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Global pattern and controls of biological nitrogen fixation under nutrient enrichment: A meta-analysis

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Abstract

Biological nitrogen (N) fixation (BNF), an important source of N in terrestrial ecosystems, plays a critical role in terrestrial nutrient cycling and net primary productivity. Currently, large uncertainty exists regarding how nutrient availability regulates terrestrial BNF and the drivers responsible for this process. We conducted a global meta-analysis of terrestrial BNF in response to N, phosphorus (P), and micronutrient (Micro) addition across different biomes (i.e, tropical/subtropical forest, savanna, temperate forest, grassland, boreal forest, and tundra) and explored whether the BNF responses were affected by fertilization regimes (nutrient-addition rates, duration, and total load) and environmental factors (mean annual temperature [MAT], mean annual precipitation [MAP], and N deposition). The results showed that N addition inhibited terrestrial BNF (by 19.0% (95% confidence interval [CI]: 17.7%–20.3%); hereafter), Micro addition stimulated terrestrial BNF (30.4% [25.7%–35.3%]), and P addition had an inconsistent effect on terrestrial BNF, i.e., inhibiting free-living N fixation (7.5% [4.4%–10.6%]) and stimulating symbiotic N fixation (85.5% [25.8%–158.7%]). Furthermore, the response ratios (i.e., effect sizes) of BNF to nutrient addition were smaller in low-latitude (<30°) biomes (8.5%–36.9%) than in mid-/high-latitude (≥30°) biomes (32.9%–61.3%), and the sensitivity (defined as the absolute value of response ratios) of BNF to nutrients in mid-/high-latitude biomes decreased with decreasing latitude ($p \le 0.009$; linear/logarithmic regression models). Fertilization regimes did not affect this phenomenon ($p > 0.05$), but environmental factors did affect it ($p < 0.001$) because MAT, MAP, and N deposition accounted for 5%–14%, 10%–32%, and 7%–18% of the variance in the BNF response ratios in cold (MAT < 15°C), low-rainfall (MAP < 2,500 mm), and low-N-deposition (<7 kg ha^{-1} year^{-1}) biomes, respectively. Overall, our meta-analysis depicts a global pattern of nutrient impacts on terrestrial BNF and indicates that certain types of global change (i.e., warming, elevated precipitation and N deposition) may reduce the sensitivity of BNF in response to nutrient enrichment in mid-/high-latitude biomes.

KEYWORDS
biological nitrogen fixation, global change, micronutrient, nitrogen, phosphorus, terrestrial ecosystems

1 | INTRODUCTION

Biological nitrogen (N) fixation (BNF), one of the pathways of N inputs performed by free-living or symbiotic N-fixing organisms, represents an important source of "new" N in terrestrial ecosystems (Cleveland et al., 1999). In many mid-/high-latitude (≥30°) ecosystems, where N is limiting and atmospheric N deposition is low, BNF plays a key role in driving ecosystem N cycling (Moyes et al., 2016; Rousk, Degboe, Michelsen, Bradley, & Bellenger, 2017; Yelenik, Perakis, & Hibbs, 2013; Zackrisson, DeLuca, Nilsson, Sellstedt, & Berglund, 2004). However, in tropical forests where soil N availability is high and soil P availability is low (Hedin, Vitousek, & Matson, 2003), N-fixing trees are still widespread (Houlton, Wang, Vitousek, & Field, 2008; Menge, Lichstein, & Angeles-Perez, 2014), and N-fixing microbes sustain high rates of N fixation across a broad range of soil N richness (Hedin, Brookshire, Menge, & Barron, 2009; Reed, Cleveland, & Townsend, 2008; Zheng et al., 2018). Although anthropogenic N deposition has to some extent reduced BNF rates in natural ecosystems (Sullivan et al., 2014; Vitousek, Menge, Reed, & Cleveland, 2013), annual rates of terrestrial BNF still reach 44–195 Tg N/year (Cleveland et al., 1999; Galloway et al., 2004; Meyerholt, Zaehle, & Smith, 2016; Vitousek et al., 2013). Given the N constraints of net primary productivity (NPP) in numerous natural ecosystems (LeBauer & Treseder, 2008; Vitousek et al., 2002), N inputs via BNF increase NPP (Dynarski & Houlton, 2018). Moreover, N fixers can increase soil mineral N in N-limited biomes and transfer the fixed N to non-N-fixing plants (Rousk, Sorensen, & Michelsen, 2017; Zanetti et al., 1997), thereby increasing carbon dioxide (CO_2) sequestration by enhancing photosynthesis (Lüscher, Hartwig, Suter, & Nösberger, 2000). Despite the important role of BNF in terrestrial ecosystems, our current knowledge of the factors controlling terrestrial BNF remains poor (Dynarski & Houlton, 2018), which has impeded our ability to accurately understand, estimate, and project terrestrial N budgets and NPP in a changing world (Meyerholt et al., 2016; Wang, Houlton, & Field, 2007).

Nutrient availability has long been considered a key regulator of BNF (Reed, Cleveland, & Townsend, 2011). There are multiple types of nutrients (e.g., N, phosphorus [P], molybdenum [Mo], iron [Fe], and vanadium [V]) that can affect BNF. Exogenous N (e.g., N fertilizer and deposited N) inputs often decrease BNF rates. For example, a single or ongoing addition of N (NH_4NO_3 or urea) was found to inhibit soil and litter BNF in forest ecosystems (Barron et al., 2008; Cusack, Silver, & McDowell, 2009; Perakis, Pett-Ridge, & Catricala, 2017; Zheng, Chen, et al., 2016). Additionally, long-term N inputs (via N fixation and/or deposition) can drive soil N accumulation, leading to declines in nodule biomass of legume trees and thus the rates of nodule N fixation (Batterman, Hedin, et al., 2013; Pearson & Vitousek, 2001; Zheng, Chen, et al., 2016). However, exogenous N inputs fail to downregulate BNF if the ecosystem remains N-limited (Reed, Cleveland, & Townsend, 2007) or if N inputs do not exceed demands (Rousk, Jones, & DeLuca, 2014). In contrast with N, P is commonly derived from rock weathering, and it becomes limiting during ecosystem development due to leaching losses and biological uptakes (Hedin et al., 2003). Exogenous P inputs (e.g., P fertilizer) often enhance BNF due to the importance of this nutrient in cell metabolism (Burns & Hardy, 1975). Because P is in short supply in many natural ecosystems, the addition of P (NaH_2PO_4 or $KH_2 PO_4$) can alleviate the P limitation of BNF, thereby increasing the rates of BNF (Reed et al., 2007; Rousk, Degboe, et al., 2017; Wurzburger & Hedin, 2016; Yelenik et al., 2013; Zheng, Li, et al., 2016). In several Panamanian tropical forests, soils with high P content exhibited high N fixation activity (Wurzburger, Bellenger, Kraepiel, & Hedin, 2012). Nevertheless, a positive P effect on BNF was not detected in several forests (Barron et al., 2008; Perakis et al., 2017), and the researchers speculated that P input alone cannot stimulate BNF, while commercial P fertilizer may contain certain non-P nutrients (e.g., Mo) that are the real drivers of BNF (Barron et al., 2008; Vitousek, 1999). Micronutrient (Micro; e.g., Mo, Fe, and V) inputs can stimulate BNF because they participate in the synthesis of nitrogenase (Hoffman, Lukoyanov, Yang, Dean, & Seefeldt, 2014). The phenomenon of Micro addition driving BNF was first observed in the laboratory (Silvester, 1989), and there are increasing studies that have documented Micro limitation of BNF in forests because the addition of Micro stimulates BNF in numerous substrates, such as soil (Barron et al., 2008), litter (Vitousek & Hobbie, 2000; Winbourne, Brewer, & Houlton, 2017), and mosses (Rousk, Degboe, et al., 2017). Although many individual studies have revealed how nutrient availability affects BNF, large uncertainty exists regarding the direction and extent of BNF in response to nutrient enrichment at terrestrial scales and the potential factors affecting BNF responses.

Two potential factors may affect BNF in response to nutrient addition. One is the fertilization regimes (i.e., nutrient-addition rates, duration, and total load; see Section 22 for details), which are known to affect the extent of nutrient impacts on ecosystem functioning (Deng, Hui, Dennis, & Reddy, 2017; Zhou, Wang, Zheng, Jiang, & Luo, 2017). For example, in a subtropical mature forest, soil and litter N fixation showed no response to an N-addition rate of 50 kg N ha^{-1} year^{-1} but showed a negative response to the N-addition rates of 100–150 kg N ha^{-1} year^{-1} (Zheng et al., 2018). Higher rates of N addition often result in higher contents of N in N fixers (e.g., moss), thereby leading to a larger decline in BNF (Gundale, Bach, & Nordin, 2013; Rousk & Michelsen, 2016b). Treatment duration also affects BNF responses. For example, Silvester (1989) reported that litter N fixation showed a slight increase (~1 nmol C_2H_4 g^{-1} hr^{-1}) after 10 hr but a large increase (>10 nmol C_2H_4 g^{-1} hr^{-1}) after 50 hr following Micro (e.g., Mo) addition. Total load of nutrient addition, which is calculated by multiplying nutrient-addition rates by duration, also affects BNF responses. For example, 3 years of N addition (total N load of 150 kg N /ha) did not affect epiphytic BNF rates in two tropical rainforests in Puerto Rico (Cusack et al., 2009), but 12 years of N addition (total N load of 1800 kg N/ha) increased epiphytic BNF rates in a subtropical forest in southern China (Zheng et al., 2018).

Environmental factors (e.g., temperature, precipitation, and N deposition) may also affect BNF responses. Temperature and precipitation are important drivers of microbial processes and nutrient cycling in terrestrial ecosystems (Hou et al., 2018; Zhou, Wang, & Luo, 2018), and they can alter the community of N-fixing organisms as well as the rates of BNF (Reed et al., 2011; Rousk & Michelsen, 2016a; Warshan et al., 2016). In many mid-/high-latitude ecosystems, warming and/or increased rainfall can partially stimulate moss BNF (Gundale, Nilsson, Bansal, & Jäderlund, 2012; Jackson, Martin, Nilsson, & Wardle, 2011; Rousk, Pedersen, Dyrnum, Michelsen, 2017; Sorensen & Michelsen, 2011), although extremely high temperature can sometimes result in water limitation on moss BNF (Lett & Michelsen, 2014). In contrast, elevated N deposition reduces BNF rates in natural ecosystems (Galloway et al., 2004; Sullivan et al., 2014), as evidenced by previous findings that mosses exhibited a decline in N fixation across N deposition gradients (Ackermann, Zackrisson, Rousk, Jones, & DeLuca, 2012; Leppänen, Salemaa, Smolander, Mäkipää, & Tiirola, 2013). In addition to regulating BNF rates, elevated N deposition and precipitation may also increase or decrease the availability of nutrients (e.g., by controlling rates of litter decomposition and N mineralization) in natural ecosystems (Jackson et al., 2011; Matson, Mcdowell, Townsend, & Vitousek, 1999). Nevertheless, it remains unclear whether temperature, precipitation, and N deposition affect the extent to which nutrients regulate terrestrial BNF.

A recent meta-analysis has revealed an N inhibition, a Mo limitation, and a lack of P impact on free-living N fixation (in soil, litter, and moss) in forests and grasslands (Dynarski & Houlton, 2018). However, there are certain N fixers (i.e., lichen and leaf epiphytes/endophytes; Benner, Conroy, Lunch, Toyoda, & Vitousek, 2007; Cusack et al., 2009; Zheng et al., 2018) and biomes (i.e., tundra and savanna; Rousk, Degboe, et al., 2017; Sanginga, Danso, Zapata, & Bowen, 1995) that have been overlooked. Furthermore, we are unclear how nutrients regulate symbiotic N fixation (performed by N-fixing legumes) in terrestrial ecosystems and whether the responses of terrestrial BNF to nutrient addition vary across biomes, compartments, and N fixation types (free-living vs. symbiotic). Importantly, large uncertainty exists regarding the potential factors that affect the responses of BNF to nutrient addition at terrestrial scales. To address these knowledge gaps, we compiled a global database of free-living N fixation (in soil, litter, moss, lichen, and leaf) and symbiotic N fixation (in legume nodule) in response to N, P, and Micro addition across different biomes (i.e., tropical/subtropical forest, savanna, temperate forest, grassland, boreal forest, and tundra) and examined whether fertilization regimes (nutrient-addition rates, duration, and total load) and environmental factors (mean annual temperature [MAT], mean annual precipitation [MAP], and background N deposition) affect the extent of BNF in response to nutrient addition. We hypothesized that N addition would inhibit terrestrial BNF but that P and Micro addition would stimulate terrestrial BNF (H1). Because humid tropical forests have higher MAT and/or MAP and are thought to be less limited by N compared to temperate/boreal ecosystems (Hedin et al., 2003), we expected that the BNF responses to nutrient addition might be different between low-latitude (<30°) and mid-/high-latitude (≥30°) biomes (H2). Furthermore, we hypothesized that fertilization regimes and environmental factors would explain the variation in the BNF responses to nutrient addition (H3). Specifically, we assumed that increased nutrient-addition rates, duration, and total load would increase the sensitivity of BNF responses given that these fertilization regimes may limit BNF responses and that increased MAT, MAP, and N deposition would reduce the sensitivity of BNF responses to exogenous nutrient inputs given that increasing rates of these environmental factors may enhance the availability of soil and litter nutrients (e.g., stimulating decomposition of organic matter and leaf litter) to N fixers.

2 | MATERIALS AND METHODS

2.1 | Data sources

Peer-reviewed journal articles were searched using Google Scholar and Web of Science with the following keywords/phrases: ("nutrient addition" or "nitrogen addition" or "N addition" or "phosphorus addition" or "P addition" or "micronutrient addition" or "fertilization" or "deposition" or "enrichment" or "limitation") and ("nitrogen fixation" or "N fixation" or "N_2 fixation" or "dinitrogen fixation" or "nitrogenase") and were further searched from the reference lists of relevant articles. Articles were selected according to the following criteria: (a) control and fertilization plots established within the same study sites, that is, same microclimate, vegetation, and soil types; (b) field studies with at least one of the three treatments, including N addition (e.g., urea, HH_4NO_3, NH_4Cl), P addition (e.g., NaH_2PO_4, KH_2PO_4, $Ca(H_2PO_4)_2$), and Micro addition (either single or combined additions of Mo, Fe, copper [Cu], manganese [Mn], boron [B], and zinc [Zn]; Figure S7a; note that studies with a combination of multiple treatments [e.g., N + P fertilization and elevated CO_2 + N fertilization] were excluded); (c) variables of BNF rates in soil, litter, moss, lichen, leaf, and plant nodule samples; (d) BNF rates measured using acetylene reduction assay (ARA) or ^{15}N tracing methods (see Data S1 for details); (e) measurements in different sites and compartments (soil, litter, moss, lichen, leaf, and nodule) or under different treatments that were considered to be independent experiments; and (f) the last time point selected if multiple time points of measurements were available (because the BNF responses may be unstable right after nutrient addition). Based on these standards, a total of 516 experiments from 53 papers published from 1961 to April 2019 were included in this synthesis (distribution of data points shown in Figure 1; data sources shown in Appendix S1).

For comparison and analysis, the nutrient-addition rates per unit area (for both continuous and single addition experiments) were transformed to the unified unit of "kg ha^{-1} $year^{-1}$," and the duration of nutrient addition (i.e., length of time between the start of treatment and sampling) was transformed to the unified unit of "year." Total load, referred to the total amount of nutrient addition during the experiments, was calculated by multiplying nutrient-addition rates by duration and expressed as the unified unit of "kg/ha." Environmental factors (MAT, MAP, and background N deposition), fertilization regimes (nutrient-addition rates, duration, and total load), and initial nutrient concentrations of soil and litter as well as latitude and longitude were collected directly from the papers

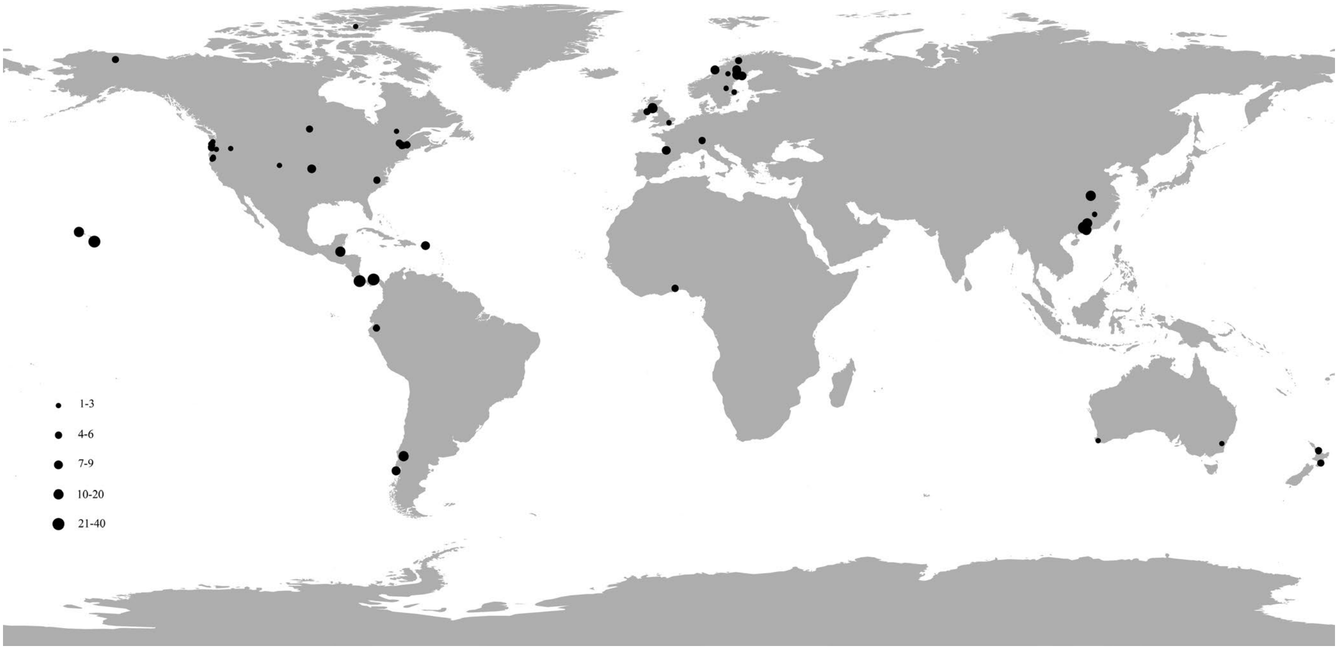

FIGURE 1 Distribution of the data points used in this meta-analysis. The sizes of the points represent the numbers of independent experiments

or indirectly from their citations. Data were obtained from tables, texts, or extracted from the figures in the papers using Origin 9.1 (OriginLab Co., Northampton) digital plugin (Digitize) software. MAT and MAP data were obtained from database (http://www.worldclim.org/) if not reported in the papers. Notably, ~60% of the selected papers (including their citations) provided N deposition data, and therefore, these papers were selected to analyze the relationships of BNF and N deposition. If the standard error (*SE*) was reported, then we calculated the standard deviation (*SD*) using the following equation:

$$SD = SE \times \sqrt{n} \tag{1}$$

where *n* is the sample size. If data were given as the means with a confidence interval (CI), then *SD* was calculated as follows:

$$SD = \frac{(\mathrm{CI_u} - \mathrm{CI_l})\sqrt{n}}{2Z_{\alpha/2}} \tag{2}$$

where $\mathrm{CI_u}$ and $\mathrm{CI_l}$ are the upper and lower limits of 95% CI, respectively, and $Z_{\alpha/2}$ is the *Z* score for a given level of significance (e.g., 1.96 when $\alpha = 0.05$). In cases that no *SD*, *SE*, or CI was given, we assigned *SE* as 1/4 of the means (Dynarski & Houlton, 2018).

2.2 | Data analysis

We conducted a meta-analysis to evaluate the responses of BNF to nutrient addition. Data were categorized into different biomes (i.e., tropical/subtropical forest, savanna, temperate forest, grassland, boreal forest, and tundra) and compartments (i.e., soil, litter, moss, lichen, leaf, and nodule). Low-latitude (<30°) biomes included tropical/subtropical forest and savanna, and mid-/high-latitude (≥30°) biomes included temperate forest, grassland, boreal forest, and tundra. Given that MAT, MAP, and N deposition rates may be different between low-latitude and mid-/high-latitude biomes, we defined two different categories of MAT (low vs. high MAT), MAP (low vs. high MAT), and N deposition (low vs. high N deposition) among the biomes. The demarcation points of the two categories of MAT, MAP, and N deposition were 15°C, 2,500 mm, and 7 kg $\mathrm{ha^{-1}\ year^{-1}}$, respectively, as determined by the median values of the available data (for MAT and MAP) or by the description of the selected articles (for N deposition). More than 60% of the data points derived from low-latitude and mid-/high-latitude biomes fell within the ranges of high MAT, MAP, and N deposition and low MAT, MAP, and N deposition, respectively (Figure S3). The effect size of nutrient addition for each observation was estimated by the natural logarithm transformed response ratio (lnRR):

$$\ln \mathrm{RR} = \ln\left(\overline{X_t}/\overline{X_c}\right) = \ln\left(\overline{X_t}\right) - \ln\left(\overline{X_c}\right) \tag{3}$$

where $\overline{X_t}$ and $\overline{X_c}$ are the means of the treatments and controls, respectively. Because the responses ratios (refer to "lnRR" throughout) of BNF to different types of nutrient (N, P, and Micro) addition are divergent (positive or negative), we defined the sensitivity of BNF to nutrient addition by the absolute value of response ratios. The variance (*v*) of response ratios was calculated as follows:

$$v = \frac{s_t^2}{n_t \overline{X_t^2}} + \frac{s_c^2}{n_c \overline{X_c^2}} \tag{4}$$

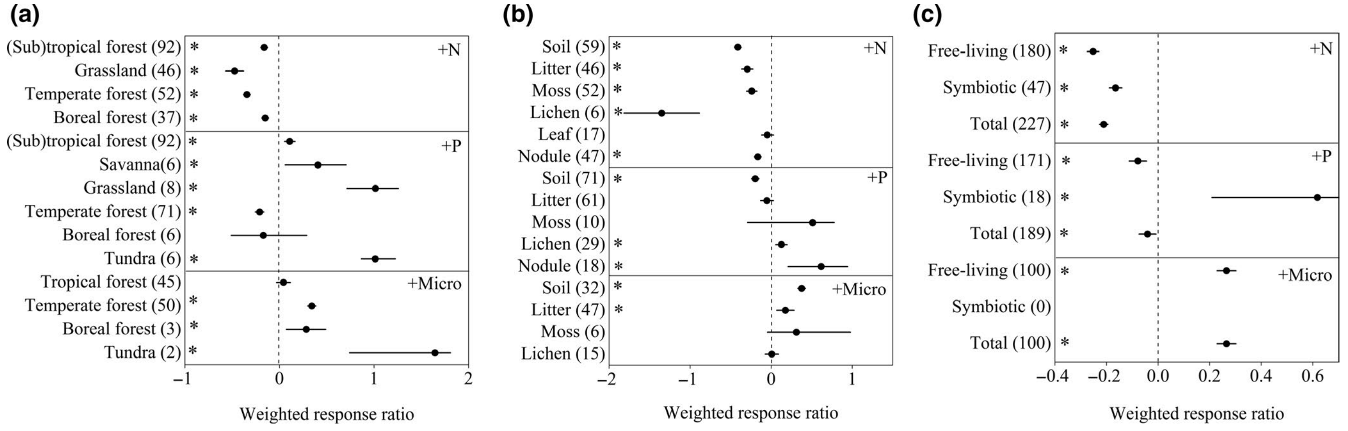

FIGURE 2 Effects of nutrient addition on biological nitrogen (N) fixation (BNF) across biomes (a), compartments (b), and N fixation types (c). +N: nitrogen addition; +P: phosphorus addition; +Micro: micronutrient addition. Total N fixation included free-living N fixation (in soil, litter, moss, lichen, and leaf) and symbiotic N fixation (in nodule). Error bars represent 95% confidence intervals (CIs). The numbers in the brackets represent sample sizes. *Represents significant responses ($p < 0.05$) that are recognized if the 95% CI does not overlap with zero. Vertical dashed lines are the reference of the response ratio of zero

where n_t and n_c are the sample sizes of the variable in the treatments and controls, respectively, and s_t and s_c are the *SD*s of the variable in the treatments and controls, respectively. We used MetaWin 2.1 software (Sinauer Associates Inc. Sunderland, MA) to calculate the weighted response ratio (RR_{++}) and 95% CI. Significant responses ($p < 0.05$) were recognized if the 95% CI did not overlap with zero. The percentage changes for the variables caused by nutrient addition were calculated as follows:

$$\text{Change}\ (\%) = \left[\exp\left(RR_{++}\right) - 1\right] \times 100\% \tag{5}$$

Previous studies have reported that BNF shows a linear (e.g., Markham, 2009) or logarithmic (e.g., Perakis et al., 2017; Reed et al., 2008; Rousk, Pedersen, et al., 2017) relationship with nutrient availability and environmental factors. Hence, linear or logarithmic regression models, which have a higher degree of fitting, were used to examine the relationships between response ratios or nutrient concentrations and fertilization regimes, environmental factors (including the full range of each environmental factor and the two categories of each environmental factor [as mentioned above]), or latitude.

3 | RESULTS

3.1 | BNF in response to nutrient addition

Nitrogen addition inhibited BNF in all the biomes, including tropical/subtropical forest (by 14.9% [95% CI: 12.2%–17.5%]; hereafter), grassland (37.8% [31.6%–43.4%]), temperate forest (29.1% [26.7%–31.5%]), and boreal forest (13.9% [11.7%–16.1%]; Figure 2a). The addition of N inhibited BNF in many substrates, including soil (by 33.7% [31.2%–36.0%]), litter (25.6% [20.3%–30.5%]), moss (21.2% [16.0%–26.1%]), lichen (74.1% [58.8%–83.7%]), and nodule (15.2% [13.1%–17.2%]; Figure 2b). Free-living, symbiotic, and total N fixation rates decreased by 22.3% [20.5%–24.0%], 15.2% [13.1%–17.2%], and 19.0% [17.7%–20.3%], respectively, following N addition (Figure 2c).

The effects of P addition on BNF varied depending on the types of ecosystem and substrate. Phosphorus addition inhibited BNF in temperate forest (by 18.9% [15.3%–22.5%]) and stimulated BNF in tropical/subtropical forest (11.5% [5.5%–17.9%]), savanna (50.2% [6.2%–102.3%]), grassland (176.0% [104.3%–251.4%]), and tundra (175.6% [137.9%–240.3%]) but did not affect BNF in boreal forest (Figure 2a). The addition of P inhibited soil BNF (by 17.8% [13.9%–21.5%]) and stimulated lichen (13.5% [5.9%–21.7%]) and nodule BNF (85.5% [23.3%–157.2%]) but did not affect litter and moss BNF (Figure 2b). Phosphorus addition inhibited free-living N fixation (by 7.5% [4.4%–10.6%]) but stimulated symbiotic N fixation (85.5% [25.8%–158.7%]). Total N fixation rates decreased following P addition though the effect was small (by 4.0% [0.8%–7.1%]; Figure 2c).

In contrast, Micro addition generally stimulated BNF, with significant effects detected in temperate forest (by 40.8% [34.9%–47.0%]), boreal forest (32.7% [7.6%–63.1%]), and tundra (418.3% [110.0%–510.0%]; Figure 2a). Regardless of ecosystem type, the addition of Micro stimulated soil (by 45.7% [39.1%–52.7%]) and litter BNF (19.2% [7.1%–32.7%]) but did not affect moss and lichen BNF (Figure 2b). Total N fixation rates (equal to free-living N fixation rates due to a lack of symbiotic N fixation data) increased by 30.4% [25.7%–35.3%] following Micro addition (Figure 2c).

The mean response ratios of BNF to nutrient addition were larger in mid-/high-latitude biomes than in low-latitude biomes, with significant differences detected for N- and P-addition scenarios ($p < 0.001$ and $p = 0.047$, respectively; Figure 3a–c). With decreasing latitude, the sensitivity of BNF to nutrient addition decreased in mid-/high-latitude biomes ($r^2 = 0.08$, $p < 0.001$; $r^2 = 0.02$, $p = 0.009$; $r^2 = 0.22$, $p < 0.001$ for N, P, and Micro addition, respectively) but did not change in low-latitude biomes ($p > 0.05$ for any nutrient treatment; Figure 3d–f). Soil and litter nutrient (N, P, and Micro)

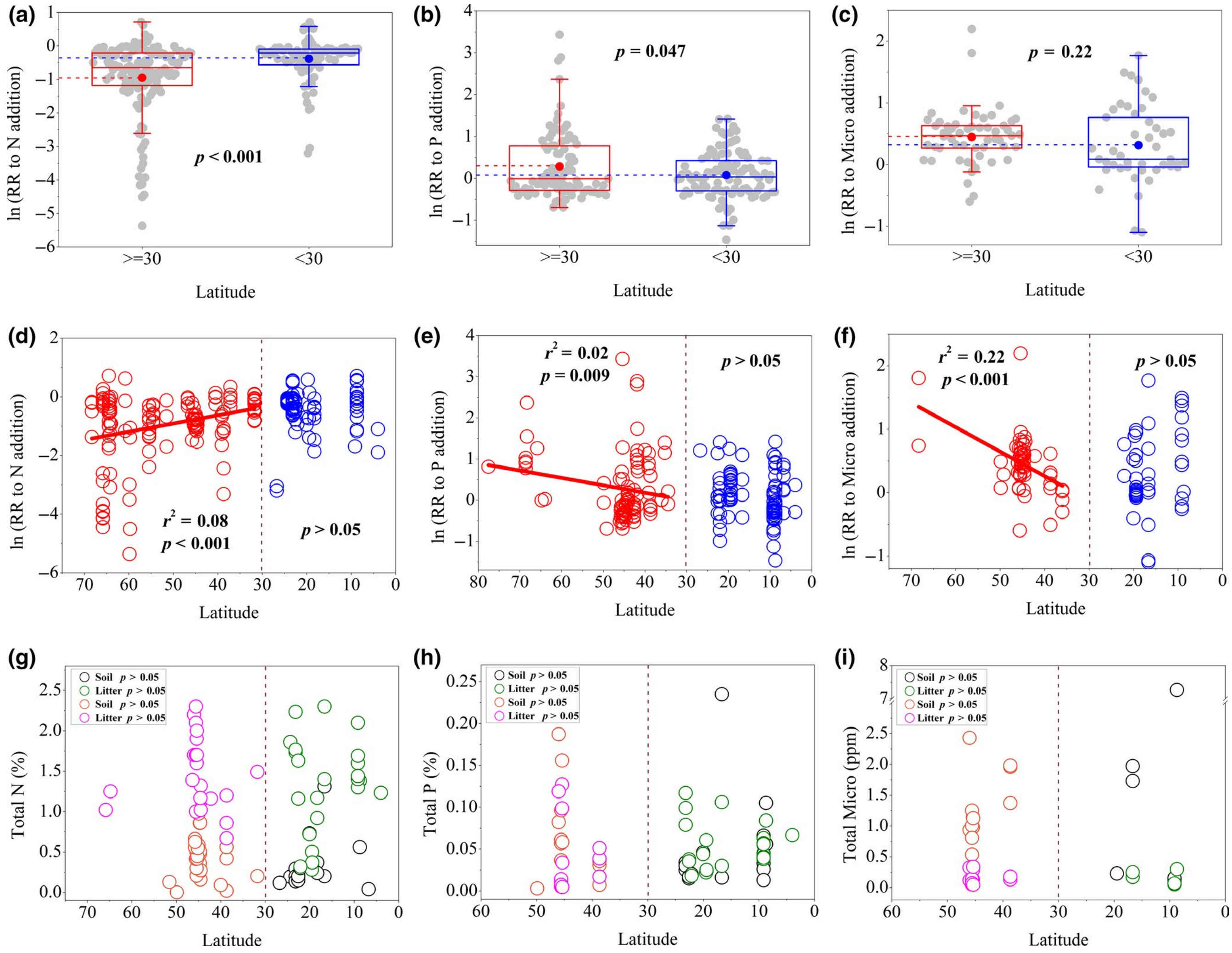

FIGURE 3 Box figures of the response ratio (RR) of biological nitrogen (N) fixation (BNF; including free-living and symbiotic N fixation) to addition of nutrients (N, phosphorus [P], or micronutrients [Micro]) (a–c); regression models of RR of BNF (free-living and symbiotic N fixation) to nutrient addition (d–f) and initial nutrient concentrations of soil and litter (g–i) against latitude in low-latitude (<30°) and mid-/high-latitude (≥30°) biomes, respectively. Each box represents the lower and upper quartiles with the medians and means shown as the central lines and solid circles, respectively. The *p* values in panels a, b, and c represent the statistical significance between the two boxes as determined using independent sample *t* test

concentrations did not change with latitude in low-latitude or mid-/high-latitude biomes ($p > 0.05$; Figure 3g–i).

3.2 | Factors affecting the BNF response to nutrient addition

Fertilization regimes (nutrient-addition rates, duration, and total load) did not explain the variance in the response ratios of BNF to nutrient addition ($p > 0.05$; linear or logarithmic regression; Table 1). In contrast, environmental factors (MAT, MAP, and N deposition) did explain the variance in the response ratios of BNF to nutrient addition (Table 1 and Figure S2). For the N-addition treatment, the sensitivity of BNF decreased across MAT ($r^2 = 0.18$, $p < 0.001$), MAP ($r^2 = 0.15$, $p < 0.001$), and N deposition ($r^2 = 0.15$, $p < 0.001$). For the P-addition treatment, the sensitivity of BNF decreased across MAT ($r^2 = 0.06$, $p = 0.004$) and MAP ($r^2 = 0.18$, $p < 0.001$) and increased across N deposition ($r^2 = 0.02$, $p < 0.001$). For the Micro-addition treatment, the sensitivity of BNF decreased across MAT ($r^2 = 0.04$, $p < 0.001$) but did not change across MAP ($p = 0.24$) and N deposition ($p = 0.73$). Overall, MAT, MAP, and N deposition had a similar effect on the response ratios of BNF to nutrient addition (which explained 4%–18%, 15%–18%, and 2%–15% of the variance in the BNF response ratios, respectively) across the biomes.

Environmental factors affected the response ratios of BNF to nutrient addition when MAT, MAP, and N deposition were low (Figure 4). Specifically, the sensitivity of BNF to N addition decreased across MAT, MAP, and N deposition in cold (MAT < 15°C; $r^2 = 0.07$, $p = 0.002$), low-rainfall (MAP < 2,500 mm; $r^2 = 0.18$, $p < 0.001$), and low-N-deposition (background N deposition <7 kg ha^{-1} year^{-1}; $r^2 = 0.18$, $p < 0.001$; Figure 4a–c) biomes, respectively. However, environmental factors did not affect the response ratios of BNF to N addition in warm (MAT ≥ 15°C; $p > 0.05$), high-rainfall

TABLE 1 Regression models of the response ratio (RR) of biological nitrogen (N) fixation (BNF; including free-living and symbiotic N fixation) to the addition of nutrients (N, phosphorus [P], and micronutrients [Micro]) against fertilization regimes (nutrient-addition rates, duration, and total load) or environmental factors (mean annual temperature [MAT], mean annual precipitation [MAP], and N deposition)

Type of independent variable	Dependent variable (*y*)	Independent variable (*x*)	Regression model	*n*	Adjust r^2	*p*
Fertilization regimes	ln(RR to N addition)	N-addition rates	—	168	—	0.62
		N duration	—	168	—	0.68
		N load	—	168	—	0.33
	ln(RR to P addition)	P-addition rates	—	99	—	0.65
		P duration	—	99	—	0.07
		P load	—	99	—	0.07
	ln(RR to Micro addition)	Micro-addition rates	—	29	—	0.83
		Micro duration	—	29	—	0.26
		Micro duration	—	29	—	0.30
Environmental factors	ln(RR to N addition)	MAT	$y = -1.41 + 0.05x$	227	0.18	<0.001
		MAP	$y = -3.76 + 0.43 \times \ln(x - 241.40)$	227	0.15	<0.001
		N deposition	$y = -1.05 + 0.03x$	149	0.15	<0.001
	ln(RR to P addition)	MAT	$y = 0.50 - 0.02x$	189	0.06	<0.001
		MAP	$y = 2.77 - 0.35 \times \ln(x - 159.02)$	189	0.18	<0.001
		N deposition	$y = 0.34 + 0.02 \times \ln(x - 0.20)$	95	0.02	<0.001
	ln(RR to Micro addition)	MAT	$y = 0.50 - 0.05 \times \ln(x - 0.20)$	100	0.04	<0.001
		MAP	—	100	—	0.24
		N deposition	—	32	—	0.73

(MAP ≥ 2,500 mm; $p > 0.05$), or high-N-deposition (background N deposition ≥ 7 kg ha^{-1} year^{-1}; $p > 0.05$) biomes. The sensitivity of BNF to P addition decreased across MAT and MAP in cold ($r^2 = 0.05$; $p = 0.02$) and low-rainfall ($r^2 = 0.32$, $p < 0.001$) biomes, respectively, but it increased across N-deposition in low-N-deposition biomes ($r^2 = 0.07$, $p = 0.01$; Figure 4d–f). The sensitivity of BNF to P addition increased across MAP in high-rainfall biomes ($r^2 = 0.05$, $p = 0.01$; Figure 4e). The sensitivity of BNF to Micro addition decreased across MAT in cold biomes ($r^2 = 0.14$, $p = 0.002$; Figure 4g). Overall, MAP affected the response ratios of BNF to nutrient addition (which explained 10%–32% of the variance in the BNF response ratios) more than MAT and N deposition did (which explained 5%–14% and 7%–18% of the variance, respectively, in the BNF response ratios) in cold, low-rainfall, and/or low-N-deposition biomes.

4 | DISCUSSION

4.1 | Terrestrial BNF in response to nutrient addition

As hypothesized (H1), total rates of terrestrial BNF (both free-living and symbiotic N fixation) decreased after N addition (Figure 2c), which extends a recent finding that terrestrial free-living N fixation declined under N enrichment (Dynarski & Houlton, 2018) and supports previous modeling estimates that BNF rates in natural ecosystems have dramatically declined due to anthropogenic N pollution (Galloway et al., 2004; Sullivan et al., 2014; Vitousek et al., 2013). It is not surprising that exogenous N inputs decrease BNF rates because the added N (e.g., NH_4^+) can inhibit nitrogenase synthesis (Bentley, 1987). Moreover, N fixers become less competitive with increasing soil N (Crews, 1999) and may therefore reduce the energy costs of BNF and obtain N from soil (Gutschick, 1981). Furthermore, we found that symbiotic N fixation was less sensitive to N inputs than was free-living N fixation, as evidenced by the smaller response ratios of BNF in nodule (15.2%) compared to those in soil, litter, moss, and lichen (21.2%–74.1%; Figure 2b). Given that there are more symbiotic N fixers (i.e., legume trees) in the tropics than in mid-/high-latitude ecosystems, our finding indicates that the sensitivity of BNF to N addition is lower in tropical/subtropical forests than in other ecosystems (14.9% vs. 13.9%–37.8%; Figure 2a), which supports a prior observation that humid tropical forests sustained high BNF rates under soil N richness (Reed et al., 2008; Zheng et al., 2018), while many temperate/boreal forests reduced BNF rates after N addition (Gundale et al., 2013; Perakis et al., 2017; Rousk & Michelsen, 2016b; Zackrisson et al., 2004). Given that free-living N fixation is a critical component of terrestrial N input (Reed et al., 2011), our finding indicates that elevated N deposition may reduce free-living N fixation more than symbiotic N fixation, which needs to be considered and integrated into the modeling of terrestrial N budgets in the future (Meyerholt et al., 2016; Wang et al., 2007). Together, our results suggest that N addition decreases terrestrial BNF regardless of biome and ecosystem compartment.

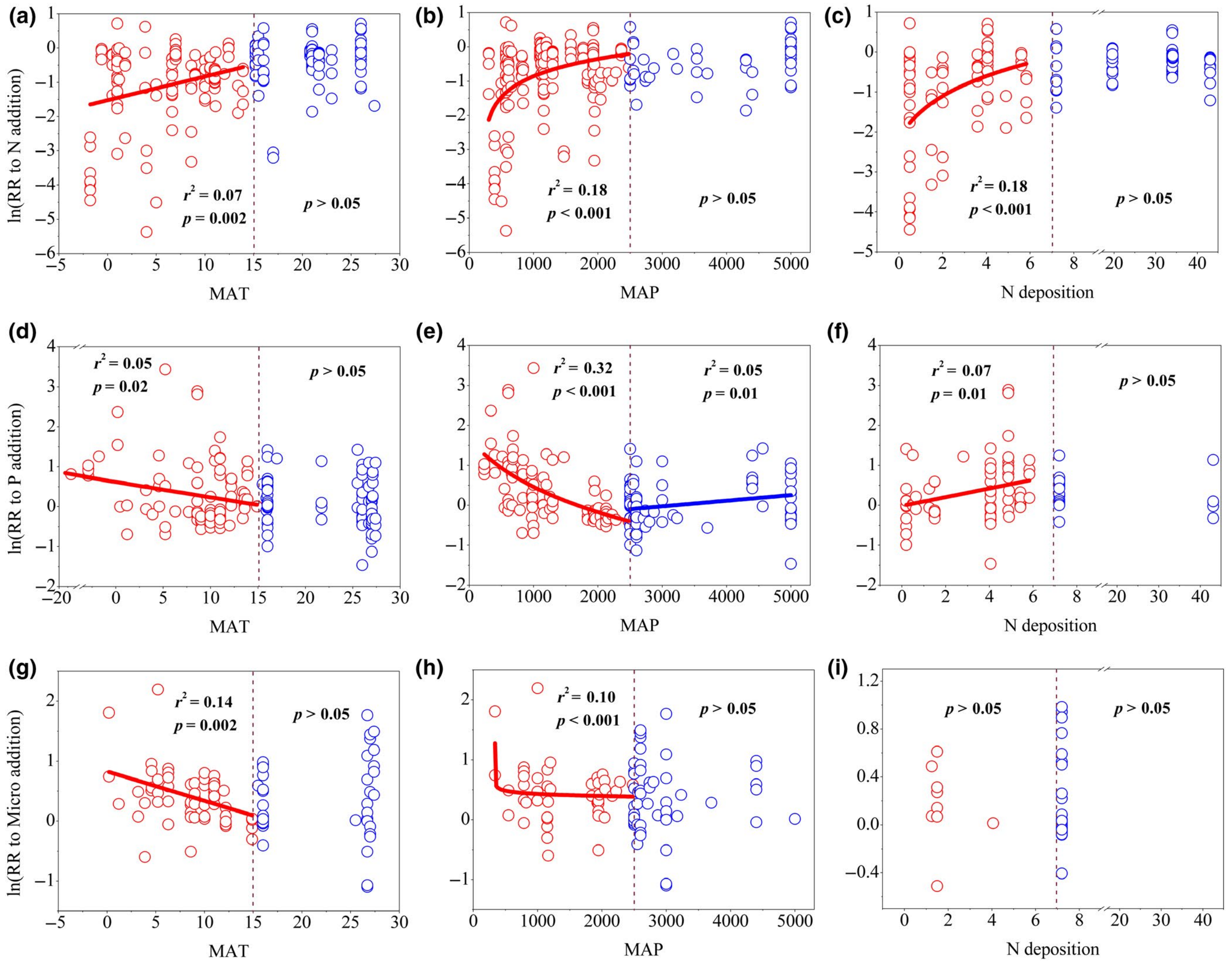

FIGURE 4 Response ratio (RR) of biological nitrogen (N) fixation (BNF; including free-living and symbiotic N fixation) to addition of nutrients (N (a–c), phosphorus [P] (d–f), or micronutrients [Micro] (g–i)) as a function of environmental factors (mean annual temperature [MAT], mean annual precipitation [MAP], and N deposition) across two different phases (low MAT [<15°C], low MAP [<2,500 mm], and low N deposition [<7 kg ha^{-1} year^{-1}] vs. high MAT [≥15°C], high MAP [≥2,500 mm], and high N deposition [≥7 kg ha^{-1} year^{-1}], respectively)

To our surprise, P addition inhibited terrestrial BNF overall, though the P-addition effect was divergent depending on N fixation type (i.e., P addition stimulated symbiotic N fixation but inhibited free-living N fixation; Figure 2c). This finding partially contradicts traditional views that increases in P supply stimulate BNF, as P is required for adenosine triphosphate (ATP) generation and for the cellular structure of N fixers (Reed et al., 2011) and, importantly, indicates that P availability limits symbiotic but not free-living N fixation at terrestrial scales (Figure 2c). Compared to a previous meta-analysis reporting a lack of significant P-addition effect on terrestrial free-living N fixation (Dynarski & Houlton, 2018), our meta-analysis, which was based on twice as much data, detected a decline in free-living N fixation after P addition (Figure 2c). However, our results did not negate the importance of P on BNF because the effects of P addition on BNF varied across biomes and ecosystem compartments. For example, P addition inhibited or did not affect BNF in temperate/boreal forest but stimulated BNF in tropical forest, savanna, grassland, and tundra (Figure 2a); moreover, P addition inhibited or did not affect soil, litter, and moss BNF but stimulated lichen and nodule BNF (Figure 2b). These divergent patterns may be explained by two mechanisms. First, the soil P status of ecosystems might affect the BNF responses to P addition (Wurzburger et al., 2012). Previous studies have indicated that P addition increases the abundance of epiphytic lichens and rates of lichen N fixation in several Hawaiian forests with P-poor soils (Benner et al., 2007) but P addition has no effect on lichen N fixation in several Chilean forests with P-rich soils (Pérez, Thomas, Silva, Aguilera, & Armesto, 2017). Second, the BNF responses to P inputs may be related to the type of N fixation. For example, because P is a key nutrient for plants, the addition of P often stimulates the growth of legume plants and thereby increases their demands for N, which further increases nodule biomass and symbiotic N fixation (Batterman, Wurzburger, & Hedin, 2013; Zheng, Li, et al., 2016). In contrast, the addition of P has no or negative effects on free-living N fixation in many forests (Barron et al., 2008; Perakis et al., 2017; Rousk, Degboe, et al., 2017; Figure S1). A potential reason for this phenomenon could be that ongoing or high P addition exerts a salt effect on free-living N fixers (e.g., mosses), leading to an osmosis imbalance and potassium (K) loss in N fixers, and thus a K limitation on BNF (Rousk, Degboe, et al., 2017). Another reason may be that P input reduces the decomposition of organic matter in several temperate forests (possibly because decomposers reduce reliance on organic matter for P after P inputs; van Huysen, Perakis, & Harmon, 2016), which leads to a decrease in C resources available to heterotrophic N fixers (Perakis et al., 2017). Hence, our findings challenge traditional views that P availability limits BNF and indicate that the role of P in BNF depends on the types of biomes and N fixers.

Consistent with our hypothesis (H1), the addition of Micro (Mo, Fe, V, etc.) increased terrestrial BNF rates (Figure 2), which extends a recent finding of Mo limitation on terrestrial free-living N fixation (Dynarski & Houlton, 2018). Moreover, we found that Micro limitation of BNF occurred in mid-/high-latitude biomes (i.e., temperate/boreal forests and tundra) but not in tropical forests (Figure 2a). This finding contrasts with prior knowledge that cold temperate and boreal soils are expected to be less limited by rock-derived nutrients (e.g., Mo; Jean, Phalyvong, Forest-Drolet, & Bellenger, 2013) and that tropical soils subjected to weathering and leaching losses often exhibit low availability of Micro (Wichard, Mishra, Myneni, Bellenger, & Kraepiel, 2009). Although previous studies have shown that Micro addition increased BNF rates in certain tropical forests (Barron et al., 2008; Vitousek & Hobbie, 2000; Winbourne et al., 2017), our results suggest that Micro may not be the key limiting factor of BNF in tropical forests overall. Among the substrates, we found that only soil and litter exhibited positive BNF responses to Micro inputs (Figure 2b), which is consistent with a recent finding that Mo addition increased BNF rates in litter but not in moss or lichen in a postvolcanic site (Pérez et al., 2017). Given that plant growth is limited by N supply (LeBauer & Treseder, 2008) and that soil and litter are important N pools supporting plant growth, our findings indicate that enhancing Micro availability in soil and litter may increase BNF and thus NPP in temperate and boreal biomes.

Although terrestrial BNF showed divergent responses to the addition of different nutrients, we found a common pattern and trend for BNF responses across latitude (Figure 3). Mean BNF response ratios to nutrient (N, P, and Micro) addition were smaller in low-latitude biomes (8.5–36.9%) than in mid-/high-latitude biomes (32.9%–61.3%; Figure 3a–c; Figure S4), which supports our hypothesis (H2). This finding, together with the decreases in the sensitivity of mid-/high-latitude BNF to nutrient addition with decreasing latitude (Figure 3d–f), indicates that low-latitude BNF is less affected by nutrient enrichment, which provides a mechanism for long-standing N-paradoxical phenomena, whereby many lowland tropical forests, expected to be limited by P rather than by N, harbor abundant N-fixing trees (Hedin et al., 2009; Menge et al., 2014) and exhibit high BNF rates compared to temperate/boreal biomes (Cleveland et al., 1999; Reed et al., 2011). However, the reasons for the decreases in the sensitivity of BNF to nutrient addition with decreasing latitude remain unclear.

4.2 | Factors affecting the BNF response to nutrient addition

To explore the factors that affect the latitude patterns of BNF in response to nutrient addition, we hypothesized that fertilization regimes might affect the extent of BNF responses (H3). There have been several lines of evidence indicating that fertilization regimes may affect the extent of BNF responses. For example, in a Swedish forest, feather mosses exert stronger negative BNF responses to high rates of N addition (50 kg N ha^{-1} year^{-1}) than to low rates of N addition (12.5 kg N ha^{-1} year^{-1}; Gundale et al., 2013). A previous study in a salt marsh grass stand in South Carolina revealed that soil N fixation increased after 2 weeks of N addition but declined after 8 weeks of N addition (Piceno & Lovell, 2000). In a Micro-addition experiment, Silvester (1989) found a larger increase in litter N fixation after 2 days of treatment compared to that after 10 hr of treatment. Barron et al. (2008) reported that N fixation of organic layers significantly increased after high doses of Micro addition (504 μg Mo/kg) and marginally increased after low doses of Micro addition (42 μg Mo/kg) in a Panamanian tropical forest. However, our study found no evidence that fertilization regimes, including nutrient-addition rates, duration, and total load, explained the variation in the BNF response to nutrient addition at terrestrial scales ($p > 0.05$; Table 1). We cannot rule out the possibility that other fertilization regimes, such as fertilizer types (e.g., NH_4NO_3 vs. urea) and forms (e.g., liquid vs. solid), affect the extent of BNF responses (Figure S7a,b). To our knowledge, there is no published study addressing why different fertilizer types and forms have different effects on the BNF responses (Figure S7a,b) and it remains unclear whether fertilizer types and forms account for the observed latitude patterns of BNF in response to nutrients (Figure 3).

We hypothesized that environmental factors might account for the BNF response to nutrient addition (H3), which is supported by our results. Our regression models showed that MAT, MAP, and N deposition explained 2%–18% of the variation in BNF response ratios ($p < 0.001$; Table 1; Figure S2). This finding advances our knowledge that environmental factors can affect not only the rates of terrestrial BNF (Galloway et al., 2004; Reed et al., 2011) but also the extent to which nutrients regulate BNF. We assumed that environmental factors would be able to explain the decreases in the sensitivity of BNF with decreases in latitude (Figure 3). Given the lower air temperature, rainfall, and N deposition rates in mid-/high-latitude regions compared to those in low-latitude areas overall, our results support the assumption, as the high sensitivity of BNF to nutrient addition observed in cold (MAT < 15°C), low-rainfall (MAP < 2,500 mm), and low-N-deposition (<7 kg ha^{-1} year^{-1}) biomes (Figure S4) decreased with increases in MAT, MAP, and N deposition ($p \le 0.02$; Figure 4). To date, several studies have revealed the variance in P and/or Mo limitation of N fixation with seasonal dynamics in temperate/boreal forests (Jean et al., 2013; Lett & Michelsen, 2014; Rousk, Degboe, et al., 2017). Although how global change directly affects BNF remains inconclusive (e.g., Gundale et al., 2012; Hungate et al., 2004; Rousk, Degboe, et al., 2017), our findings offer new insight that the variance in certain environmental factors (i.e., elevated temperature, precipitation, and N deposition) may lead to declines in the nutrient constraints (i.e., the N inhibition and the P and Micro limitation) of BNF in mid-/high-latitude biomes. Furthermore, we explored the potential mechanisms underlying this phenomenon as follows.

Among the tested environmental factors, temperature had a dominant effect on mid-/high-latitude BNF responses to nutrient addition. Increases in MAT reduced the sensitivity of BNF to nutrient (N, P, and Micro) addition in cold biomes (MAT < 15°C) but not in warm biomes (MAT ≥ 15°C; Figure 4). A potential mechanism for this phenomenon is that biological processes are limited by temperature in cold biomes (Jean et al., 2013; Markham, 2009) such that elevated temperature may have increased the supply of soil and litter nutrients (e.g., via decomposition of leaf litter and organic matter; Aerts, 2006) to N fixers in cold biomes and thus reduced the sensitivity of N fixers to exogenous nutrient inputs. Another possible mechanism is that increasing temperature stimulates the growth of woody plants and increases shading of forest canopies in cold biomes, which result in light limitation on N fixers (e.g., mosses; Gundale et al., 2012). Given that light regulates BNF more strongly than nutrients (e.g., N; Taylor & Menge, 2018), it is possible that N fixers increase investment in light acquisition rather than in nutrient acquisition under light-limiting conditions. Moreover, we found that increases in MAT explained 7%–93% of the decreases in free-living and/or symbiotic BNF response ratios in cold biomes and 7%–65% of the decreases in BNF response ratios in grasslands and in temperate and boreal forests (Figures S5 and S6), which supports our finding that elevated temperature reduces the sensitivity of N fixers to nutrient addition in mid-/high-latitude biomes.

Similarly, increasing precipitation reduced the sensitivity of BNF to nutrient (N and P) addition in low-rainfall biomes (MAP < 2,500 mm; Figure 4), which is supported by our result that increases in MAP explained 2%–34% of the decreases in free-living and/or symbiotic BNF response ratios in low-rainfall biomes and 11%–87% of the decreases in BNF response ratios in grasslands and in temperate and boreal forests (Figures S5 and S6). There is a potential mechanism responsible for this phenomenon. Specifically, as moisture is a limiting factor of nutrient cycling in low-rainfall biomes, elevated precipitation increases nutrient (e.g., N, P, and Micro) release through the pathways of litter decomposition and mineralization of organic matter (Jackson et al., 2011; Jean et al., 2013) and increase the mobility of soil nutrients (N, P, and Micro), which become readily available to N fixers (Winbourne et al., 2017). Therefore, N fixers may reduce dependence on exogenous nutrients and the sensitivity to exogenous nutrient addition. In contrast, precipitation intensified the P limitation of BNF in high-rainfall biomes (Figure 4; Figure S5), though the reason for this phenomenon is not clear. We propose that humid tropical forests are typically limited by P due to chronic leaching losses of P (Hedin et al., 2003) and that increased rainfall may lead to greater losses of soil P, which aggravates the P limitation of BNF.

Background N deposition affected the sensitivity of BNF to N and P addition in low-N-deposition biomes (<7 kg ha^{-1} year^{-1}), and the effects varied depending on the type of nutrients added (Figure 4). On the one hand, elevated N deposition alleviated the inhibition of N on BNF (Figure 4c), indicating a lower sensitivity of N fixers to added N under high N deposition scenarios. This finding is consistent with a recent finding that free-living N fixation showed a minor response to experimental N addition in a subtropical forest that experienced high N deposition (Zheng et al., 2018). A potential mechanism is that chronic high N deposition may lead to a shift in N-fixing microbes that adapt to N-rich environments (Piceno & Lovell, 2000). On the other hand, elevated N deposition intensified the P limitation of BNF (Figure 4f), which was expected because high N deposition can result in ecosystem N/P imbalance and induce soil acidification, thereby reducing the availability of P (Matson et al., 1999). However, it is interesting that the elevated P limitation of BNF induced by N deposition occurred (Figure S6) but that P addition alone did not increase BNF rates in temperate forests (Figure 2). This finding indicates a potential interaction between N and P inputs on BNF. Because BNF is regulated by multiple nutrients (Reed et al., 2011) and current knowledge is limiting with regard to how multiple nutrients simultaneously affect terrestrial BNF (Dynarski & Houlton, 2018), we suggest that more studies are needed to assess the effects of multiple nutrients on BNF in natural ecosystems.

Overall, our study found a negative effect of N addition, a positive effect of Micro addition, and an inconsistent effect of P addition on terrestrial BNF across different biomes, compartments, and N fixation types, which extends a recent meta-analysis reporting the responses of free-living N fixation to nutrient availability in forests and grasslands (Dynarski & Houlton, 2018). Moreover, we found that temperature, precipitation, and N deposition affected BNF in response to nutrient addition in mid-/high-latitude (≥30°) biomes, which provides new insight that environmental factors affect terrestrial BNF responses to nutrient enrichment. There are several limitations or implications of our study. First, our study focuses on the environmental factors of temperature, precipitation, and N deposition (which only explained 5%–32% of the variance in the BNF responses), while other factors (e.g., light intensity: Gundale et al., 2012; Taylor & Menge, 2018; CO_2 concentrations: Hungate et al., 2004; seasonal dynamics: Jean et al., 2013; Rousk, Degboe, et al., 2017) that may affect the growth of N fixers were not evaluated and deserve further research. Second, many Micro-addition studies focus on the role of Mo and its combination with other micronutrients, and our knowledge regarding how non-Mo micronutrients (e.g., Fe and V) regulate BNF in natural ecosystems remains poor (Figure S7a). We suggest that comparative studies of the importance of different micronutrients (e.g., Mo vs. Fe and Mo vs. V) in regulating BNF are needed in the future. Third, most of the studies measured BNF using the ARA method, and relatively few studies measured BNF using the ^{15}N tracing method (Figure S7c). Different measurement methods may lead to differences in the extent of BNF responses to nutrients (Figure S7c), and the reason for this phenomenon needs to be explored. Fourth, a meta-analysis addresses the direction and extent of a variable in response to treatments at large scales, but it cannot avoid the bias caused by subjective factors (e.g., anthropogenic selection of nutrient-addition doses). For example, many humid tropical forests are N-rich (or experience high N deposition) such that researchers apply a high dose of N addition to detect a response, while many boreal/arctic ecosystems are N-poor (or experience low N deposition) such that researchers mainly apply a low dose of N addition. Although this bias cannot be avoided, our study examined the BNF responses in different biomes separately and detected nonsignificant relationships of the BNF responses with fertilization regimes. We suggest that future studies of N deposition and BNF should also consider low doses of N addition in tropical ecosystems because many experiments have applied N-addition rates that are too high (e.g., 150 kg N ha^{-1} $year^{-1}$) to reflect the real N-deposition level (only up to ~60 kg N ha^{-1} $year^{-1}$ in several high-N-pollution areas; Galloway et al., 2008). Finally, our findings indicate that certain types of global change (i.e., warming, elevated precipitation and N deposition) lead to declines in the nutrient constraints of BNF in mid-/high-latitude biomes, which does not necessarily mean that global change will increase BNF in these biomes. For example, several recent studies in high-latitude ecosystems have reported that warming increases BNF (Rousk & Michelsen, 2016a; Rousk, Pedersen, et al., 2017), but extreme or chronic warming inhibits BNF (Gundale et al., 2012; Lett & Michelsen, 2014). Our study reveals a global pattern of terrestrial BNF under nutrient enrichment and its potential relationships with temperature, precipitation, and N deposition, but how terrestrial BNF responses directly to global change remains largely unclear.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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SUPPORTING INFORMATION

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