

Isotopic evidence for oligotrophication of terrestrial ecosystems

Joseph M. Craine^{1*}, Andrew J. Elmore², Lixin Wang³, Julieta Aranibar⁴, Marijn Bauters^{5,6}, Pascal Boeckx⁵, Brooke E. Crowley^{7,8}, Melissa A. Dawes⁹, Sylvain Delzon¹⁰, Alex Fajardo¹¹, Yunting Fang¹², Lei Fujiyoshi¹³, Alan Gray¹⁴, Rossella Guerrieri¹⁵, Michael J. Gundale¹⁶, David J. Hawke¹⁷, Peter Hietz¹⁸, Mathieu Jonard¹⁹, Elizabeth Kearsley²⁰, Tanaka Kenzo²¹, Mikhail Makarov²², Sara Marañón-Jiménez^{23,24,25}, Terrence P. McGlynn^{26,27}, Brenden E. McNeil²⁸, Stella G. Mosher⁷, David M. Nelson², Pablo L. Peri²⁹, Jean Christophe Roggy³⁰, Rebecca Sanders-DeMott^{31,32}, Minghua Song³³, Paul Szpak³⁴, Pamela H. Templer³¹, Dewidine Van der Colff³⁵, Christiane Werner³⁶, Xingliang Xu³², Yang Yang³⁷, Guirui Yu^{38,39} and Katarzyna Zmudczyńska-Skarbek⁴⁰

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

¹Jonah Ventures, Boulder, CO, USA. ²Appalachian Laboratory, University of Maryland Center for Environmental Science, Frostburg, MD, USA.

³Department of Earth Sciences, Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA. ⁴Facultad de Ciencias Exactas y Naturales

(FCEN-UNCuyo), Instituto Argentino de Nivología, Glaciología y Ciencias Ambientales (IANIGLA-CONICET), Mendoza, Argentina. ⁵MB Isotope

Bioscience Laboratory – ISOFYS, Ghent University, Ghent, Belgium. ⁶CAVElab, Computational and Applied Vegetation Ecology, Ghent University, Ghent,

Belgium. ⁷Department of Geology, University of Cincinnati, Cincinnati, OH, USA. ⁸Department of Anthropology, University of Cincinnati, OH, USA.

⁹Swiss Federal Institute for Forest, Snow and Landscape Research – WSL Forest soils and Biogeochemistry, Birmensdorf, Switzerland. ¹⁰BIOGECO, INRA

University of Bordeaux, Pessac, France. ¹¹Centro de Investigación en Ecosistemas de la Patagonia, Coyhaique, Chile. ¹²CAS Key Laboratory of Forest Ecology

and Management, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China. ¹³Research Institute for Humanity and Nature, Kyoto,

Japan. ¹⁴NERC Centre for Ecology and Hydrology, Penicuik, UK. ¹⁵Centre for Ecological Research and Forestry Applications, Barcelona, Spain. ¹⁶Department

of Forest Ecology and Management, Swedish University of Agricultural Sciences, Umeå, Sweden. ¹⁷Department of Science and Primary Industries, Ara

Institute of Canterbury, Christchurch, New Zealand. ¹⁸Institute of Botany, University of Natural Resources and Life Sciences, Vienna, Austria.

¹⁹Earth and Life Institute, Université Catholique de Louvain, Louvain-la-Neuve, Belgium. ²⁰Computational and Applied Vegetation Ecology lab, Department

of Environment, Ghent University, Ghent, Belgium. ²¹Department of Plant Ecology, Forestry and Forest Products Research Institute, Tsukuba, Japan.

²²Soil Science Department, Moscow M.V. Lomonosov State University, Moscow, Russia. ²³Department of Ecology, Faculty of Sciences, University of

Granada, Granada, Spain. ²⁴CREAF, Barcelona, Spain. ²⁵CSIC, Global Ecology Unit CREAF-CSIC-UAB, Barcelona, Spain. ²⁶Department of Biology, California

State University Dominguez Hills, Carson, CA, USA. ²⁷Department of Entomology, Natural History Museum of Los Angeles County, Los Angeles, CA,

USA. ²⁸Department of Geology and Geography, West Virginia University, Morgantown, WV, USA. ²⁹Instituto Nacional de Tecnología Agropecuaria,

CONICET Universidad Nacional de la Patagonia Austral, Río Gallegos, Argentina. ³⁰INRA, UMR Ecologie des Forêts de Guyane, Campus Agronomique,

Kourou, Guyane Française, France. ³¹Department of Biology, Boston University, Boston, MA, USA. ³²Department of Natural Resources and the Environment,

University of New Hampshire, Durham, NH, USA. ³³Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences

and Natural Resources Research, Chinese Academy of Sciences, Beijing, China. ³⁴Department of Anthropology, Trent University, Peterborough, Ontario,

Canada. ³⁵South African National Biodiversity Institute, Cape Town, South Africa. ³⁶Ecosystem Physiology, University of Freiburg, Freiburg, Germany.

³⁷Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.

³⁸Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy

of Sciences, Beijing, China. ³⁹College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, China. ⁴⁰Faculty of Biology,

Department of Vertebrate Ecology and Zoology, University of Gdańsk, Gdańsk, Poland. *e-mail: josephmcraine@gmail.com

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

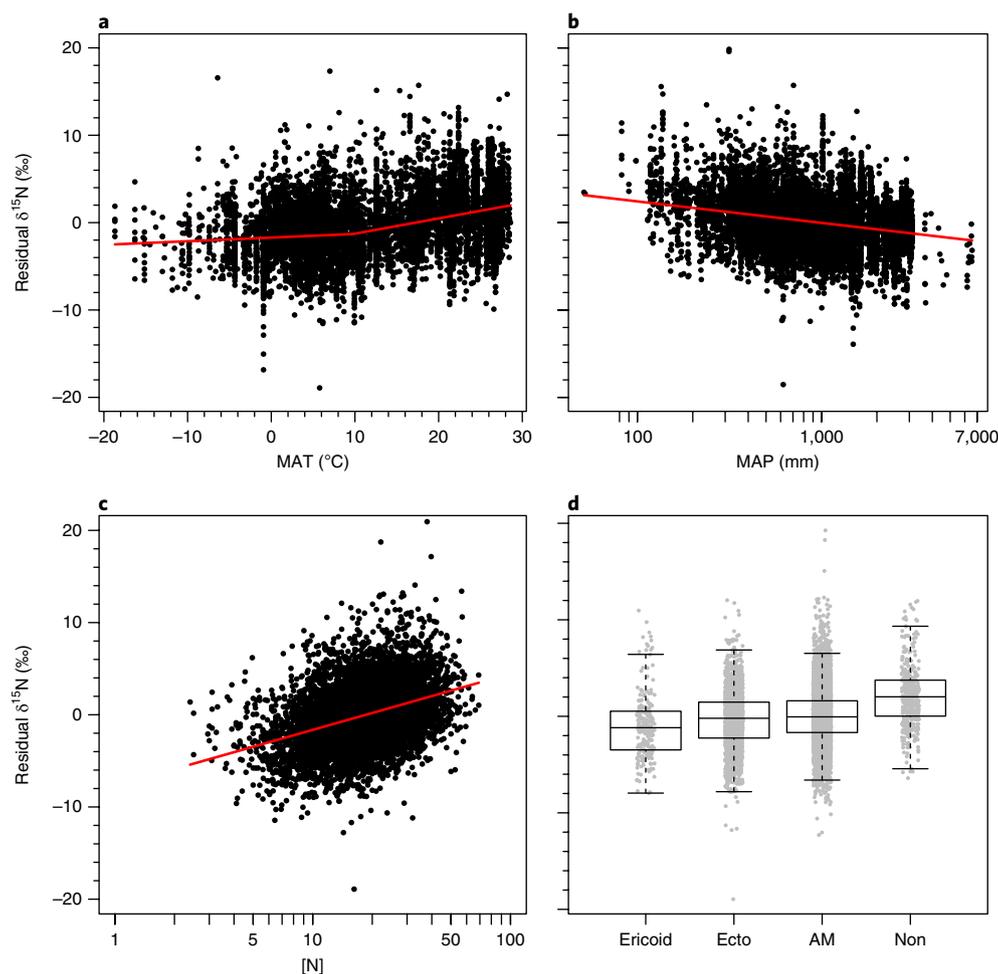


Fig. 1 | Relationships between residual foliar $\delta^{15}\text{N}$ of non- N_2 -fixing species and predictors. a–d, Included are MAT (a), log MAP (b), log [N] (c) and mycorrhizal type (d) after standardizing for the other three factors. All data were summarized by species for a given location before 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

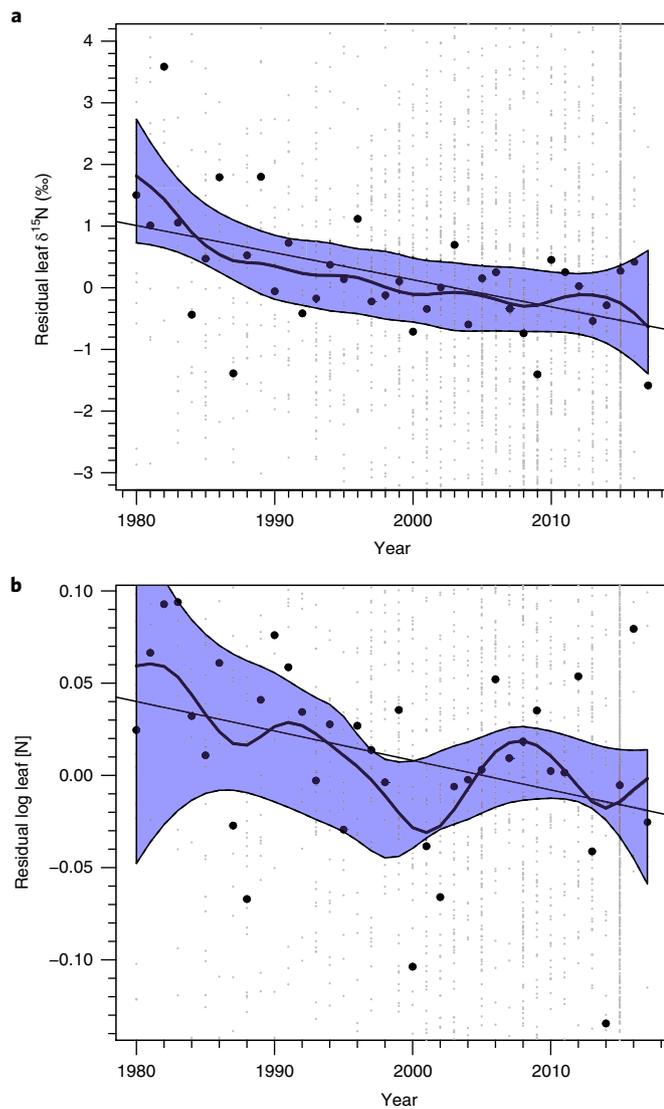


Fig. 2 | Results of the regressions of residual foliar $\delta^{15}\text{N}$ and residual log-transformed foliar [N]. **a, b.** Results for residual foliar $\delta^{15}\text{N}$ (**a**) and residual log-transformed foliar [N] (**b**) shown after accounting for site climate, mycorrhizal type and identity 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.

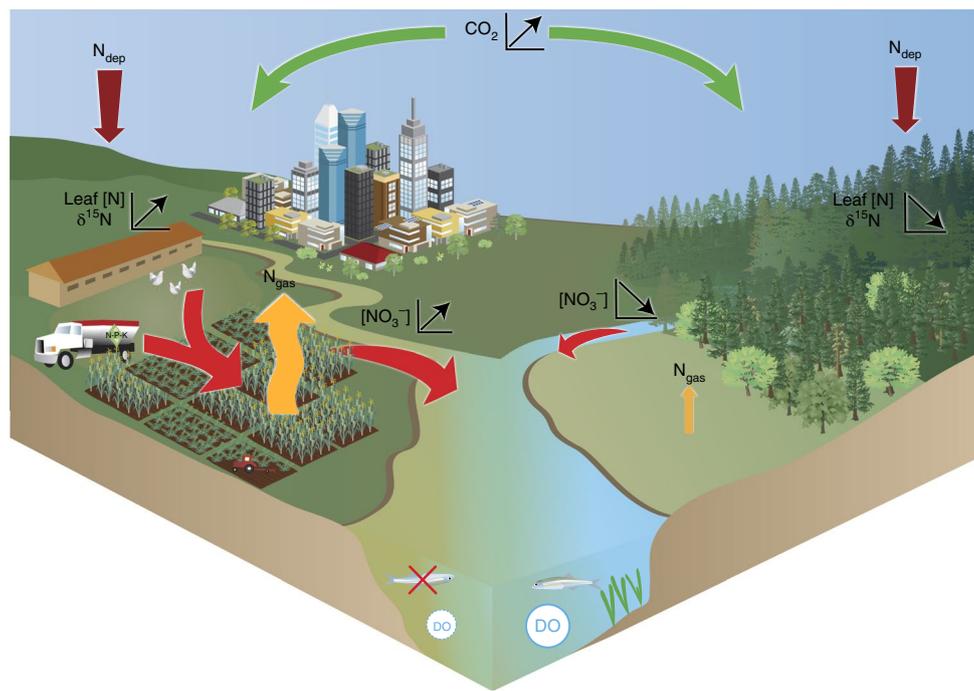


Fig. 3 | Conceptual diagram summarizing N availability bifurcation hypothesis for global terrestrial ecosystems. The left half of the diagram represents the most anthropogenically influenced areas. These ecosystems are becoming more eutrophic; represented are the increases in N availability, foliar N 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>).

Received: 31 May 2018; Accepted: 12 September 2018;

Published online: 22 October 2018

References

1. Rockström, J. et al. A safe operating space for humanity. *Nature* **461**, 472–475 (2009).
2. Steffen, W. et al. Planetary boundaries: guiding human development on a changing planet. *Science* **347**, 1259855 (2015).
3. Fowler, D. et al. The global nitrogen cycle in the twenty-first century. *Philos. Trans. R. Soc. B* **368**, 20130164 (2013).
4. Clark, C. M., Morefield, P. E., Gilliam, F. S. & Pardo, L. H. Estimated losses of plant biodiversity in the United States from historical N deposition (1985–2010). *Ecology* **94**, 1441–1448 (2013).
5. de Vries, W., Kros, J., Kroeze, C. & Seitzinger, S. P. Assessing planetary and regional nitrogen boundaries related to food security and adverse environmental impacts. *Curr. Opin. Environ. Sustain.* **5**, 392–402 (2013).
6. Sinha, E., Michalak, A. & Balaji, V. Eutrophication will increase during the 21st century as a result of precipitation changes. *Science* **357**, 405–408 (2017).
7. Vitousek, P. M. Human domination of Earth's ecosystems. *Science* **277**, 494–499 (1997).
8. Luo, Y. et al. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *Bioscience* **54**, 731–739 (2004).
9. Gill, R. A. et al. Nonlinear grassland responses to past and future atmospheric CO₂. *Nature* **417**, 279–282 (2002).

10. Norby, R. J., Warren, J. M., Iversen, C. M., Medlyn, B. E. & McMurtrie, R. E. CO₂ enhancement of forest productivity constrained by limited nitrogen availability. *Proc. Natl Acad. Sci. USA* **107**, 19368–19373 (2010).
11. Feng, Z. et al. Constraints to nitrogen acquisition of terrestrial plants under elevated CO₂. *Glob. Change Biol.* **21**, 3152–3168 (2015).
12. Elmore, A. J., Nelson, D. M. & Craine, J. M. Earlier springs are causing reduced nitrogen availability in North American eastern deciduous forests. *Nat. Plants* **2**, 16133 (2016).
13. Smith, B. et al. Implications of incorporating N cycling and N limitations on primary production in an individual-based dynamic vegetation model. *Biogeosciences* **11**, 2027–2054 (2014).
14. Bobbink, R. et al. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol. Appl.* **20**, 30–59 (2010).
15. Craine, J. M., Elmore, A. & Angerer, J. P. Long-term declines in dietary nutritional quality for North American cattle. *Environ. Res. Lett.* **12**, 044019 (2017).
16. Stiling, P. & Cornelissen, T. How does elevated carbon dioxide (CO₂) affect plant–herbivore interactions? A field experiment and meta-analysis of CO₂-mediated changes on plant chemistry and herbivore performance. *Glob. Change Biol.* **13**, 1823–1842 (2007).
17. Craine, J. M. et al. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytol.* **183**, 980–992 (2009).
18. Craine, J. M. et al. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant Soil* **1**, 1–26 (2015).
19. Garten, C. T. Variation in foliar ¹⁵N abundance and the availability of soil-nitrogen on Walker Branch watershed. *Ecology* **74**, 2098–2113 (1993).
20. Werner, G. D., Cornwell, W. K., Sprent, J. I., Kattge, J. & Kiers, E. T. A single evolutionary innovation drives the deep evolution of symbiotic N₂-fixation in angiosperms. *Nat. Commun.* **5**, 4087 (2014).
21. Brundrett, M. C. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* **320**, 37–77 (2009).
22. Craine, J. M. et al. Convergence of soil nitrogen isotopes across global climate gradients. *Sci. Rep.* **5**, 8280 (2015).
23. Hietz, P. et al. Long-term change in the nitrogen cycle of tropical forests. *Science* **334**, 664–666 (2011).
24. Decina, S. M., Templer, P. H., Hutrya, L. R., Gately, C. K. & Rao, P. Variability, drivers, and effects of atmospheric nitrogen inputs across an urban area: emerging patterns among human activities, the atmosphere, and soils. *Sci. Total Environ.* **609**, 1524–1534 (2017).
25. McLaughlan, K. K. et al. Centennial-scale reductions in nitrogen availability in temperate forests of the United States. *Sci. Rep.* **7**, 7856 (2017).
26. Jonard, M. et al. Tree mineral nutrition is deteriorating in Europe. *Glob. Change Biol.* **21**, 418–430 (2015).
27. McLaughlan, K. K., Ferguson, C. J., Wilson, I. E., Ocheltree, T. W. & Craine, J. M. Thirteen decades of foliar isotopes indicate declining nitrogen availability in central North American grasslands. *New Phytol.* **187**, 1135–1145 (2010).
28. Ziska, L. H. et al. Rising atmospheric CO₂ is reducing the protein concentration of a floral pollen source essential for North American bees. *Proc. Biol. Sci.* **283**, 20160414 (2016).
29. Gruneberg, E., Ziche, D. & Wellbrock, N. Organic carbon stocks and sequestration rates of forest soils in Germany. *Glob. Change Biol.* **20**, 2644–2662 (2014).
30. Durán, J. et al. Climate change decreases nitrogen pools and mineralization rates in northern hardwood forests. *Ecosphere* **7**, e01251 (2016).
31. Eshleman, K. N., Sabo, R. D. & Kline, K. M. Surface water quality is improving due to declining atmospheric N deposition. *Environ. Sci. Technol.* **47**, 12193–12200 (2013).
32. Sabo, R. D. et al. Watershed-scale changes in terrestrial nitrogen cycling during a period of decreased atmospheric nitrate and sulfur deposition. *Atmos. Environ.* **146**, 271–279 (2016).
33. Lucas, R. W. et al. Long-term declines in stream and river inorganic nitrogen (N) export correspond to forest change. *Ecol. Appl.* **26**, 545–556 (2016).
34. Bernal, S., Hedin, L. O., Likens, G. E., Gerber, S. & Buso, D. C. Complex response of the forest nitrogen cycle to climate change. *Proc. Natl Acad. Sci. USA* **109**, 3406–3411 (2012).
35. Nordhaus, T., Shellenberger, M. & Blomqvist, L. *The Planetary Boundaries Hypothesis. A Review of the Evidence* (Breakthrough Institute, Oakland, 2012).
36. Hallmann, C. A. et al. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE* **12**, e0185809 (2017).
37. IPCC *Climate Change 2014: Synthesis Report* (eds Core Writing Team, Pachauri, R. K. & Meyer, L. A.) (IPCC, 2014).
38. New, M., Lister, D., Hulme, M. & Makin, I. A high-resolution data set of surface climate over global land areas. *Clim. Res.* **21**, 1–25 (2002).
39. Rosseel, Y. lavaan: an R package for structural equation modeling. *J. Stat. Softw.* **48**, 1–36 (2012).
40. Grace, J. B. *Structural Equation Modeling and Natural Systems* (Cambridge Univ. Press, Cambridge, 2006).
41. Eldridge, D. J., Wang, L. & Ruiz-Colmenero, M. Shrub encroachment alters the spatial patterns of infiltration. *Ecohydrology* **8**, 83–93 (2015).
42. Lu, X., Wang, L. & McCabe, M. F. Elevated CO₂ as a driver of global dryland greening. *Sci. Rep.* **6**, 20716 (2016).
43. Anderson, J. T. & Gezon, Z. J. Plasticity in functional traits in the context of climate change: a case study of the subalpine forb *Boechera stricta* (Brassicaceae). *Glob. Change Biol.* **21**, 1689–1703 (2015).
44. Aranibar, J. N., Goiran, S. B., Guevara, A. & Villagra, P. E. Carbon and nitrogen dynamics in a sandy groundwater-coupled ecosystem in the Monte Desert, indicated by plant stable isotopes. *J. Arid Environ.* **102**, 58–67 (2014).
45. Averill, C. & Finzi, A. C. Increasing plant use of organic nitrogen with elevation is reflected in nitrogen uptake rates and ecosystem δ¹⁵N. *Ecology* **92**, 883–891 (2011).
46. Bai, E., Boutton, T. W., Liu, F., Ben Wu, X. & Archer, S. R. Variation in woody plant δ¹³C along a topoedaphic gradient in a subtropical savanna parkland. *Oecologia* **156**, 479–489 (2008).
47. Bai, E. et al. Spatial variation of the stable nitrogen isotope ratio of woody plants along a topoedaphic gradient in a subtropical savanna. *Oecologia* **159**, 493–503 (2009).
48. Bai, S. H., Sun, F., Xu, Z. & Blumfield, T. J. Ecophysiological status of different growth stage of understory *Acacia leiocalyx* and *Acacia disparrima* in an Australian dry sclerophyll forest subjected to prescribed burning. *J. Soils Sediments* **13**, 1378–1385 (2013).
49. Bansal, S., Nilsson, M.-C. & Wardle, D. A. Response of photosynthetic carbon gain to ecosystem retrogression of vascular plants and mosses in the boreal forest. *Oecologia* **169**, 661–672 (2012).
50. Baptist, F. et al. ¹³C and ¹⁵N allocations of two alpine species from early and late snowmelt locations reflect their different growth strategies. *J. Exp. Bot.* **60**, 2725–2735 (2009).
51. Baraloto, C. et al. Decoupled leaf and stem economics in rain forest trees. *Ecol. Lett.* **13**, 1338–1347 (2010).
52. Bauer, G. A. et al. in *Carbon and Nitrogen Cycling in European Forest Ecosystems* (ed. Schulze, E.-D.) 189–214 (Springer, Berlin, 2000).
53. Bauters, M. et al. Parallel functional and stoichiometric trait shifts in South-American and African forest communities with elevation. *Biogeosci. Discuss.* **2017**, 1–27 (2017).
54. Bazot, S., Fresneau, C., Damesin, C. & Barthes, L. Contribution of previous year's leaf N and soil N uptake to current year's leaf growth in sessile oak. *Biogeosciences* **13**, 3475–3484 (2016).
55. Beyschlag, W., Hanisch, S., Friedrich, S., Jentsch, A. & Werner, C. ¹⁵N natural abundance during early and late succession in a middle-European dry acidic grassland. *Plant Biol.* **11**, 713–724 (2009).
56. Blonder, B., Baldwin, B. G., Enquist, B. J. & Robichaux, R. H. Variation and macroevolution in leaf functional traits in the Hawaiian silversword alliance (Asteraceae). *J. Ecol.* **104**, 219–228 (2016).
57. Blumenthal, S. A., Chritz, K. L., Rothman, J. M. & Cerling, T. E. Detecting intraannual dietary variability in wild mountain gorillas by stable isotope analysis of feces. *Proc. Natl Acad. Sci. USA* **109**, 21277–21282 (2012).
58. Blumenthal, S. A., Rothman, J. M., Chritz, K. L. & Cerling, T. E. Stable isotopic variation in tropical forest plants for applications in primatology. *Am. J. Primatol.* **78**, 92 (2016).
59. Boeckx, P., Paulino, L., Oyarzun, C., van Cleemput, O. & Godoy, R. Soil δ¹⁵N patterns in old-growth forests of southern Chile as integrator for N-cycling. *Isotopes Environ. Health Stud.* **41**, 249–259 (2005).
60. Brearley, F. Q. Nitrogen stable isotopes indicate differences in nitrogen cycling between two contrasting Jamaican montane forests. *Plant Soil* **367**, 465–476 (2013).
61. Brearley, F. Q., Fine, P. V. A. & Perreijn, K. Does nitrogen availability have greater control over the formation of tropical heath forests than water stress? A hypothesis based on nitrogen isotope ratios. *Acta Amazon.* **41**, 589–592 (2011).
62. Burton, J. I., Perakis, S. S., McKenzie, S. C., Lawrence, C. E. & Puettmann, K. J. Intraspecific variability and reaction norms of forest understory plant species traits. *Funct. Ecol.* **31**, 1881–1893 (2017).
63. Cardon, Z. G., Stark, J. M., Herron, P. M. & Rasmussen, J. A. Sagebrush carrying out hydraulic lift enhances surface soil nitrogen cycling and nitrogen uptake into inflorescences. *Proc. Natl Acad. Sci. USA* **110**, 18988–18993 (2013).
64. Carr, A. S., Chase, B. M., Boom, A. & Medina-Sanchez, J. Stable isotope analyses of rock hyrax faecal pellets, hyraceum and associated vegetation in southern Africa: implications for dietary ecology and palaeoenvironmental reconstructions. *J. Arid Environ.* **134**, 33–48 (2016).
65. Chen, C., Li, J., Wang, G. & Shi, M. Accounting for the effect of temperature in clarifying the response of foliar nitrogen isotope ratios to atmospheric nitrogen deposition. *Sci. Total Environ.* **609**, 1295–1302 (2017).

66. Compton, J. E., Hooker, T. D. & Perakis, S. S. Ecosystem N distribution and $\delta^{15}\text{N}$ during a century of forest regrowth after agricultural abandonment. *Ecosystems* **10**, 1197–1208 (2007).
67. Correa, S. B., Winemiller, K. & Cardenas, D. Isotopic variation among Amazonian floodplain woody plants and implications for food-web research. *Biota Neotrop.* **16**, e20150078 (2016).
68. Courty, P.-E. et al. Carbon and nitrogen metabolism in mycorrhizal networks and mycoheterotrophic plants of tropical forests: a stable isotope analysis. *Plant Physiol.* **156**, 952–961 (2011).
69. Couto-Vazquez, A. & Gonzalez-Prieto, S. J. Effects of biotic and abiotic factors on $\delta^{15}\text{N}$ in young *Pinus radiata*. *Eur. J. For. Res.* **133**, 631–637 (2014).
70. Couto-Vázquez, A. & González-Prieto, S. J. Effects of climate, tree age, dominance and growth on $\delta^{15}\text{N}$ in young pinewoods. *Trees* **24**, 507–514 (2010).
71. Craine, J. M. et al. Grazing and landscape controls on nitrogen availability across 330 South African savanna sites. *Austral Ecol.* **34**, 731–740 (2009).
72. Craine, J. M., Towne, E. G., Ocheltree, T. W. & Nippert, J. B. Community traitscape of foliar nitrogen isotopes reveals N availability patterns in a tallgrass prairie. *Plant Soil* **356**, 395–403 (2012).
73. Crowley, B. E., McGoogan, K. C. & Lehman, S. M. Edge effects on foliar stable isotope values in a Madagascan tropical dry forest. *PLoS ONE* **7**, e44538 (2012).
74. Crowley, B. E., Rasoazanabary, E. & Godfrey, L. R. Stable isotopes complement focal individual observations and confirm dietary variability in reddish-gray mouse lemurs (*Microcebus griseorufus*) from southwestern Madagascar. *Am. J. Phys. Anthropol.* **155**, 77–90 (2014).
75. Crowley, B. E. et al. Explaining geographical variation in the isotope composition of mouse lemurs (*Microcebus*). *J. Biogeogr.* **38**, 2106–2121 (2011).
76. Dahlin, K. M., Asner, G. P. & Field, C. B. Environmental and community controls on plant canopy chemistry in a Mediterranean-type ecosystem. *Proc. Natl Acad. Sci. USA* **110**, 6895–6900 (2013).
77. Dawes, M. A., Schleppei, P., Hattenschwiler, S., Rixen, C. & Hagedorn, F. Soil warming opens the nitrogen cycle at the alpine treeline. *Glob. Change Biol.* **23**, 421–434 (2017).
78. Diaz, F. P., Frugone, M., Gutierrez, R. A. & Latorre, C. Nitrogen cycling in an extreme hyperarid environment inferred from $\delta^{15}\text{N}$ analyses of plants, soils and herbivore diet. *Sci. Rep.* **6**, 22226 (2016).
79. Dominguez, T. F., Martinelli, L. A. & Ehleringer, J. R. Ecophysiological traits of plant functional groups in forest and pasture ecosystems from eastern Amazonia, Brazil. *Plant Ecol.* **193**, 101–112 (2007).
80. Dominguez, M. T. et al. Relationships between leaf morphological traits, nutrient concentrations and isotopic signatures for Mediterranean woody plant species and communities. *Plant Soil* **357**, 407–424 (2012).
81. Elmore, A. J., Craine, J. M., Nelson, D. M. & Guinn, S. M. Continental scale variability of foliar nitrogen and carbon isotopes in *Populus balsamifera* and their relationships with climate. *Sci. Rep.* **7**, 7759 (2017).
82. Ens, E., Hutley, L. B., Rossiter-Rachor, N. A., Douglas, M. M. & Setterfield, S. A. Resource-use efficiency explains grassy weed invasion in a low-resource savanna in north Australia. *Front. Plant Sci.* **6**, 560 (2015).
83. Evaristo, J. & McDonnell, J. J. Carbon, nitrogen, and water stable isotopes in plant tissue and soils across a moisture gradient in Puerto Rico. *Hydrol. Process.* **31**, 1558–1559 (2017).
84. Fajardo, A. & Gundale, M. J. Combined effects of anthropogenic fires and land-use change on soil properties and processes in Patagonia, Chile. *For. Ecol. Manage.* **357**, 60–67 (2015).
85. Falxa-Raymond, N., Palmer, M. I., McPhearson, T. & Griffin, K. L. Foliar nitrogen characteristics of four tree species planted in New York City forest restoration sites. *Urban Ecosyst.* **17**, 807–824 (2014).
86. Falxa-Raymond, N., Patterson, A. E., Schuster, W. S. F. & Griffin, K. L. Oak loss increases foliar nitrogen, $\delta^{15}\text{N}$ and growth rates of *Betula lenta* in a northern temperate deciduous forest. *Tree Physiol.* **32**, 1092–1101 (2012).
87. Fang, Y. et al. Nitrogen deposition and forest nitrogen cycling along an urban-rural transect in southern China. *Glob. Change Biol.* **17**, 872–885 (2011).
88. Feng, Z., Brumme, R., Xu, Y. J. & Lamersdorf, N. Tracing the fate of mineral N compounds under high ambient N deposition in a Norway spruce forest at Solling/Germany. *For. Ecol. Manage.* **255**, 2061–2073 (2008).
89. Finger, R. A. et al. Effects of permafrost thaw on nitrogen availability and plant-soil interactions in a boreal Alaskan lowland. *J. Ecol.* **104**, 1542–1554 (2016).
90. Frenette-Dussault, C., Shipley, B., Léger, J. F., Meziane, D. & Hingrat, Y. Functional structure of an arid steppe plant community reveals similarities with Grime's C-S-R theory. *J. Veg. Sci.* **23**, 208–222 (2012).
91. Fujiyoshi, L. et al. Spatial variations in larch needle and soil ^{15}N at a forest-grassland boundary in northern Mongolia. *Isotopes Environ. Health Stud.* **53**, 54–69 (2017).
92. Gao, J., Zhao, P., Shen, W., Rao, X. & Hu, Y. Physiological homeostasis and morphological plasticity of two tree species subjected to precipitation seasonal distribution changes. *Perspect. Plant Ecol. Evol. Syst.* **25**, 1–19 (2017).
93. Gao, J. et al. Suppression of nighttime sap flux with lower stem photosynthesis in Eucalyptus trees. *Int. J. Biometeorol.* **60**, 545–556 (2016).
94. Godfrey, L. R. et al. What did Hadropithecus eat, and why should paleoanthropologists care? *Am. J. Primatol.* **78**, 1098–1112 (2016).
95. Golluscio, R., Faigon, A. & Tanke, M. Spatial distribution of roots and nodules, and $\delta^{15}\text{N}$ evidence of nitrogen fixation in *Adesmia volckmanni*, a Patagonian leguminous shrub. *J. Arid Environ.* **67**, 328–335 (2006).
96. Gos, P. et al. Relative contribution of soil, management and traits to co-variations of multiple ecosystem properties in grasslands. *Oecologia* **180**, 1001–1013 (2016).
97. Gray, A. et al. Does geographic origin dictate ecological strategies in *Acacia senegal* (L.) Willd.? Evidence from carbon and nitrogen stable isotopes. *Plant Soil* **369**, 479–496 (2013).
98. Große-Stoltenberg, A., Hellmann, C., Thiele, J., Oldeland, J. & Werner, C. Invasive acacias differ from native dune species in the hyperspectral/biochemical trait space. *J. Veg. Sci.* **29**, 325–335 (2018).
99. Guerrieri, R., Lepine, L., Asbjornsen, H., Xiao, J. & Ollinger, S. V. Evapotranspiration and water use efficiency in relation to climate and canopy nitrogen in US forests. *J. Geophys. Res. Biogeosci.* **121**, 2610–2629 (2016).
100. Gundale, M. J., Deluca, T. H. & Nordin, A. Bryophytes attenuate anthropogenic nitrogen inputs in boreal forests. *Glob. Change Biol.* **17**, 2743–2753 (2011).
101. Gundale, M. J., From, F., Bach, L. H. & Nordin, A. Anthropogenic nitrogen deposition in boreal forests has a minor impact on the global carbon cycle. *Glob. Change Biol.* **20**, 276–286 (2014).
102. Gundale, M. J., Hyodo, F., Nilsson, M.-C. & Wardle, D. A. Nitrogen niches revealed through species and functional group removal in a boreal shrub community. *Ecology* **93**, 1695–1706 (2012).
103. Gurmesa, G. A. et al. Nitrogen input ^{15}N signatures are reflected in plant ^{15}N natural abundances in subtropical forests in China. *Biogeosciences* **14**, 2359–2370 (2017).
104. Haberer, K. et al. Effects of long-term free-air ozone fumigation on $\delta^{15}\text{N}$ and total N in *Fagus sylvatica* and associated mycorrhizal fungi. *Plant Biol.* **9**, 242–252 (2007).
105. Hall, S. J., Hale, R. L., Baker, M. A., Bowling, D. R. & Ehleringer, J. R. Riparian plant isotopes reflect anthropogenic nitrogen perturbations: robust patterns across land use gradients. *Ecosphere* **6**, 1137–1146 (2015).
106. Hamerlynck, E. P. & McAuliffe, J. R. Growth and foliar $\delta^{15}\text{N}$ of a Mojave desert shrub in relation to soil hydrological dynamics. *J. Arid Environ.* **74**, 1569–1571 (2010).
107. Havik, G., Catenazzi, A. & Holmgren, M. Seabird nutrient subsidies benefit non-nitrogen fixing trees and alter species composition in South American coastal dry forests. *PLoS ONE* **9**, e86381 (2014).
108. Hawke, D. et al. Foliar and soil N and $\delta^{15}\text{N}$ as restoration metrics at Pūtaringamotu Riccarton Bush, Christchurch city. *J. R. Soc. N. Z.* **47**, 319–335 (2017).
109. Hellmann, C., Grosse-Stoltenberg, A., Laustroer, V., Oldeland, J. & Werner, C. Retrieving nitrogen isotopic signatures from fresh leaf reflectance spectra: disentangling $\delta^{15}\text{N}$ from biochemical and structural leaf properties. *Front. Plant Sci.* **6**, 307 (2015).
110. Hellmann, C., Grosse-Stoltenberg, A., Thiele, J., Oldeland, J. & Werner, C. Heterogeneous environments shape invader impacts: integrating environmental, structural and functional effects by isoscapes and remote sensing. *Sci. Rep.* **7**, 4118 (2017).
111. Hellmann, C., Rascher, K. G., Oldeland, J. & Werner, C. Isoscapes resolve species-specific spatial patterns in plant-plant interactions in an invaded Mediterranean dune ecosystem. *Tree Physiol.* **36**, 1460–1470 (2016).
112. Hellmann, C. et al. Impact of an exotic N_2 -fixing *Acacia* on composition and N status of a native Mediterranean community. *Acta Oecol.* **37**, 43–50 (2011).
113. Hellmann, C., Werner, C. & Oldeland, J. A spatially explicit dual-isotope approach to map regions of plant-plant interaction after exotic plant invasion. *PLoS ONE* **11**, e0159403 (2016).
114. Hobbie, E. A., Rice, S. F., Weber, N. S. & Smith, J. E. Isotopic evidence indicates saprotrophy in post-fire *Morchella* in Oregon and Alaska. *Mycologia* **108**, 638–645 (2016).
115. Hobbie, J. E. et al. Mycorrhizal fungi supply nitrogen to host plants in Arctic tundra and boreal forests: ^{15}N is the key signal. *Can. J. Microbiol.* **55**, 84–94 (2009).
116. Hofmeister, J., Hosek, J., Buzek, F. & Rolecek, J. Foliar N concentration and $\delta^{15}\text{N}$ signature reflect the herb layer species diversity and composition in oak-dominated forests. *Appl. Veg. Sci.* **15**, 318–328 (2012).

117. Hofmocker, K. S. et al. Sources of increased N uptake in forest trees growing under elevated CO₂: results of a large-scale ¹⁵N study. *Glob. Change Biol.* **17**, 3338–3350 (2011).
118. Hogberg, P. et al. Recovery of ectomycorrhiza after 'nitrogen saturation' of a conifer forest. *New Phytol.* **189**, 515–525 (2011).
119. Hogberg, P. & Alexander, I. J. Roles of root symbioses in African woodland and forest: evidence from ¹⁵N abundance and foliar analysis. *J. Ecol.* **83**, 217–224 (1995).
120. Hoogmoed, M., Cunningham, S. C., Baker, P., Beringer, J. & Cavagnaro, T. R. N-fixing trees in restoration plantings: effects on nitrogen supply and soil microbial communities. *Soil Biol. Biochem.* **77**, 203–212 (2014).
121. Houle, D., Moore, J. D., Ouimet, R. & Marty, C. Tree species partition N uptake by soil depth in boreal forests. *Ecology* **95**, 1127–1133 (2014).
122. Hudson, J. M. G., Henry, G. H. R. & Cornwell, W. K. Taller and larger: shifts in Arctic tundra leaf traits after 16 years of experimental warming. *Glob. Change Biol.* **17**, 1013–1021 (2011).
123. Hyodo, F., Kusaka, S., Wardle, D. A. & Nilsson, M.-C. Changes in stable nitrogen and carbon isotope ratios of plants and soil across a boreal forest fire chronosequence. *Plant Soil* **364**, 315–323 (2013).
124. Ingram, L. J. & Adams, M. A. Does season and grazing influence the ^δ¹³C and ^δ¹⁵N of C4 native grasses in semi-arid rangelands of the Pilbara region of NW Australia? *Agric. Ecosyst. Environ.* **236**, 277–284 (2017).
125. Jiang, C. & Zhang, X. N isotopes and N cycle in the TieShanPing subtropical forest ecosystem, southwestern China. *Environ. Monit. Assess.* **154**, 301–308 (2009).
126. Kahmen, A., Wanek, W. & Buchmann, N. Foliar ^δ¹⁵N values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient. *Oecologia* **156**, 861–870 (2008).
127. Kang, H. et al. Variation in foliar ^δ¹⁵N among oriental oak (*Quercus variabilis*) stands over eastern China: patterns and interactions. *J. Geochem. Explor.* **110**, 8–14 (2011).
128. Kearsley, E. et al. Functional community structure of African monodominant *Gilbertiodendron dewevrei* forest influenced by local environmental filtering. *Ecol. Evol.* **7**, 295–304 (2017).
129. Kenzo, T., Tanaka-Oda, A., Mastuura, Y. & Hinzman, L. D. Morphological and physicochemical traits of leaves of different life-forms of various broadleaf woody plants in interior Alaska. *Can. J. For. Res.* **46**, 1475–1482 (2016).
130. Kleinebecker, T. et al. Evidence from the real world: ¹⁵N natural abundances reveal enhanced nitrogen use at high plant diversity in Central European grasslands. *J. Ecol.* **102**, 456–465 (2014).
131. Klopatek, J. M., Barry, M. J. & Johnson, D. W. Potential canopy interception of nitrogen in the Pacific Northwest, USA. *For. Ecol. Manage.* **234**, 344–354 (2006).
132. Koba, K. et al. ^δ¹⁵N of soil N and plants in a N-saturated, subtropical forest of southern China. *Rapid Commun. Mass Spectrom.* **24**, 2499–2506 (2010).
133. Koch, P. L. & Fox, L. R. Browsing impacts on the stable isotope composition of chaparral plants. *Ecosphere* **8**, e01686 (2017).
134. Korner, C., Leuzinger, S., Riedl, S., Siegwolf, R. T. & Streule, L. Carbon and nitrogen stable isotope signals for an entire alpine flora, based on herbarium samples. *Alp. Bot.* **126**, 153–166 (2016).
135. Kranabetter, J. & Meeds, J. Tree ring ^δ¹⁵N as validation of space-for-time substitution in disturbance studies of forest nitrogen status. *Biogeochemistry* **134**, 201–215 (2017).
136. Kranabetter, J. M., Dube, S. & Lilles, E. An investigation into the contrasting growth response of lodgepole pine and white spruce to harvest-related soil disturbance. *Can. J. For. Res.* **47**, 340–348 (2016).
137. Kranabetter, J. M. & MacKenzie, W. H. Contrasts among mycorrhizal plant guilds in foliar nitrogen concentration and ^δ¹⁵N along productivity gradients of a boreal forest. *Ecosystems* **13**, 108–117 (2010).
138. Kuang, Y. et al. Nitrogen deposition influences nitrogen isotope composition in soil and needles of *Pinus massoniana* forests along an urban-rural gradient in the Pearl River Delta of south China. *J. Soils Sediments* **11**, 589–595 (2011).
139. Ladd, B., Pepper, D. A. & Bonser, S. P. Competition intensity at local versus regional spatial scales. *Plant Biol.* **12**, 772–779 (2010).
140. Laiolo, P., Carlos Illera, J., Melendez, L., Segura, A. & Ramon Obeso, J. Abiotic, biotic, and evolutionary control of the distribution of C and N isotopes in food webs. *Am. Nat.* **185**, 169–182 (2015).
141. Laughlin, D. C., Fule, P. Z., Huffman, D. W., Crouse, J. & Laliberte, E. Climatic constraints on trait-based forest assembly. *J. Ecol.* **99**, 1489–1499 (2011).
142. LeDuc, S. D., Rothstein, D. E., Yermakov, Z. & Spaulding, S. E. Jack pine foliar ^δ¹⁵N indicates shifts in plant nitrogen acquisition after severe wildfire and through forest stand development. *Plant Soil* **373**, 955–965 (2013).
143. Li, Y., Xue, J., Clinton, P. W. & Dungey, H. S. Genetic parameters and clone by environment interactions for growth and foliar nutrient concentrations in radiata pine on 14 widely diverse New Zealand sites. *Tree Genet. Genomes* **11**, 10 (2015).
144. Liu, X., Wang, G., Li, J. & Wang, Q. Nitrogen isotope composition characteristics of modern plants and their variations along an altitudinal gradient in Dongling Mountain in Beijing. *Sci. China Ser. D Earth Sci.* **53**, 128–140 (2010).
145. Liu, X. et al. Foliar ^δ¹³C and ^δ¹⁵N values of C₃ plants in the Ethiopia Rift Valley and their environmental controls. *Chin. Sci. Bull.* **52**, 1265–1273 (2007).
146. Ma, L. et al. Ecophysiological and foliar nitrogen concentration responses of understory *Acacia* spp. and *Eucalyptus* sp. to prescribed burning. *Environ. Sci. Pollut. R.* **22**, 10254–10262 (2015).
147. Makarov, M. I. et al. Determinants of ¹⁵N natural abundance in leaves of co-occurring plant species and types within an alpine lichen heath in the Northern Caucasus. *Arct. Antarct. Alp. Res.* **46**, 581–590 (2014).
148. Maranon-Jimenez, S., Castro, J., Ignacio Querejeta, J., Fernandez-Ondono, E. & Allen, C. D. Post-fire wood management alters water stress, growth, and performance of pine regeneration in a Mediterranean ecosystem. *For. Ecol. Manage.* **308**, 231–239 (2013).
149. Matsushima, M., Choi, W.-J. & Chang, S. X. White spruce foliar ^δ¹³C and ^δ¹⁵N indicate changed soil N availability by understory removal and N fertilization in a 13-year-old boreal plantation. *Plant Soil* **361**, 375–384 (2012).
150. Mayor, J. R., Schuur, E. A. G., Mack, M. C., Hollingsworth, T. N. & Baath, E. Nitrogen isotope patterns in Alaskan black spruce reflect organic nitrogen sources and the activity of ectomycorrhizal fungi. *Ecosystems* **15**, 819–831 (2012).
151. Mayor, J. R., Wright, S. J., Schuur, E. A. G., Brooks, M. E. & Turner, B. L. Stable nitrogen isotope patterns of trees and soils altered by long-term nitrogen and phosphorus addition to a lowland tropical rainforest. *Biogeochemistry* **119**, 293–306 (2014).
152. McGlynn, T. P. et al. Spurious and functional correlates of the isotopic composition of a generalist across a tropical rainforest landscape. *BMC Ecol.* **9**, 23–23 (2009).
153. McLaughlan, K. K., Craine, J. M., Nippert, J. B. & Ocheltree, T. W. Lack of eutrophication in a tallgrass prairie ecosystem over 27 years. *Ecology* **95**, 1225–1235 (2014).
154. Medina, E., Cuevas, E. & Lugo, A. E. Substrate chemistry and rainfall regime regulate elemental composition of tree leaves in karst forests. *Forests* **8**, 182 (2017).
155. Menge, D. N. L., Baisden, W. T., Richardson, S. J., Peltzer, D. A. & Barbour, M. M. Declining foliar and litter ^δ¹⁵N diverge from soil, epiphyte and input ^δ¹⁵N along a 120,000 yr temperate rainforest chronosequence. *New Phytol.* **190**, 941–952 (2011).
156. Menyailo, O. V., Makarov, M. I. & Cheng, C. H. Isotopic composition of carbon (^δ¹³C) and nitrogen (^δ¹⁵N) in foliage and soil as a function of tree species. *Dokl. Biol. Sci.* **456**, 209–211 (2014).
157. Mercado, A. R. Jr, Van Noordwijk, M. & Cadisch, G. Positive nitrogen balance of *Acacia mangium* woodlots as fallows in the Philippines based on ¹⁵N natural abundance data of N₂ fixation. *Agroforest. Syst.* **81**, 221–233 (2011).
158. Meyer, W. M. III & Yeung, N. W. Trophic relationships among terrestrial molluscs in a Hawaiian rain forest: analysis of carbon and nitrogen isotopes. *J. Trop. Ecol.* **27**, 441–445 (2011).
159. Morford, S. L., Houlton, B. Z. & Dahlgren, R. A. Increased forest ecosystem carbon and nitrogen storage from nitrogen rich bedrock. *Nature* **477**, 78–81 (2011).
160. Msanne, J. et al. Ecophysiological responses of native invasive woody *Juniperus virginiana* L. to resource availability and stand characteristics in the semiarid grasslands of the Nebraska Sandhills. *Photosynthetica* **55**, 219–230 (2017).
161. Murphy, B. P. & Bowman, D. M. J. S. The carbon and nitrogen isotope composition of Australian grasses in relation to climate. *Funct. Ecol.* **23**, 1040–1049 (2009).
162. Nielsen, J. A., Frew, R. D., Whigham, P. A., Callaway, R. M. & Dickinson, K. J. M. Thyme travels: ¹⁵N isoscapes of *Thymus vulgaris* L. invasion in lightly grazed pastoral communities. *Austral Ecol.* **41**, 28–39 (2016).
163. Ogaya, R. & Penuelas, J. Changes in leaf ^δ¹³C and ^δ¹⁵N for three Mediterranean tree species in relation to soil water availability. *Acta Oecol.* **34**, 331–338 (2008).
164. Pasquini, S. C. & Santiago, L. S. Nutrients limit photosynthesis in seedlings of a lowland tropical forest tree species. *Oecologia* **168**, 311–319 (2012).
165. Pellegrini, A. F. A., Hoffmann, W. A. & Franco, A. C. Carbon accumulation and nitrogen pool recovery during transitions from savanna to forest in central Brazil. *Ecology* **95**, 342–352 (2014).
166. Perakis, S. S. & Kellogg, C. H. Imprint of oaks on nitrogen availability and ^δ¹⁵N in California grassland-savanna: a case of enhanced N inputs? *Plant Ecol.* **191**, 209–220 (2007).
167. Perakis, S. S., Tepley, A. J. & Compton, J. E. Disturbance and topography shape nitrogen availability and ^δ¹⁵N over long-term forest succession. *Ecosystems* **18**, 573–588 (2015).

168. Perakis, S. S., Sinkhorn, E. R. & Compton, J. E. $\delta^{15}\text{N}$ constraints on long-term nitrogen balances in temperate forests. *Oecologia* **167**, 793–807 (2011).
169. Peri, P. L. et al. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope composition in plant and soil in Southern Patagonia's native forests. *Glob. Change Biol.* **18**, 311–321 (2012).
170. Pillar, V. D. & Sosinski, E. E. Jr An improved method for searching plant functional types by numerical analysis. *J. Veg. Sci.* **14**, 323–332 (2003).
171. Pons, T. L., Perreijn, K., van Kessel, C. & Werger, M. J. A. Symbiotic nitrogen fixation in a tropical rainforest: ^{15}N natural abundance measurements supported by experimental isotopic enrichment. *New Phytol.* **173**, 154–167 (2007).
172. Powers, J. S. & Tiffin, P. Plant functional type classifications in tropical dry forests in Costa Rica: leaf habit versus taxonomic approaches. *Funct. Ecol.* **24**, 927–936 (2010).
173. Priyadarshini, K. V. R. et al. Overlap in nitrogen sources and redistribution of nitrogen between trees and grasses in a semi-arid savanna. *Oecologia* **174**, 1107–1116 (2014).
174. Ren, H. et al. Exacerbated nitrogen limitation ends transient stimulation of grassland productivity by increased precipitation. *Ecol. Monogr.* **87**, 457–469 (2017).
175. Roa-Fuentes, L. L., Templer, P. H. & Campo, J. Effects of precipitation regime and soil nitrogen on leaf traits in seasonally dry tropical forests of the Yucatan Peninsula, Mexico. *Oecologia* **179**, 585–597 (2015).
176. Roberts, P., Blumenthal, S. A., Dittus, W., Wedage, O. & Lee-Thorp, J. A. Stable carbon, oxygen, and nitrogen, isotope analysis of plants from a South Asian tropical forest: implications for primatology. *Am. J. Primatol.* **79**, e22656 (2017).
177. Roggy, J. C. et al. Complementary N uptake strategies between tree species in tropical rainforest. *Int. Sch. Res. Notices* **2014**, 427194 (2014).
178. Roggy, J. C., Prevost, M. F., Garbaye, J. & Domenach, A. M. Nitrogen cycling in the tropical rain forest of French Guiana: comparison of two sites with contrasting soil types using $\delta^{15}\text{N}$. *J. Trop. Ecol.* **15**, 1–22 (1999).
179. Rosado, B. H. P. & de Mattos, E. A. Interspecific variation of functional traits in a CAM-tree dominated sandy coastal plain. *J. Veg. Sci.* **21**, 43–54 (2010).
180. Ruiz-Navarro, A., Barbera, G. G., Albaladejo, J. & Querejeta, J. I. Plant $\delta^{15}\text{N}$ reflects the high landscape-scale heterogeneity of soil fertility and vegetation productivity in a Mediterranean semiarid ecosystems. *New Phytol.* **212**, 1030–1043 (2016).
181. Salmon, V. G. et al. Nitrogen availability increases in a tundra ecosystem during five years of experimental permafrost thaw. *Glob. Change Biol.* **22**, 1927–1941 (2016).
182. Santiago, L., Silvera, K., Andrade, J. & Dawson, T. Functional strategies of tropical dry forest plants in relation to growth form and isotopic composition. *Environ. Res. Lett.* **12**, 115006 (2017).
183. Schimann, H. et al. Differing nitrogen use strategies of two tropical rainforest late successional tree species in French Guiana: evidence from ^{15}N natural abundance and microbial activities. *Soil Biol. Biochem.* **40**, 487–494 (2008).
184. Scott, E. E., Perakis, S. S. & Hibbs, D. E. $\delta^{15}\text{N}$ patterns of Douglas-fir and red alder riparian forests in the Oregon coast range. *Forest Sci.* **54**, 140–147 (2008).
185. Selmants, P. C. & Hart, S. C. Substrate age and tree islands influence carbon and nitrogen dynamics across a retrogressive semiarid chronosequence. *Glob. Biogeochem. Cycles* **22**, GB1021 (2008).
186. Serbin, S. P., Singh, A., McNeil, B. E., Kingdon, C. C. & Townsend, P. A. Spectroscopic determination of leaf morphological and biochemical traits for northern temperate and boreal tree species. *Ecol. Appl.* **24**, 1651–1669 (2014).
187. Shen, J. et al. Relationships of leaf nitrogen concentration and $\delta^{15}\text{N}$ value in *Humulus scandens* with atmospheric NH_3 and NO_2 . *J. China Agric. Univ.* **15**, 84–88 (2010).
188. Silva, L. C. R., Gomez-Guerrero, A., Doane, T. A. & Horwath, W. R. Isotopic and nutritional evidence for species- and site-specific responses to N deposition and elevated CO_2 in temperate forests. *J. Geophys. Res. Biogeosci.* **120**, 1110–1123 (2015).
189. Smith, K. R., Mathias, J. M., McNeil, B. E., Peterjohn, W. T. & Thomas, R. B. Site-level importance of broadleaf deciduous trees outweighs the legacy of high nitrogen (N) deposition on ecosystem N status of Central Appalachian red spruce forests. *Plant Soil* **408**, 343–356 (2016).
190. Song, M., Djagbletye, G., Nkrumah, E. E. & Huang, M. Patterns in leaf traits of leguminous and non-leguminous dominant trees along a rainfall gradient in Ghana. *J. Plant Ecol.* **9**, 69–76 (2016).
191. Soper, F. M., Boutton, T. W. & Sparks, J. P. Investigating patterns of symbiotic nitrogen fixation during vegetation change from grassland to woodland using fine scale $\delta^{15}\text{N}$ measurements. *Plant Cell Environ.* **38**, 89–100 (2015).
192. Soper, F. M. et al. Natural abundance ($\delta^{15}\text{N}$) indicates shifts in nitrogen relations of woody taxa along a savanna-woodland continental rainfall gradient. *Oecologia* **178**, 297–308 (2015).
193. Stephan, K., Kavanagh, K. L. & Koyama, A. Comparing the influence of wildfire and prescribed burns on watershed nitrogen biogeochemistry using ^{15}N natural abundance in terrestrial and aquatic ecosystem components. *PLoS ONE* **10**, e0119560 (2015).
194. Szpak, P., White, C. D., Longstaffe, F. J., Millaire, J.-F. & Vasquez Sanchez, V. F. Carbon and nitrogen isotopic survey of northern Peruvian plants: baselines for paleodietary and paleoecological studies. *PLoS ONE* **8**, e53763 (2013).
195. Tanaka-Oda, A. et al. Variation in leaf and soil $\delta^{15}\text{N}$ in diverse tree species in a lowland dipterocarp rainforest, Malaysia. *Trees* **30**, 509–522 (2016).
196. Tanaka-Oda, A., Kenzo, T., Toriyama, J. & Matsuura, Y. Variability in the growth rates and foliage $\delta^{15}\text{N}$ values of black spruce trees across a slope gradient in the Alaskan Interior. *Can. J. For. Res.* **46**, 1483–1490 (2016).
197. Tang, B., Yin, C., Yang, H., Sun, Y. & Liu, Q. The coupling effects of water deficit and nitrogen supply on photosynthesis, WUE, and stable isotope composition in *Picea asperata*. *Acta Physiol. Plant.* **39**, 148 (2017).
198. Templer, P. H. et al. Fog as a source of nitrogen for redwood trees: evidence from fluxes and stable isotopes. *J. Ecol.* **103**, 1397–1407 (2015).
199. Thorpe, A. S., Perakis, S., Catricala, C. & Kaye, T. N. Nutrient limitation of native and invasive N_2 -fixing plants in northwest prairies. *PLoS ONE* **8**, e84593 (2013).
200. Van der Colff, D., Dreyer, L. L., Valentine, A. & Roets, F. Comparison of nutrient cycling abilities between the invasive *Acacia mearnsii* and the native *Virgilia divaricata* trees growing sympatrically in forest margins in South Africa. *S. Afr. J. Bot.* **111**, 358–364 (2017).
201. Viani, R. A. G., Rodrigues, R. R., Dawson, T. E. & Oliveira, R. S. Functional differences between woodland savannas and seasonally dry forests from south-eastern Brazil: evidence from ^{15}N natural abundance studies. *Austral Ecol.* **36**, 974–982 (2011).
202. Wang, A. et al. Variations in nitrogen-15 natural abundance of plant and soil systems in four remote tropical rainforests, southern China. *Oecologia* **174**, 567–580 (2014).
203. Wang, C. et al. Aridity threshold in controlling ecosystem nitrogen cycling in arid and semi-arid grasslands. *Nat. Commun.* **5**, 4799 (2014).
204. Wang, L. & Macko, S. A. Constrained preferences in nitrogen uptake across plant species and environments. *Plant Cell Environ.* **34**, 525–534 (2011).
205. Wang, L., D'Odorico, P., O'Halloran, L. R., Caylor, K. & Macko, S. Combined effects of soil moisture and nitrogen availability variations on grass productivity in African savannas. *Plant Soil* **328**, 95–108 (2010).
206. Wang, L., D'Odorico, P., Ries, L. & Macko, S. A. Patterns and implications of plant-soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in African savanna ecosystems. *Quat. Res.* **73**, 77–83 (2010).
207. Wang, L., Okin, G. S., D'Odorico, P., Caylor, K. K. & Macko, S. A. Ecosystem-scale spatial heterogeneity of stable isotopes of soil nitrogen in African savannas. *Landsc. Ecol.* **28**, 685–698 (2013).
208. Wang, L., Okin, G. S., Wang, J., Epstein, H. & Macko, S. A. Predicting leaf and canopy ^{15}N compositions from reflectance spectra. *Geophys. Res. Lett.* **34**, L02401 (2007).
209. Wang, L., Shaner, P.-J. L. & Macko, S. Foliar $\delta^{15}\text{N}$ patterns along successional gradients at plant community and species levels. *Geophys. Res. Lett.* **34**, L16403 (2007).
210. Watkins, J. E. Jr, Rundel, P. W. & Cardelus, C. L. The influence of life form on carbon and nitrogen relationships in tropical rainforest ferns. *Oecologia* **153**, 225–232 (2007).
211. Werner, C. & Máguas, C. Carbon isotope discrimination as a tracer of functional traits in a Mediterranean macchia plant community. *Funct. Plant Biol.* **37**, 467–477 (2010).
212. Williams, M., Shimabokuro, Y. E. & Rastetter, E. B. *LBA-ECO CD-09 Soil and Vegetation Characteristics, Tapajos National Forest, Brazil* (Oak Ridge National Laboratory Distributed Active Archive Center, 2012).
213. Woodcock, P. et al. Assessing trophic position from nitrogen isotope ratios: effective calibration against spatially varying baselines. *Naturwissenschaften* **99**, 275–283 (2012).
214. Wu, T. & Huang, J. Effects of grazing on the $\delta^{15}\text{N}$ values of foliage and soil in a typical steppe ecosystem in Inner Mongolia, China. *J. Plant Ecol. (Chinese Version)* **34**, 160–169 (2010).
215. Xiao, L., Yang, H., Sun, B., Li, X. & Guo, J. Stable isotope compositions of recent and fossil sun/shade leaves and implications for palaeoenvironmental reconstruction. *Rev. Palaeobot. Palynol.* **190**, 75–84 (2013).
216. Yang, Y. et al. Vegetation and soil ^{15}N natural abundance in alpine grasslands on the Tibetan Plateau: patterns and implications. *Ecosystems* **16**, 1013–1024 (2013).
217. Yang, Y., Siegwolf, R. T. W. & Koerner, C. Species specific and environment induced variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in alpine plants. *Front. Plant Sci.* **6**, 423 (2015).

218. Yao, F. Y., Wang, G. A., Liu, X. J. & Song, L. Assessment of effects of the rising atmospheric nitrogen deposition on nitrogen uptake and long-term water-use efficiency of plants using nitrogen and carbon stable isotopes. *Rapid Commun. Mass Spectrom.* **25**, 1827–1836 (2011).
219. Ye, L. et al. Contrasting impacts of grass species on nitrogen cycling in a grazed Sudanian savanna. *Acta Oecol.* **63**, 8–15 (2015).
220. Zhang, H.-Y. et al. Impacts of leguminous shrub encroachment on neighboring grasses include transfer of fixed nitrogen. *Oecologia* **180**, 1213–1222 (2016).
221. Zhao, L. et al. The effects of short-term rainfall variability on leaf isotopic traits of desert plants in sand-binding ecosystems. *Ecol. Eng.* **60**, 116–125 (2013).
222. Zmudzynska-Skarbek, K., Barcikowski, M., Zwolicki, A., Iliszko, L. & Stempniewicz, L. Variability of polar scurvygrass *Cochlearia groenlandica* individual traits along a seabird influenced gradient across Spitsbergen tundra. *Polar Biol.* **36**, 1659–1669 (2013).
223. Sparks, J. A. & Crowley, B. E. Where did people forage in prehistoric Trinidad? Testing the utility of isotopic tools for tracking terrestrial resource use. *J. Archaeol. Sci. Rep.* **19**, 968–978 (2018).
224. Mosher, S. *Carbon Isotope Discrimination and Nitrogen Isotope Values Indicate that Increased Relative Humidity from Fog Decreases Plant Water Use Efficiency in a Subtropical Montane Cloud Forest*. MSc thesis, Univ. Cincinnati (2015).

Acknowledgements

Funding for this research was in part provided by the BRIDGE Project (ANR-2006 Biodiversity Call) and from an ‘Investissement d’Avenir’ grant managed by the Agence Nationale de la Recherche (CEBA, grant no. ANR-10-LABX-0025) (J.C.R.); by the

European Research Council through the Advanced Grant Project TREEPEACE (grant no. FP7-339728) and the Cluster of Excellence COTE (grant no. ANR-10-LABX-45) (S.D.); by NASA project no. NNX12AK56G and EU MSCA individual fellowship (project no. 705432) (R.G.); by COILEX (grant no. CGL2008-01671), ECOLPIN (grant no. AGL2011-24296) and EU MSCA individual fellowship (project no. 750252) (S.M.J.); and by the Russian Science Foundation (grant no. 16-14-10208) (M.M.). No funding was provided to J.M.C. or A.J.E. in support of this research.

Author contributions

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.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41559-018-0694-0>.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to J.M.C.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2018

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a | Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated
- Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection

Data analysis

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

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