

Global Biogeochemical Cycles[®]



RESEARCH ARTICLE

10.1029/2026GB009098

Key Points:

- More carbon (C) is invested in biological nitrogen fixation (BNF) at higher latitudes and in biomes that are less productive
- Patterns of C investment in BNF are driven largely by mosses, biocrusts, shrubs, and herbs, overriding patterns in trees
- Investment in symbiotic biological nitrogen fixation is 10× higher in agricultural versus natural biomes

Supporting Information:

Supporting Information may be found in the online version of this article.

Correspondence to:

D. N. L. Menge,
dm2972@columbia.edu

Citation:

Menge, D. N. L., Reis Ely, C. R., Perakis, S. S., Kou-Giesbrecht, S., Cleveland, C. C., Reed, S. C., et al. (2026). Global carbon investment in terrestrial biological nitrogen fixation. *Global Biogeochemical Cycles*, 40, e2026GB009098. <https://doi.org/10.1029/2026GB009098>

Received 27 JAN 2026

Accepted 13 JUL 2026

Author Contributions:

Conceptualization: Duncan N. L. Menge, Carla R. Reis Ely, Steven S. Perakis, Sian Kou-Giesbrecht, Cory C. Cleveland, Sasha C. Reed, Benton N. Taylor

Funding acquisition: Duncan N. L. Menge, Steven S. Perakis, Cory C. Cleveland, Sasha C. Reed, Jennifer L. Funk, Kevin L. Griffin

Investigation: Duncan N. L. Menge, Carla R. Reis Ely

Methodology: Duncan N. L. Menge, Carla R. Reis Ely, Steven S. Perakis

Visualization: Duncan N. L. Menge, Carla R. Reis Ely

© 2026 UT-Battelle LLC and The Author(s). This article has been contributed to by U.S. Government employees and their work is in the public domain in the USA.

This is an open access article under the terms of the [Creative Commons Attribution License](#), which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Global Carbon Investment in Terrestrial Biological Nitrogen Fixation

Duncan N. L. Menge¹ , Carla R. Reis Ely^{2,3}, Steven S. Perakis⁴ , Sian Kou-Giesbrecht⁵ , Cory C. Cleveland⁶, Sasha C. Reed⁷ , Benton N. Taylor⁸, Sarah A. Batterman^{9,10,11} , Timothy E. Crews¹², Katherine A. Dynarski⁶ , Jennifer L. Funk¹³, Maga Gei¹⁴ , Kevin L. Griffin^{1,15,16} , Michael J. Gundale¹⁷, David F. Herridge¹⁸, Sarah E. Jovan¹⁹, Mark B. Peoples²⁰, Johannes Piipponen²¹, Emilio Rodríguez-Caballero^{22,23} , Verity G. Salmon²⁴ , Fiona M. Soper²⁵, Anika P. Staccone²⁶, Bettina Weber^{23,27} , Amelia A. Wolf²⁸, and Nina Wurzburger²⁹

¹Department of Ecology, Evolution, and Environmental Biology, Columbia University, New York, NY, USA, ²Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR, USA, ³Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA, ⁴U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Corvallis, OR, USA, ⁵School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC, Canada, ⁶Department of Ecosystem and Conservation Sciences, W.A. Franke College of Forestry and Conservation, University of Montana, Missoula, MT, USA, ⁷U.S. Geological Survey, Southwest Biological Science Center, Moab, UT, USA, ⁸Department of Organismic and Evolutionary Biology, Harvard University, Roslindale, MA, USA, ⁹Cary Institute of Ecosystem Studies, Millbrook, NY, USA, ¹⁰School of Geography, University of Leeds, Leeds, UK, ¹¹Smithsonian Tropical Research Institute, Ancon, Panama, ¹²The Land Institute, Salina, KS, USA, ¹³Department of Plant Sciences, University of California, Davis, Davis, CA, USA, ¹⁴Association for Tropical Biology and Conservation, Minneapolis, MN, USA, ¹⁵Department of Earth and Environmental Science, Columbia University, New York, NY, USA, ¹⁶Division of Biology & Paleoenvironment, Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY, USA, ¹⁷Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, Umea, Sweden, ¹⁸School of Environmental and Rural Science, University of New England, Armidale, NSW, Australia, ¹⁹USDA Forest Service, PNW Research Station, Portland, OR, USA, ²⁰CSIRO Agriculture and Food, Canberra, ACT, Australia, ²¹Water and Development Research Group, Aalto University, Espoo, Finland, ²²Departamento de Agronomía, Universidad de Almería, Almería, Spain, ²³Multiphase Chemistry Department, Max Planck Institute for Chemistry, Mainz, Germany, ²⁴Environmental Science Division and Climate Change Science Institute, Oak Ridge National Laboratory, Oak Ridge, TN, USA, ²⁵Department of Biology and School of the Environment, McGill University, Montreal, QC, Canada, ²⁶Earthshot Labs, Mill Valley, CA, USA, ²⁷Division of Plant Sciences, Institute for Biology, University of Graz, Graz, Austria, ²⁸Department of Integrative Biology, University of Texas at Austin, Austin, TX, USA, ²⁹Odum School of Ecology, University of Georgia, Athens, GA, USA

Abstract Biological nitrogen (N) fixation (BNF) provides the N needed to produce proteins and other biological building blocks, helping feed humanity and mitigate climate change. Due to its high energetic cost compared to other forms of N acquisition, biotic investment in BNF indicates N limitation. Globally gridded BNF flux data provide an opportunity to determine the energetic investment in BNF across ecosystems, which would help reconcile conflicting indicators of N limitation. Here, we use a new BNF synthesis to quantify the relative importance of BNF in different N-fixing niches, in different biomes, and across the globe by calculating the fraction of net primary productivity (NPP) invested in BNF and the fraction of plant N acquisition provided by BNF. Larger fractions of non-agricultural NPP were invested in BNF in less-productive, higher-latitude biomes. This pattern was driven by biocrusts and mosses. Similarly, non-agricultural symbiotic N-fixing plants invested relatively more of their own NPP in BNF in less-productive, higher-latitude biomes. This symbiotic plant pattern was driven by shrubs and herbs, overriding the opposite pattern in trees. Symbiotic plants also acquired a higher fraction of their N from BNF at higher latitudes and in less productive biomes. Investments in symbiotic BNF were 10× higher in agricultural (2.9% of NPP) versus natural (0.29%) biomes, providing 26% versus 3.1% of ecosystem-scale plant N acquisition. These results support the paradigm of strong N limitation at higher latitudes, help understand the rarity of N-fixing trees at higher latitudes, underscore the dominance of human activity, and inform terrestrial biosphere models.

Plain Language Summary Similar to plants in a garden, plants and microbes in nature often do not have enough nitrogen. Some microbes and plant-microbe symbioses can access the nitrogen gas that makes up 78% of air. This “nitrogen fixation” provides fertilizer, but requires a lot of energy. This energy comes from

Writing – original draft: Duncan

N. L. Menge

Writing – review & editing: Duncan

N. L. Menge, Carla R. Reis Ely, Steven

S. Perakis, Sian Kou-Giesbrecht, Cory

C. Cleveland, Sasha C. Reed, Benton

N. Taylor, Sarah A. Batterman, Timothy

E. Crews, Katherine A. Dynarski, Jennifer

L. Funk, Maga Gei, Kevin L. Griffin,

Michael J. Gundale, David F. Herridge,

Sarah E. Jovan, Mark B. Peoples,

Johannes Piipponen, Emilio Rodríguez-

Caballero, Verity G. Salmon, Fiona

M. Soper, Anika P. Staccone,

Bettina Weber, Amelia A. Wolf,

Nina Wurzbürger

carbohydrates (a “carbon cost”), which ultimately come from photosynthesis. Because of this carbon cost, investment in nitrogen fixation indicates a need for nitrogen. We quantified the amount of carbon spent on nitrogen fixation by plants and microbes across the globe. Investment in nitrogen fixation was higher at higher latitudes and in biomes that were less productive, implying that nitrogen was scarcer in these areas.

Furthermore, 10 times more carbon was invested in nitrogen fixation in agricultural systems compared to natural systems, underscoring humans' impact.

1. Introduction

Biological nitrogen (N) fixation (BNF), the primary natural input of N to ecosystems, is essential to life and to human well-being (Vitousek et al., 2013). By converting dinitrogen gas to biologically useful N, BNF provides the N needed to make proteins, nucleic acids, and other biological building blocks. BNF provides a third of agricultural N inputs (Reis Ely et al., 2025a), helping to feed the global population while causing less environmental damage than synthetic fertilizers (Crews & Peoples, 2005; Jensen et al., 2012). BNF helps mitigate global climate change, supplying new N needed to support plant growth and carbon (C) storage (Batterman et al., 2013; Kou-Giesbrecht & Arora, 2022; Sulman et al., 2019), although the representation of BNF and its interactions with C remain a major uncertainty in terrestrial biosphere models (Davies-Barnard et al., 2020; Kou-Giesbrecht & Arora, 2022; Kou-Giesbrecht et al., 2023, 2025; Meyerholt et al., 2020).

The BNF process is energy-intensive, requiring more carbohydrates than other forms of N acquisition because of the needs to break the triple bond of N_2 and to maintain low oxygen concentrations (Gutschick, 1978, 1981; Tjepkema & Winship, 1980). As a consequence of this C cost and other resource requirements, BNF is typically most beneficial when N limitation is severe, suggesting that investment in BNF indicates N limitation (Menge, Batterman, Hedin, et al., 2017; Vitousek & Howarth, 1991, though see McCulloch et al., 2026, Menge et al., 2009 for reasons for sustained BNF when N is not limiting). However, BNF fluxes alone do not convey the degree of N limitation, or the importance of BNF more broadly, without additional context. For example, a BNF flux of $1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ is more important in arctic tundra than in a humid tropical rainforest due to the vastly lower rates of primary productivity and resulting nutrient demands in tundra.

A promising metric for quantifying the importance of BNF is the fraction of C generated via primary productivity that is invested in BNF, which is analogous to the proportion of income spent on food in economics (Chai & Moneta, 2010). The proportion of C from primary productivity that is allocated to BNF can be applied at multiple scales—the globe, biomes, grid cells, and individual organisms—and can complement other indirect approaches used to infer N limitation (Sullivan et al., 2014). It also applies across all N-fixing niches (Reis Ely et al., 2025a). In symbiotic plants such as acacia, alder, clover, lupine, and soybean, carbohydrates flow directly from the plants that produced them to the N-fixing prokaryotes living in root nodules. In associative and free-living N fixers such as cyanobacteria living in sphagnum mosses or free-living bacteria living in soil, carbohydrates come either directly from photosynthesis (for autotrophs like cyanobacteria) or from consuming organic matter that originated from primary productivity. Half a century ago, Gutschick (1978) conducted a back-of-the-envelope calculation of this metric at the global scale, estimating that 2.5% of C from terrestrial gross primary productivity (GPP) was allocated to BNF. Given that GPP is approximately double net primary productivity (NPP; Collalti & Prence, 2019; Jung et al., 2020; Running & Zhao, 2021), Gutschick's estimate translates to approximately 5% of NPP. A later model-based effort using CLM4.0-FUN2.0, a terrestrial biosphere model, reported values for symbiotic BNF rather than all BNF, estimating that 0.24% of terrestrial GPP and 0.79% of terrestrial net primary productivity (NPP) were invested in symbiotic BNF (Shi et al., 2016). A more recent model using ELM-FUN3.0, another terrestrial biosphere model, estimated that 0.002% of terrestrial NPP was invested in symbiotic BNF (Braghiere et al., 2022). Similar approaches have estimated the global importance of other pathways of nutrient acquisition, suggesting that 6.7% (Hawkins et al., 2023), 2.4% (Shi et al., 2016), or 5.9% (Braghiere et al., 2022) of global net primary productivity (NPP) is invested in mycorrhizal fungi, and that 9% of GPP is exported from plant roots to soil as root exudates (Chari et al., 2024).

Another promising metric for quantifying the importance of BNF is the fraction of plant N acquisition from BNF. In fact, at the scale of individual N-fixing plants, the fraction of N acquisition from BNF is one of the common ways to measure BNF (Shearer & Kohl, 1986; Soper, Taylor, et al., 2021). It is less common to quantify the

fraction of ecosystem-scale N acquisition from BNF, but a number of modeling studies (Cleveland et al., 2013; Shi et al., 2016; Sulman et al., 2019) provide a blueprint. Similar to the fraction of productivity invested in BNF, the fraction of plant N acquisition from BNF can be applied at multiple levels of organization, from individual plants to groups of N-fixing plants to all plants, and at multiple spatial scales, from individual plants to grid cells to biomes to the globe. Although the concepts for these metrics have existed for decades (Gutschick, 1978; Shearer & Kohl, 1986), they have not been used to assess the relative importance of BNF or the relative degree of N limitation across ecosystems or different types of N fixers, in large part due to a lack of data.

Our understanding of BNF (Cleveland et al., 1999; Davies-Barnard & Friedlingstein, 2020; Reis Ely et al., 2025a) and plant productivity (Running & Zhao, 2021) has advanced tremendously in recent years. In particular, a recent synthesis greatly reduced the uncertainty of global BNF by incorporating data on the abundance distributions of N fixers, which had previously been the largest source of uncertainty in global BNF syntheses (Reis Ely et al., 2025a). The synthesis also quantified the global spatial distributions of BNF from the different N-fixing niches that occur in ecosystems (Reis Ely et al., 2025a). Along with recent well-constrained estimates of NPP (Running & Zhao, 2021) and GPP (Jung et al., 2020), these new BNF data provide the opportunity to quantify the relative importance of BNF from each N-fixing niche in each location across the globe.

The relative importance of BNF and the relative degree of N limitation across and within ecosystems have long been sources of debate, in part because of data limitations, and in part because ecosystems are highly heterogeneous. For example, although some global syntheses suggest that tropical forests are less N limited than higher latitude forests (Du et al., 2020), tropical forests vary widely in N availability (Townsend et al., 2008), and other syntheses suggest widespread N limitation in the tropics (Wright, 2019). BNF plays a key role in this latitudinal conundrum: the ability to form N-fixing symbioses is thought to be an adaptation to N limitation, but N-fixing trees are rare at higher latitudes, where N limitation is common (MenMenge, Batterman, Hedin, et al., 2017; Menge, Batterman, Liao, et al., 2017; Steidinger et al., 2019). The relative importance of BNF and the relative degree of N limitation within individual ecosystems are also complicated. Within each ecosystem, BNF occurs in many biogeochemical niches with distinct environmental conditions. For example, symbioses with vascular plants, associations with mosses, lichens, and biocrusts, and free-living N fixers in litter, soil, and wood (Cleveland et al., 1999, 2022) each have differing N demands and access to different N pools (Cleveland et al., 2022; Menge & Hedin, 2009; Reed et al., 2008). Consequently, the relative importance of BNF in these N-fixing niches varies greatly within sites (Menge & Hedin, 2009; Reed et al., 2008), and focusing on one N-fixing niche might belie the ecosystem-level importance of BNF. Human activity, particularly the planting of agricultural legumes that house N-fixing bacteria (Herridge et al., 2008; Peoples et al., 2021), further contributes to heterogeneity by circumventing the ecological and evolutionary forces that shape natural ecosystems. Therefore, the relative degree of N limitation and the relative importance of BNF, across and within ecosystems, have remained elusive.

Here, we used a spatially explicit data set of global terrestrial BNF spanning all major N-fixing niches (Reis Ely et al., 2025a) along with spatially explicit data sets of global primary productivity (Jung et al., 2020; Running & Zhao, 2021) to quantify two metrics of the importance of BNF across and within ecosystems: the fraction of primary productivity invested in BNF and the fraction of plant N acquisition supplied by BNF. We focused mostly on values based on NPP, though we also calculated values based on GPP. Following Reis Ely et al. (2025a), we grouped N-fixing niches into symbioses with vascular plants (trees, shrubs, herbs in natural biomes, and herbs in crops and cultivated pastures; hereafter, “symbiotic plants,” even though mosses are also plants), associative N fixers (mosses, lichens, and biocrusts; Weber et al., 2022), and free-living N fixers (in soil, leaf litter, and decomposing wood; Cleveland et al., 2022). We addressed four specific questions: (a) What fraction of C from NPP is invested in BNF in different natural N-fixing niches? (b) How does C investment in natural BNF vary across space and among biomes? (c) How does C investment in agricultural BNF compare to that in natural BNF? (d) How does the fraction of plants' N acquisition that comes from BNF vary across space, among natural biomes, and between agricultural and natural biomes? We addressed these questions at two levels of organization: the ecosystem scale (NPP_{ecosys} and $N_{acquisition_{ecosys}}$) and the scale of N-fixing symbioses, using symbiotic plants' own productivity (NPP_{symb} and $N_{acquisition_{symb}}$; see Section 2). The ecosystem-scale calculations integrate the abundance of N fixers with their BNF activity, whereas the symbiosis-scale calculations focus on BNF activity independent of the abundance of N fixers.

2. Methods

2.1. Global Data Sets

The global data sets we used are at 1° resolution, aggregated from finer resolution using the bilinear method. For GPP and NPP we used FluxCom (Jung et al., 2020) and MODIS (Running & Zhao, 2021) data averaged from 2001–2015 and 2001–2020, respectively. The FluxCom and MODIS data sets agree well at the global scale (Seiler et al., 2022).

We used BNF data from a recent bottom-up synthesis (Reis Ely et al., 2025a, 2025b). The synthesis combined 1,177 published BNF rates with global maps of the relative abundance of N fixers to construct gridded BNF estimates for symbiotic N-fixing trees, shrubs, and herbs in natural biomes (here, natural symbiotic plants); symbiotic N-fixing herbs in cultivated pastures and crops (agricultural symbiotic plants); biocrusts, non-biocrust mosses, and epiphytic lichens (associative N fixers); and soil, leaf litter, and decomposing wood (free-living N fixers). These grouping names are imperfect—for example, N-fixing lichens are true symbioses rather than associative relationships, biocrusts include lichens and mosses, and mosses are also plants—but they make sense ecologically. Symbioses with vascular plants are vastly different than mosses or lichens, for instance, and the N fixers in biocrusts (Abed et al., 2010; Weber et al., 2022), mosses (Holland-Moritz et al., 2021), and lichens (Rösch & Bothe, 2009), which are primarily autotrophic cyanobacteria, have easier access to new photosynthate than the primarily heterotrophic free-living N fixers in soil (Rösch & Bothe, 2009), leaf litter (Purahong et al., 2016), and decomposing wood (Groß et al., 2022).

We used the IGBP (International Geosphere-Biosphere Programme) biome classification scheme (Friedl & Sulla-Menashe, 2019). Following Reis Ely et al. (2025a), we assigned IGBP biomes “Cropland” and “Cropland/Natural Mosaic” as Croplands, and assigned 27% of each “Grassland” grid cell between 67°N and 67°S as Cultivated Pasture. We resampled the biome classification scheme from 0.004° to 1° resolution using the nearest neighbor method. We converted values from kg or Mg ha^{−1} yr^{−1} to Tg or Pg yr^{−1} using the land area per grid cell.

2.2. C Cost per N Fixed: Symbiotic Plants

The C cost per BNF has been studied in legumes, particularly agricultural legumes, since the 1970s (Atkins, 1984; Gutschick, 1981; Layzell et al., 1979; Minchin & Witty, 2005; Schubert, 1982; Vance, 2008; Witty et al., 1983). These studies have considered many types of C cost, including nitrogenase and hydrogenase activity, ammonia assimilation, ancillary C metabolism, transport of N, maintenance of low O₂, and growth and maintenance of nodules (Atkins, 1984; Gutschick, 1981; Jensen et al., 2012; Layzell et al., 1979; Minchin & Witty, 2005; Schubert, 1982; Vance, 2008; Witty et al., 1983). Some studies (Atkins, 1984; Gutschick, 1981; Jensen et al., 2012) compared the costs of BNF to the costs to nitrate assimilation; others focused on the C costs of BNF itself. We focused on the C costs of BNF itself rather than differences between the C costs of BNF and nitrate assimilation. Presenting the C costs of BNF itself allows for easier comparisons to other C costs, such as the C costs of acquiring N through mycorrhizae (Hawkins et al., 2023).

Citing measurements from herbaceous legumes, Gutschick (1981) reported 8–12 g glucose per g N fixed, which equates to 3.2–4.8 g C per g N fixed. Atkins (1984) reported 2.9–6.1 g C per g N fixed from theory, 1.4–5.4 for *Vigna radiata*, 2.0–2.3 for *V. unguiculata*, and 3.9–5.0 for *Lupinus albus*, each across a range of growth stages. Minchin and Witty (2005), after synthesizing theoretical estimates at 2.8–4.8 g C per g N fixed, examined nodule respiratory costs across 18 studies of *Acacia* and herbaceous legumes. They suggested that the lower values were erroneous, suggesting 3–5 g C per g N fixed as the most reasonable range for respiratory costs (Minchin & Witty, 2005). Vance (2008), summarizing 30 legume studies, reported 6–7 g C per g N fixed. Modeling studies that have estimated C costs of BNF (Holland et al., 2023; Layzell et al., 1988) have reported similar values, such as 4.6 and 4.1 g C per g N fixed for soybean.

The existing estimates of the C costs of BNF in actinorhizal species are similar to those for legumes. Tjepkema and Winship (1980) reported that nodule respiratory costs of BNF varied across species, but not consistently between rhizobial (legume) and actinorhizal symbioses. Translating their values from mol CO₂ per mol C₂H₄ to g C per g N fixed using 3 mol C₂H₄ per mol N₂ (Soper, Simon, & Jauss, 2021), their species-level means ranged from 3.6 to 11.2 g C per g N fixed; averages across species were 5.9 and 5.5 g C per g N fixed for actinorhizal species and legumes, respectively (Tjepkema & Winship, 1980). Ruess et al. (2013), using ¹⁵N₂ isotopes and CO₂ fluxes to measure respiratory costs in *Alnus tenuifolia*, reported 4.2 g C per g N in control plots.

Ecosystem and land models have drawn on these estimates to incorporate C costs per unit N fixed for symbiotic plants. For example, MEL (Rastetter et al., 2001) used 9.12 (6–14) g C per g N fixed, and CASA-CNP (Wang et al., 2007), CROPGRO (Boote et al., 2008), and O-CN (Meyerholt et al., 2020) all used 6 g C per g N fixed. Fisher et al. (2010) used a temperature-dependent range of 7.5–12.5 g C per g N fixed in FUN. However, they cited Gutschick (1981), who, as stated above, reported 8–12 g glucose per g N fixed, which equates to 3.2–4.8 g C per g N fixed.

Summarizing the above information, theoretical and empirical average estimates are in the range of 3–6 g C per g N fixed. Models have generally used higher values (6–14 g C per g N fixed). We suspected that the mean of the literature values (4.4) was slightly too low for a variety of reasons. Minchin and Witty (2005) pointed out that some of the low estimates used faulty methods. Some of the values included only respiratory costs, which are the majority of costs (Atkins, 1984) but not the only costs. Furthermore, these measurements were typically made in laboratory conditions, and there is evidence that the C cost is higher under suboptimal conditions such as lower temperatures because nodule maintenance respiration is more sustained than BNF at low temperatures (Gutschick, 1982; Ruess et al., 2013). For these reasons, we used a value higher than empirical mean of 4.4 g C per g N fixed. However, given the paucity of empirical estimates above 6 g C per g N fixed, we used a central value that was only slightly above the empirical average: 5 g C per g N fixed. We also reported results for low and high values equal to 4 and 6 g C per g N fixed, respectively. There is variation across species, growth stages, bacterial strains, and environmental conditions (Atkins, 1984; Gutschick, 1981, 1982; Minchin & Witty, 2005; Tjepkema & Winship, 1980), but there is no clear evidence that the C cost per unit N fixed differs between actinorhizal and rhizobial N fixers, across biomes, or between agricultural and natural legumes. Therefore, we used the same central value and range—5 (4–6) g C per g N fixed—for all symbiotic plants (Table S1 in Supporting Information S1).

2.3. C Cost per N Fixed: Associative and Free-Living N Fixers

Less work has focused on C costs per unit N fixed in associative and free-living N fixers. Based on theoretical calculations and a synthesis, Gutschick (1978, 1982) reported that free-living heterotrophic N fixers had higher C costs than symbiotic plants because of low respiratory efficiencies in making ATP, high respiration rates to protect nitrogenase from oxygen, and a greater need to re-synthesize nitrogenase due to oxygen damage. Based on these arguments and efficiency values for heterotrophic N fixers in the literature, Gutschick (1978) estimated 20 g C per g N fixed for free-living N fixers. Hill (1992, cited in Jones et al., 2003; Unkovich & Baldock, 2008; Zuberer, 2021) estimated C costs per N fixed for multiple taxa of free-living heterotrophic bacteria grown in chemostats under a range of oxygen conditions, derived from comparisons of growth efficiencies of bacteria grown with ammonium versus N_2 as the only N sources. They reported a median and range of 42 (5.3–117) g C per g N fixed. A model of free-living N fixation in the rhizosphere (Jones et al., 2003) assumed 10 g C per g N fixed if the C substrate was glucose. Given this spread, we used 20 (5–42) g C per g N fixed for free-living N fixers (Table S1 in Supporting Information S1).

Gutschick (1978) assumed that respiration costs of autotrophic N fixers (“associative” in our scheme) and heterotrophic (“free-living” in our scheme) fixers were similar, though higher autotrophic relative to heterotrophic BNF rates in similar environmental conditions (Cooper & Scherer, 2012) and estimates from marine cyanobacteria (Brauer et al., 2013) suggest lower costs in autotrophic N fixers. For example, the cyanobacteria *Cyanothece* grown at a range of temperatures used 1–4.5 mol O_2 per mol ethylene fixed (Brauer et al., 2013), which, using 3 mol ethylene per mol N_2 (Soper, Simon, & Jaus, 2021) and a respiratory quotient of 1 mol C per mol O_2 , translates to 1.3–5.8 g C per g N fixed. A study of the cyanobacteria *Crocospaera watsonii*, which did not report absolute cost values, noted that costs were much higher at ambient oxygen concentrations (20%) than at 5% O_2 (Großkopf & LaRoche, 2012), implying higher costs in natural conditions for associative N fixers than for symbiotic plants that tightly regulate oxygen conditions near the nodule (Gutschick, 1978, 1982; Zuberer, 2021). Given the limited information for associative N fixers, we used 12 g C per g N fixed (between the values from Gutschick, 1978; Brauer et al., 2013) as our central value, and 5 (our central value for symbiotic BNF) and 20 g C per g C fixed (Gutschick, 1978) as our range (Table S1 in Supporting Information S1).

2.4. Calculations for C Investment in BNF for Global Data Sets

The calculation for p_{ij} , the percentage of C flux invested in BNF in grid cell i for N-fixing niche j , is:

$$p_{ij} = \frac{\text{BNF}_{ij} * \text{C cost per BNF}_j}{\text{C flux}_i} * 100 \quad (1)$$

We used this approach for each grid cell, including those with agricultural biomes. The BNF values for each N-fixing niche (trees, shrubs, herbs in natural biomes, herbs in crops, herbs in cultivated pastures, mosses, lichens, biocrusts, soil, leaf litter, and decomposing wood) were from Reis Ely et al. (2025a). The C costs per BNF are described in the previous sections. The C fluxes we used for Equation 1 were GPP and NPP at two scales: total ecosystem-level C fluxes ($\text{GPP}_{\text{ecosys}}$, $\text{NPP}_{\text{ecosys}}$) and only the GPP and NPP from symbiotic N-fixing plants (GPP_{symb} and NPP_{symb}) (see below). For the version where we used GPP_{symb} and NPP_{symb} , there were also j subscripts in the C flux terms in Equations 1–3.

To aggregate across grid cells to the biome or global scale, we summed C costs and C fluxes before applying Equation 1. For niche j in biome k containing grid cells i_k :

$$p_{kj} = \frac{\sum_{i_k} \text{BNF}_{ij} * \text{C cost per BNF}_j}{\sum_{i_k} \text{C flux}_i} * 100. \quad (2)$$

We aggregated across N-fixing niches similarly. For example, to calculate the total C investment in BNF in biome k , we calculated:

$$p_k = \frac{\sum_j \sum_{i_k} \text{BNF}_{ij} * \text{C cost per BNF}_j}{\sum_j \sum_{i_k} \text{C flux}_i} * 100. \quad (3)$$

To calculate the N fixer-specific C investment in BNF, we needed to estimate GPP and NPP of growth form l (trees, shrubs, or herbs) and the fraction of each growth form's GPP and NPP from N fixers. The equation for symbiotic N fixers' C fluxes (GPP_{symb} or NPP_{symb}) is:

$$\text{C flux}_{il, \text{N fixer}} = \text{C flux}_i * \text{growthformfrac}_{il} * \text{fixerfrac}_{il}. \quad (4)$$

C flux_i is the ecosystem-level C flux ($\text{GPP}_{\text{ecosys}}$ or $\text{NPP}_{\text{ecosys}}$) in grid cell i . The term $\text{growthformfrac}_{il}$ is the fraction of the C flux in grid cell i from plant growth form l (trees, shrubs, or herbs). We did not know of a published source for this quantity, so we estimated it based on the definitions of the IGBP biomes (Table S2 in Supporting Information S1). The term fixerfrac_{il} is the fraction of each growth form l 's C flux in grid cell i from N fixers. We assumed that fixerfrac_{il} was related to the relative abundance of N fixers (Reis Ely et al., 2025a), but that N fixers have 50% higher C fluxes per relative abundance than average plants. This assumption of a 50% higher C flux per relative abundance in N fixers comes from N fixers' high foliar N concentrations and the link between foliar N concentration and net photosynthesis. Foliar %N was higher in N fixers than in non-fixers by 20% and 33% in tropical trees (Fyllas et al., 2009; Nasto et al., 2014), 70% in grassland plants (Cheaib et al., 2025), and 50%–120% in different global regions (Adams et al., 2016). Due to its role in Rubisco and other biochemical machinery, increased foliar N concentration typically implies higher net photosynthesis (Farquhar et al., 1980), although the relationship is concave-down rather than proportional after accounting for differences in leaf mass per area (Osnas et al., 2013). Given that foliar N is at least 50% higher in N fixers than in non-fixers, on average, but that the link to C fluxes is less than proportional, we assumed 50% higher C fluxes in N fixers. Note that this assumption does not imply that N-fixing plants have 50% higher growth rates than non-fixing plants because the excess C can be invested in processes other than growth, including but not limited to BNF, mycorrhizal uptake, and root exudation. The abundance data we used originated from Steidinger et al. (2019) for trees, Bruehlheide et al. (2019) for shrubs and natural herbs, FAO (2021) and Piipponen et al. (2022) for herbs in cultivated pastures, and FAO (2021) for crops. Because the separation of GPP_{symb} and NPP_{symb} into trees versus shrubs versus herbs was uncertain, we focused on the aggregated values across all symbiotic plants.

2.5. Calculation of the Fraction of Plant N Acquisition From BNF

We calculated the percent of plant N acquisition from BNF at both the ecosystem scale (the percent of all plant N acquisition from BNF) and the symbiosis scale (the percent of symbiotic N-fixing plants' N acquisition from BNF). We were unable to reliably estimate N acquisition for associative or free-living N fixers, so we only calculated the fraction of N acquisition from BNF for vascular plants.

The percent f_i of ecosystem-scale plant N acquisition from BNF in grid cell i was:

$$f_i = \frac{\text{Symbiotic plant BNF}_i}{\text{Plant N acquisition}_i} * 100. \quad (5)$$

The BNF data came from Reis Ely et al. (2025a). For ecosystem-scale plant N acquisition, we used the approach from Cleveland et al. (2013), which used $\text{NPP}_{\text{ecosys}}$, the fractional allocation of NPP to four tissue types, and the mass-based C:N ratio and resorption efficiency of each tissue type.

$$\text{Plant N acquisition}_i = \sum_m \frac{\text{NPP}_i C_{im}}{\text{CN}_{im}} (1 - \% \text{resorp}_{im}). \quad (6)$$

In Equation 6, NPP_i is $\text{NPP}_{\text{ecosys}}$ in grid cell i (Running & Zhao, 2021), C_{im} is the fraction of $\text{NPP}_{\text{ecosys}}$ allocated to tissue type m for the biome in grid cell i , CN_{im} is the mass-based C:N ratio of tissue type m for the biome in grid cell i , and $\% \text{resorp}_{im}$ is the resorption efficiency of tissue type m for the biome in grid cell i . The tissue types are leaves, stems, coarse roots, and fine roots. For all tissue types aside from leaves, we assumed no resorption (Cleveland et al., 2013).

Aside from NPP, all data used in Equation 6 (fractions of $\text{NPP}_{\text{ecosys}}$ allocated to tissue types, C:N ratios, and resorption efficiencies) came from Table S1 in Cleveland et al. (2013), with the following amendments. We used the mean of their Wet Savanna and Savanna values for our Savanna values. We used their Shrubland values for our Barren values. We used their Grassland values for our Cultivated Pasture and Cropland biomes, except that we assumed 33% lower C:N (roughly equivalent to 50% higher %N) in Croplands due to the higher N content in croplands compared to natural systems.

Cleveland et al. (2013) had a different focus than our study, so they did not report the % of N acquisition from BNF, but they provided the necessary components needed to calculate it at the biome level. To calculate the % of N acquisition_{ecosys} from BNF from their data ($f_{k,\text{Clevelanddata}}$), we divided their symbiotic BNF by the sum of symbiotic BNF, N deposition, and N mineralization, each at the biome scale (indexed k , as above):

$$f_{k,\text{Clevelanddata}} = \frac{\text{Symbiotic plant BNF}_k}{\text{Symbiotic plant BNF}_k + \text{N deposition}_k + \text{N mineralization}_k} * 100. \quad (7)$$

To calculate symbiotic N fixer-specific N acquisition (N acquisition_{symb}), we used the assumptions described in the previous subsection (Equation 4) to estimate NPP_{symb} in each grid cell, then we used Equations 5 and 6 to calculate the % that came from BNF. We assumed that N fixers' N demand per C was 50% higher than average, reflecting the high N concentration in N fixer tissues (Adams et al., 2016; Cheaib et al., 2025; Fyllas et al., 2009; Nasto et al., 2014).

2.6. Field-Grown and Greenhouse-Grown Plants

To provide a check on our global data sets, we used data from field (Carreras Pereira et al., 2023; Menge et al., 2023a) and greenhouse (Menge et al., 2015, 2024) studies where we had quantified symbiotic BNF.

In the field study (Carreras Pereira et al., 2023; Menge et al., 2023a), we grew *Robinia pseudoacacia* in New York, *Alnus rubra* in Oregon, and *Gliricidia sepium*, *Casuarina equisetifolia*, *Acacia koa*, and *Morella faya* in Hawaii, all in open fields. We planted bare-root seedlings, which grew for 4–5 years. We established fertilization treatments, but here we only used controls. In New York we harvested aboveground and belowground tissues. In Oregon we harvested aboveground tissues and used allometric relationships to quantify belowground tissues. In Hawaii we harvested aboveground tissue and did not quantify belowground tissues. We measured BNF by

labeling soils with ^{15}N and comparing N isotopes in N-fixing trees to non-fixing neighbors (Soper, Taylor, et al., 2021). The BNF metrics we used were $\%N_{\text{dfa}}$, the percentage of the plant's N from BNF, and the total mass of N fixed that went into all tissues (New York and Oregon) or aboveground tissues (Hawaii).

In the greenhouse studies, we grew herbaceous seedlings at Chapman College and Stanford University (Menge et al., 2015), and temperate and tropical tree seedlings as well as *Glycine max* (soybean) at Barnard College and the University of California Davis (Menge et al., 2024). The approach was similar to the field experiment, with the following differences. Instead of non-fixing species as reference plants, we used uninoculated (non-fixing) seedlings of the N-fixing species. We typically used nine or 10 fertilization treatments. We harvested plants within a year, then quantified biomass and BNF.

To calculate C investment in BNF in our field-grown and greenhouse-grown species, we used a version of Equation 1 for individual plants. The BNF data we used in Equation 1 were the mass of N fixed throughout the plant's lifetime. We assumed the C cost per unit N fixed was 5 (4–6) g C g N^{-1} (see above). To calculate GPP_{symb} and NPP_{symb} for individual plants, we assumed that accumulated biomass C was half of the plant's NPP, and that NPP was half of GPP. We also reported $\%N_{\text{dfa}}$ to compare to the global estimates of the % of N acquisition_{symb} from BNF.

2.7. Statistical Analyses and Uncertainty

We conducted analyses in R (Garnier et al., 2023; Hijmans, 2023; Pierce, 2023; R Core Team, 2022). To evaluate trends across productivity, we regressed the percentage of C fluxes invested in BNF against productivity, using biome-level values. We used nonlinear least squares regression, using an exponentially declining function: $y = a + be^{-kx} + \epsilon$. If the nonlinear regression did not converge, we used linear regression. For the trends with latitude, we used linear regressions with one datapoint for each degree of latitude. The latitudinal trend for biocrusts was clearly unimodal, though we did not conduct a formal analysis for unimodality or other nonmonotonic trends.

Each of the values in our calculations was uncertain. The high ends of the ranges we used for the C cost per unit N fixed were 20%, 67%, and 110% higher than the central values for symbiotic, associative, and free-living BNF, respectively. The upper 68% confidence interval (analogous to +1 SE) of total terrestrial BNF was 18% higher than the mean (Reis Ely et al., 2025a). The upper end of the ensemble spread for FLUXCOM GPP was 7% higher than the mean (Jung et al., 2020). Our value for global N acquisition (1,127 Tg N yr^{-1}) was 5% higher than one recent estimate (Peng et al., 2023; 950 Tg N yr^{-1} of N uptake from Peng et al., 2023 combined with 120 Tg N yr^{-1} of BNF from Reis Ely et al., 2025a gives 1,070 Tg N yr^{-1} of N acquisition) and 6% lower than another (Schlesinger & Bernhardt, 2013). Because these metrics of uncertainty are fundamentally different (range vs. confidence intervals vs. ensemble spread vs. comparison across studies), formally combining them would not be straightforward. Therefore, we assumed that the uncertainty for the C cost per N fixed was largest and did not propagate uncertainty from our other data sources.

The results for the proportions of N fixers' C fluxes (GPP_{symb} and NPP_{symb}) invested in BNF (Equations 1–3 with j subscripts on the C flux terms) and the proportion of N fixers' N acquisition from BNF (Equation 5 for N fixers' N acquisition rather than all plant N acquisition) are inversely proportional to the assumption of a higher C flux per relative abundance in N fixers than in non-fixers (which was assumed to be 50%, as described above). Therefore, the sensitivity to this assumption can be evaluated with simple multiplication. For example, if the C flux per relative abundance is 20% higher in N fixers than non-fixers rather than 50% higher, then the proportion of NPP_{symb} invested in BNF (Equation 3) would be multiplied by 1.5/1.2 (to undo the assumption of 50% higher and replace it with an assumption of 20% higher). In this scenario, a result listed as 5.4% of NPP_{symb} invested in BNF would change to $5.4\% \times 1.5/1.2 = 6.8\%$ of NPP_{symb} invested in BNF. Similarly, a result listed as 24% of N fixers' N acquisition from BNF would change to $24\% \times 1.5/1.2 = 30\%$. Note that, unlike the symbiosis-scale results, the ecosystem-level results do not depend on the assumption of higher C flux per relative abundance in N fixers compared to non-fixers.

3. Results

3.1. C Investment in Natural BNF Across Niches

After excluding agricultural and non-terrestrial biomes (see Section 2), $\text{NPP}_{\text{ecosys}}$ and BNF in natural terrestrial biomes were 48 Pg C yr^{-1} (Running & Zhao, 2021) and 65 Tg N yr^{-1} (Reis Ely et al., 2025a) (Table 1 and Figure

Table 1

Fractions of Global Ecosystem-Scale Gross (GPP_{ecosys}) and Net Primary Productivity (NPP_{ecosys}) Invested in Biological Nitrogen Fixation (BNF)

N-fixing niche(s)	GPP ^a	NPP ^a	GPP ^a	NPP ^a	BNF ^a	BNF ^a	C invested in BNF ^b	% of GPP ^b _{ecosys}	% of NPP ^b _{ecosys}
	Pg C yr ⁻¹		Mg C ha ⁻¹ yr ⁻¹		Tg N yr ⁻¹	kg N ha ⁻¹ yr ⁻¹	Pg C yr ⁻¹	% invested in BNF	
Total	104	57	8.2	4.5	120	9.4	1.1 (0.52–1.8)	1.0 (0.50–1.7)%	1.9 (0.90–3.2)%
• Natural	87	48	8.2	4.5	65	6.1	0.76 (0.29–1.4)	0.87 (0.34–1.6)%	1.6 (0.61–2.9)%
• Agricultural	17	9.2	8.1	4.4	56	27	0.31 (0.23–0.42)	1.8 (1.3–2.5)%	3.4 (2.5–4.6)%
Free-living	104	57	8.2	4.5	25	2.0	0.50 (0.13–1.1)	0.48 (0.12–1.0)%	0.87 (0.22–1.8)%
• Natural	87	48	8.2	4.5	23	2.1	0.46 (0.11–0.96)	0.52 (0.13–1.1)%	0.94 (0.24–2.0)%
◦ Soil	87	48	8.2	4.5	17	1.6	0.33 (0.083–0.70)	0.38 (0.096–0.80)%	0.69 (0.17–1.4)%
◦ Leaf litter	87	48	8.2	4.5	2.8	0.26	0.056 (0.014–0.12)	0.064 (0.016–0.13)%	0.12 (0.029–0.24)%
◦ Decomposing wood	87	48	8.2	4.5	3.3	0.31	0.066 (0.017–0.14)	0.076 (0.019–0.16)%	0.14 (0.034–0.29)%
• Agricultural (cropland)	12	6.3	9.4	4.8	2.3	1.7	0.046 (0.012–0.097)	0.37 (0.093–0.78)%	0.73 (0.18–1.5)%
Symbiotic plants	104	57	8.2	4.5	82	6.4	0.41 (0.33–0.49)	0.39 (0.31–0.47)%	0.71 (0.57–0.85)%
• Natural	87	48	8.2	4.5	28	2.7	0.14 (0.11–0.17)	0.16 (0.13–0.20)%	0.29 (0.23–0.35)%
◦ Trees	87	48	8.2	4.5	12	1.1	0.060 (0.048–0.072)	0.069 (0.055–0.083)%	0.12 (0.10–0.15)%
◦ Shrubs	87	48	8.2	4.5	5.7	0.54	0.029 (0.023–0.034)	0.033 (0.026–0.039)%	0.059 (0.047–0.071)%
◦ Herbs	87	48	8.2	4.5	11	1.0	0.053 (0.043–0.064)	0.061 (0.049–0.073)%	0.11 (0.088–0.13)%
• Agricultural	17	9.2	8.1	4.4	54	25	0.27 (0.21–0.32)	1.6 (1.3–1.9)%	2.9 (2.3–3.5)%
◦ Crop herbs	12	6.3	9.4	4.8	34	26	0.17 (0.14–0.20)	1.4 (1.0–1.6)%	2.7 (2.1–3.2)%
◦ Pasture herbs	4.7	2.8	6.0	3.6	20	25	0.098 (0.078–0.12)	2.1 (1.7–2.5)%	3.5 (2.8–4.1)%
Associative	87	48	8.2	4.5	13	1.3	0.16 (0.067–0.27)	0.18 (0.077–0.31)%	0.33 (0.14–0.55)%
• Biocrusts	87	48	8.2	4.5	11	1.1	0.14 (0.057–0.23)	0.16 (0.066–0.26)%	0.29 (0.12–0.48)%
• Mosses	87	48	8.2	4.5	1.8	0.17	0.021 (0.089–0.036)	0.024 (0.010–0.041)%	0.044 (0.018–0.074)%
• Lichens	87	48	8.2	4.5	0.1	0.01	0.0012 (0.0005–0.0020)	0.001 (0.001–0.002)%	0.002 (0.001–0.004)%

^a GPP_{ecosys} and NPP_{ecosys} from the relevant land area are listed for all rows even though GPP and NPP do not come from free-living N fixers, which are primarily heterotrophs. Per area rates are averages across all natural (non-agricultural) land area for natural sources of BNF, all agricultural area for agricultural sources, or all land area for combined sources. Data sources were Reis Ely et al. (2025a, 2025b, 2025c) for BNF, Jung et al. (2020) for GPP, and Running and Zhao (2021) for NPP. ^bValues shown outside parentheses used the central C costs: 5, 12, and 20 g C per g N fixed for symbiotic plants, associative BNF, and free-living BNF, respectively (see Section 2, Table S1 in Supporting Information S1). Values shown inside parentheses show ranges of results from using the low (4, 5, and 5 g C per g N fixed) and high (6, 20, and 42 g C per g N fixed) C costs for symbiotic plants, associative BNF, and free-living BNF, respectively.

S1 in Supporting Information S1). For each N-fixing niche in each grid cell, we multiplied the BNF flux by the C cost per g N fixed to calculate the total C invested in BNF, which we divided by NPP_{ecosys} to estimate the percent of NPP_{ecosys} invested in BNF (see Section 2 for details). Carbon costs are higher for free-living BNF than for symbiotic plants due to respiratory inefficiencies and oxygen damage, but are also less certain (see Section 2 for details), resulting in wider uncertainty values in these results (Tables 1–4).

Globally, our central estimate is that 1.6% of natural terrestrial NPP_{ecosys} was invested in natural BNF (Table 1, Figures 1a and 2a). Of the 1.6%, 0.94% came from free-living N fixers (in soil, litter, and decomposing wood), 0.33% came from associative N fixers (biocrusts, mosses, and lichens), and 0.29% came from symbiotic plants (trees, shrubs, and natural herbs; Table 1, Figures 1b–1d, 2b and Figures S2–S4 in Supporting Information S1). Free-living (23 Tg N yr⁻¹) and associative fixers (13 Tg N yr⁻¹) each fixed less N than symbiotic plants (28 Tg N yr⁻¹; Reis Ely et al., 2025a), but the higher C costs per g N fixed in free-living and associative N fixers explained their similar or higher investment.

Next, we asked what fraction of symbiotic plants' own productivity (NPP_{symb}) was invested in BNF. We estimated NPP_{symb} using NPP_{ecosys} (Running & Zhao, 2021) and the relative abundance of symbiotic plants (Reis Ely et al., 2025a; see Section 2 for details). Natural symbiotic plants invested 3.6% of their NPP_{symb} in BNF (Table 2 and Figures 3a, 3b). Although trees accounted for a total BNF flux (12 Tg N yr⁻¹) that was higher than shrubs

Table 2
Fractions of Global Symbiotic Plant GPP_{symb} and NPP_{symb} Invested in BNF

N-fixing niche(s)	GPP	NPP	GPP^a	NPP^a	BNF	BNF^a	% of GPP_{symb}^b	% of NPP_{symb}^b
	Pg C yr^{-1}		Mg C $ha^{-1} yr^{-1}$		Tg N yr^{-1}	kg N $ha^{-1} yr^{-1}$	% invested in BNF	
Total	14	7.5	1.1	0.59	82	6.4	2.9 (2.3–3.5)%	5.4 (4.4–6.5)%
• Natural	7.7	4.0	0.72	0.37	28	2.7	1.9 (1.5–2.2)%	3.6 (2.9–4.3)%
• Trees ^c	4.8	2.3	0.45	0.22	12	1.1	1.3 (1.0–1.5)%	2.6 (2.1–3.1)%
• Shrubs ^c	0.54	0.34	0.051	0.032	5.7	0.54	5.3 (4.2–6.3)%	8.5 (6.8–10)%
• Herbs ^c	2.3	1.3	0.22	0.13	11	1.0	2.3 (1.8–2.7)%	4.0 (3.2–4.7)%
• Agricultural	6.5	3.5	3.1	1.7	54	25	4.1 (3.3–5.0)%	7.6 (6.1–9.1)%

^aPer area rates are averages across all natural (non-agricultural) land area for natural sources of BNF, all agricultural area for agricultural sources, or all land area for combined sources. BNF data were from Reis Ely et al. (2025a, 2025b, 2025c).

^bValues shown outside parentheses used the central C costs: 5, 12, and 20 g C per g N fixed for symbiotic plants, associative BNF, and free-living BNF, respectively (see Section 2 and Table S1 in Supporting Information S1). Values shown inside parentheses show ranges of results from using the low (4, 5, and 5 g C per g N fixed) and high (6, 20, and 42 g C per g N fixed) C costs for symbiotic plants, associative BNF, and free-living BNF, respectively. ^cValues of % GPP_{symb} and NPP_{symb} for trees (or shrub or herb) are percentages of N-fixing tree (or shrub or herb) GPP_{symb} and NPP_{symb} rather than percentages of all N-fixing plant GPP_{symb} and NPP_{symb} , which is shown in Figure 3b.

(5.7 Tg N yr^{-1}) and similar to natural herbs (11 Tg N yr^{-1}), trees invested the lowest fraction of their NPP_{symb} in BNF: 2.6%, compared to 4.0% for herbs and 8.5% for shrubs (Table 2). To put these global estimates in context, we compared them to field- and greenhouse-grown symbiotic plants for which we had rigorously measured BNF (Menge et al., 2015, 2023a, 2024). For six species of open-field-grown 4–5-year-old trees and 22 species of greenhouse-grown seedlings, average NPP_{symb} invested in BNF ranged from 2.9% to 9.2% and 0.69%–16%, respectively (Table S3 in Supporting Information S1). Reviews of herbaceous legumes (Atkins, 1984; Vance, 2008) reported 3%–30%. These local-scale empirical estimates indicate that our global estimates are plausible and that there is wide variation across species, growth forms, and environmental conditions.

3.2. C Investment in Natural BNF Across Space

The fraction of NPP_{ecosys} invested in BNF varied substantially across the land surface (Figure 1). Forests and savannas, including the two natural biomes with the highest total productivity and BNF fluxes (savannas and evergreen broadleaf forests), invested relatively less NPP_{ecosys} in BNF (0.71%–1.3%; Table 3 and Figure 2a) than the global average (1.6%; Table 1 and Figure 2a). Natural grasslands, shrublands, and barren lands invested relatively more (2.6%, 3.2%, and 4.0%, respectively; Table 3 and Figure 2a). In grasslands and shrublands, the high relative values were driven by multiple N-fixing niches, whereas in barren systems they were driven mostly by biocrusts (Table 3 and Figure 2a). Larger fractions of NPP_{ecosys} were invested in BNF at intermediate and higher latitudes than at lower latitudes, driven by latitudinal patterns in soil, shrubs, herbs, biocrusts, and mosses, which outweighed the opposite latitudinal pattern in trees and decomposing wood (Table 5 and Figure 2b).

The biome-level breakdown (Table 3 and Figure 2a), latitudinal patterns (Figure 2b), and map (Figure 1) suggested that a greater proportion of NPP_{ecosys} was invested in BNF in less productive areas. Regression analysis confirmed this observation (Figure 2c). Furthermore, it showed that the pattern was driven by biocrusts and mosses (Figure 2f); there was no relationship between NPP and the proportion of NPP_{ecosys} invested in BNF for free-living N fixers (Figure 2d) or symbiotic plants (Figure 2e).

N-fixing plants invested a greater proportion of their own C (NPP_{symb}) in BNF in some biomes (Table 4 and Figures 3a, 3b), with a similar distribution as the ecosystem-scale calculations. Symbiotic plants invested a lower fraction of their NPP_{symb} in BNF in evergreen broadleaf forests (2.6%) and savannas (3.2%), where N-fixing trees were relatively common (Reis Ely et al., 2025a), than in natural grasslands (4.3%), the other forest biomes (4.5%–7.1%), and shrublands (9.5%; Table 4 and Figure 3b). The fraction of NPP_{symb} invested in BNF appeared high in barren ecosystems, though the miniscule areal fluxes of NPP_{symb} and BNF in barren ecosystems (Table 4 and Figure 3b) cast doubt on the validity of the estimate. Similar to ecosystem-scale productivity (Figure 2b), a

Table 3
Fractions of Global Ecosystem-Scale GPP_{ecosys} and NPP_{ecosys} Invested in BNF, Split by Biome

Biome and niche grouping	GPP^a		NPP^a		BNF^a		C invested in BNF^b		% of GPP_{ecosys}^b	
	Pg C yr ⁻¹		Mg C ha ⁻¹ yr ⁻¹		Tg N yr ⁻¹	kg N ha ⁻¹ yr ⁻¹	Pg C yr ⁻¹		% invested in BNF	% of NPP_{ecosys}^b
Evergreen Needleleaf Forest	2.0	1.3	8.8	5.7	0.83	3.6	0.0094 (0.0037–0.017)		0.46 (0.18–0.84)%	0.71 (0.28–1.3)%
• Free-living	2.0	1.3	8.8	5.7	0.31	1.3	0.0062 (0.0016–0.013)		0.30 (0.076–0.64)%	0.47 (0.12–0.99)%
• Symbiotic plants	2.0	1.3	8.8	5.7	0.44	1.9	0.0022 (0.0018–0.0026)		0.11 (0.086–0.13)%	0.17 (0.13–0.20)%
• Associative	2.0	1.3	8.8	5.7	0.080	0.34	0.00096 (0.00040–0.0016)		0.047 (0.020–0.078)%	0.073 (0.030–0.12)%
Evergreen Broadleaf Forest	28	14	23	11	15	12	0.18 (0.066–0.33)		0.64 (0.24–1.2)%	1.3 (0.49–2.5)%
• Free-living	28	14	23	11	6.8	5.6	0.14 (0.034–0.28)		0.49 (0.12–1.0)%	1.0 (0.25–2.1)%
• Symbiotic plants	28	14	23	11	8.0	6.6	0.040 (0.032–0.048)		0.14 (0.12–0.17)%	0.30 (0.24–0.35)%
• Associative	28	14	23	11	0.11	0.092	0.0013 (0.0006–0.0022)		0.005 (0.002–0.008)%	0.010 (0.004–0.016)%
Deciduous Broadleaf Forest	3.5	1.9	12	6.7	1.8	6.1	0.016 (0.0075–0.027)		0.44 (0.21–0.76)%	0.80 (0.39–1.4)%
• Free-living	3.5	1.9	12	6.7	0.44	1.5	0.0088 (0.0022–0.018)		0.25 (0.062–0.52)%	0.45 (0.11–0.95)%
• Symbiotic plants	3.5	1.9	12	6.7	1.3	4.5	0.0065 (0.0052–0.0078)		0.18 (0.15–0.22)%	0.33 (0.27–0.40)%
• Associative	3.5	1.9	12	6.7	0.02	0.069	0.00024 (0.00010–0.00040)		0.007 (0.003–0.011)%	0.012 (0.005–0.021)%
Mixed Forest	4.4	2.7	9.8	5.9	1.9	4.2	0.024 (0.0085–0.045)		0.54 (0.19–1.0)%	0.89 (0.32–1.7)%
• Free-living	4.4	2.7	9.8	5.9	0.92	2.0	0.018 (0.0046–0.039)		0.42 (0.11–0.88)%	0.69 (0.17–1.5)%
• Symbiotic plants	4.4	2.7	9.8	5.9	0.90	2.0	0.0045 (0.0036–0.0054)		0.10 (0.082–0.12)%	0.17 (0.14–0.20)%
• Associative	4.4	2.7	9.8	5.9	0.05	0.11	0.00060 (0.00025–0.0010)		0.014 (0.006–0.023)%	0.023 (0.009–0.038)%
Savannas	31	17	11	6.3	17	6.1	0.19 (0.075–0.35)		0.61 (0.24–1.1)%	1.1 (0.43–2.0)%
• Free-living	31	17	11	6.3	6.7	2.4	0.13 (0.034–0.28)		0.43 (0.11–0.90)%	0.77 (0.19–1.6)%
• Symbiotic plants	31	17	11	6.3	9.4	3.4	0.047 (0.038–0.057)		0.15 (0.12–0.18)%	0.27 (0.22–0.32)%
• Associative	31	17	11	6.3	0.72	0.26	0.0086 (0.0036–0.014)		0.028 (0.012–0.046)%	0.050 (0.021–0.083)%
Shrublands	3.4	2.5	2.4	1.8	7.6	5.5	0.079 (0.035–0.14)		2.4 (1.0–4.1)%	3.2 (1.4–5.4)%
• Free-living	3.4	2.5	2.4	1.8	1.3	0.92	0.026 (0.0064–0.054)		0.76 (0.19–1.6)%	1.0 (0.26–2.2)%
• Symbiotic plants	3.4	2.5	2.4	1.8	3.2	2.3	0.016 (0.013–0.019)		0.47 (0.38–0.57)%	0.64 (0.51–0.76)%
• Associative	3.4	2.5	2.4	1.8	3.2	2.3	0.038 (0.016–0.063)		1.1 (0.47–1.9)%	1.5 (0.63–2.5)%
Natural Grasslands	13	7.8	5.6	3.4	16	7.0	0.21 (0.076–0.38)		1.6 (0.59–3.0)%	2.6 (0.97–4.9)%
• Free-living	13	7.8	5.6	3.4	5.8	2.5	0.12 (0.029–0.24)		0.90 (0.22–1.9)%	1.5 (0.37–3.1)%
• Symbiotic plants	13	7.8	5.6	3.4	4.9	2.1	0.025 (0.020–0.029)		0.19 (0.15–0.23)%	0.31 (0.25–0.38)%
• Associative	13	7.8	5.6	3.4	5.4	2.4	0.065 (0.027–0.11)		0.51 (0.21–0.84)%	0.83 (0.35–1.4)%
Barren	1.9	0.97	0.95	0.48	3.2	1.6	0.039 (0.016–0.066)		2.0 (0.83–3.5)%	4.0 (1.6–6.8)%
• Free-living	1.9	0.97	0.95	0.48	0.23	0.11	0.0046 (0.0012–0.0097)		0.24 (0.060–0.51)%	0.47 (0.12–0.99)%
• Symbiotic plants	1.9	0.97	0.95	0.48	0.19	0.095	0.0010 (0.0008–0.0011)		0.050 (0.040–0.060)%	0.098 (0.078–0.12)%
• Associative	1.9	0.97	0.95	0.48	2.8	1.4	0.033 (0.014–0.056)		1.8 (0.73–2.9)%	3.4 (1.4–5.7)%
Cultivated Pastures ^c	4.7	2.8	6.0	3.6	20	25	0.098 (0.078–0.12)		2.1 (1.7–2.5)%	3.5 (2.8–4.1)%
Croplands	12	6.3	9.4	4.8	36	27	0.22 (0.15–0.30)		1.7 (1.2–2.4)%	3.4 (2.3–4.7)%
• Free-living	12	6.3	9.4	4.8	2.3	1.7	0.046 (0.012–0.097)		0.37 (0.093–0.78)%	0.73 (0.18–1.5)%
• Symbiotic plants	12	6.3	9.4	4.8	34	26	0.17 (0.14–0.20)		1.4 (1.0–1.6)%	2.7 (2.1–3.2)%

^a GPP_{ecosys} and NPP_{ecosys} from the relevant land area are listed for all rows even though they only come from autotrophs. Per area rates are averages across the natural (non-agricultural) land area within each biome for all biomes except crops + crop/natural mosaic, which are averages across the (agricultural) land area in these biomes. Data sources were Reis Ely et al. (2025a, 2025b, 2025c) for BNF, Jung et al. (2020) for GPP, and Running and Zhao (2021) for NPP. ^bValues shown outside parentheses used the central C costs: 5, 12, and 20 g C per g N fixed for symbiotic plants, associative BNF, and free-living BNF, respectively (see Section 2 and Table S1 in Supporting Information S1). Values shown inside parentheses show ranges of results from using the low (4, 5, and 5 g C per g N fixed) and high (6, 20, and 42 g C per g N fixed) C costs for symbiotic plants, associative BNF, and free-living BNF, respectively. ^cCultivated pastures only have data for symbiotic plants. Croplands are a combination of the International Geosphere-Biosphere Programme (IGBP) biomes Croplands and Crop/Natural Mosaic (Friedl & Sulla-Menashe, 2019).

Table 4
Fractions of Symbiotic Plant GPP_{symb} and NPP_{symb} Invested in BNF, Split by Biome

Biome	GPP^a	NPP^a	GPP^a	NPP^a	BNF^a	BNF^a	% of GPP_{symb}^b	% of NPP_{symb}^b
	Pg C yr ⁻¹		Mg C ha ⁻¹ yr ⁻¹		Tg N yr ⁻¹	kg N ha ⁻¹ yr ⁻¹	% invested in BNF	
Evergreen Needleleaf Forest	0.051	0.031	0.22	0.13	0.44	1.9	4.3 (3.5–5.2)%	7.1 (5.7–8.5)%
Evergreen Broadleaf Forest	3.3	1.5	2.7	1.3	8.0	6.6	1.2 (0.98–1.5)%	2.6 (2.1–3.1)%
Deciduous Broadleaf Forest	0.30	0.12	1.0	0.43	1.3	4.5	2.2 (1.7–2.6)%	5.3 (4.2–6.3)%
Mixed Forest	0.16	0.10	0.37	0.22	0.90	2.0	2.7 (2.2–3.3)%	4.5 (3.6–5.4)%
Savannas	2.7	1.5	0.99	0.53	9.4	3.4	1.7 (1.4–2.1)%	3.2 (2.6–3.9)%
Shrublands	0.23	0.17	0.16	0.12	3.2	2.3	7.0 (5.6–8.4)%	9.5 (7.6–11)%
Natural Grasslands	0.93	0.57	0.41	0.25	4.9	2.1	2.6 (2.1–3.2)%	4.3 (3.5–5.2)%
Barren	0.010	0.0053	0.0052	0.0026	0.19	0.095	9.2 (7.4–11)%	18 (14–22)%
Cultivated Pastures	2.8	1.7	3.6	2.2	20	25	3.5 (2.8–4.2)%	5.8 (4.6–6.9)%
Cropland	3.7	1.8	2.8	1.4	34	26	4.6 (3.7–5.6)%	9.2 (7.4–11)%

^aBNF data were from Reis Ely et al. (2025a, 2025b, 2025c). ^bValues shown outside parentheses used the central C costs: 5, 12, and 20 g C per g N fixed for symbiotic plants, associative BNF, and free-living BNF, respectively (see Section 2 and Table S1 in Supporting Information S1). Values shown inside parentheses show ranges of results from using the low (4, 5, and 5 g C per g N fixed) and high (6, 20, and 42 g C per g N fixed) C costs for symbiotic plants, associative BNF, and free-living BNF, respectively.

larger fraction of NPP_{symb} was invested in BNF at mid and high latitudes (Table 5 and Figure 3c). This pattern was driven by shrubs and herbs, counteracting trees, which invested relatively more symbiotic N-fixing plant NPP in BNF at lower latitudes (Table 5 and Figure 3c). Symbiotic plants invested relatively more of their own NPP in BNF in less productive biomes (Figure 3d), similar to the ecosystem-level trend for all BNF (Figure 2c) but unlike the ecosystem-level trend for symbiotic BNF (Figure 2e). The reason the symbiosis-level (Figure 3d) and ecosystem-level (Figure 2e) trends across productivity differ for symbiotic BNF is that symbiotic N-fixing plants are relatively less abundant in low-productivity biomes, where biocrusts and mosses are relatively more common.

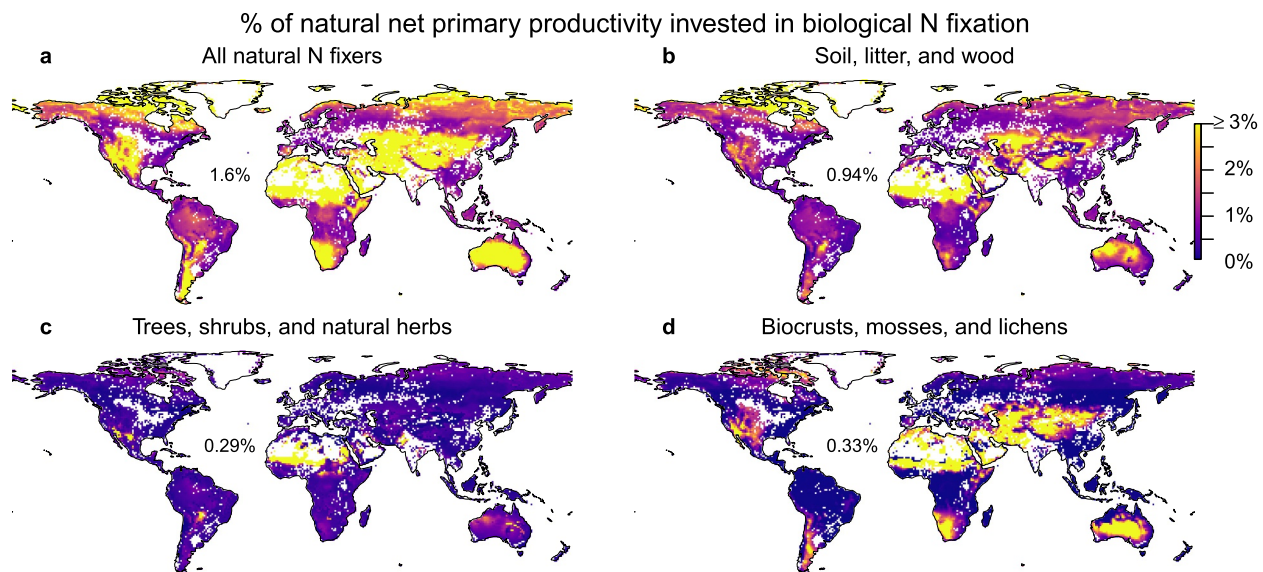


Figure 1. Global maps of ecosystem-scale carbon investment in natural biological N fixation (BNF). The color of each grid cell shows the percentage of ecosystem-scale net primary productivity (NPP_{ecosys}) invested in (a) all natural BNF, (b) free-living BNF (in soil, leaf litter, and decomposing wood), (c) symbiotic BNF (trees, shrubs, and natural herbs), and (d) associative BNF (biocrusts, mosses, and lichens). Numbers printed on each panel are the global values (Table 1). White grid cells have been masked out because they are aquatic, agricultural, or have negligible NPP (e.g., ice-covered areas and parts of the Sahara Desert). The color scale is linear but truncated for visibility (i.e., all values above 3 are shown as 3). All results in this figure used the central values of the C cost per unit N fixed (see Section 2, Table S1 in Supporting Information S1).

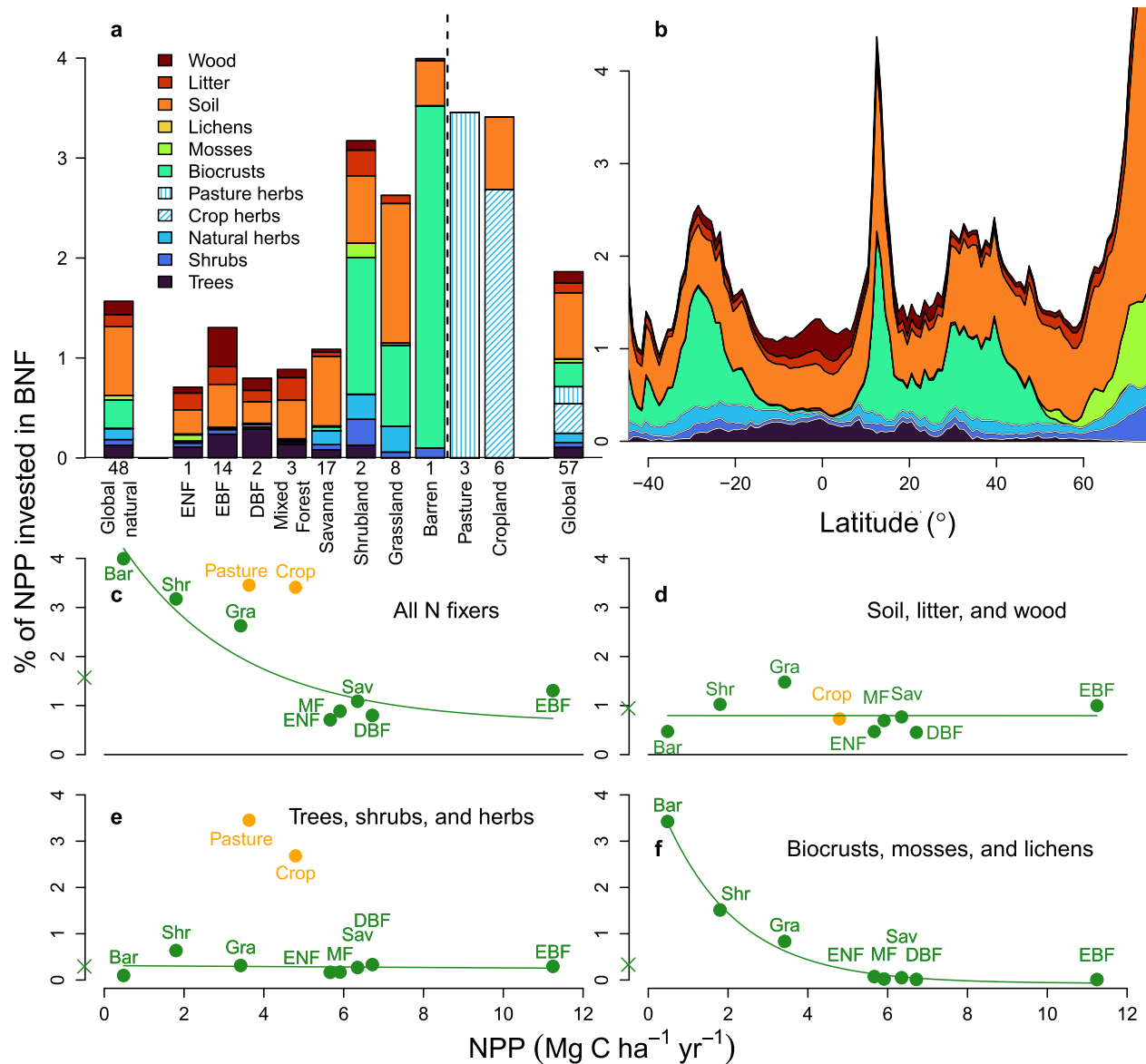


Figure 2. Ecosystem-scale carbon investment in BNF across terrestrial biomes, N-fixing niches, latitude, and productivity. Percentages of NPP_{ecosys} invested in BNF are shown across biomes (a) and latitude (b) separated by N-fixing niche, and across NPP_{ecosys} separated by biome and N-fixing niche grouping (c–f). Lichens are often so small that they are invisible. Biome-level NPP_{ecosys} fluxes ($Pg\ C\ yr^{-1}$; Table 3) are printed under each bar (a). Note that these are in different units than the per-area units in panels (c–f). Biome (Friedl & Sulla-Menashe, 2019) abbreviations (a) are: Evergreen Needleleaf Forest (ENF), Evergreen Broadleaf Forest (EBF), and Deciduous Broadleaf Forest (DBF). Bars to the left of the dashed line in panel (a) exclude agricultural biomes. The latitudinal display (b) omits agricultural biomes and latitudes for which there are fewer than 10^7 ha of land. Trends across NPP_{ecosys} are shown for (c) all N fixers, (d) free-living N fixers (in soil, leaf litter, and decomposing wood), (e) symbiotic plants (trees, shrubs, and herbs), and (f) associative N fixers (biocrusts, mosses, and lichens). The green curves (c, f) or lines (d, e) show regression fits to the natural biome values. Green Xs on the vertical axes show the global natural values (Table 1 and Figure 1). All results in this figure used the central values of the C cost per unit N fixed (see Section 2 and Table S1 in Supporting Information S1).

3.3. C Investment in Agricultural BNF

A higher fraction of NPP_{ecosys} was invested in BNF in agricultural biomes, 3.4%, than in natural biomes, 1.6% (Table 1 and Figure 2a), with similar fractions in cultivated pastures (3.5%) and crops (3.4%; Table 3, Figures 2b–2e, and Figure S5 in Supporting Information S1). The higher fractions in agricultural biomes were not due to free-living N fixers, which invested a slightly lower fraction of NPP in BNF in agricultural biomes (0.73%) versus in natural biomes (0.94%; Table 1), nor were they due to associative N fixers, for which we had no data in agricultural biomes (resulting in values of 0% vs. 0.33% in agricultural vs. natural biomes; Table 1). In fact, given

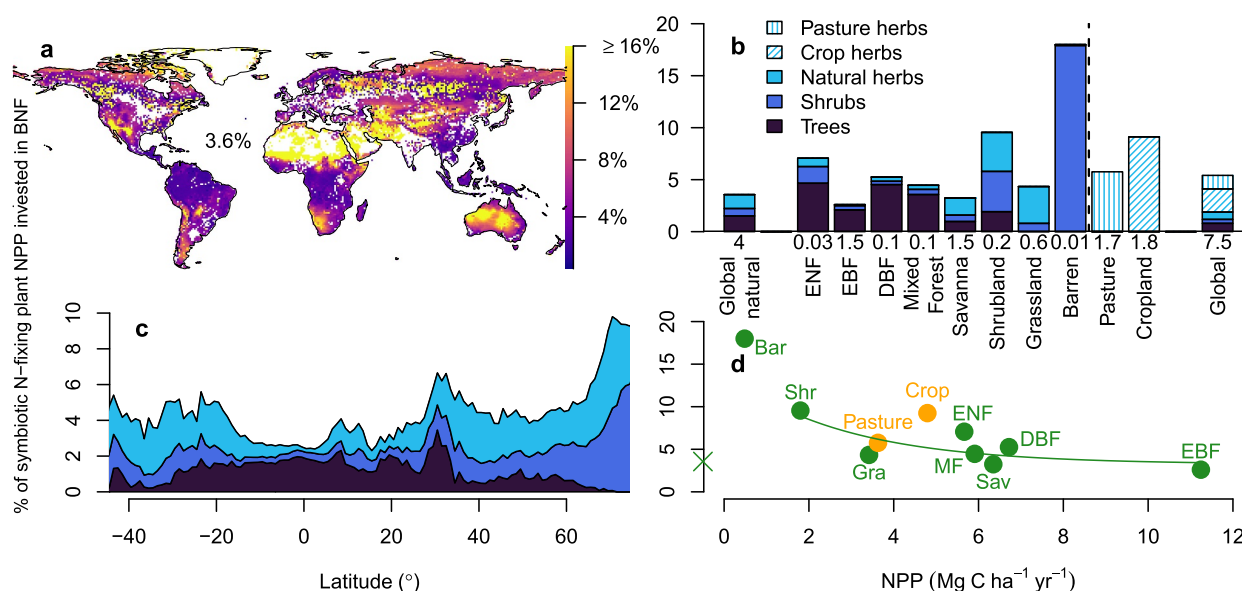


Figure 3. Investment of symbiotic N-fixing plants' own NPP (NPP_{symb}) in BNF. The percentages are shown across (a) one degree grid cells, (b) biomes and N-fixing niches, (c) latitude and N-fixing niches, and (d) NPP_{ecosys} . Agricultural plants are not shown in panels (a, c). The difference between this figure and Figures 1 and 2 is that the response variable in this figure is the fraction of productivity from symbiotic plants (NPP_{symb}) invested in BNF, whereas Figures 1 and 2 shows the fraction of productivity from all plants (NPP_{ecosys}) invested in BNF. The differences between the growth-form-specific values in panels (b, c) and the values in Table 2 are due to the fact that the values in panels (b, c) are fractions of total N-fixing plant NPP, whereas the values in Table 2 are fractions of N-fixing tree (or shrub or herb) NPP. The value for Barren in panels (b, d) is less reliable than the other values due to the extremely low per-area rates of NPP_{symb} and BNF in the Barren biome (Table 4), and thus is not included in the regression in panel (d), although it would strengthen the trend.

that our estimates for litter BNF (a subset of free-living BNF) and associative BNF in agricultural systems were 0% due to a lack of data, the disparity in total investment in BNF between agricultural and natural biomes is likely even greater than we reported.

Rather, the larger fractions we reported in agricultural biomes came from the 10× higher investment in BNF by symbiotic plants (2.9% in agricultural biomes vs. 0.29% in natural biomes; Table 1). This large disparity primarily reflected the higher abundance of symbiotic plants in agricultural systems due to intentional planting of legumes. All symbiotic BNF in crops and cultivated pastures come from legumes, all of which (for crops) or many of which (for cultivated pastures) were planted intentionally by humans. The secondary cause of higher investment in symbiotic BNF in agricultural systems, particularly in crops, was a greater fraction of symbiotic plants' own C (NPP_{symb}) allocated to BNF. N-fixing herbs in cultivated pastures and croplands invested 5.8% and 9.2% of their NPP_{symb} in BNF, respectively, compared to 4.3% in natural grasslands (Table 4 and Figures 3b, 3d). It was unclear whether the approximately two-fold higher investment of NPP_{symb} in BNF in crops resulted from humans selecting for high BNF rates, greater resource availability (e.g., water, light, nutrients), or some other reason.

Including agricultural investment in BNF in the global total raised the fraction of NPP_{ecosys} invested in BNF from 1.6% in natural systems to 1.9% overall (Table 1 and Figure 2a). This 19% increase in C investment due to agriculture is notable, but is far less than the near doubling of global BNF fluxes due to agriculture (65 Tg N yr⁻¹ natural BNF vs. 120 Tg N yr⁻¹ total) (Reis Ely et al., 2025a). The reason that agricultural BNF does not double the C investment in BNF is that nearly all agricultural BNF is symbiotic, which has a relatively low C cost, whereas the relatively more expensive free-living and associative BNF account for over half of natural BNF (Reis Ely et al., 2025a; Table 1).

The pioneering estimate of global energetic investment in BNF from the 1970s, 2.5% of GPP_{ecosys} (Gutschick, 1978), included agriculture. Gutschick's estimate was 2.5× our central estimate for the global total (1.0% of GPP_{ecosys} , corresponding to 1.9% of NPP_{ecosys} ; Table 1). Our lower value reflects more robust estimates of both BNF and GPP. Our BNF flux was lower than Gutschick's (120 vs. 140 Tg N yr⁻¹) but less weighted toward symbiotic (68% vs. 80%). We also used a lower C cost per N fixed for associative BNF (12 vs. 20 g C g N⁻¹; Table S1 in Supporting Information S1). However, these effects canceled out: the total C invested in BNF was 1.1 Pg

Table 5

Regressions for the Percentages of Ecosystem-Scale and Symbiotic Plant GPP and NPP Invested in BNF as Functions of the Absolute Value of Latitude

Response variable ^a	Equation ^b	Adj. R^2	P value
% $GPP_{ecosystem}$ invested in all natural BNF	$+0.016^a latitude + 0.56$	0.39	<0.0001
% $NPP_{ecosystem}$ invested in all natural BNF	$+0.012^a latitude + 1.4$	0.13	<0.0001
• % $GPP_{ecosystem}$ invested in natural free-living BNF	$+0.012^a latitude + 0.23$	0.55	<0.0001
• % $NPP_{ecosystem}$ invested in natural free-living BNF	$+0.011^a latitude + 0.68$	0.30	<0.0001
◦ % $GPP_{ecosystem}$ invested in natural soil BNF	$+0.012^a latitude + 0.11$	0.61	<0.0001
◦ % $NPP_{ecosystem}$ invested in natural soil BNF	$+0.013^a latitude + 0.40$	0.42	<0.0001
◦ % $GPP_{ecosystem}$ invested in leaf litter BNF	$+0.0013^a latitude + 0.031$	0.50	<0.0001
◦ % $NPP_{ecosystem}$ invested in leaf litter BNF	$+0.0011^a latitude + 0.084$	0.26	<0.0001
◦ % $GPP_{ecosystem}$ invested in decomposing wood BNF	$-0.0013^a latitude + 0.095$	0.46	<0.0001
◦ % $NPP_{ecosystem}$ invested in decomposing wood BNF	$-0.0030^a latitude + 0.20$	0.55	<0.0001
• % $GPP_{ecosystem}$ invested in natural symbiotic plant BNF	$+0.00089^a latitude + 0.14$	0.05	0.0052
• % $NPP_{ecosystem}$ invested in natural symbiotic plant BNF	$-0.00086^a latitude + 0.31$	0.03	0.029
◦ % $GPP_{ecosystem}$ invested in tree BNF	$-0.0015^a latitude + 0.10$	0.68	<0.0001
◦ % $NPP_{ecosystem}$ invested in tree BNF	$-0.0032^a latitude + 0.20$	0.74	<0.0001
◦ % $GPP_{ecosystem}$ invested in shrub BNF	$+0.0012^a latitude + 0.0071$	0.39	<0.0001
◦ % $NPP_{ecosystem}$ invested in shrub BNF	$+0.0013^a latitude + 0.031$	0.30	<0.0001
◦ % $GPP_{ecosystem}$ invested in natural herb BNF	$+0.0012^a latitude + 0.033$	0.25	<0.0001
◦ % $NPP_{ecosystem}$ invested in natural herb BNF	$+0.0010^a latitude + 0.080$	0.14	<0.0001
• % $GPP_{ecosystem}$ invested in associative BNF	$+0.0030^a latitude + 0.19$	0.06	0.0025
• % $NPP_{ecosystem}$ invested in associative BNF	$+0.0024^a latitude + 0.39$	<0.01	0.15
◦ % $GPP_{ecosystem}$ invested in biocrust BNF	$-0.00090^a latitude + 0.26$	<0.01	0.39
◦ % $NPP_{ecosystem}$ invested in biocrust BNF	$-0.0026^a latitude + 0.48$	<0.01	0.14
◦ % $GPP_{ecosystem}$ invested in moss BNF	$+0.0039^a latitude - 0.072$	0.41	<0.0001
◦ % $NPP_{ecosystem}$ invested in moss BNF	$+0.0050^a latitude - 0.088$	0.41	<0.0001
◦ % $GPP_{ecosystem}$ invested in epiphytic lichen BNF	$+0.00002^a latitude + 0.0008$	0.11	<0.0001
◦ % $NPP_{ecosystem}$ invested in epiphytic lichen BNF	$+0.00001^a latitude + 0.0019$	0.02	0.083
• % $GPP_{symbiosis}$ invested in natural symbiotic plant BNF	$+0.053^a latitude + 0.96$	0.67	<0.0001
• % $NPP_{symbiosis}$ invested in natural symbiotic plant BNF	$+0.049^a latitude + 2.8$	0.48	<0.0001
◦ % $GPP_{symbiosis}$ invested in tree BNF	$-0.0094^a latitude + 0.97$	0.34	<0.0001
◦ % $NPP_{symbiosis}$ invested in tree BNF	$-0.024^a latitude + 2.0$	0.48	<0.0001
◦ % $GPP_{symbiosis}$ invested in shrub BNF	$+0.029^a latitude - 0.14$	0.69	<0.0001
◦ % $NPP_{symbiosis}$ invested in shrub BNF	$+0.035^a latitude + 0.010$	0.66	<0.0001
◦ % $GPP_{symbiosis}$ invested in natural herb BNF	$+0.033^a latitude + 0.13$	0.65	<0.0001
◦ % $NPP_{symbiosis}$ invested in natural herb BNF	$+0.038^a latitude + 0.64$	0.61	<0.0001

^aThese equations use the central C costs: 5, 12, and 20 g C per g N fixed for symbiotic plants, associative BNF, and free-living BNF, respectively (Table S1 in Supporting Information S1). ^bWe weighted the datapoints by the land area in each degree of latitude; unweighted regressions yielded similar results.

C yr⁻¹ in both our calculation (Table 1) and Gutschick's calculation (Gutschick, 1978). Therefore, the primary reason for the difference was our higher GPP_{ecosys} value (104 vs. 44 Pg C yr⁻¹).

The CLM4.0-FUN2.0 and ELM-FUN3.0 model-based estimates of C investment in BNF also included agricultural BNF, though they only reported C investment in symbiotic BNF (Braghiere et al., 2022; Shi et al., 2016). The global estimate from CLM4.0-FUN2.0, 0.40 Pg C yr⁻¹ (0.79% of NPP) spent on symbiotic BNF (Shi

et al., 2016), was quite similar to our values of $0.41 \text{ Pg C yr}^{-1}$ and 0.71% of NPP spent on symbiotic BNF (Table 1). However, according to our data, the majority of C investment in symbiotic BNF came from agricultural N fixers ($0.27 \text{ Pg C yr}^{-1}$), whereas in CLM, as in other terrestrial biosphere models, the large majority of BNF came from natural symbiotic N fixers (Kou-Giesbrecht et al., 2025). In FUN, BNF is down-regulated when N supply is high (Fisher et al., 2010), such as in heavily fertilized agricultural soils. Our results, along with other papers using the same BNF data set (Kou-Giesbrecht et al., 2025; Reis Ely et al., 2025a, 2025b, 2025c), highlight that planting legumes leads to high rates of BNF, which has a major impact on the global C and N cycles. The ELM-FUN3.0 model had orders of magnitude lower overall C investment in symbiotic BNF ($0.0008 \text{ Pg C yr}^{-1}$, 0.002% of NPP) (Braghiere et al., 2022). In ELM-FUN3.0, which also includes the possibility of P limitation, nearly all plant C allocated to N uptake is spent on direct root uptake and mycorrhizal uptake, and nearly all BNF is nonsymbiotic (Braghiere et al., 2022).

3.4. Fraction of Plant N Acquisition From BNF

To address our final question, we divided BNF by plant N acquisition, for both ecosystem-scale N acquisition and symbiotic plants' own N acquisition. We calculated plant N acquisition (at both scales) in each grid cell as the N needed to support NPP—calculated from NPP; allocation to leaves, stems, coarse roots, and fine roots; and the C:N ratio of each tissue—minus N resorption (Cleveland et al., 2013; see Section 2 for details). At the global scale, BNF directly supplied 3.1% of ecosystem-scale N acquisition in natural biomes (Table 6 and Figures 4a, 4c). The fraction was nearly an order of magnitude higher (26%) in agricultural biomes, raising the global total to 7.3% of ecosystem-scale N acquisition (Table 6 and Figure 4c). The nearly 10× higher fraction in agricultural biomes was driven by the same factors as the 10× higher relative C investment in symbiotic BNF: the high abundance of legumes in agriculture and a 2–3× higher fraction of N fixers' own N acquisition from BNF (47% in cultivated pastures and 75% in croplands vs. 24% in natural systems; Table 6). In general, the patterns of the fraction of plant N acquisition from BNF across biomes, latitude, and productivity (Figure 4) were similar to the analogous patterns of plant C investment in BNF (Figures 1–3).

The percent of ecosystem-scale N acquisition from BNF in natural biomes generated in our analysis was lower than many model-based estimates. According to the values in Cleveland et al. (2013), 13% of ecosystem-scale N acquisition came from BNF in natural biomes, fourfold greater than the value here (3.1%; Table 6), with particularly large differences in savannas, deciduous broadleaf forest, and evergreen broadleaf forest (Table 6). Studies using the terrestrial biosphere models LM3-SNAP (Sulman et al., 2019) and CLASSIC (Kou-Giesbrecht & Arora, 2022) estimated that 5.0% (Sulman et al., 2019) and 3.2% (Kou-Giesbrecht & Arora, 2022) of natural global ecosystem-scale N acquisition came from BNF, closer to the 3.1% in our results. Xu-Ri and Prentice (2017) took a different approach, modeling the new nitrogen needed by plants after soil N supply runs out in the terrestrial biosphere model DyN-LPJ. For natural, unpolluted systems, which were the focus of their study, this new nitrogen comes mostly but not entirely from BNF. Their model estimated that 25%–30% of global ecosystem-scale N acquisition came from new nitrogen (Xu-Ri & Prentice, 2017), nearly an order of magnitude higher than the 3.1% from BNF we report here for natural ecosystems. A study using CLM4.0-FUN2.0 (Shi et al., 2016) estimated 6.7% of global N acquisition came from symbiotic BNF. Given that CLM4.0-FUN2.0 included agriculture, our comparable value (for all sources of BNF) is 7.3%, which is quite close. As above, though, we note that in CLM, the fraction from BNF was lower in agriculture than in natural systems (Kou-Giesbrecht et al., 2025), whereas in our data the fraction from BNF was much higher in agriculture (26%) than in natural systems (3.1%). Braghiere et al. (2022), using ELM-FUN3.0, reported nearly 0% of global N acquisition from symbiotic BNF, including agriculture, well under our values for all systems (7.3%) and natural systems (3.1%). All of these other studies used modeled estimates of symbiotic BNF, whereas our rates were based on BNF measurements (Reis Ely et al., 2025a).

In addition to ecosystem-scale plant N acquisition, we also quantified the fraction of symbiotic N-fixing plants' own N acquisition that came from BNF, which is conceptually analogous to isotopic measurements of $\%N_{\text{dfa}}$ (Shearer & Kohl, 1986), but at larger scales (grid cell, biome, and globe) than the individual plant-based measurements of $\%N_{\text{dfa}}$. Globally, 24% of natural symbiotic plants' N acquisition came from BNF, with more at higher latitudes and in biomes with lower N acquisition (Table 6 and Figures 4b, 4d, 4f, 4h). The results for the barren biome were unreliable due to the extremely low symbiotic plant N acquisition and BNF fluxes (Table 6 and Figures 4b, 4d). Symbiotic plants in cultivated pastures and croplands derived 47% and 75% of their N acquisition from BNF, respectively, raising the global average from 24% for natural symbiotic plants to 40% for all symbiotic

Table 6
Fractions of Plant N Acquisition Supplied by Symbiotic Plant BNF

Biome	N acquisition BNF ^a		N acquisition BNF ^a		% acquisition from BNF	
	Tg N yr ⁻¹		kg N ha ⁻¹ yr ⁻¹		This study	Cleveland et al. (2013)
Total (ecosystem scale)	1127	82	88	6.4	7.3%	N/A
• Natural (ecosystem scale)	925	28	87	2.7	3.1%	13%
◦ Evergreen Needleleaf Forest	13	0.44	55	1.9	3.5%	1.1%
◦ Evergreen Broadleaf Forest	289	8.0	241	6.6	2.8%	11%
◦ Deciduous Broadleaf Forest	33	1.3	115	4.5	3.9%	20%
◦ Mixed Forest	29	0.90	65	2.0	3.1%	4.6%
◦ Savanna	371	9.4	135	3.4	2.5%	24%
◦ Shrublands	43	3.1	31	2.3	7.4%	4.5%
◦ Natural Grasslands	128	4.9	56	2.1	3.8%	10%
◦ Barren	17	0.19	8.3	0.095	1.1%	N/A
• Agricultural (ecosystem scale)	202	54	96	25	26%	N/A
◦ Cultivated Pastures	47	20	60	25	42%	N/A
◦ Croplands + Crop/Natural Mosaic	156	34	118	26	22%	N/A
Total (symbiosis scale)	206	82	16	6.4	40%	
• Natural (symbiosis scale)	119	28	11	2.7	24%	
◦ Evergreen Needleleaf Forest	0.45	0.44	1.9	1.9	98%	
◦ Evergreen Broadleaf Forest	49	8.0	41	6.6	16%	
◦ Deciduous Broadleaf Forest	3.2	1.3	11	4.5	41%	
◦ Mixed Forest	1.7	0.90	3.7	2.0	54%	
◦ Savanna	46	9.4	17	3.4	20%	
◦ Shrublands	4.3	3.2	3.1	2.3	74%	
◦ Natural Grasslands	14	4.9	6.1	2.1	35%	
◦ Barren ^b	0.14	0.19	0.068	0.095	140% ^b	
• Agricultural (symbiosis scale)	87	54	41	25	61%	
◦ Cultivated Pastures	42	20	54	25	47%	
◦ Croplands + Crop/Natural Mosaic	45	34	34	26	75%	

^aBNF data were from Reis Ely et al. (2025a, 2025b, 2025c). ^b% of N acquisition from BNF is unreliable for the Barren biome due to the extremely low symbiotic plant N acquisition and BNF fluxes.

plants. For comparison, field-based isotopic measurements of %N_{dff} for natural symbiotic herbs average 84%–89% in forested biomes and 52%–54% in other biomes (Reis Ely et al., 2025a). Field-derived averages from agricultural legumes range across crop and country from 44% to 78% (Peoples et al., 2021; Reis Ely et al., 2025a). Field-based %N_{dff} values from woody plants can range from 0% to nearly 100% within individual studies (Menge et al., 2023a), but we are unaware of an estimate of the global average for woody plants, so it is difficult to compare our results to other values in the literature. Isotopic estimates have well-established challenges (Shearer & Kohl, 1986; Soper, Taylor, et al., 2021), so these values from the literature provide a comparison to our estimates rather than a validation.

4. Discussion

Our results highlight the importance of BNF in high latitude and low productivity regions of the world, consistent with the view that higher latitudes are strongly N limited (Du et al., 2020). This is analogous to Engel's law in economics, which shows that higher proportions of income (analogous to productivity) are spent on food (analogous to BNF) when income (productivity) is lower (Chai & Moneta, 2010). At the ecosystem scale, this

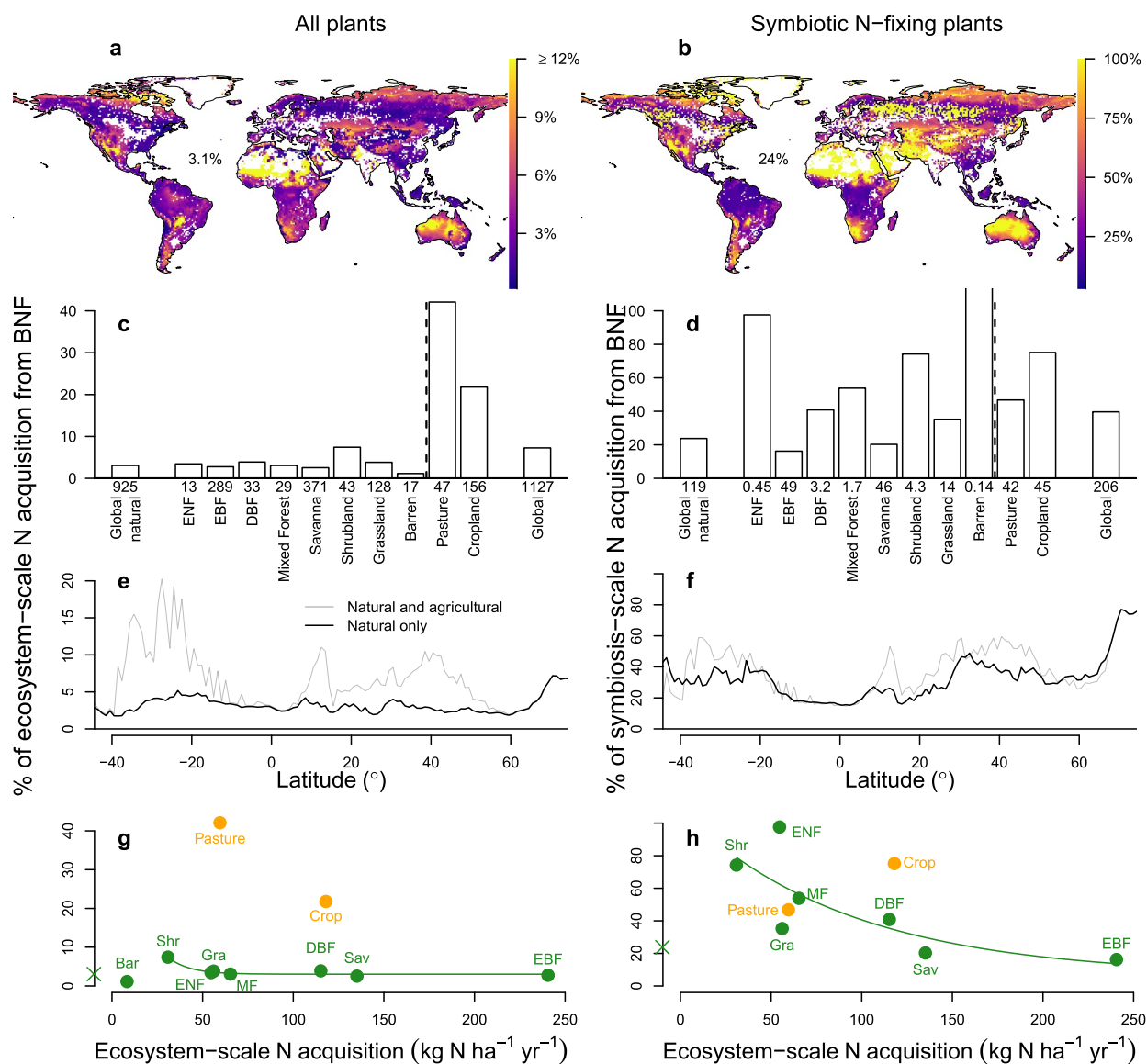


Figure 4. Percentages of plant N acquisition supplied by BNF. N acquisition is the new N (e.g., soil uptake or BNF) needed to support productivity. (a, b) Maps, (c, d) biome-level distributions, (e, f) latitudinal distributions, and (g, h) trends across ecosystem-scale N acquisition are shown for the percentage of (a, c, e, g) ecosystem-scale N acquisition and (b, d, f, h) symbiotic plant N acquisition from BNF. The color scale of (a) is linear but truncated. Only natural plants are shown in (a, b) and to the left of the dashed vertical lines in (c, d). Numbers below bars show Tg N yr⁻¹ of (c) ecosystem-scale and (d) symbiotic plant N acquisition in each biome. The Barren value in (d, h) extends above the scale, but is less reliable than the other values due to the extremely low per-area rates of symbiotic plant N acquisition and BNF (Table 6), and thus is not included in regressions (g, h). Including the Barren values would weaken the regression in panel (g) and strengthen the regression in panel (h). In panels (e, f), only latitudes with at least 10⁷ ha of land area are shown. The panels are analogous to Figure 1c (a); Figure 3a (b); the combined blue patterns in Figure 2a (c), Figure 3b (d), Figure 2b (e), Figure 3c (f); Figure 2e (g); and Figure 3d (h).

result likely reflects the effects of cold temperatures, aridity, low litter quality, incipient soil development, and other factors that constrain decomposition, soil N mineralization, and soil N availability (Davidson & Janssens, 2006; Liu et al., 2017), as well as the temporal asynchrony between N supply and N demand in many of these systems, which can promote N losses (Augustine & McNaughton, 2004; Dijkstra et al., 2012; Khosh et al., 2017). Relaxing our assumption that the C cost per N fixed is constant within each N-fixing niche would likely strengthen this conclusion, given that BNF is more costly at low temperatures (Gutschick, 1982; Ruess et al., 2013). Additional research into C cost values and how they vary is clearly needed.

The patterns we observed across different N-fixing niches add critical context to a long-standing question in ecology: why, given the prevalence of N limitation outside the tropics, are N-fixing trees much rarer there than in

tropical biomes (Menge, Batterman, Hedin, et al., 2017; Menge, Batterman, Liao, et al., 2017; Vitousek & Howarth, 1991)? Our results confirm that N-fixing trees invest proportionally more C in BNF in the tropics, consistent with the pattern observed for N-fixing tree abundance. However, our results show that other N-fixing niches—shrubs, herbs, mosses, and soil—show the opposite latitudinal pattern, and that the strength of their latitudinal trends outweighs the latitudinal trend in N-fixing trees. When all N-fixing niches are considered collectively, larger fractions of ecosystem-scale and symbiotic N-fixing plant productivity are invested in BNF at higher latitudes, as expected given the prevalence of N limitation, resolving the apparent paradox (Menge, Batterman, Hedin, et al., 2017; Menge, Batterman, Liao, et al., 2017; Vitousek & Howarth, 1991). These findings suggest the need to study why the dominant symbiotic N-fixing plants switch from trees to shrubs and herbs at higher latitudes, and why the importance of associative BNF relative to symbiotic BNF increases at higher latitudes and at lower productivity.

Our results also advance our understanding of the degree to which human modifications of the global N cycle (Reis Ely et al., 2025a; Vitousek et al., 1997, 2013) affect the global C cycle (Jensen et al., 2012; Menegat et al., 2022). The production and transportation of N fertilizers releases $0.13 \text{ Pg CO}_2\text{-C yr}^{-1}$ ($0.47 \text{ Pg CO}_2 \text{ yr}^{-1}$; Menegat et al., 2022), accounting for 1.1% of anthropogenic CO_2 emissions (Friedlingstein et al., 2025). This amount is notable, but our results suggest that CO_2 emissions from agricultural BNF are approximately twice as high (assuming that most C invested in BNF is respired). Although the global N input flux from agricultural legume BNF is approximately half the flux from synthesizing fertilizers (54 vs. 105 Tg N yr^{-1}) (Reis Ely et al., 2025a), the higher C cost per N fixed for legume BNF compared to the Haber-Bosch process means that agricultural legumes accounted for $0.27 \text{ Pg C yr}^{-1}$ (Table 1; cf. Jensen et al., 2012). Subtracting the BNF-related CO_2 emissions that would have occurred naturally in regions that are now croplands and pasture lands (using the per-area BNF rate of herbs in natural grasslands) lowers the anthropogenic contribution of agricultural legumes only slightly, to $0.25 \text{ Pg C yr}^{-1}$, which is approximately twice the C flux from the production and transportation of N fertilizers. We want to be clear that the comparison of CO_2 emissions stemming directly from agricultural BNF versus the Haber-Bosch process, though intriguing, is only a small part of the overall environmental implications of these N fixation processes. Importantly, legume-based BNF recycles recently photosynthesized C rather than fossil C (Jensen et al., 2012) and has other environmental advantages over synthetic fertilizers, including stimulating soil C storage and reducing N_2O emissions (Crews & Peoples, 2005; Jensen et al., 2012; Menegat et al., 2022). The full accounting of CO_2 and C-N interactions is complicated, but nonetheless, our results emphasize that agricultural BNF plays a significant role in the global C cycle.

Finally, our results are valuable for terrestrial biosphere models, which have identified interactions between BNF and C as an area in need of improvement (Davies-Barnard et al., 2020; Kou-Giesbrecht & Arora, 2022; Kou-Giesbrecht et al., 2023, 2025; Meyerholt et al., 2020). One of the ways that terrestrial biosphere models incorporate BNF is by modeling BNF as a function of NPP (Kou-Giesbrecht et al., 2023, 2025). Some models assume that the fraction of NPP allocated to BNF is constant, whereas others assume that the fraction of NPP allocated to BNF declines with NPP (Kou-Giesbrecht et al., 2023). We show that, at the biome scale, the fraction of ecosystem-scale NPP allocated to BNF is constant for symbiotic and free-living BNF but declines with productivity for associative BNF and total BNF. Furthermore, we show exactly how the fraction of NPP allocated to BNF varies across space (latitude and grid cells), biomes, and N-fixing niches. Given the starkly different patterns we observed between N-fixing niches, and given that the controls on BNF differ across N-fixing niches (Cleveland et al., 2022; Menge & Hedin, 2009; Reed et al., 2008), it is a promising sign that some terrestrial biosphere models are starting to distinguish between symbiotic BNF and free-living BNF (e.g., Kou-Giesbrecht et al., 2023). Whereas some models represent BNF as a function of NPP, other models allow C investment in symbiotic BNF to vary as a function of the relative costs of BNF versus soil N acquisition (Braghiere et al., 2022; Fisher et al., 2010; Kou-Giesbrecht et al., 2023, 2025; Shi et al., 2016). These models show that flexible C investment in BNF can overcome N limitation to CO_2 fertilization (Kou-Giesbrecht & Arora, 2022; Sulman et al., 2019), but they produce remarkably high C investment in BNF at elevated CO_2 (Kou-Giesbrecht et al., 2025), and they rarely distinguish between different N-fixing niches (but see Braghiere et al., 2022; Kou-Giesbrecht & Arora, 2022; Kou-Giesbrecht et al., 2023). Our results provide benchmarks for C investment in BNF, both overall and across N-fixing niches. Given that our results directly address current challenges in modeling BNF and C-N interactions, they provide opportunities to refine future projections of climate change.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Availability Statement

Code for this manuscript is published at Dryad (Menge, 2026): <https://doi.org/10.5061/dryad.tqjq2bwbn>. All the data used in this manuscript are from Jung et al. (2020) (data at <https://www.bgc-jena.mpg.de/geodb/projects/Ho-me.php>), Menge et al. (2015, 2023a, 2024) (data publications at Menge et al., 2023b; Menge, 2024), Reis Ely et al. (2025a) (data publication at Reis Ely et al., 2025c), Reis Ely et al. (2025b), and Running and Zhao (2021). Some of the data in Reis Ely et al. (2025a, 2025c) originate from Bruehlheide et al. (2019), FAO (2021), Piipponen et al. (2022), or Steidinger et al. (2019). Some of the data are typed directly in our code, originating from Cleveland et al. (2013) and a synthesis of multiple sources on carbon costs of biological N fixation (see Section 2).

Acknowledgments

This research was supported in part by an appointment to the USGS Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the U.S. Department of Interior (DOI). The views and conclusions in this article represent those of the authors from ORAU/ORISE, USFS and UESPA. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. Funding was provided by the United States Geological Survey Powell Center Grant (G19AC00036 to SSP, DNLM, SCR, CCC), the National Science Foundation (DEB-1457650 to DNLM, SSP; DEB-1457444 to JLF; IOS-2129542 to DNLM, KLG), the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (Grant 819202) (JP), the FEDER/Ministerio de Ciencia e Innovación-Agencia Estatal de Investigación (Spain) PID2021-127631NA-I00 and RYC2020-030762-I (ERC), the U.S. Department of Energy Biological Environmental Research Program, NGEE-Arctic (VGS), and the Natural Environment Research Council Grants NE/M019497/1 and NE/S009663/1 (SAB). We thank Michelle Wong and two peer reviewers for useful feedback.

References

- Abed, R. M., Al Kharusi, S., Schramm, A., & Robinson, M. D. (2010). Bacterial diversity, pigments and nitrogen fixation of biological desert crusts from the Sultanate of Oman. *FEMS Microbiology Ecology*, 72(3), 418–428. <https://doi.org/10.1111/j.1574-6941.2010.00854.x>
- Adams, M. A., Turnbull, T. L., Sprent, J. I., & Buchmann, N. (2016). Legumes are different: Leaf nitrogen, photosynthesis, and water use efficiency. *Proceedings of the National Academy of Sciences*, 113(15), 4098–4103. <https://doi.org/10.1073/pnas.1523936113>
- Atkins, C. A. (1984). Efficiencies and inefficiencies in the legume-Rhizobium symbiosis. *Plant and Soil*, 82(3), 273–284. <https://doi.org/10.1007/BF02184267>
- Augustine, D. J., & McNaughton, S. J. (2004). Temporal asynchrony in soil nutrient dynamics and plant production in a semiarid ecosystem. *Ecosystems*, 7(8), 829–840. <https://doi.org/10.1007/s10021-004-0253-1>
- Batterman, S. A., Hedin, L. O., van Breugel, M., Ransijn, J., Craven, D. J., & Hall, J. S. (2013). Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature*, 502(7470), 224–227. <https://doi.org/10.1038/nature12525>
- Boote, K. J., Hoogenboom, G., Jones, J. W., & Ingram, K. T. (2008). Modeling nitrogen fixation and its relationship to nitrogen uptake in the CROPGRO model. In *Quantifying and understanding plant nitrogen uptake for systems modeling*. CRC Press. <https://doi.org/10.1201/9781420052978>
- Braghiere, R. K., Fisher, J. B., Allen, K., Brzostek, E., Shi, M., Yang, X., et al. (2022). Modeling global carbon costs of plant nitrogen and phosphorus acquisition. *Journal of Advances in Modeling Earth Systems*, 14(8), e2022MS003204. <https://doi.org/10.1029/2022MS003204>
- Brauer, V. S., Stomp, M., Rosso, C., van Beusekom, S. A. M., Emmerich, B., Stal, L. J., & Huisman, J. (2013). Low temperature delays timing and enhances the cost of nitrogen fixation in the unicellular cyanobacterium *Cyanothece*. *ISME Journal*, 7(11), 2105–2115. <https://doi.org/10.1038/ismej.2013.103>
- Bruehlheide, H., Dengler, J., Jiménez-Alfaro, B., Purschke, O., Kennekens, S. M., Chytrý, M., et al. (2019). sPlot – A new tool for global vegetation analyses. *Journal of Vegetation Science*, 30(2), 161–186. <https://doi.org/10.1111/jvs.12710>
- Carreras Pereira, K. A., Wolf, A. A., Kou-Giesbrecht, S., Akana, P. R., Funk, J. L., & Menge, D. N. L. (2023). Allometric relationships for eight species of 4–5 year old nitrogen-fixing and non-fixing trees. *PLoS ONE*, 18(8), e0289679. <https://doi.org/10.1371/journal.pone.0289679>
- Chai, A., & Moneta, A. (2010). Retrospectives: Engel's curves. *The Journal of Economic Perspectives*, 24(1), 225–240. <https://doi.org/10.1257/jep.24.1.225>
- Chari, N. R., Tumber-Dávila, S. J., Phillips, R. P., Bauerle, T. L., Brunn, M., Hafner, B. D., et al. (2024). Estimating the global root exudate carbon flux. *Biogeochemistry*, 167(7), 895–908. <https://doi.org/10.1007/s10533-024-01161-z>
- Cheib, A., Waring, E. F., McNellis, R., Perkowski, E. A., Martina, J. P., Seabloom, E. W., et al. (2025). Soil nitrogen supply exerts largest influence on leaf nitrogen in environments with the greatest leaf nitrogen demand. *Ecology Letters*, 28(1), e70015. <https://doi.org/10.1111/ele.70015>
- Cleveland, C. C., Houlton, B. Z., Smith, W. K., Marklein, A. R., Reed, S. C., Parton, W., et al. (2013). Patterns of new versus recycled primary production in the terrestrial biosphere. *Proceedings of the National Academy of Sciences*, 110(31), 12733–12737. <https://doi.org/10.1073/pnas.1302768110>
- Cleveland, C. C., Reis, C. R. G., Perakis, S. S., Dynarski, K. A., Batterman, S. A., Crews, T. E., et al. (2022). Exploring the role of cryptic nitrogen fixers in terrestrial ecosystems: A frontier in nitrogen cycling research. *Ecosystems*, 25(8), 1653–1669. <https://doi.org/10.1007/s10021-022-00804-2>
- Cleveland, C. C., Townsend, A. R., Schimel, D. S., Fisher, H., Howarth, R. W., Hedin, L. O., et al. (1999). Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. *Global Biogeochemical Cycles*, 13(2), 623–645. <https://doi.org/10.1029/1999GB900014>
- Collalti, A., & Prentice, I. C. (2019). Is NPP proportional to GPP? Waring's hypothesis 20 years on. *Tree Physiology*, 39, 1473–1483. <https://doi.org/10.1093/treephys/tpz034>
- Cooper, J. E., & Scherer, H. W. (2012). Nitrogen fixation. In P. Marschner (Ed.), *Marschner's mineral nutrition of higher plants* (3rd ed., pp. 389–408). Elsevier. <https://doi.org/10.1016/C2009-0-63043-9>
- Crews, T. E., & Peoples, M. B. (2005). Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems? *Nutrient Cycling in Agroecosystems*, 72(2), 101–120. <https://doi.org/10.1007/s10705-004-6480-1>
- Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, 440(7081), 165–173. <https://doi.org/10.1038/nature04514>
- Davies-Barnard, T., & Friedlingstein, P. (2020). The global distribution of biological nitrogen fixation in terrestrial natural ecosystems. *Global Biogeochemical Cycles*, 34(3), e2019GB006387. <https://doi.org/10.1029/2019GB006387>
- Davies-Barnard, T., Meyerholt, J., Zaehle, S., Friedlingstein, P., Brovkin, V., Fan, Y., et al. (2020). Nitrogen cycling in CMIP6 land surface models: Progress and limitations. *Biogeosciences*, 17(20), 5129–5148. <https://doi.org/10.5194/bg-17-5129-2020>
- Dijkstra, F. A., Augustine, D. J., Brewer, P., & von Fischer, J. C. (2012). Nitrogen cycling and water pulses in semiarid grasslands: Are microbial and plant processes temporally asynchronous? *Oecologia*, 170(3), 799–808. <https://doi.org/10.1007/s00442-012-2336-6>

- Du, E., Terrer, C., Pellegrini, A. F. A., Ahlstrom, A., van Lissa, C. J., Zhao, X., et al. (2020). Global patterns of terrestrial nitrogen and phosphorus limitation. *Nature Geoscience*, 13(3), 221–226. <https://doi.org/10.1038/s41561-019-0530-4>
- FAO, FAOSTAT. (2021). *License*. CC BY-NC-SA 3.0 IGO. Retrieved from <https://www.fao.org/faostat/en/#data>
- Farquhar, G. D., von Caemmerer, S., & Berry, J. A. (1980). A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta*, 149(1), 78–90. <https://doi.org/10.1007/BF00386231>
- Fisher, J. B., Sitch, S., Malhi, Y., Fisher, R. A., Huntingford, C., & Tan, S.-Y. (2010). Carbon cost of plant nitrogen acquisition: A mechanistic, globally applicable model of plant nitrogen uptake, retranslocation, and fixation. *Global Biogeochemical Cycles*, 24(1), GB1014. <https://doi.org/10.1029/2009GB003621>
- Friedl, M. A., & Sulla-Menashe, D. (2019). MCD12Q1 MODIS/Terra+Aqua land cover type yearly L3 global 500m SIN grid V006 [Dataset]. *NASA EOSDIS Land Processes Distributed Active Archive Center*. <https://doi.org/10.5067/MODIS/MCD12Q1.006>
- Friedlingstein, P., O'Sullivan, M., Jones, M. W., Andrew, R. M., Hauck, J., Landschützer, P., et al. (2025). Global carbon budget 2024. *Earth System Science Data*, 17(3), 965–1039. <https://doi.org/10.5194/essd-17-965-2025>
- Fyllas, N. M., Patiño, S., Baker, T. R., Bielefeld Nardoto, G., Martinelli, L. A., Quesada, C. A., et al. (2009). Basin-wide variations in foliar properties of Amazonian forest: Phylogeny, soils and climate. *Biogeosciences*, 6(11), 2677–2708. <https://doi.org/10.5194/bg-6-2677-2009>
- Garnier, S., Ross, N., Rudis, R., Camargo, A. P., Sciani, M., & Scherer, C. (2023). viridis(Lite)—Colorblind-Friendly Color Maps for R. viridis package version 0.6.4. Retrieved from <https://CRAN.R-project.org/package=viridis>
- Groß, C., Hossen, S., Hartmann, H., Noll, M., & Borken, W. (2022). Biological nitrogen fixation and nifH gene abundance in deadwood of 13 different tree species. *Biogeochemistry*, 161(3), 353–371. <https://doi.org/10.1007/s10533-022-00986-w>
- Großkopf, T., & LaRoche, J. (2012). Direct and indirect costs of dinitrogen fixation in *Crocospaera watsonii* WH8501 and possible implications for the nitrogen cycle. *Frontiers in Microbiology*, 3, 236. <https://doi.org/10.3389/fmicb.2012.00236>
- Gutschick, V. P. (1978). Energy and nitrogen fixation. *BioScience*, 28(9), 571–575. <https://doi.org/10.2307/1307512>
- Gutschick, V. P. (1981). Evolved strategies in nitrogen acquisition by plants. *American Naturalist*, 118(5), 607–637. <https://doi.org/10.1086/283858>
- Gutschick, V. P. (1982). Energetics of microbial fixation of dinitrogen. In *Microbes and engineering aspects* (pp. 109–168). Springer-Verlag. <https://doi.org/10.1007/3-540-11019-4>
- Hawkins, H. J., Cargill, R. I. M., Van Nuland, M. E., Hagen, S. C., Field, K. J., Sheldrake, M., et al. (2023). Mycorrhizal mycelium as a global carbon pool. *Current Biology*, 33(11), R560–R573. <https://doi.org/10.1016/j.cub.2023.02.027>
- Herridge, D. F., Peoples, M. B., & Boddy, R. M. (2008). Global inputs of biological nitrogen fixation in agricultural systems. *Plant and Soil*, 311, 1–18. <https://doi.org/10.1007/s11104-008-9668-3>
- Hijmans, R. (2023). raster: Geographic data analysis and modeling. R package version, 3, 6–20. Retrieved from <https://CRAN.R-project.org/package=raster>
- Hill, S. (1992). Physiology of nitrogen fixation in free-living heterotrophs. In G. Stacy, R. H. Burris, & H. J. Evans (Eds.), *Biological nitrogen fixation* (pp. 87–134). <https://doi.org/10.1007/978-1-0412-0242-1>
- Holland, B. L., Matthews, M. L., Bota, P., Sweetlove, L. J., Long, S. P., & di Cenzo, G. C. (2023). A genome-scale metabolic reconstruction of soybean and *Bradyrhizobium diazoefficiens* reveals the cost-benefit of nitrogen fixation. *New Phytologist*, 240(2), 744–756. <https://doi.org/10.1111/nph.19203>
- Holland-Moritz, H., Stuart, J. E. M., Lewis, L. R., Miller, S. N., Mack, M. C., Ponciano, J. M., et al. (2021). The bacterial communities of Alaskan mosses and their contributions to N₂-fixation. *Microbiome*, 9, 1–14. <https://doi.org/10.1186/s40168-021-01001-4>
- Jensen, E. S., Peoples, M. B., Boddey, R. M., Gresshoff, P. M., Hauggard-Nielsen, H., Alves, B. J. R., et al. (2012). Legumes for mitigation of climate change and provision of feedstock for biofuel and biorefineries. A review. *Agronomy for Sustainable Development*, 32(2), 329–364. <https://doi.org/10.1007/s13593-011-0056-7>
- Jones, D. L., Farrar, J., & Giller, K. E. (2003). Associative nitrogen fixation and root exudation – What is theoretically possible in the rhizosphere? *Symbiosis*, 35, 19–38.
- Jung, M., Schwalm, C., Migliavacca, M., Walther, S., Camps-Valls, G., Koirala, S., et al. (2020). Scaling carbon fluxes from eddy covariance sites to globe: Synthesis and evaluation of the FLUXCOM approach. *Biogeosciences*, 17(5), 1343–1365. <https://doi.org/10.5194/bg-17-1343-2020>
- Khosh, M. S., McClelland, J. W., Jacobson, A. D., Douglas, T. A., Barker, A. J., & Lehn, G. O. (2017). Seasonality of dissolved nitrogen from spring melt to fall freezeup in Alaskan Arctic tundra and mountain streams. *Journal of Geophysical Research: Biogeosciences*, 122(7), 1718–1737. <https://doi.org/10.1002/2016JG003377>
- Kou-Giesbrecht, S., & Arora, V. (2022). Representing the dynamics response of vegetation to nitrogen limitation via biological nitrogen fixation in the CLASSIC land model. *Global Biogeochemical Cycles*, 36(6), e2022GB007341. <https://doi.org/10.1029/2022GB007341>
- Kou-Giesbrecht, S., Arora, V. K., Seiler, C., Arneeth, A., Falk, S., Jain, A. K., et al. (2023). Evaluating nitrogen cycling in terrestrial biosphere models: A disconnect between the carbon and nitrogen cycles. *Earth System Dynamics*, 14(4), 767–795. <https://doi.org/10.5194/esd-14-767-2023>
- Kou-Giesbrecht, S., Reis Ely, C. R., Perakis, S. S., Cleveland, C. C., Menge, D. N. L., Reed, S. C., et al. (2025). Overestimated biological nitrogen fixation translates to an exaggerated CO₂ fertilization effect in Earth system models. *Proceedings of the National Academy of Sciences*, 122(48), e2514628122. <https://doi.org/10.1073/pnas.2514628122>
- Layzell, D. B., Gaito, S. T., & Hunt, S. (1988). Model of gas exchange and diffusion in legume nodules. I. Calculation of gas exchange rates and the energy cost of N₂ fixation. *Planta*, 173(1), 117–127. <https://doi.org/10.1007/BF00394946>
- Layzell, D. B., Rainbird, R. M., Atkins, C. A., & Pate, J. S. (1979). Economy of photosynthate use in nitrogen-fixing legume nodules: Observations on two contrasting symbioses. *Plant Physiology*, 64(5), 888–891. <https://doi.org/10.1104/pp.64.5.888>
- Liu, Y., Wang, C., He, N., Wen, X., Gao, Y., Li, S., et al. (2017). A global synthesis of the rate and temperature sensitivity of soil nitrogen mineralization: Latitudinal patterns and mechanisms. *Global Change Biology*, 23(1), 455–464. <https://doi.org/10.1111/gcb.13372>
- McCulloch, L. A., Taylor, B. N., Wurzbarger, N., & Prescott, C. E. (2026). Rethinking symbiotic nitrogen fixation: Could surplus carbon drive unexpected patterns of resource allocation? *Plant and Soil*, 521(1), 1–8. <https://doi.org/10.1007/s11104-026-08391-0>
- Menegat, S., Ledo, A., & Tirado, R. (2022). Greenhouse gas emissions from global production and use of nitrogen synthetic fertilisers in agriculture. *Scientific Reports*, 12(1), 14490. <https://doi.org/10.1038/s41598-022-18773-w>
- Menge, D. N. L. (2024). Data from: Nitrogen fixation and fertilization have similar effects on biomass allocation in nitrogen-fixing plants [Dataset]. *Dryad*. <https://doi.org/10.5061/dryad.cnp5hqc9s>
- Menge, D. N. L. (2026). Code for: Global carbon investment in terrestrial biological nitrogen fixation. *Dryad*. <https://doi.org/10.5061/dryad.tqjq2bwbn>
- Menge, D. N. L., Batterman, S. A., Hedin, L. O., Liao, W., Pacala, S. W., & Taylor, B. N. (2017). Why are nitrogen-fixing trees rare at higher compared to lower latitudes? *Ecology*, 98(12), 3127–3140. <https://doi.org/10.1002/ecy.2034>

- Menge, D. N. L., Batterman, S. A., Liao, W., Taylor, B. N., Lichstein, J. W., & Ángeles-Pérez, G. (2017). Nitrogen-fixing tree abundance in higher-latitude North America is not constrained by diversity. *Ecology Letters*, 20(7), 842–851. <https://doi.org/10.1111/ele.12778>
- Menge, D. N. L., & Hedin, L. O. (2009). Nitrogen fixation in different biogeochemical niches along a 120,000-year chronosequences in New Zealand. *Ecology*, 90(8), 2190–2201. <https://doi.org/10.1890/08-0877.1>
- Menge, D. N. L., Levin, S. A., & Hedin, L. O. (2009). Facultative versus obligate nitrogen fixation strategies and their ecosystem consequences. *American Naturalist*, 174(4), 465–477. <https://doi.org/10.1086/605377>
- Menge, D. N. L., Wolf, A. A., & Funk, J. L. (2015). Diversity of nitrogen fixation strategies in Mediterranean legumes. *Nature Plants*, 1(6), 1–5. <https://doi.org/10.1038/nplants.2015.64>
- Menge, D. N. L., Wolf, A. A., Funk, J. L., Perakis, S. S., Akana, P. R., Arkebauer, R., et al. (2023a). Tree symbioses sustain nitrogen fixation despite excess nitrogen supply. *Ecological Monographs*, 93(2), e1562. <https://doi.org/10.1002/ecm.1562>
- Menge, D. N. L., Wolf, A. A., Funk, J. L., Perakis, S. S., Akana, P. R., Arkebauer, R., et al. (2023b). Data for: Tree symbioses sustain nitrogen fixation despite excess nitrogen supply [Dataset]. *Dryad*. <https://doi.org/10.5061/dryad.fbg79cnxk>
- Menge, D. N. L., Wolf, A. A., Funk, J. L., Perakis, S. S., & Carreras Pereira, K. A. (2024). Nitrogen fixation and fertilization have similar effects on biomass allocation in nitrogen-fixing plants. *Ecology and Evolution*, 14(9), e70309. <https://doi.org/10.1002/eec3.70309>
- Meyerholt, J., Sickel, K., & Zaehle, S. (2020). Ensemble projections elucidate effects of uncertainty in terrestrial nitrogen limitation on future carbon uptake. *Global Change Biology*, 26(7), 3978–3996. <https://doi.org/10.1111/gcb.15114>
- Minchin, F. R., & Witty, J. F. (2005). Respiratory/carbon costs of symbiotic nitrogen fixation in legumes. In *Plant respiration: From cell to ecosystem*. Springer. https://doi.org/10.1007/1-4020-3589-6_11
- Nasto, M. K., Alvarez-Clare, S., Lekberg, Y., Sullivan, B. W., Townsend, A. R., & Cleveland, C. C. (2014). Interactions among nitrogen fixation and soil phosphorus acquisition strategies in lowland tropical rain forests. *Ecology Letters*, 17(10), 1282–1289. <https://doi.org/10.1111/ele.12335>
- Osnas, J. L. D., Lichstein, J. W., Reich, P. B., & Pacala, S. W. (2013). Global leaf trait relationships: Mass, area, and the leaf economics spectrum. *Science*, 340(6133), 741–744. <https://doi.org/10.1126/science.1231574>
- Peng, Y., Prentice, I. C., Bloomfield, K. J., Campioli, M., Guo, Z., Sun, Y., et al. (2023). Global terrestrial nitrogen uptake and nitrogen use efficiency. *Journal of Ecology*, 111(12), 2676–2693. <https://doi.org/10.1111/1365-2745.14208>
- Peoples, M. B., Giller, K. E., Jensen, E. S., & Herridge, D. F. (2021). Quantifying country-to-global scale nitrogen fixation for grain legumes I. Reliance on nitrogen fixation for growth of soybean, groundnut and pulses. *Plant and Soil*, 469(1–2), 1–14. <https://doi.org/10.1007/s1104-021-05167-6>
- Pierce, D. (2023). ncdf4: Interface to Unidata netCDF (Version 4 or earlier) Format Data files. *R package version 1.21*. Retrieved from <https://CRAN.R-project.org/package=ncdf4>
- Piipponen, J., Jalava, M., de Leeuw, J., Rizayeva, A., Godde, C., Cramer, G., et al. (2022). Global trends in grassland carrying capacity and relative stocking density of livestock. *Global Change Biology*, 28(12), 3902–3919. <https://doi.org/10.1111/gcb.16174>
- Purahong, W., Wubet, T., Lentendu, G., Schloter, M., Pecyna, M. J., Kapturska, D., et al. (2016). Life in leaf litter: Novel insights into community dynamics of bacteria and fungi during litter decomposition. *Molecular Ecology*, 25(16), 4059–4074. <https://doi.org/10.1111/mec.13739>
- Rastetter, E. B., Vitousek, P. M., Field, C., Shaver, G. R., Herbert, D., & Ågren, G. I. (2001). Resource optimization and symbiotic nitrogen fixation. *Ecosystems*, 4, 369–388. <https://doi.org/10.1007/s10021-001-0018-z>
- R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from <https://www.R-project.org/>
- Reed, S. C., Cleveland, C. C., & Townsend, A. R. (2008). Tree species control rates of free-living nitrogen fixation in a tropical rain forest. *Ecology*, 89(10), 2924–2934. <https://doi.org/10.1890/07-1430.1>
- Reis Ely, C. R., Perakis, S. S., Cleveland, C. C., Menge, D. N. L., Reed, S. C., Taylor, B. N., et al. (2025a). Global terrestrial biological nitrogen fixation and its modification by agriculture. *Nature*, 643(8072), 705–711. <https://doi.org/10.1038/s41586-025-09201-w>
- Reis Ely, C. R., Perakis, S. S., Cleveland, C. C., Menge, D. N. L., Reed, S. C., Batterman, S. A., et al. (2025b). A global dataset of terrestrial biological nitrogen fixation. *Scientific Data*, 12(1), 1362. <https://doi.org/10.1038/s41597-025-05131-4>
- Reis Ely, C. R., Perakis, S. S., Cleveland, C. C., Menge, D. N. L., Reed, S. C., Taylor, B. N., et al. (2025c). Global gridded dataset of terrestrial biological nitrogen fixation across natural and agricultural biomes. *U.S. Geological Survey data release*. <https://doi.org/10.5066/P13THKNR>
- Rösch, C., & Bothe, H. (2009). Diversity of total, nitrogen-fixing and denitrifying bacteria in an acid forest soil. *European Journal of Soil Science*, 60(6), 883–894. <https://doi.org/10.1111/j.1365-2389.2009.01167.x>
- Ruess, R. W., Anderson, M. D., McFarland, J. M., Kielland, K., Olson, K., & Taylor, D. L. (2013). Ecosystem-level consequences of symbiont partnerships in an N-fixing shrub from interior Alaskan floodplains. *Ecological Monographs*, 83(2), 177–194. <https://doi.org/10.1890/12-0782.1>
- Running, S., & Zhao, M. (2021). MODIS/Terra net primary production gap-filled yearly L4 Global 500m SIN grid V061 [Dataset]. *NASA EOSDIS Land Processes DAAC*. <https://doi.org/10.5067/MODIS/MOD17A3HGF.061>
- Schlesinger, W. H., & Bernhardt, E. S. (2013). *Biogeochemistry: An analysis of global change* (3rd ed.). Elsevier. <https://doi.org/10.1016/C2010-0-66291-2>
- Schubert, K. R. (1982). The energetics of biological nitrogen fixation. In *Workshop summary, American Society of Plant Physiologists*.
- Seiler, C., Melton, J. R., Arora, V. K., Sitch, S., Friedlingstein, P., Anthoni, P., et al. (2022). Are terrestrial biosphere models fit for simulating the global land carbon sink? *Journal of Advances in Modeling Earth Systems*, 14(5), e2021MS002946. <https://doi.org/10.1029/2021MS002946>
- Shearer, G., & Kohl, D. H. (1986). N₂-fixation in field settings: Estimations based on natural ¹⁵N abundance. *Functional Plant Biology*, 13(6), 699–756. <https://doi.org/10.1071/PP9860699>
- Shi, M., Fisher, J. B., Brzostek, E. R., & Phillips, R. P. (2016). Carbon cost of plant nitrogen acquisition: Global carbon cycle impact from an improved plant nitrogen cycle in the Community Land Model. *Global Change Biology*, 22(3), 1299–1314. <https://doi.org/10.1111/gcb.13131>
- Soper, F. M., Simon, C., & Jauss, V. (2021). Measuring nitrogen fixation by the acetylene reduction assay (ARA): Is 3 the magic ratio? *Biogeochemistry*, 152(2–3), 345–351. <https://doi.org/10.1007/s10533-021-00761-3>
- Soper, F. M., Taylor, B. N., Winbourne, J. B., Wong, M. Y., Dynarski, K. A., Reis, C. R. G., et al. (2021). A roadmap for sampling and scaling biological nitrogen fixation in terrestrial ecosystems. *Methods in Ecology and Evolution*, 12(6), 1122–1137. <https://doi.org/10.1111/2041-210X.13586>
- Steidinger, B. S., Crowther, T. W., Liang, J., Van Nuland, M. E., Werner, G. D. A., Reich, P. B., et al. (2019). Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. *Nature*, 569(7756), 404–408. <https://doi.org/10.1038/s41586-019-1128-0>
- Sullivan, B. W., Alvarez-Clare, S., Castle, S. C., Porder, S., Reed, S. C., Schreng, L., et al. (2014). Assessing nutrient limitation in complex forested ecosystems: Alternatives to large-scale fertilization experiments. *Ecology*, 95(3), 668–681. <https://doi.org/10.1890/13-0825.1>

- Sulman, B. N., Shevliakova, E., Brzostek, E. R., Kivlin, S. N., Malyshev, S., Menge, D. N. L., & Zhang, X. (2019). Diverse mycorrhizal associations enhance terrestrial C storage in a global model. *Global Biogeochemical Cycles*, 33(4), 501–523. <https://doi.org/10.1029/2018GB005973>
- Tjepkema, J. D., & Winship, L. J. (1980). Energy requirement for nitrogen fixation in actinorhizal and legume root nodules. *Science*, 209(4453), 279–281. <https://doi.org/10.1126/science.7384801>
- Townsend, A. R., Asner, G. P., & Cleveland, C. C. (2008). The biogeochemical heterogeneity of tropical forests. *Trends in Ecology & Evolution*, 23(8), 424–431. <https://doi.org/10.1016/j.tree.2008.04.009>
- Unkovich, M., & Baldock, J. (2008). Measurement of asymbiotic N₂ fixation in Australian agriculture. *Soil Biology and Biochemistry*, 40(12), 2915–2921. <https://doi.org/10.1016/j.soilbio.2008.08.021>
- Vance, C. P. (2008). Carbon and nitrogen metabolism in legume nodules. In M. J. Dilworth, E. K. James, J. I. Sprent, & W. E. Newton (Eds.), *Nitrogen-fixing leguminous symbioses. Nitrogen fixation: Origins, applications, and research progress* (Vol. 7, pp. 293–320). Springer. https://doi.org/10.1007/978-1-4020-3548-7_10
- Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., et al. (1997). Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications*, 7(3), 737–750. [https://doi.org/10.1890/1051-0761\(1997\)007\[0737:HAOTGN\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2)
- Vitousek, P. M., & Howarth, R. W. (1991). Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry*, 13(2), 87–115. <https://doi.org/10.1007/BF00002772>
- Vitousek, P. M., Menge, D. N. L., Reed, S. C., & Cleveland, C. C. (2013). Biological nitrogen fixation: Rates, patterns and ecological controls in terrestrial ecosystems. *Philosophical Transactions of the Royal Society B*, 368(1621), 20130119. <https://doi.org/10.1098/rstb.2013.0119>
- Wang, Y.-P., Houlton, B. Z., & Field, C. B. (2007). A model of biogeochemical cycles of carbon, nitrogen, and phosphorus including symbiotic nitrogen fixation and phosphatase production. *Global Biogeochemical Cycles*, 21(1), GB1018. <https://doi.org/10.1029/2006GB002797>
- Weber, B., Belnap, J., Büdel, B., Antoninka, A. J., Barger, N. N., Chaudhary, V. B., et al. (2022). What is a biocrust? A refined, contemporary definition for a broadening research community. *Biological Reviews*, 97(5), 1768–1785. <https://doi.org/10.1111/brv.12862>
- Witty, J. F., Minchin, F. R., & Sheehy, J. E. (1983). Carbon costs of nitrogenase activity in legume root nodules determined using acetylene and oxygen. *Journal of Experimental Botany*, 34(8), 951–963. <https://doi.org/10.1093/jxb/34.8.951>
- Wright, S. J. (2019). Plant responses to nutrient addition experiments conducted in tropical forests. *Ecological Monographs*, 89(4), e01382. <https://doi.org/10.1002/ecm.1382>
- Xu-Ri, & Prentice, I. C. (2017). Modelling the demand for new nitrogen fixation by terrestrial ecosystems. *Biogeosciences*, 14(7), 2003–2017. <https://doi.org/10.5194/bg-14-2003-2017>
- Zuberer, D. A. (2021). Biological dinitrogen (N₂) fixation: Introduction and non-symbiotic. In T. J. Gentry, J. J. Fuhrmann, & D. A. Zuberer (Eds.), *Principles and applications of soil microbiology* (3rd ed., pp. 423–453). Elsevier. <https://doi.org/10.1016/B978-0-12-820202-9.00016-2>

Supporting Information for

Title: Global carbon investment in terrestrial biological nitrogen fixation

Authors: Duncan N. L. Menge^{1*}, Carla R. Reis Ely^{2,3}, Steven S. Perakis⁴, Sian Kou-Giesbrecht⁵, Cory C. Cleveland⁶, Sasha C. Reed⁷, Benton N. Taylor⁸, Sarah A. Batterman^{9,10,11}, Timothy E. Crews¹², Katherine A. Dynarski⁶, Jennifer L. Funk¹³, Maga Gei¹⁴, Kevin L. Griffin^{1,15,16}, Michael J. Gundale¹⁷, David F. Herridge¹⁸, Sarah E. Jovan¹⁹, Mark B. Peoples²⁰, Johannes Piipponen²¹, Emilio Rodríguez-Caballero^{22,23}, Verity G. Salmon²⁴, Fiona M. Soper²⁵, Anika P. Staccone²⁶, Bettina Weber^{23,27}, Amelia A. Wolf²⁸, and Nina Wurzburger²⁹

Affiliations:

¹ Department of Ecology, Evolution, and Environmental Biology, Columbia University; New York, NY 10027, USA.

² Department of Forest Ecosystems and Society, Oregon State University; Corvallis, OR 97331, USA.

³ Oak Ridge Institute for Science and Education, Oak Ridge, TN 37830, USA

⁴ US Geological Survey, Forest and Rangeland Ecosystem Science Center; Corvallis, OR 97331, USA.

⁵ Department of Earth and Environmental Sciences, Dalhousie University; Halifax, NS B3H 4R2, Canada.

⁶ Department of Ecosystem and Conservation Sciences, W.A. Franke College of Forestry and Conservation, University of Montana; Missoula, MT 59812, USA.

⁷ US Geological Survey, Southwest Biological Science Center; Moab, UT 84532, USA.

24 ⁸ Department of Organismic and Evolutionary Biology, Harvard University; 1300
25 Centre St., Roslindale, MA 02131, USA.

26 ⁹ Cary Institute of Ecosystem Studies; Millbrook, NY 12545, USA.

27 ¹⁰ School of Geography, University of Leeds; Leeds, UK.

28 ¹¹ Smithsonian Tropical Research Institute; Ancon, Panama.

29 ¹² The Land Institute; Salina, KS 67401, USA.

30 ¹³ Department of Plant Sciences, University of California, Davis; Davis, CA 95616, USA.

31 ¹⁴ Association for Tropical Biology and Conservation; Minneapolis, MN 55405, USA.

32 ¹⁵ Department of Earth and Environmental Science, Columbia University; New York, NY
33 10027, USA.

34 ¹⁶ Division of Biology & Paleoenvironment, Lamont-Doherty Earth Observatory of
35 Columbia University; Palisades, NY 10027, USA.

36 ¹⁷ Department of Forest Ecology and Management, Swedish University of Agricultural
37 Sciences; 90183 Umea, Sweden.

38 ¹⁸ School of Environmental and Rural Science, University of New England; Armidale, NSW
39 2351, Australia.

40 ¹⁹ USDA Forest Service, PNW Research Station; Portland, OR, USA.

41 ²⁰ CSIRO Agriculture and Food, GPO Box 1700; Canberra, ACT 2601, Australia.

42 ²¹ Water and Development Research Group, Aalto University; Espoo, Finland.

43 ²² Departamento de Agronomía, Universidad de Almería; Almería, Spain.

44 ²³ Multiphase Chemistry Department, Max Planck Institute for Chemistry; Mainz, Germany.

45 ²⁴ Environmental Science Division and Climate Change Science Institute, Oak Ridge
46 National Laboratory; Oak Ridge, TN 37831, USA.

²⁵ Department of Biology and School of the Environment, McGill University; Montreal, QC
H3A 1B1, Canada.

²⁶ Earthshot Labs; Mill Valley, CA, USA.

²⁷ Division of Plant Sciences, Institute for Biology, University of Graz; Graz, Austria.

²⁸ Department of Integrative Biology, University of Texas at Austin, Austin, TX 78712,
USA.

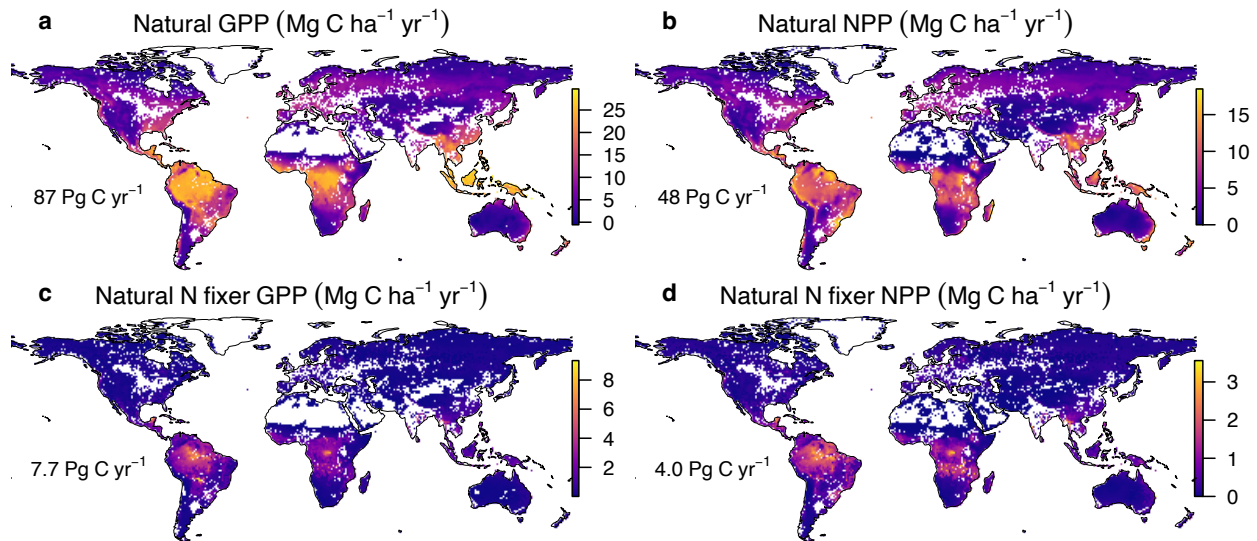
²⁹ Odum School of Ecology, University of Georgia; Athens, GA 30602, USA.

*Corresponding author. Email: dm2972@columbia.edu

Contents of this file:

Figures S1 to S5

Tables S1 to S3



59

60 **Figure S1. Global maps of $\text{GPP}_{\text{ecosys}}$, $\text{NPP}_{\text{ecosys}}$, GPP_{symp} , and NPP_{symp} in natural biomes.**

61 These data were used to calculate the values in Figs. 1–3, Figs. S2–S5, and Tables 1–6.

62 Ecosystem-scale natural (a) GPP ($\text{GPP}_{\text{ecosys}}$) (Jung et al., 2020) and (b) NPP ($\text{NPP}_{\text{ecosys}}$) (Running
 63 & Zhao, 2021) are shown along with (c) GPP_{symp} and (d) NPP_{symp} , which are the C fluxes
 64 associated with productivity from symbiotic N-fixing plants (symbiotic trees, shrubs, and herbs
 65 combined) in natural biomes. Numbers printed on panels are global annual fluxes.

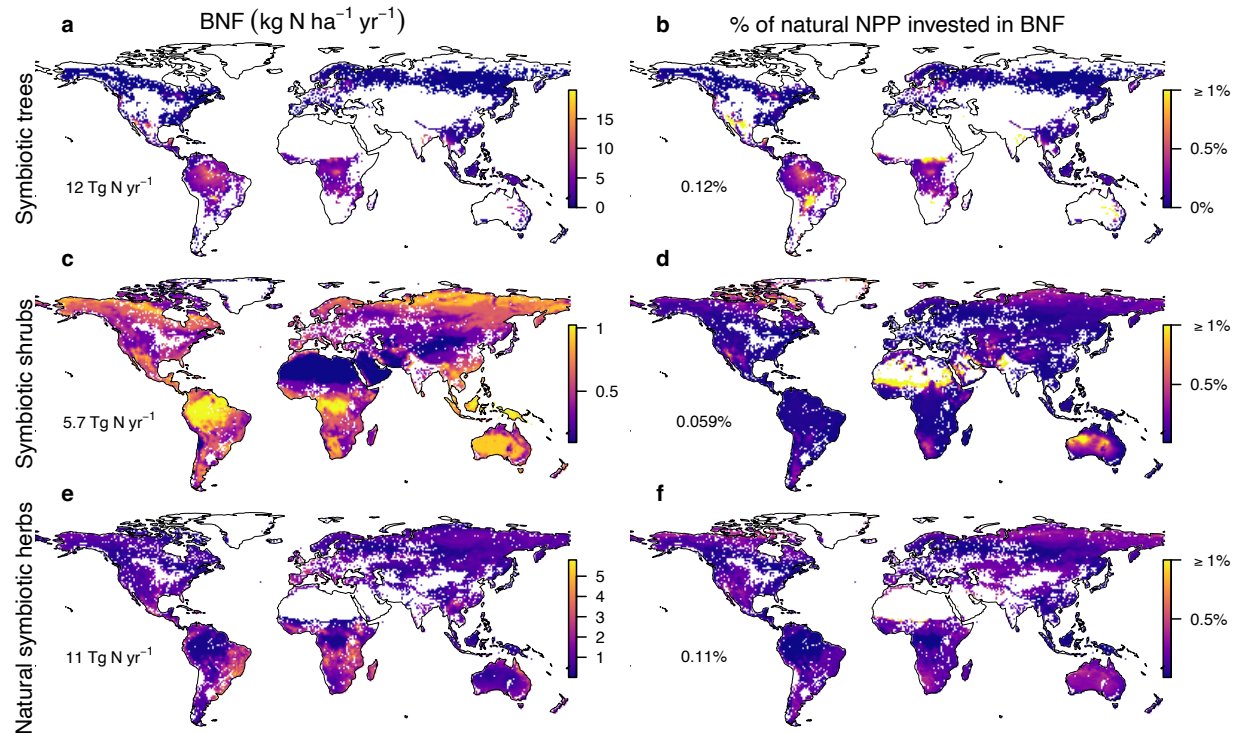


Figure S2. Global maps of BNF and ecosystem-scale C investment in BNF by symbiotic trees, shrubs, and natural herbs.

The color of each grid cell shows BNF from symbiotic (a) trees, (c) shrubs, and (e) natural herbs; and the percentage of the grid cell's NPP_{ecosys} invested in BNF from (b) trees, (d) shrubs, and (f) natural herbs. Numbers printed on each panel are the global totals. White grid cells are biomes that have been masked out because they are agricultural or have no data. Color scales are linear but truncated. BNF data are from Reis Ely et al. (2025).

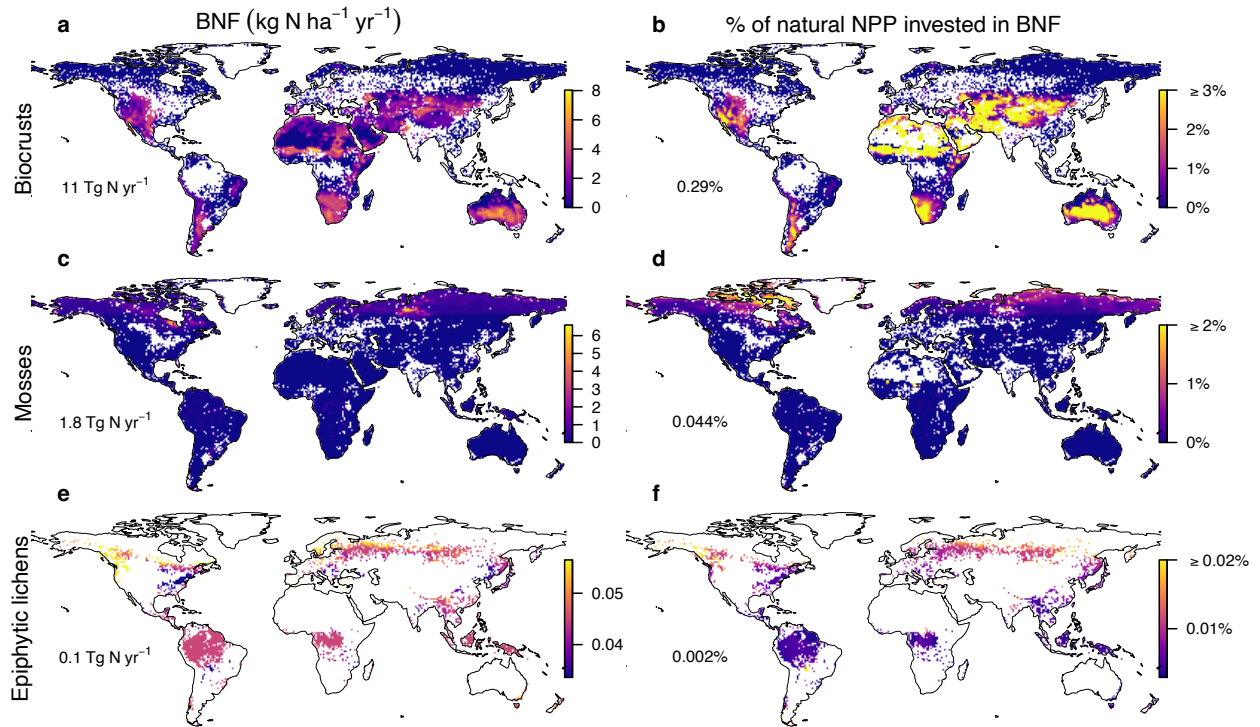
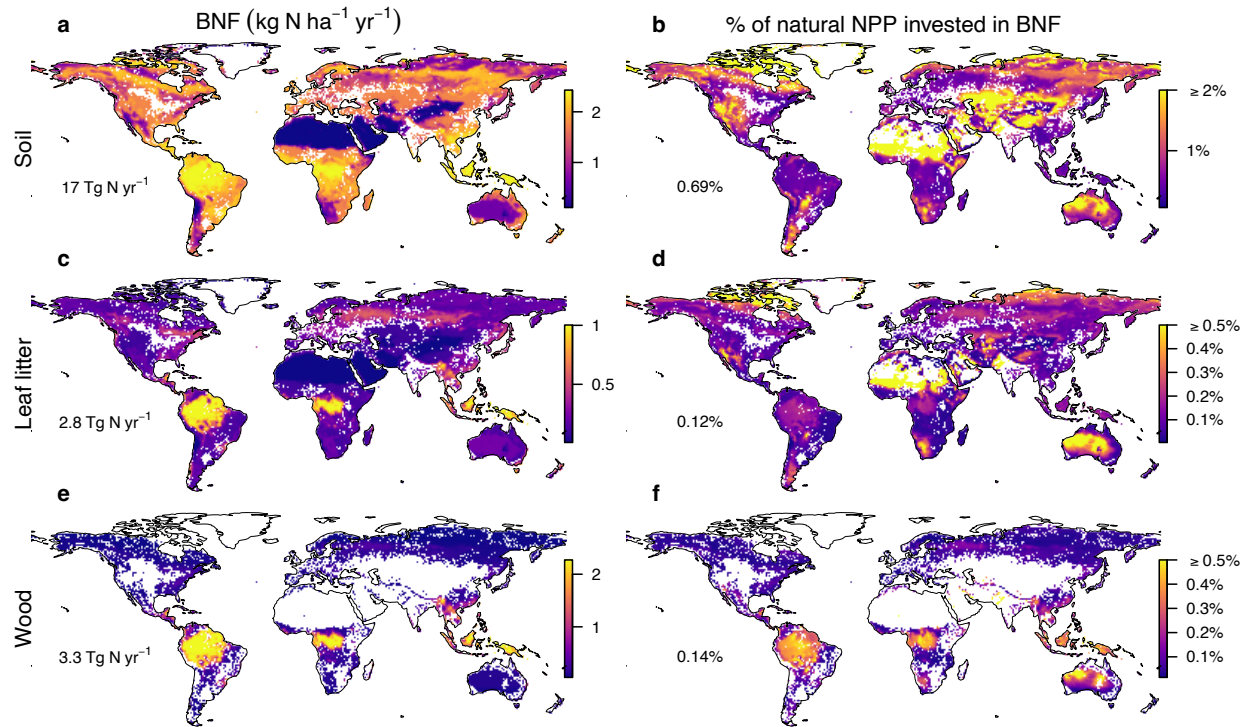


Figure S3. Global maps of BNF and ecosystem-scale C investment in BNF by biocrusts, mosses, and epiphytic lichens. Details as in Fig. S2 except that data are for biocrusts, mosses, and lichens instead of symbiotic trees, shrubs, and natural herbs. BNF data are from Reis Ely et al. (2025).



79

80 **Figure S4. Global maps of BNF and ecosystem-scale C investment in BNF in soils, leaf**
 81 **litter, and decomposing wood in natural biomes.** Details as in Fig. S2 except that data are for
 82 soils, leaf litter, and decomposing wood instead of symbiotic trees, shrubs, and herbs. BNF data
 83 are from Reis Ely et al. (2025).

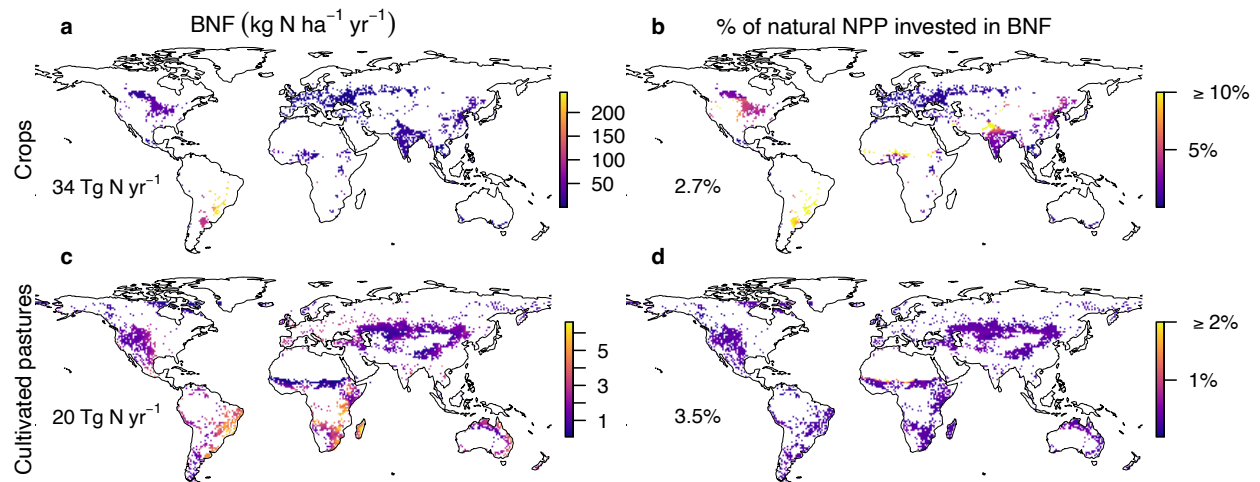


Figure S5. Global maps of BNF and ecosystem-scale C investment in BNF by leguminous herbs in crops and cultivated pastures. Details as in Fig. S2 except that the data are for agricultural instead of natural BNF and $\text{NPP}_{\text{ecosys}}$, and that the BNF data are for herbs in crops and cultivated pastures instead of symbiotic trees, shrubs, and natural herbs. BNF data are from Reis Ely et al. (2025).

91 **Table S1: Carbon cost per unit nitrogen fixed used in this work.**

Group	Central value (g C g N ⁻¹)	Low value (g C g N ⁻¹)	High value (g C g N ⁻¹)	Gutschick value*
Plant symbioses	5	4	6	4.8
Associative BNF	12	5	20	20
Free-living BNF	20	5	42	20

92 *Used by Gutschick (1978).

93 **Table S2: Assumptions about the percentages of $GPP_{\text{ecosystem}}$ and $NPP_{\text{ecosystem}}$ from each**
 94 **plant growth form in each biome.**

Biome	% from trees	% from shrubs	% from herbs
Evergreen Needleleaf Forest	90	5	5
Deciduous Needleleaf Forest	90	5	5
Evergreen Broadleaf Forest	90	5	5
Deciduous Broadleaf Forest	90	5	5
Mixed Forest	90	5	5
Savannas	30	20	50
Shrublands	15	80	5
Natural Grasslands	0	0	100
Cultivated Pastures	0	0	100
Barren	0	75	5

95

96 **Table S3: Fractions of $GPP_{\text{symbiosis}}$ and $NPP_{\text{symbiosis}}$ invested in BNF from field-grown and**
97 **greenhouse-grown plants.**

Location and species*	Symbiotic type	Growth form	% of GPP _{symbiosis} [‡]	% of NPP _{symbiosis} [‡]	%N _{dfa}
Units (N fixers)	% invested in BNF				
Field-grown trees					
• <i>Robinia pseudoacacia</i>	Rhizobial	Tree	4.6 (3.7–5.5)%	9.2 (7.4–11)%	90%
• <i>Gliricidia sepium</i> [†]	Rhizobial	Tree	3.6 (2.9–4.3)%	7.2 (5.8–8.6)%	78%
• <i>Acacia koa</i> [†]	Rhizobial	Tree	2.1 (1.7–2.6)%	4.3 (3.4–5.1)%	50%
• <i>Alnus rubra</i>	Actinorhizal	Tree	2.5 (2.0–3.0)%	5.1 (4.1–6.1)%	97%
• <i>Casuarina equisetifolia</i> [†]	Actinorhizal	Tree	2.2 (1.8–2.7)%	4.4 (3.5–5.3)%	87%
• <i>Morella faya</i> [†]	Actinorhizal	Tree	1.5 (1.2–1.8)%	2.9 (2.4–3.5)%	56%
Barnard greenhouse seedlings					
	Rhizobial	Tree	1.8 (1.4–2.2)%	3.6 (2.9–4.3)%	28%
• <i>Acacia farnesiana</i>	Rhizobial	Tree	1.5 (1.2–1.7)%	2.9 (2.3–3.5)%	28%
• <i>Acacia koa</i>	Rhizobial	Tree	1.5 (1.2–1.8)%	3.0 (2.4–3.7)%	24%
• <i>Albizia julibrissin</i>	Rhizobial	Tree	0.71 (0.57–0.85)%	1.4 (1.1–1.7)%	11%
• <i>Enterolobium cyclocarpum</i>	Rhizobial	Tree	2.4 (1.9–2.9)%	4.9 (3.9–5.8)%	40%
• <i>Gliricidia sepium</i>	Rhizobial	Tree	2.6 (2.1–3.1)%	5.2 (4.2–6.2)%	42%
• <i>Leucaena leucocephala</i>	Rhizobial	Tree	0.46 (0.37–0.55)%	0.92 (0.73–1.1)%	9.1%
• <i>Robinia pseudoacacia</i>	Rhizobial	Tree	3.2 (2.5–3.8)%	6.4 (5.1–7.6)%	46%
• <i>Sophora chrysophylla</i>	Rhizobial	Tree	0.67 (0.54–0.80)%	1.3 (1.1–1.6)%	14%
UC Davis greenhouse seedlings					
	Mixed	Mixed	3.9 (3.1–4.7)%	7.9 (6.3–9.4)%	59%
• <i>Acacia farnesiana</i>	Rhizobial	Tree	4.9 (3.9–5.9)%	9.8 (7.8–12)%	80%
• <i>Acacia koa</i>	Rhizobial	Tree	5.2 (4.2–6.3)%	10 (8.4–13)%	78%
• <i>Gliricidia sepium</i>	Rhizobial	Tree	7.4 (6.0–8.9)%	15 (12–18)%	92%
• <i>Robinia pseudoacacia</i>	Rhizobial	Tree	7.9 (6.3–9.5)%	16 (13–19)%	86%
• <i>Alnus acuminata</i>	Actinorhizal	Tree	3.9 (3.1–4.7)%	7.8 (6.2–9.4)%	80%
• <i>Alnus rubra</i>	Actinorhizal	Tree	2.4 (1.9–2.9)%	4.9 (3.9–5.8)%	52%

•	<i>Casuarina equisetifolia</i>	Actinorhizal	Tree	2.4 (1.9–2.9)%	4.9 (3.9–5.8)%	56%
•	<i>Elaeagnus angustifolia</i>	Actinorhizal	Tree	1.7 (1.4–2.1)%	3.5 (2.8–4.2)%	14%
•	<i>Morella cerifera</i>	Actinorhizal	Tree	4.2 (3.4–5.0)%	8.4 (6.7–10)%	77%
•	<i>Morella faya</i>	Actinorhizal	Tree	2.6 (2.1–3.1)%	5.2 (4.1–6.2)%	57%
•	<i>Glycine max</i> (soybean)	Rhizobial	Herb (crop)	2.4 (1.9–2.9)%	4.8 (3.8–5.8)%	36%

Stanford greenhouse seedlings

•	<i>Astragalus douglasii</i>	Rhizobial	Herb	3.3 (2.6–3.9)%	6.5 (5.2–7.8)%	50%
•	<i>Trifolium hirtum</i>	Rhizobial	Herb	4.0 (3.2–4.8)%	8.0 (6.4–9.6)%	78%
•	<i>Vicia americana</i>	Rhizobial	Herb	7.7 (6.2–9.3)%	15 (12–19)%	93%
•	<i>Vicia sativa</i>	Rhizobial	Herb	7.0 (5.6–8.5)%	14 (11–17)%	90%

Chapman greenhouse seedlings

•	<i>Medicago polymorpha</i>	Rhizobial	Herb	1.6 (1.3–1.9)%	3.1 (2.5–3.8)%	27%
•	<i>Melilotus officinalis</i>	Rhizobial	Herb	1.3 (1.0–1.5)%	2.5 (2.0–3.0)%	23%
•	<i>Trifolium repens</i>	Rhizobial	Herb	0.35 (0.28–0.42)%	0.69 (0.55–0.83)%	6.8%
•	<i>Trifolium willdenovii</i>	Rhizobial	Herb	1.1 (0.87–1.3)%	2.2 (1.7–2.6)%	17%

*Field-grown plants only include control treatments. Field-grown plants were planted in open fields in New York (*Robinia*), Oregon (*Alnus*), and Hawaii (*Gliricidia*, *Casuarina*, *Acacia*, and *Morella*), and harvested 4–5 years after planting. All greenhouse data include multiple fertilization treatments, ranging from low N and P to high N and P. Data sources are Menge et al. (2015, 2023, 2024) and Carreras Pereira et al. (2023).

[†]GPP_{symbiosis}, NPP_{symbiosis}, and BNF for field-grown plants in Hawaii only represent aboveground tissues.

[‡]Values shown outside parentheses use the central C costs: 5, 12, and 20 g C per g N fixed for symbiotic plants, associative BNF, and free-living BNF, respectively. Values shown inside parentheses show ranges of results from using the low (4, 5, and 5 g C per g N fixed) and high (6, 20, and 42 g C per g N fixed) C costs for symbiotic plants, associative BNF, and free-living BNF, respectively.