

Isotopic evidence for oligotrophication of terrestrial ecosystems

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Human societies depend on an Earth system that operates within a constrained range of nutrient availability, yet the recent trajectory of terrestrial nitrogen (N) availability is uncertain. Examining patterns of foliar N concentrations and isotope ratios ($\delta^{15}\text{N}$) from more than 43,000 samples acquired over 37 years, here we show that foliar N concentration declined by 9% and foliar $\delta^{15}\text{N}$ declined by 0.6–1.6‰. Examining patterns across different climate spaces, foliar $\delta^{15}\text{N}$ declined across the entire range of mean annual temperature and mean annual precipitation tested. These results suggest declines in N supply relative to plant demand at the global scale. In all, there are now multiple lines of evidence of declining N availability in many unfertilized terrestrial ecosystems, including declines in $\delta^{15}\text{N}$ of tree rings and leaves from herbarium samples over the past 75–150 years. These patterns are consistent with the proposed consequences of elevated atmospheric carbon dioxide and longer growing seasons. These declines will limit future terrestrial carbon uptake and increase nutritional stress for herbivores.

Human societies depend on a resilient, accommodating Earth system with a constrained range of environmental conditions^{1,2}, yet there is a fundamental uncertainty about the trajectory of N availability in terrestrial ecosystems. On the one

hand, as more and more reactive N has been fixed by humans over time³, planetary boundaries for N fluxes are thought to have been exceeded, generating wide-scale aquatic and terrestrial eutrophication as well as contributing to terrestrial acidification and aquatic

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anoxia. Exceeding the planetary N boundary is thought to be destabilizing the Earth system^{1,2,4-7}. In contrast, atmospheric carbon dioxide (CO₂) concentrations have increased by >40% over the past 150 years. Elevated atmospheric CO₂ could be reducing N availability in terrestrial ecosystems as plant N demand increases more than N supplies and/or higher ratios of carbon (C) to N in plants induce greater microbial N limitation⁸⁻¹¹. Longer growing seasons associated with climate warming could also be increasing plant N demand more than supply in some ecosystems, also reducing N availability¹². With no global databases to directly assess changes in terrestrial N availability, the lack of resolution of the trajectory of terrestrial N cycling constrains our ability to predict future terrestrial C uptake¹³, plant biodiversity¹⁴ and herbivore performance^{15,16}. For example, increasing N availability would generally increase plant productivity in unfertilized ecosystems, reduce plant biodiversity and increase the growth of herbivores as protein limitation is reduced. Decreasing N availability generally leads to opposite effects. Given the central role of N in so many ecosystem properties, a basic N trajectory is required to increase the certainty with which regulatory decisions can be made and predict future terrestrial ecosystem function.

To test whether there is evidence of global-scale increases or decreases in terrestrial N availability, we compiled a data set of 43,015 measurements of foliar N concentrations ([N]) and $\delta^{15}\text{N}$. Measurements were restricted to terrestrial rooted plants that were not directly fertilized and were collected between 1980 and 2017 (Supplementary Figs. 1–3). Similar to foliar [N], foliar $\delta^{15}\text{N}$ scales positively with N availability relative to plant N demand within an ecosystem¹⁷⁻¹⁹ as plants experiencing higher N availability acquire soil N that is more enriched in ^{15}N . When N availability is high, there is a greater relative importance of loss pathways that strongly fractionate against ^{15}N ; for example, denitrification. Also, plants acquire less N from mycorrhizal fungi, which transfer ^{15}N -depleted N to plants. Our data set is over 30,000 data points larger than previously assembled¹⁷, providing a better representation of global ecosystems and climate space as well as representing an additional 12 years of data to examine temporal trends (Supplementary Figs. 1 and 2). All samples used in analyses were associated with mean climate data and assigned a mycorrhizal type and N₂-fixing capacity based on provided data or literature syntheses^{20,21}. Data were then averaged to provide a single foliar $\delta^{15}\text{N}$ value for each species at a given site in a given year before determining relationships between foliar $\delta^{15}\text{N}$ and climate, mycorrhizal type and foliar [N]. We then tested whether foliar $\delta^{15}\text{N}$ and foliar [N] increased or decreased over time across sites with regression and structural equation modelling.

Results

Examining patterns for 38,646 unsummarized measurements of foliar $\delta^{15}\text{N}$ from non-N₂-fixing species, mean foliar $\delta^{15}\text{N}$ was 0.4‰ and varied by over 40‰ across all samples (−20.6‰ to +21.4‰). As observed in a previous synthesis¹⁷, using data summarized by species at each site for each year ($n = 10,827$), foliar $\delta^{15}\text{N}$ increased with increasing mean annual temperature (MAT) and decreasing mean annual precipitation (MAP) (Fig. 1). With these data, $\delta^{15}\text{N}$ began increasing with increasing MAT above $9.9 \pm 1.0^\circ\text{C}$, which was similar to the 8.5°C break point observed in the full data set of the first synthesis¹⁷ and the 9.8°C break point observed for a global synthesis of soil organic matter $\delta^{15}\text{N}$ ²². As observed previously, in the current synthesis, foliar $\delta^{15}\text{N}$ increased with increasing log-transformed foliar [N] and differed among species with different mycorrhizal associations (Fig. 1). Non-mycorrhizal species still had the highest foliar $\delta^{15}\text{N}$, and ericoid species the lowest (Fig. 1). Foliar [N] was highest under cold, wet climates and also differed significantly among mycorrhizal types, with non-mycorrhizal and arbuscular species having the highest N concentrations (19.2 mg g^{-1}) and ericoid species the lowest (12.2 mg g^{-1}). After accounting for climate, foliar [N] and mycorrhizal type, there were significant

differences among continents in foliar $\delta^{15}\text{N}$. For example, samples from Europe were $0.9 \pm 0.2\%$ lower than expected based on climate, mycorrhizal type and [N], whereas samples from South America were $1.0 \pm 0.2\%$ more than expected (Supplementary Table 1, Supplementary Fig. 4). For foliar [N], leaves from a typical plant with the geometric mean [N] of 17.3 mg g^{-1} would be 10.7 mg g^{-1} if the plant were from Australia and 19.5 mg g^{-1} from Asia if one held mycorrhizal type and climate constant and only varied the continent of origin (Supplementary Table 1).

After standardizing values for differences in climate, mycorrhizal type, foliar [N] and continent, there was no significant trend in residual $\delta^{15}\text{N}$ over time when comparing at the same foliar [N] over time ($-0.003 \pm 0.007\% \text{ y}^{-1}$; $P = 0.62$, $n = 2,301$; Supplementary Table 1). However, log-transformed foliar [N] did decline over time ($-0.0012 \pm 0.0003 \text{ y}^{-1}$; $P = 0.001$, $n = 2,301$; Supplementary Table 1). This rate of decline is the equivalent of a typical plant with the geometric mean [N] of 17.3 mg g^{-1} in 1980 declining 9% to 15.6 mg g^{-1} in 2017. Given this declining foliar [N] over time and the relationship between foliar [N] and $\delta^{15}\text{N}$, we ran additional regression models that standardized foliar $\delta^{15}\text{N}$ for climate, mycorrhizal type and continent, but did not hold foliar [N] constant over time. Not holding foliar [N] constant over time, residual foliar $\delta^{15}\text{N}$ declined by $1.6 \pm 0.5\%$ ($P < 0.001$) over 37 years (Fig. 2).

To test how changes in foliar [N] were affecting the trend in $\delta^{15}\text{N}$ given that both were changing over time, a structural equation model (SEM) further examined the role of foliar [N] in determining changes in $\delta^{15}\text{N}$ and the changes in $\delta^{15}\text{N}$ that were independent of trends in foliar [N]. Doing so allows partitioning of direct effects of time on foliar $\delta^{15}\text{N}$ from indirect effects that occur through changes in foliar [N]. Working with site-level residual foliar $\delta^{15}\text{N}$ and foliar [N] for non-N₂-fixing species after standardizing for climate, mycorrhizal type and continent, taking into account [N]-dependent and -independent pathways, the SEM resulted in a lower estimate of the decline in foliar $\delta^{15}\text{N}$ in non-N₂-fixing species than the regression with the sums of effects via the two pathways generating an estimated decline of 0.6% over 37 years (Supplementary Fig. 5, Supplementary Table 2). With this approach, 70% of the decline occurred independent of changes in [N], while the remainder was due to the decline in [N].

To explore patterns of changes in foliar $\delta^{15}\text{N}$ and [N] over time across climates for non-N₂-fixing species, we ran a series of SEMs using subsets of the data created by serially adjusting the minimum and maximum MAT for inclusion in the SEM. Examining the coefficients for SEMs across ranges of MAT, plants in hot regions have been more likely to increase in foliar [N] over time than plants from cold regions (Supplementary Fig. 6). Yet, the [N]-independent changes in foliar $\delta^{15}\text{N}$ were consistently negative across the entire range of MAT tested (Supplementary Fig. 6). As a result, after calculating [N]-dependent and [N]-independent pathways of changes in foliar $\delta^{15}\text{N}$, foliar $\delta^{15}\text{N}$ declined across the entire range of MAT tested and the decline was probably of the highest magnitude in hotter regions. Employing a similar approach for MAP, regions with intermediate MAP were most likely to experience increased [N] (Supplementary Fig. 7), but the strength of the [N]-independent declines in foliar $\delta^{15}\text{N}$ led to declines in foliar $\delta^{15}\text{N}$ across the entire range of MAP examined (Supplementary Fig. 7).

N₂-fixing plants showed similar patterns over time in site-level foliar $\delta^{15}\text{N}$ as non-N₂-fixers, although it is uncertain whether these patterns result from increasing N₂ fixation or reduced $\delta^{15}\text{N}$ of acquired N due to declining N availability. Across 4,369 samples (Supplementary Fig. 8), mean foliar $\delta^{15}\text{N}$ for N₂-fixers was 2.0‰ and varied by over 24‰. Probably reflecting their greater access to N, the geometric mean foliar [N] for N₂-fixing species was 25.8 mg g^{-1} as opposed to just 17.3 mg g^{-1} for non-N₂-fixers. Foliar $\delta^{15}\text{N}$ increased with increasing MAT, decreasing MAP and increasing foliar [N] (Supplementary Fig. 9). Potentially signifying greater

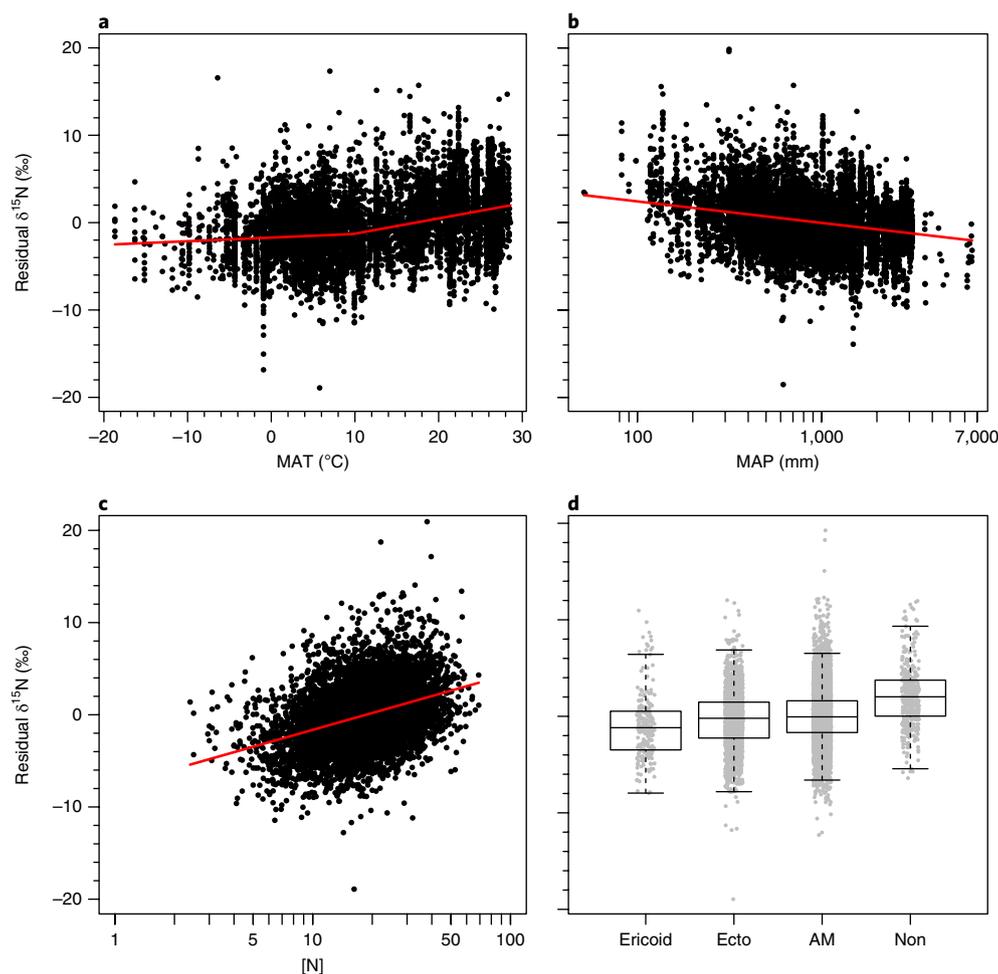


Fig. 1 | Relationships between residual foliar $\delta^{15}\text{N}$ of non- N_2 -fixing species and predictors. a–d, Included are MAT (a), log MAP (b), log [N] (c) and mycorrhizal type (d) after standardizing for the other three factors. All data were summarized by species for a given location before the regression. Each point represents an average foliar $\delta^{15}\text{N}$ for a given species at a given location in a given year. $n = 10,827$. Ericoid, 2,921; ectomycorrhizal (Ecto), 8,039; arbuscular (AM), 25,960; non-mycorrhizal (Non), 1,726.

N_2 -fixation, [N] was increasing for N_2 -fixers between 1980 and 2017 (Supplementary Table 1). However, leaf $\delta^{15}\text{N}$ still declined by 1.4‰ over the 37 years. Using the same SEM used for non- N_2 -fixers, taking into account the increases in N as well as N-independent decline, foliar $\delta^{15}\text{N}$ declined by 0.8‰. This decline is similar to the rate for non- N_2 -fixers (Supplementary Fig. 10, Supplementary Table 3), but it should be noted that sample size was lower for non- N_2 -fixers ($n = 379$ for data averaged by site and year).

Discussion

Based on evidence that foliar $\delta^{15}\text{N}$ reflects soil N availability to plants in non- N_2 -fixing species, the most parsimonious interpretation of the decline in foliar [N] and foliar $\delta^{15}\text{N}$ for non- N_2 -fixing species is that the ratio of plant N demand to soil N supply is increasing in unfertilized ecosystems across the Earth; that is, N availability to plants is declining. Within this general global pattern, there are individual examples of local to regional increases in N availability occurring^{23–25}. We also reveal evidence that some climatic regions might be experiencing complicated responses, with foliar [N] increasing while foliar $\delta^{15}\text{N}$ decreased. Yet, patterns consistent with broad-scale oligotrophication in unfertilized terrestrial ecosystems are documented in numerous other studies. Evidence of oligotrophication includes regional declines in grass and tree foliar N concentrations^{26,27}, reduced dietary protein in cattle on rangelands¹⁵, reduced pollen protein concentrations²⁸, increased soil C:N²⁹,

reduced N mineralization rates in forests³⁰ and reductions in NO_3^- in forest streams^{31–34}. In addition, declines in $\delta^{15}\text{N}$ of tree rings and leaves from herbarium samples indicate reduction of plant N availability in forests and grasslands over the past 75–150 years^{12,25,27}. It should be noted that these corroborating patterns have been quantified for North America and Europe. Similar research has yet to be conducted to begin to examine other lines of evidence on other continents.

The mechanism behind terrestrial oligotrophication cannot be directly tested here, but progressive N limitation associated with elevated atmospheric CO_2 and/or longer growing seasons due to climate change are likely candidates due to the global extent of the oligotrophication. Although N deposition does affect terrestrial ecosystem N cycling, North American tree ring and herbarium studies support the timing of the initiation of the decline in N availability to predate broad-scale increases in N deposition^{25,27} and declines in foliar [N] are unlikely to be explained by greater N deposition rates globally. Further N isotope research on tree rings and herbarium specimens as well as more extensive syntheses of foliar [N] data could elucidate regional variation in changes in N availability over time, as well as further explore the timing of declines before the years examined here.

Considered alongside these other lines of evidence, our results suggest that most terrestrial ecosystems are undergoing oligotrophication, even while other ecosystems—particularly intensively

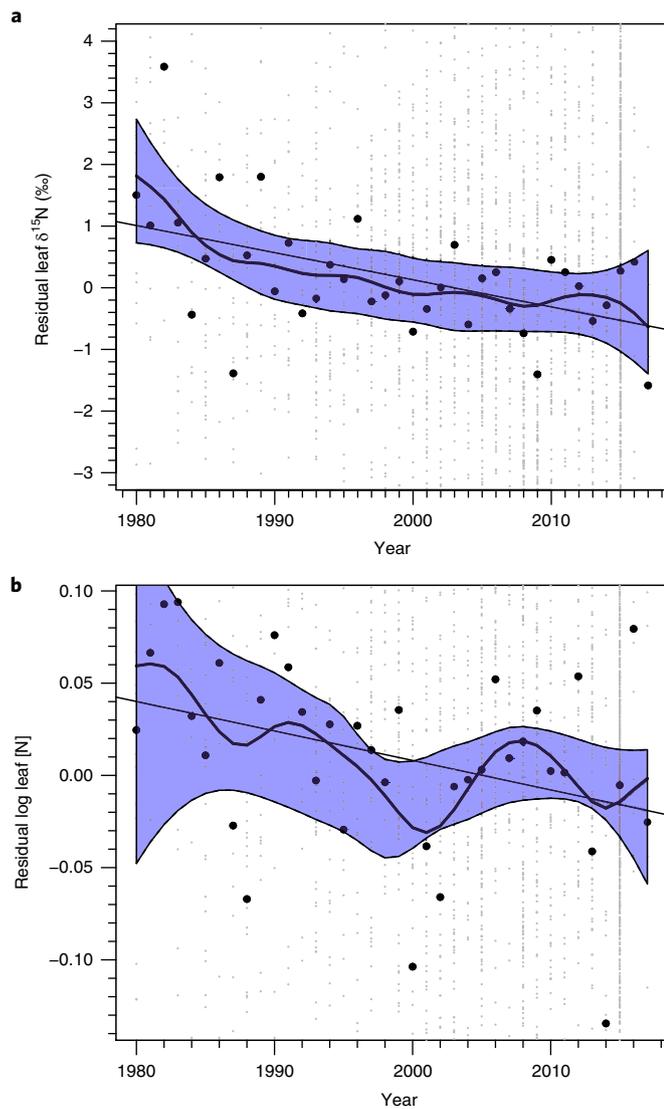


Fig. 2 | Results of the regressions of residual foliar $\delta^{15}\text{N}$ and residual log-transformed foliar [N]. a,b, Results for residual foliar $\delta^{15}\text{N}$ (**a**) and residual log-transformed foliar [N] (**b**) shown after accounting for site climate, mycorrhizal type and identity of continent from which samples were collected. Included are means for each year (black symbols) and site-averaged data (grey symbols). The thin black line represents the regression line on average residual $\delta^{15}\text{N}$ for each year ($y = 88.07 - 0.044x$, $r^2 = 0.25$, $P < 0.001$ (**a**); $y = 3.20 - 0.0016x$, $r^2 = 0.12$, $P = 0.03$ (**b**)) and blue area represents the 95% confidence interval for the less-smoothed curve of average residual values (thick black line).

fertilized terrestrial ecosystems, urban areas and their downstream receiving waters—are eutrophying (Fig. 3). This global bifurcation of N availability raises questions about whether humanity has exceeded a true planetary boundary for N availability³⁵. Broad-scale reductions of anthropogenic N fixation would certainly reduce impacts on aquatic ecosystems, but at the same time could also further constrain terrestrial C uptake as atmospheric CO_2 concentrations continue to increase and N deposition rates fall¹³. Given projected increases in atmospheric CO_2 concentrations and global temperatures over the next century, it is likely that this oligotrophication will continue and the C:N stoichiometry of most terrestrial ecosystems will continue to increase. It remains to be tested whether reduced plant protein accompanying oligotrophication can explain reductions in production in herbivores³⁶, but declining N availability

to plants should cascade up to herbivores^{15,16}. Even if atmospheric CO_2 is eventually stabilized at low enough levels to mitigate the most serious impacts of climate change³⁷, many terrestrial ecosystems will increasingly display signs of too little reactive N as opposed to too much. Preventing these declines in N availability further emphasizes the need to reduce anthropogenic CO_2 emissions.

Methods

Data acquisition. Data for this synthesis included data from the 2009 synthesis and published and unpublished data acquired from other researchers. To identify data not included in the 2009 synthesis, Web of Science was searched with the terms ‘(nitrogen isotope or 15-N) and (leaf or leaves or foliar)’ for papers published between 2006 and the present. Web of Science was also searched for papers that cited Craine et al., 2009, and these were examined using the same criteria. Google Scholar was searched using similar criteria and the first 300 articles were examined. Articles that potentially were associated with foliar $\delta^{15}\text{N}$ data were downloaded and examined to see whether foliar $\delta^{15}\text{N}$ data were collected. Articles were excluded if foliar $\delta^{15}\text{N}$ data were limited to: (1) urban areas, (2) agricultural ecosystems, (3) non-control samples of manipulative experiments, (4) non-vascular plants, (5) fertilized plants, (6) semi-aquatic or aquatic plants or (7) sites with $\text{MAP} < 50$ mm. For those papers that did not provide data online, we contacted the corresponding author using the provided email and requested the data. First emails were sent on October 3, 2017. Failures to respond within two weeks or bounced emails resulted in searching the web for a different email or contacting another author on the paper. If an author was successfully contacted, we asked whether they had additional appropriate foliar $\delta^{15}\text{N}$ data that could be provided to the project. Data were included in the final synthesis if data points were associated with the year the data were collected, if there were location data to generate site latitude and longitude, if there was sufficient identity of the species from which the leaves were collected to identify the mycorrhizal type and N_2 -fixing status of the plant and if both foliar N concentration and foliar $\delta^{15}\text{N}$ were recorded. Out of 146 researchers we attempted to contact, a total of 108 researchers responded that they would send data and 100 sent data by January 10, 2018. Along with data acquired from Dryad and TRY databases, 180 new data sets were added to the 78 data sets of the original paper.

For each data point in the database, we assigned a MAT and MAP based on its geographic location from New et al.³⁸. Original climate data were maintained if data from the first database did not have location data associated with them. N_2 -fixing status of each species was assigned based on the study by Werner and colleagues²⁰. Any Fabaceae species not listed in the reference was assumed to be N_2 -fixing unless the majority of the species in the genus were non- N_2 fixers. Family identification was assigned from theplantlist.org or ITIS. Mycorrhizal type of the species was derived from the study by Brundrett²¹ using family- and genus-level assignments.

Data analysis. Statistical analyses were preregistered with Open Science Framework on 3 October 2017 (<https://osf.io/thnyf>) to reduce post-analysis bias in choosing statistical methods. Analyses were constructed with the knowledge of patterns from the 2009 analyses, but without access to any subsequent data. All analyses were conducted in R 3.3.2. All data for a given species at a given site within a year were averaged. All samples within each 0.1° latitude and longitude were considered to be from the same site. Statistical analyses were run separately for N_2 - and non- N_2 -fixing species. Our general regression approach was to test for trends in foliar $\delta^{15}\text{N}$ after standardizing for as many covariates as possible that could be skewing the temporal trend due to differential sampling. To accomplish this, we conducted a set of regressions with species-averaged data followed by a regression with site-averaged data. In the first set of regressions, foliar $\delta^{15}\text{N}$ (averaged by site, species and year) was regressed against log-transformed MAP, log-transformed foliar [N] and mycorrhizal type. Differences among mycorrhizal types were assessed with pairwise contrasts on least-squares means. The residuals of this regression were then subjected to a segmented regression to identify the break point between MAT and foliar $\delta^{15}\text{N}$. A subsequent regression of foliar $\delta^{15}\text{N}$ (averaged for site, species and year) then included MAT, the interaction between MAT and a categorical variable representing whether sites had MAT above or below the break point, log-transformed MAP, log-transformed foliar [N] and mycorrhizal type. Outliers were considered as those points having a Cook's $D > 1$, but no points met this threshold for this data set. To test whether foliar $\delta^{15}\text{N}$ increased or decreased over time, we ran a second regression on the residual foliar $\delta^{15}\text{N}$ from the previous analysis after averaging the residuals by year and site. The regression tested the averaged residuals against year of sampling and the identity of the continent from which the sample was taken. Continent was included to ensure that any trends over time were not due to differential sampling over time between continents, which had been previously observed as explaining variation in foliar $\delta^{15}\text{N}$ independent of climate. A similar set of regressions were run with log-transformed foliar [N] as the response and without foliar [N] as a predictor. After observing that foliar [N] declined, additional regressions were performed that paralleled the foliar $\delta^{15}\text{N}$ regressions but did not include log foliar [N] in the regression model in order to quantify how foliar $\delta^{15}\text{N}$ changed without standardizing for the declines in log foliar [N]. In this model, the regression was not weighted by sample size for each year, in accordance with all other models.

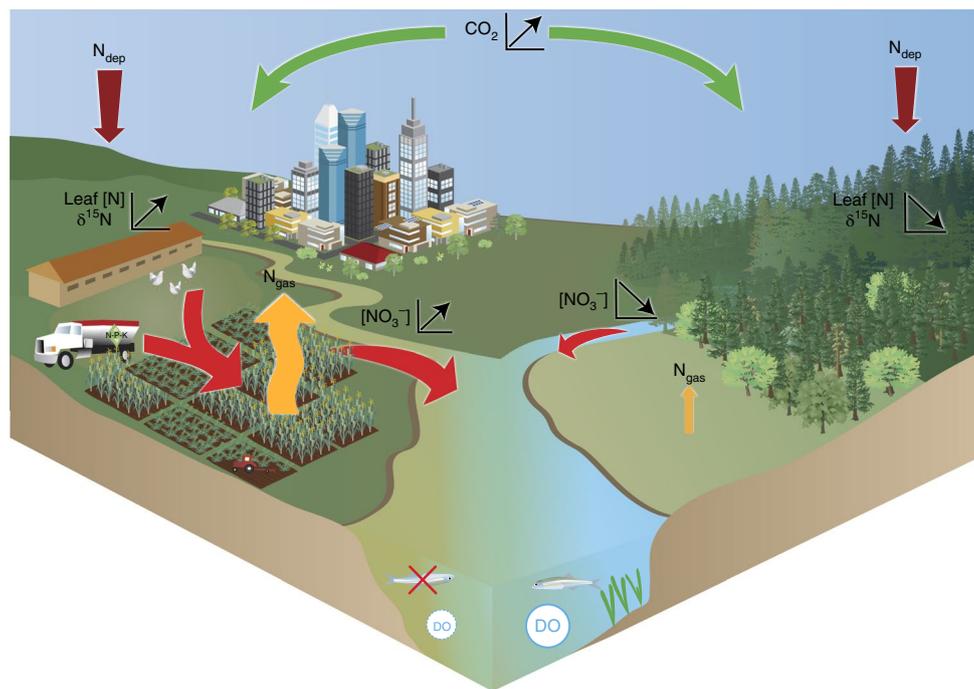


Fig. 3 | Conceptual diagram summarizing N availability bifurcation hypothesis for global terrestrial ecosystems. The left half of the diagram represents the most anthropogenically influenced areas. These ecosystems are becoming more eutrophic; represented are the increases in N availability, foliar N concentrations, gaseous N loss and transfers to aquatic ecosystems as well as declining dissolved oxygen (DO) levels in waters. The right half of the diagram represents the least anthropogenically influenced ecosystems, which are becoming more oligotrophic. Here, any increased N deposition is overwhelmed by the consequences of increasing atmospheric CO₂ concentrations on N availability. These ecosystems are demonstrating oligotrophication with declining N availability, declining foliar N concentrations, declining gaseous N loss and reduced transfers to aquatic ecosystems. N_{dep}, N deposition; N_{gas}, gaseous N emissions. Credit: Diagram created by Brianne Walsh, University of Maryland Center for Environmental Science, Integration and Application Network

Structural equation modelling. The first set of regressions were designed to test for trends in foliar $\delta^{15}\text{N}$ after standardizing foliar $\delta^{15}\text{N}$ with respect to foliar [N]. To account for trends in [N] occurring over time when assessing the potential causes of changes in $\delta^{15}\text{N}$ over time (which cannot be assessed using regression methods), we also conducted an SEM that used site-level residual $\delta^{15}\text{N}$ after accounting for MAT and its break point, log-transformed MAP, mycorrhizal type and identity of the continent from which samples were taken. The SEM then assessed the influence of time on foliar [N] as well as time and [N] on foliar $\delta^{15}\text{N}$.

Post-registration, the SEM was altered to correct an oversight. The final SEM used residual foliar [N] after standardizing for MAT, log MAP, mycorrhizal type and continent as opposed to unstandardized foliar $\delta^{15}\text{N}$. The SEM was also altered from its original pre-registered formulation to use site-averaged data rather than unsummarized data, which was another initial oversight. As an exploratory exercise, we also ran a series of SEMs that explored the response of foliar $\delta^{15}\text{N}$ as a function of climate. In the first set of SEMs, data were subset by MAT, serially incrementing the lower bound from -5 to 18°C and the upper bound from 10°C more than the lower bound up to 28°C . All combinations were tested with MAT bounds incremented by 1°C between runs. In the second set of SEMs, data were subset by log MAP, serially varying the lower bound from 2 to 3.1 and the upper bound from 0.4 more than the lower bound to 3.6. log MAP bounds were incremented by 0.1 and the SEMs explored responses in MAP from 100 mm to 3981 mm. SEM models were implemented in the R programming language using the Lavaan package version 0.5–23.1097³⁹. No modification indices were used.

Because all possible pathways between all variables were accounted for in the SEM—that is, the models were ‘just identified’ or saturated—we could not test the significance of our models^{22,40–42}, but do report significance values and unstandardized coefficients for each path. Future research could test more complex SEMs that are not saturated, which would allow for estimation of the significance of a model as a whole. Given that we could not estimate the significance of the model, one should not rely on the SEM as support for the causal structure that was tested, but instead as a technique for generating the individual parameters that were used to assess N-dependent and N-independent rates of decline in $\delta^{15}\text{N}$.

Data sources. Articles from which published data were derived are listed in refs ^{23,43–224}. A table of the papers examined and their ultimate inclusion or exclusion can be found in Supplementary Table 4.

Reporting summary. Further information on experimental research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data sets generated during and/or analysed during the current study are available in the Dryad repository (<https://doi.org/10.5061/dryad.v2k2607>). All codes used for statistical analyses and figure generation are available on Dryad (<https://doi.org/10.5061/dryad.v2k2607>).

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J.M.C. and A.J.E. conceived of the research, conducted analyses, generated figures and prepared the original draft. All authors contributed data and provided comments on manuscripts.

Competing interests

Although J.M.C. is an owner of Jonah Ventures, a for-profit DNA sequencing company, the authors declare no competing interests in the publication of this research.

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Study description

In 2009, we published a paper that quantified relationships between foliar delta15N and a) climate relationships, b) foliar N concentrations, and c) mycorrhizal associations. Data for the paper were collected through 2006. Since then, over a decade of data has accumulated on foliar delta15N, but there has been no analysis to assess whether these relationships have held constant or changed. For example, rising atmospheric CO2 concentrations might be reducing N availability to plants, which would cause declines in both foliar N concentrations and delta15N. Alternatively, increased atmospheric N deposition could cause increases in both.

All hypotheses will separately tested for N2-fixing and non-N2-fixing plants.

H1) Have there been significant shifts in the relationships between foliar delta15N and a) mean annual temperature, b) mean annual precipitation, c) foliar N concentrations, and d) mycorrhizal associations? More specifically, has there been a significant change in the mean estimates for each parameter for data collected before 2006 and those collected after 2006?

H2) After accounting for mean annual temperature, mean annual precipitation, foliar N concentrations, and mycorrhizal association, has foliar delta15N either increased or decreased over time for N2-fixing and non-N2-fixing plants?

H3) After accounting for mean annual temperature, mean annual precipitation, and mycorrhizal association, has foliar delta15N either increased or decreased over time for N2-fixing and non-N2-fixing plants??

H4) How are any trends in foliar N concentrations contributing to any trends in foliar delta15N?

Research sample

An individual sample would be considered a single value of foliar d15N and [N] collected from a species at a given site in a given year.

Sampling strategy

Data were acquired from the literature and by contacting coauthors for unpublished data. Data collection proceeded until all data identified as appropriate had been collected to a certain date. Individual researchers were contacted via email twice for their data to limit the effort to acquire any one data set.

Data collection

Data were acquired by first identifying extant published data sets. wer

First, we searched Web of Science with the terms "(nitrogen isotope or 15-N) and (leaf or leaves or foliar)" for years 2006-present. Articles that potentially were associated with foliar 15N data were flagged for downloading and examination to see if foliar 15N data were collected. Articles were excluded if data are limited to:

- 1) urban areas
- 2) agricultural ecosystems
- 3) non-control samples of manipulative experiments
- 4) non-vascular plants
- 5) fertilized plants
- 6) semi-aquatic or aquatic plants
- 7) sites with mean annual precipitation $\leq 50\text{ mm}$

Planted trees were included if they were from plantations older than 5 years and met the above requirements.

Web of Science was searched for relevant datasets to included any papers that cite Craine et al. 2009.

In addition to identifying these datasets on Web of Science, we also searched Google Scholar using the same search terms and criteria to look for relevant articles.

After identifying articles that potentially report collection of foliar 15N data, we downloaded each article to examine whether relevant foliar 15N data were collected. For those that were, we first saw whether the data had been provided in an on-line repository. If not, we contacted the corresponding author and requested the data. Failures to respond were handled by contacting other authors on the paper. Unsuccessful attempts to request data will be noted in a log.

If an author was successfully contacted, we asked the researcher whether they have additional appropriate foliar 15N data that could be provided to the project.

Data were included in the final synthesis if data points were associated with a date of collection (at least to year), location data that could be used to generate site latitude and longitude, the identity of the species from which the leaves were collected, and both foliar N concentration and foliar d15N.

In parallel to obtaining data sets identified in the literature, additional datasets were sought from Dryad (datadryad.org) using the search terms "nitrogen isotope" and "15N".

Timing and spatial scale	Data were included if they were collected between 1980 and 2017. First emails were sent on October 3, 2017. Data collection ended January 10, 2018.
Data exclusions	For data that met the above criteria, no data were excluded from the analyses.
Reproducibility	All data were collected and analyzed once.
Randomization	All data were pre-assigned to mycorrhizal groups and climate parameters were assessed based on geographic location.
Blinding	All data were acquired before any statistical analyses were run. No blinding is involved in this study.
Did the study involve field work?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Unique biological materials
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants

Methods

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging