.007‰$ yr^{-1}, $P < 0.001$), saprotrophic fungi ($-0.039 \pm 0.009‰$ yr^{-1}, $P < 0.001$), and EM plants ($-0.028 \pm 0.005‰$ yr^{-1}, $P < 0.001$). The only difference in the slopes emerged among plants: EM foliar $\delta^{15}N$ declined significantly less than AM foliar $\delta^{15}N$ ($P = 0.026$) (Fig. 3).

Spatial foliar $\delta^{15}N$ analysis: potential controls of foliar $\delta^{15}N$ and EM fungal N retention across the United States of America

The final model explained 59% of the variation in foliar $\delta^{15}N$ across the United States (marginal $r^2 = 0.10$/conditional $r^2 = 0.59$). Foliar $\delta^{15}N$ was strongly and positively related to foliar N concentrations, and negatively related to atmospheric CO_2 concentrations (Table 1). The interaction between mycorrhizal type and N deposition was significant ($P < 0.001$), while the interaction between mycorrhizal type and CO_2 was not. Specifically, AM foliar $\delta^{15}N$ was significantly and negatively related to N deposition, while EM foliar $\delta^{15}N$ had no significant relationship with N deposition. Foliar $\delta^{15}N$ was also significantly related to soil N content, clay fraction, and silt fraction. Furthermore, the proportion of EM rootstocks was also strongly negatively related to foliar $\delta^{15}N$.

Changes in EM foliar ^{15}N depletion across both studies

We calculated differences in foliar $\delta^{15}N$ between EM and AM plants (EM $\delta^{15}N$–AM $\delta^{15}N$; $\delta^{15}N_{EM-AM}$) in both datasets.

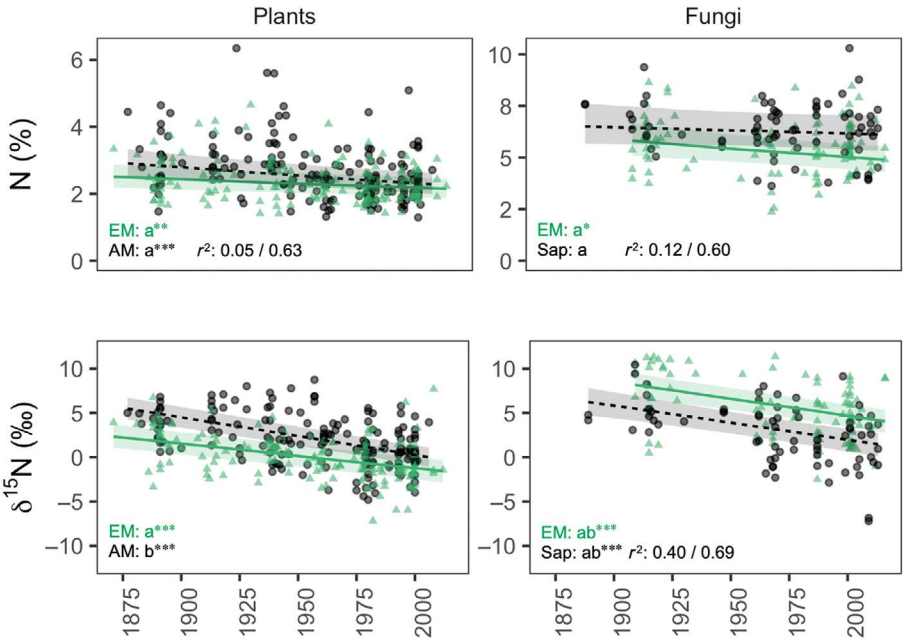

Fig. 3 Temporal trends in measured and modeled [N] and $\delta^{15}N$ among ectomycorrhizal (EM) plants, arbuscular mycorrhizal (AM) plants, EM fungi, and saprotrophic (Sap) fungi with 80% prediction intervals associated with fixed effects. Asterisks indicate significant temporal trends (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$), while letters denote differences in slope between groups. Green triangles, solid line = EM; black circles, dotted line = non-EM. Marginal and conditional r^2 values are given for each of the three models (plant [N], fungal [N], and $\delta^{15}N$) in the bottom right of the corresponding panels.

$\delta^{15}N_{EM-AM}$ increased from $-3.39 \pm 1.38‰$ in 1871 ($P = 0.014$) to $-1.27 \pm 1.32‰$ in 2016 ($P = 0.34$; Fig. 4a). Across the United States, we compared EM and AM foliar $\delta^{15}N$ at the minimum level of N deposition represented in the dataset (1.8 kg N ha^{-1} yr^{-1}) and the third quartile level of N deposition (6 kg N ha^{-1} yr^{-1}) to avoid making predictions at levels of N deposition with low coverage for AM plants (Fig. 2b). Across the United States, the $\delta^{15}N_{EM-AM}$ increased from $-1.17 \pm 0.44‰$ at 1.8 kg N ha^{-1} yr^{-1} ($P = 0.009$) to $0.06 \pm 0.30‰$ at 6 kg N ha^{-1} yr^{-1} ($P = 0.83$) (Fig. 4b).

Discussion

Both EM and AM plants in Minnesota exhibited comparable declines in foliar [N] over historical increases in atmospheric CO_2 concentrations, despite concomitant N deposition (Fig. 3). This does not support the hypothesis that AM plants are favored by N deposition because their mode of N acquisition is better adapted to inorganic N environments, which is enhanced under N deposition (Averill *et al.*, 2018). At the same time, our results also do not support the hypothesis that EM plants are advantaged over AM plants in a rising CO_2 world because they have better access to organic N pools (Terrer *et al.*, 2016). Instead, we found that both groups appear increasingly N-limited despite inputs from N deposition. These findings likely reflect that rising CO_2 and N deposition (alongside other environmental shifts that have occurred in Minnesota such as rising temperatures) interact to influence plant N status, complicating predictions from experiments that typically isolate CO_2 and N deposition (Mohan *et al.*, 2014).

We admit that foliar N and $\delta^{15}N$ are not perfect proxies for plant N status. For example, foliar N concentrations may belie changes in N content (Jonard *et al.*, 2015) or in N use efficiency under changing environmental conditions (Smith, 2022). Similarly, foliar $\delta^{15}N$ may simply reflect the isotopic composition of deposited N (Hiltbrunner *et al.*, 2019). The design of our study, however, addresses these concerns in two ways: (1) supplementing foliar N trajectories with those of fungal sporocarps and (2) conducting an additional analysis of foliar $\delta^{15}N$ among EM and AM plants across the United States to compare how spatial variation in N deposition and temporal variation in CO_2 concentrations have influenced foliar $\delta^{15}N$. We show that (1) declining plant [N] was matched by declining [N] in EM fungal sporocarps, consistent with declining N availability over time to plants and the fungi that partially moderate N supply (Fig. 3). This result represents the first record of declining [N] in EM fungi over historical timescales. We also found that (2) declining foliar $\delta^{15}N$ was linked to both CO_2 rise and local N deposition, although the latter was true among AM plants alone (Table 1). Together, these findings add a new line of evidence for widespread terrestrial N oligotrophication (Mason *et al.*, 2022).

It is possible that long-term trajectories of EM fungal [N] and $\delta^{15}N$ may not closely track EM foliar [N] and $\delta^{15}N$. For example, EM fungi have been shown in some cases to constrain N availability to plants through N immobilization (Näsholm *et al.*, 2013; Hasselquist *et al.*, 2016). Under rising CO_2, inorganic N pools may be insufficient to satisfy increased plant N demand from longer growing seasons and/or increased photosynthesis (CO_2 fertilization effect) (Elmore *et al.*, 2016; Craine *et al.*, 2018), thereby increasing plant reliance on EM fungi for organic N acquisition (Terrer *et al.*, 2016; Pellitier *et al.*, 2021). If rising CO_2 stimulates EM fungal growth, this should increase EM fungal N immobilization and therefore increase plant N limitation, thus decoupling EM plant and fungal N status when using tissue [N] as an index (Dong *et al.*, 2018). The comparable declines in [N] among EM fungi and plants that we observed,

Table 1 Summary of fixed effects in mixed model of ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) foliar $\delta^{15}N$ across the United States (using data from Craine *et al*., 2018), grouped by climate features (MAP, mean annual precipitation; MAT, mean annual temperature; NPP, net primary productivity), land cover features (Herbaceous, herbaceous land cover; Developed, built and agricultural land cover; EM Rootstock Proportion, proportion of EM rootstocks), location, edaphic features, plant features (log(Leaf N), log-transformed leaf nitrogen concentrations; MT : AM, arbuscular mycorrhizal, MT : EM, ectomycorrhizal), global change drivers, and interactions between global change drivers (N deposition, nitrogen deposition; CO_2, atmospheric carbon dioxide) and mycorrhizal type.

Predictor	Estimate	SE	*P*-value
MAP	5.73E-04	5.29E-04	0.280
MAT	9.55E-02	5.84E-02	0.103
NPP	1.50E-04	1.67E-04	0.369
Herbaceous cover	7.51E-03	1.15E-02	0.514
Developed land cover	5.09E-03	9.43E-03	0.590
EM rootstock proportion	**−1.26E+00**	**4.32E-01**	**0.004**
Latitude	1.91E-02	8.54E-02	0.823
Longitude	−2.31E-03	2.39E-02	0.924
Soil N content	**−3.12E-03**	**1.15E-03**	**0.007**
Soil organic carbon stock	1.28E-03	7.10E-04	0.072
Soil pH	4.89E-02	2.75E-02	0.076
Soil Clay Fraction	**−7.93E-03**	**2.34E-03**	**0.001**
Soil Silt Fraction	**4.92E-03**	**1.89E-03**	**0.010**
log(Leaf Nitrogen Concentration)	**3.89E+00**	**4.98E-01**	**< 0.001**
MT : AM	7.53E+00	6.36E+00	0.237
MT : EM	1.85E-01	5.62E+00	0.974
N Deposition × MT : AM[a]	**−3.09E-01**	**1.02E-01**	**0.002**
N Deposition × MT : EM[b]	−1.43E-03	8.74E-02	0.987
CO_2 × MT : AM[a]	**−4.49E-02**	**1.16E-02**	**< 0.001**
CO_2 × MT : EM[a]	**−3.02E-02**	**7.69E-03**	**< 0.001**

Significant factors are bolded, significant interactions are indicated by superscript letters.

however, is not consistent with this scenario (Fig. 3). Specifically, both EM fungi and plants exhibit declining [N] over a 40% increase in atmospheric CO_2 concentrations. We stress that our study is situated in temperate forests, which differ from the Fennoscandian boreal forests from which evidence that EM fungi maintain plant N limitation largely derives (Högberg *et al*., 2017).

Tissue $\delta^{15}N$ declined in both leaves and sporocarps (Fig. 3). This finding represents the first record of declining $\delta^{15}N$ in fungal tissues, supplementing similar records from lake sediments, tree rings, and leaves (Mason *et al*., 2022). Foliar $\delta^{15}N$ decline, however, was steeper in AM plants than in EM plants in Minnesota. The significant interaction between mycorrhizal type and N deposition rates in the model of foliar $\delta^{15}N$ across the United States may explain this slope difference (Table 1). Specifically, N deposition was negatively related to AM but not EM $\delta^{15}N$ (Table 1). This interaction may arise from differences in N source: if deposited N is low in $\delta^{15}N$, this signal may show up faster in AM plants relying on 'newer' inorganic N compared with EM plants acquiring significant portions of their N from older, organic sources. This explanation is complicated by evidence that plant tissue $\delta^{15}N$ does not reflect the isotopic signature of deposited N, but instead may reflect N deposition-mediated changes in soil biogeochemical processes (Savard *et al*., 2023). Indeed, this interaction, which resulted in a reduction of the ^{15}N depletion characteristic of EM leaves relative to AM leaves (Fig. 4b), may reflect a change in EM N transfer dynamics that reduces the ^{15}N depletion in foliar N. If plants obtain a smaller proportion of N from EM fungi or EM fungal N retention in mycelia decreases, foliar ^{15}N depletion in EM plants should decrease (Hobbie & Högberg, 2012). Both of these explanations would be plausible under N deposition, which may reduce EM plant reliance on organic N acquired by EM fungi, or reduce EM fungal growth, lessening N sequestrations in nonmobile pools that drive ^{15}N depletion in foliar N (Lilleskov *et al*., 2002, 2019; Hobbie & Högberg, 2012).

The stability of the [N] of saprotrophic fungal sporocarps over 14 decades is intriguing. Historical declines in plant tissue $\delta^{15}N$ are often attributed to decreased soil N availability limiting the microbial activities that drive soil ^{15}N enrichment (Craine *et al*., 2015). Organisms relying on soil N like saprotrophic fungi may then exhibit declining N status over time. In our data, however, this was not the case. It is important to note that of the 71 saprotrophic fungal specimens analyzed in this study, the two best represented species (*Lycoperdon pyriforme* and *Psathyrella candolleana*, $n = 31$) are wood rotters. Because these fungi were likely reliant on wood for N supply, their N trajectories may not track soil N availability. Terrestrial saprotrophs may be more sensitive to changes in soil N availability; however, investigating this further was not possible in this study given limited species-level collections. Future study of saprotrophic fungal N trajectories

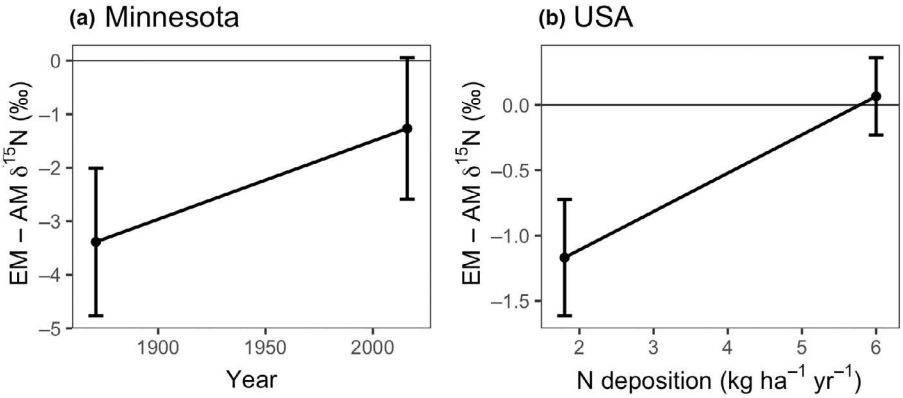

Fig. 4 Changes in ^{15}N depletion in ectomycorrhizal (EM) vs arbuscular mycorrhizal (AM) leaves across (a) 145 yr environmental change in Minnesota, USA (b) atmospheric nitrogen deposition rates across the United States. Error bars represent standard errors, estimated using the function ggeffect in the GGEFFECTS package (Lüdecke, 2018).

over time at the species level should help to better understand how decomposers that both moderate and are subject to changing soil N availability have responded to historical environmental change.

Conclusions

Herbarium specimens contain rich information about ecosystem responses to historical environmental change. By analyzing collections from a region that has experienced concomitant atmospheric N deposition and CO_2 rise, we found that foliar N trajectories did not diverge by mycorrhizal type as expected from results based on short-term experimentation. Instead, EM fungi as well as EM and AM plants exhibited declining tissue N concentrations despite regional N deposition, adding a new line of evidence in support of widespread terrestrial N oligotrophication (Mason *et al.*, 2022). Smaller declines in EM foliar $\delta^{15}N$ than in AM foliar $\delta^{15}N$ in Minnesota may be explained by divergent responses to N deposition, evidenced by the significant interaction between N deposition and mycorrhizal type in the reanalysis of previously published foliar $\delta^{15}N$ data from across the United States. These differential responses likely reflect differences in N acquisition between mycorrhizal types and warrant further investigation.

Acknowledgements

This work was supported by an NSF RUBC Postdoctoral Fellowship (DEB 1611856) to LC Cline and an NSF grant (DEB 2019518) to PG Kennedy. We are grateful to G. Weiblen for specimen access from the Bell Museum at the University of Minnesota. We thank A. Tang and E. Andrews for help processing herbarium samples, J. Sooksa-nguan and C. Loopstra for assistance running the IRMS, and colleagues at the University of Minnesota for thoughtful discussions of this work.

Competing interests

None declared.

Author contributions

LCC, TJM, JLMG and PGK conceived of the study. LCC coordinated the herbarium sampling and generated data from herbarium specimens. TJM conducted statistical analyses with inputs from PGK and EAH. TJM wrote the manuscript with guidance from PGK and EAH. All authors contributed to manuscript revisions. TJM and LCC contributed equally to this work.

ORCID

Lauren C. Cline https://orcid.org/0000-0002-8529-1497
Jessica L. M. Gutknecht https://orcid.org/0000-0001-7667-5272
Erik A. Hobbie https://orcid.org/0000-0002-1629-6307
Peter G. Kennedy https://orcid.org/0000-0003-2615-3892
Talia J. Michaud https://orcid.org/0000-0002-7295-6808

Data availability

The data that support the findings of this study are available in the Data Repository for the University of Minnesota (DRUM), doi: 10.13020/DRXN-9M88, and Dryad, doi: 10.5061/dryad.v2k2607.

References

Alberton O, Kuyper TW, Gorissen A. 2007. Competition for nitrogen between *Pinus sylvestris* and ectomycorrhizal fungi generates potential for negative feedback under elevated CO_2. *Plant and Soil* **296**: 159.

Arnolds E. 1991. Decline of ectomycorrhizal fungi in Europe. *Agriculture, Ecosystems & Environment* **35**: 209–244.

Averill C, Dietze MC, Bhatnagar JM. 2018. Continental-scale nitrogen pollution is shifting forest mycorrhizal associations and soil carbon stocks. *Global Change Biology* **24**: 4544–4553.

Bailey RG. 2014. *Ecoregions: the ecosystem geography of the oceans and continents.* New York, NY, USA: Springer.

Baldrian P, López-Mondéjar R, Kohout P. 2023. Forest microbiome and global change. *Nature Reviews Microbiology* **21**: 487–501.

Barceló M, van Bodegom PM, Soudzilovskaia NA. 2023. Fine-resolution global maps of root biomass carbon colonized by arbuscular and ectomycorrhizal fungi. *Scientific Data* **10**: 56.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using LME4. *Journal of Statistical Software* **67**: 1–48.

Belmecheri S, Lavergne A. 2020. Compiled records of atmospheric CO_2 concentrations and stable carbon isotopes to reconstruct climate and derive plant ecophysiological indices from tree rings. *Dendrochronologia* **63**: 125748.

Clark CM, Phelan J, Doraiswamy P, Buckley J, Cajka JC, Dennis RL, Lynch J, Nolte CG, Spero TL. 2018. Atmospheric deposition and exceedances of critical loads from 1800–2025 for the conterminous United States. *Ecological Applications* **28**: 978–1002.

Craine JM, Brookshire ENJ, Cramer MD, Hasselquist NJ, Koba K, Marin-Spiotta E, Wang L. 2015. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant and Soil* **396**: 1–26.

Craine JM, Elmore AJ, Aidar MPM, Bustamante M, Dawson TE, Hobbie EA, Kahmen A, Mack MC, McLauchlan KK, Michelsen A *et al.* 2009. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytologist* **183**: 980–992.

Craine JM, Elmore AJ, Wang L, Aranibar J, Bauters M, Boeckx P, Crowley BE, Dawes MA, Delzon S, Fajardo A *et al.* 2018. Isotopic evidence for oligotrophication of terrestrial ecosystems. *Nature Ecology & Evolution* **2**: 1735–1744.

Craine JM, Elmore AJ, Wang L, Aranibar J, Bauters M, Boeckx P, Crowley BE, Dawes MA, Delzon S, Fajardo A *et al.* 2019. Data from: Isotopic evidence for oligotrophication of terrestrial ecosystems. (v.1, p. 6505354 bytes). *Dryad* [dataset]. doi: 10.5061/DRYAD.V2K2607.

Dong Y, Wang Z, Sun H, Yang W, Xu H. 2018. The response patterns of arbuscular mycorrhizal and ectomycorrhizal symbionts under elevated CO_2: a meta-analysis. *Frontiers in Microbiology* **9**: 1248.

Elmore AJ, Nelson DM, Craine JM. 2016. Earlier springs are causing reduced nitrogen availability in North American eastern deciduous forests. *Nature Plants* **2**: 16133.

Fernandez CW, See CR, Kennedy PG. 2019. Decelerated carbon cycling by ectomycorrhizal fungi is controlled by substrate quality and community composition. *bioRxiv*. doi: 10.1101/716555.

Fox J, Weisberg S. 2018. *An R companion to applied regression.* Thousand Oaks, CA, USA: Sage Publications.

Frey SD. 2019. Mycorrhizal fungi as mediators of soil organic matter dynamics. *Annual Review of Ecology, Evolution, and Systematics* **50**: 237–259.

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA *et al.* 2004. nitrogen cycles: past, present, and future. *Biogeochemistry* **70**: 153–226.

Hasselquist NJ, Metcalfe DB, Inselsbacher E, Stangl Z, Oren R, Näsholm T, Högberg P. 2016. Greater carbon allocation to mycorrhizal fungi reduces tree nitrogen uptake in a boreal forest. *Ecology* **97**: 1012–1022.

Hiltbrunner E, Körner C, Meier R, Braun S, Kahmen A. 2019. Data do not support large-scale oligotrophication of terrestrial ecosystems. *Nature Ecology & Evolution* **3**: 1285–1286.

Hobbie EA, Chen J, Hasselquist NJ. 2019. Fertilization alters nitrogen isotopes and concentrations in ectomycorrhizal fungi and soil in pine forests. *Fungal Ecology* **39**: 267–275.

Hobbie EA, Colpaert JV. 2003. Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. *New Phytologist* **157**: 115–126.

Hobbie EA, Högberg P. 2012. Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. *New Phytologist* **196**: 367–382.

Högberg P, Johannisson C, Yarwood S, Callesen I, Näsholm T, Myrold DD, Högberg MN. 2011. Recovery of ectomycorrhiza after 'nitrogen saturation' of a conifer forest. *New Phytologist* **189**: 515–525.

Högberg P, Näsholm T, Franklin O, Högberg MN. 2017. Tamm review: on the nature of the nitrogen limitation to plant growth in Fennoscandian boreal forests. *Forest Ecology and Management* **403**: 161–185.

Jonard M, Fürst A, Verstraeten A, Thimonier A, Timmermann V, Potočić N, Waldner P, Benham S, Hansen K, Merilä P *et al.* 2015. Tree mineral nutrition is deteriorating in Europe. *Global Change Biology* **21**: 418–430.

Karst J, Wasyliw J, Birch JD, Franklin J, Chang SX, Erbilgin N. 2021. Long-term nitrogen addition does not sustain host tree stem radial growth but doubles the abundance of high-biomass ectomycorrhizal fungi. *Global Change Biology* **27**: 4125–4138.

Knowles JE, Frederick C. 2016. *MERTOOLS: tools for analyzing mixed effect regression models*. R package v.0.3.0.

Kranabetter JM, Harman-Denhoed R, Hawkins BJ. 2019. Saprotrophic and ectomycorrhizal fungal sporocarp stoichiometry (C : N : P) across temperate rainforests as evidence of shared nutrient constraints among symbionts. *New Phytologist* **221**: 482–492.

Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. LMERTEST package: tests in linear mixed effects models. *Journal of Statistical Software* **82**: 1–26.

Lilleskov EA, Hobbie EA, Fahey TJ. 2002. Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. *New Phytologist* **154**: 219–231.

Lilleskov EA, Hobbie EA, Horton TR. 2011. Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. *Fungal Ecology* **4**: 174–183.

Lilleskov EA, Kuyper TW, Bidartondo MI, Hobbie EA. 2019. Atmospheric nitrogen deposition impacts on the structure and function of forest mycorrhizal communities: a review. *Environmental Pollution* **246**: 148–162.

Lindahl BD, Tunlid A. 2015. Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. *New Phytologist* **205**: 1443–1447.

Lüdecke D. 2018. GGEFFECTS: tidy data frames of marginal effects from regression models. *Journal of Open Source Software* **3**: 772.

Mason RE, Craine JM, Lany NK, Jonard M, Ollinger SV, Groffman PM, Fulweiler RW, Angerer J, Read QD, Reich PB *et al.* 2022. Evidence, causes, and consequences of declining nitrogen availability in terrestrial ecosystems. *Science* **376**: eabh3767.

McLauchlan KK, Ferguson CJ, Wilson IE, Ocheltree TW, Craine JM. 2010. Thirteen decades of foliar isotopes indicate declining nitrogen availability in central North American grasslands. *New Phytologist* **187**: 1135–1145.

Mohan JE, Cowden CC, Baas P, Dawadi A, Frankson PT, Helmick K, Hughes E, Khan S, Lang A, Machmuller M *et al.* 2014. Mycorrhizal fungi mediation of terrestrial ecosystem responses to global change: mini-review. *Fungal Ecology* **10**: 3–19.

Näsholm T, Högberg P, Franklin O, Metcalfe D, Keel SG, Campbell C, Hurry V, Linder S, Högberg MN. 2013. Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? *New Phytologist* **198**: 214–221.

Pellitier PT, Ibáñez I, Zak DR, Argiroff WA, Acharya K. 2021. Ectomycorrhizal access to organic nitrogen mediates CO_2 fertilization response in a dominant temperate tree. *Nature Communications* **12**: 5403.

Phillips RP, Brzostek E, Midgley MG. 2013. The mycorrhizal-associated nutrient economy: A new framework for predicting carbon-nutrient couplings in temperate forests. *New Phytologist* **199**: 41–51.

R Core Team. 2023. *R: the R project for statistical computing*. [WWW document] URL https://www.r-project.org/ [accessed 12 June 2023].

Savard MM, Marion J, Bégin C, Laganière J. 2023. On the significance of long-term trends in tree-ring N isotopes – the interplay of soil conditions and regional NOx emissions. *Science of the Total Environment* **857**: 159580.

Shah F, Nicolás C, Bentzer J, Ellström M, Smits M, Rineau F, Canbäck B, Floudas D, Carleer R, Lackner G *et al.* 2016. Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. *New Phytologist* **209**: 1705–1719.

Smith B. 2022. Declining global leaf nitrogen content: Smart resource use by flexible plants? *New Phytologist* **235**: 1683–1685.

Smith SE, Read DJ. 2010. *Mycorrhizal symbiosis*. London, UK: Academic Press.

Soudzilovskaia NA, Vaessen S, Barcelo M, He J, Rahimlou S, Abarenkov K, Brundrett MC, Gomes S, Merckx V, Tedersoo L. 2019. FUNGALROOT: global online database of plant mycorrhizal associations. (p. 717488). *bioRxiv*. doi: 10.1101/717488.

Steidinger BS, Crowther TW, Liang J, Van Nuland ME, Werner GDA, Reich PB, Nabuurs GJ, De-Miguel S, Zhou M, Picard N *et al.* 2019. Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. *Nature* **569**: 404–408.

Terrer C, Vicca S, Hungate BA, Phillips RP, Prentice IC. 2016. Mycorrhizal association as a primary control of the CO_2 fertilization effect. *Science* **353**: 72–74.

Teste FP, Jones MD, Dickie IA. 2020. Dual-mycorrhizal plants: their ecology and relevance. *New Phytologist* **225**: 1835–1851.

Supporting Information

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

Fig. S1 Analytical control between UC Davis Stable Isotope Facility and UMN Stable Isotope Facility for herbarium specimens analyzed in this study.

Methods S1 Herbarium specimen selection criteria, analysis protocols, and data quality control.

Table S1 Summary of species-level herbarium collections from Minnesota analyzed in this study, with reference to their species, genus, and ecological group.

Table S2 Summary of species-level foliar data analyzed from across the United States, with reference to their species, genus, and mycorrhizal type.

Table S3 Description of spatial data layers used in the analysis of foliar data across the United States.

Please note: Wiley is not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.