Review Paper

Patterns and mechanisms of responses by soil microbial communities to nitrogen addition

Zhenghu Zhou [a], Chuankuan Wang [a, *], Mianhai Zheng [b], Lifen Jiang [c], Yiqi Luo [c]

[a] Center for Ecological Research, Northeast Forestry University, 26 Hexing Road, Harbin 150040, China
[b] Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China
[c] Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK 73019, USA

ABSTRACT

Anthropogenic nitrogen (N) deposition is expected to increase substantially and continuously in the future. Soil N availability regulates microbial communities and the decomposition and formation of soil organic matter, which have great impacts on global carbon (C) cycling. We conducted a meta-analysis based on 454 N-addition experiments in order to synthesize the patterns and mechanisms of responses by soil microbial communities to N addition in various biomes (i.e., boreal forest, temperate forest, tropical/subtropical forest, grassland, and desert). Results showed that the effects of N addition on the total microbial biomass varied depending on biome types, methodologies (fumigation—extraction technique vs. total phospholipid fatty acid), and N-addition rates. Nitrogen addition consistently decreased the microbial C:N and fungi to bacteria ratio (F:B), but increased Gram positive bacteria to Gram negative bacteria ratio (GP:GN) among biome types and N-addition rates. Nitrogen addition increased soil N availability and thereby resulted in soil acidification. Regression technique and principal component analyses showed that the shifts in the F:B and GP:GN mainly resulted from enhanced N availability due to N addition rather than soil acidification. When the N addition rate is lower than 100 kg N ha^{-1} year^{-1}, about ten times higher than of global normal rate, the positive response of microbial growth was found. Overall, these findings revised the previous notion that N addition inhibited the microbial growth. Microbial species shifts might accentuate or mitigate the effects of alterations in microbial biomass at the ecosystem level, highlighting the critical role of microbial community composition in soil ecosystem functions under N deposition scenarios.

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1. Introduction

Anthropogenic nitrogen (N) deposition is expected to increase substantially and continuously in the future (Dentener et al., 2006; Galloway et al., 2008). The worldwide N deposition has the potential to constrain the accumulation of anthropogenic CO_2 in the Earth's atmosphere by increasing ecosystem carbon (C) storage, thereby slowing the pace of climate warming (LeBauer and Treseder, 2008; Xia and Wan, 2008; Zak et al., 2017). Soil microbes, regulated by soil N availability, are capable of changing the terrestrial C cycling by decomposition and formation of soil organic matter (SOM) (Cotrufo et al., 2013; Xu et al., 2016, 2017). For example, the substrate with a high N content can be rapidly decomposed by microorganisms at the initial stage, resulting in large accumulation of microbial products and concomitant formation of stable SOM. In contrast, for the substrate with a low N content, more C tends to be respired rather than stored in soils as stable SOM (Manzoni et al., 2012; Cotrufo et al., 2013; Xu et al., 2014). However, it remains unclear how N addition regulates soil microbial biomass and community composition among various terrestrial ecosystems, which constrains our understanding of soil C cycling in response to N deposition.

Nitrogen addition is supposed to increase microbial biomass via increasing the C or/and N resource availability. Specifically, most terrestrial ecosystems in the world are limited by N, as evidenced by the positive responses of above-ground (29% increase; LeBauer and Treseder, 2008) and below-ground plant growth (35.5% increase; Xia and Wan, 2008) to N additions. Besides the quantity of

litter inputs, N addition can significantly increase litter N concentration by 36% at the global scale (Yuan and Chen, 2015). Several previous meta-analyses reported that N addition increased soil N availability and dissolved organic C by more than 110% and 10%, respectively (Liu and Greaver, 2010; Lu et al., 2011a, b; Yue et al., 2016). Nevertheless, N addition also results in soil acidification, leading to leaching of base cations (e.g., Mg^{2+}, Ca^{2+}, and Na^+) and mobilization of Al^{3+} (Treseder, 2008). Consequently, microbial growth may be constrained due to the reduced availability of Mg^{2+}, Ca^{2+}, or Na^+ and the toxicity of Al^{3+} in soils (Treseder, 2008; Chen et al., 2015).

Microbial community composition is also sensitive to variations in soil N availability and soil pH (Six et al., 2006; Högberg et al., 2007; Rousk et al., 2010; Fanin et al., 2013; Waring et al., 2013; Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015; Zhou and Wang, 2015). Fungi dominates in the decomposition of the SOM with a low nutrient content, because their nutrient demands and metabolic activities are low, compared to bacteria (Fierer et al., 2003; Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015; Zhou et al., 2017a). Consequently, N addition may decrease the fungi to bacteria ratio (F:B). Meanwhile, the soil acidification induced by N addition is likely to increase the F:B, because fungi have higher adaptability of the soils with high H^+ concentration than bacteria (Högberg et al., 2007; Rousk et al., 2010; Chen et al., 2015) due to their thick and interlinked peptidoglycan cell wall (Schimel et al., 2007; Nielsen and Ball, 2015). Through these two opposite processes, the ultimate effect of N addition on soil microbial biomass and community composition remains unclear and needs validation across different ecosystems.

To date, there have been eight meta-analyses regarding the effects of simulated N deposition on microbial biomass or/and community composition (i.e., Treseder, 2008; Janssens et al., 2010; Liu and Greaver, 2010; Lu et al., 2011a, b; Geisseler and Scow, 2014; Geisseler et al., 2016; Yue et al., 2016). However, no consistent conclusions have been reached yet. Five of the eight meta-analyses emphasized the central tendency of ecosystem C or N pools and associated C or N processes under N addition (Janssens et al., 2010; Liu and Greaver, 2010; Lu et al., 2011a, b; Yue et al., 2016). Two studies examined the responses of microbial biomass to N addition in agricultural systems (Geisseler and Scow, 2014; Geisseler et al., 2016). These seven studies predominantly focused on microbial biomass C (MBC) rather than community composition. Only one study synthesized the responses of both microbial biomass and community composition to N addition (Treseder, 2008), but her data set compiled almost one decade ago included only 29 studies. Moreover, about 89% of the Earth's vegetation receives N input of <10 kg N ha^{-1} year^{-1} currently (Dentener et al., 2006). Summarizing from previous meta-analyses (LeBauer and Treseder, 2008; Xia and Wan, 2008; Janssens et al., 2010; Peng et al., 2017), however, simulated N deposition experiments were generally applied very high N addition rates. Given the N-addition experiments have been booming recently, it allows us to compile the global data and explore generalities of soil microbial communities in response to N addition across various ecosystems and geographical gradients, and figure out what is actually happening in ecosystems.

In this meta-analysis, we established a global data set by retrieving peer-reviewed papers published till November 2016, including 454 simulated N-deposition experiments from 134 papers that covered various natural ecosystems (i.e., boreal forest, temperate forest, tropical/subtropical forest, grassland, and desert; Text S1; Table S1; Figs. S1 and S2). Our objectives were to explore general responses of soil microbial communities to N addition and the underlying mechanisms.

2. Materials and methods

2.1. Sources of data

The peer-reviewed articles reporting effects of N addition on soil microbes in terrestrial ecosystems were collected globally by searching the Web of Science (http://apps.webofknowledge.com), Google Scholar (https://scholar.google.com), and China National Knowledge Infrastructure (CNKI, http://www.cnki.net) till November 2016. The keywords and terms used for the literature online-searching were "(nitrogen deposition OR nitrogen addition OR nitrogen enrichment OR nitrogen fertilizer OR nitrogen amendment OR nitrogen elevated) AND (microbial biomass OR microbial communities OR fungi OR bacteria) AND (soil)". The articles satisfying the following criteria were included in this meta-analysis: (1) only field experiments were included; (2) experimental and control plots must be established within the same experimental site, i.e., same microclimate, vegetation and soil types between the treatments; and (3) the N addition rates were clearly reported. A total of 454 N addition experiments from 134 papers met the criteria above and were included in the meta-analysis (Text S1; Table S1; Figs. S1 and S2).

Similar to a previous meta-analysis by Treseder (2008), we only adopted the microbial biomass measured with the chloroform fumigation—extraction technique and the total phospholipid fatty acid (PLFA) analysis, which are the most commonly used methods for microbial biomass measurements. Therefore, microbial biomass C (MBC), microbial biomass N (MBN), and total PLFA were used to estimate the microbial total biomass. The biomass of fungi (the term of 'fungi' is referred to saprotrophic fungi in order to examine whether N addition reduces the abundance of decomposer fungi), bacteria, Gram positive bacteria (GP), Gram negative bacteria (GN), actinomycetes, and arbuscular mycorrhizal fungi were measured by taxa-specific PLFA. For the community composition, the data set included the microbial C:N, fungi to bacteria ratio (F:B), and Gram positive bacteria to Gram negative bacteria ratio (GP:GN).

The matched soil pH, soil organic C (SOC), soil total N, soil dissolved organic C (DOC), soil available N (aN, sum of NO_3^- and NH_4^+), and location (i.e., latitude and longitude) were also collected. Finally, the relevant environmental variables (mean annual temperature (MAT) and mean annual precipitation (MAP)) were also obtained directly from the papers, cited papers, or extracted from the data base at http://www.worldclim.org/ using the location information if not reported.

2.2. Data acquisition and analysis

The raw data were obtained numerically from the tables, texts, or extracted from the figures in the original papers with the Origin 7.0 digital plugin (Digitize) software. If the mean and standard error (SE) were reported, then the standard deviation (SD) was calculated as:

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

where n is the sample size. If the mean and confidence interval (CI) were given, the SD was computed as:

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

where CI_u and 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 (i.e., 1.96 at $\alpha = 0.05$). In the cases that no SE, SD, or CI was reported, the SD was assigned as 1/10 of the mean (Luo et al., 2006).

To maximize the comparability, (1) we categorized the data into

different biomes, i.e., boreal forests, temperate forests, tropical/subtropical forests, grasslands, and deserts; and (2) the experiments were further binned by 50 kg N ha^{-1} year^{-1} of N-addition rate intervals, i.e., <50 kg N ha^{-1} year^{-1}, 50—100 kg N ha^{-1} year^{-1}, 100—150 kg N ha^{-1} year^{-1}, 150—200 kg N ha^{-1} year^{-1}, and >200 kg N ha^{-1} year^{-1}.

Following Rosenberg et al. (2000), we conducted the meta-analysis to evaluate responses of soil microbes to N addition using MetaWin 2.1 software (Sinauer Associates Inc., Sunderland, MA, USA). Specifically, the effect of N addition was estimated for each observation as the natural logarithm transformed (ln) response ratio (RR):

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

where $\overline{X_t}$ and $\overline{X_c}$ are means of the concerned variable in the treatment and control groups, respectively. The variance (v) was calculated as:

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

where n_t and n_c are the sample sizes of the variable in the N-addition treatment and control, respectively; s_t and s_c are the SDs of the variable in the treatment and control, respectively. The weighted mean response ratio (RR$_{++}$) was calculated from the RR of individual pairwise comparison between the treatment and control:

$$RR_{++} = \frac{\sum_{i=1}^{m} \sum_{j=1}^{k} w_{ij} RR_{ij}}{\sum_{i=1}^{m} \sum_{j=1}^{k} w_{ij}} \tag{5}$$

where m is the number of groups (e.g., biomes types, N-addition rates), and k is the number of comparisons in the ith group. The SE of the RR$_{ij}$(s(RR$_{++}$)) was calculated as:

$$s(RR_{++}) = \sqrt{\frac{1}{\sum_{i=1}^{m} \sum_{j=1}^{k} w_{ij}}} \tag{6}$$

where w_{ij} (weighting factor) was estimated as:

$$w_{ij} = \frac{1}{v} \tag{7}$$

If the number of data points used for assessing RR$_{++}$ of a concerned variable was greater than 20, the 95% CI was calculated as:

$$95\% \, CI = RR_{++} \pm 1.96 s(RR_{++}) \tag{8}$$

If the number of data points was less than 20, the bootstrapping method was used to obtain the lowest and highest 2.5% values as the bootstrap confidence based on 5000 iterations (Janssens et al., 2010). If the 95% CI overlapped with zero, then it was considered as an insignificant N-induced response. The percentage changes in the variables induced by N addition were measured by:

$$Effect \, size \, (\%) = (\exp(RR_{++}) - 1) \times 100\% \tag{9}$$

Principal component analyses (PCA) were conducted in order to identify the multivariate effects of soil acidification, increased N availability, climate factors (latitude, MAT, and MAP), and N addition regimes (N addition rate and study duration) on the RRs of microbial biomass (pooled MBC and total PLFA) and community composition (F:B and GP:GN) and their interactions.

3. Results

3.1. Effects of N addition on soil properties

A total of 454 N-addition experiments from 134 articles were collected in this study (Text S1; Table S1; Figs. S1 and S2) with more than half of the data points published in the past three years (i.e., 2014—2016; Fig. S2). Only two points added N at the rate of 5 kg N ha^{-1} year^{-1}, five points added N at the rate of 10 kg N ha^{-1} year^{-1}, and more frequent rates were 50 (19%), 100 (22%), and >150 (28%) kg N ha^{-1} year^{-1} (Fig. S2). Overall, 98% of the studies applied N at rates in excess of 10 kg N ha^{-1} year^{-1}.

Across all studies, N addition resulted in soil acidification (Table 1), which was remarkably enhanced by the N-addition rates (Fig. S3). Study duration was not significantly correlated with the RR of pH (Fig. S3). Nitrogen addition significantly increased soil available N by 92.8%, soil DOC by 12.4%, organic C by 4.8%, and total N by 8.6% (Table 1). The RR of available N increased as the N-addition rates and experimental duration increased (Fig. S3).

3.2. Effects of N addition on microbial communities across all experiments

Across all individual studies, N addition reduced MBC by 5.6% (Fig. 1) but stimulated MBN by 3.2% (Fig. 1). However, the 95% CI of the effect size of the total PLFA overlapped the zero (Fig. 1). The biomass of fungi, arbuscular mycorrhizal fungi, and actinomycetes were reduced by 7.1%, 6.1%, and 2.3%, respectively, after the N addition (Fig. 1). Nitrogen addition significantly increased the biomass of total bacteria and GP bacteria by 5.8% and 15.8%, respectively (Fig. 1). However, N addition had no significant effect on the GN bacteria biomass (Fig. 1). Additionally, N addition shifted the microbial community composition, with reduced microbial C:N and F:B by 8.2% and 10.0%, respectively, but increased GP:GN by 10.5% (Fig. 1).

3.3. Factors affecting responses of microbial communities

3.3.1. Biome types

The MBC and total PLFA tended to respond oppositely to N addition for each of biome types (Figs. 2a, c). Specifically, N addition decreased the MBC but increased the total PLFA in temperate forests and grasslands, while it increased the MBC but decreased the total PLFA in tropical/subtropical forests. Nitrogen addition increased the MBN in all biomes except for grasslands (Fig. 2b), and decreased the fungal biomass by 56.2%, 7.3%, 2.2%, 8.7%, and 23.2% in boreal forests, temperate forests, tropical/subtropical forests, grasslands, and deserts, respectively (Fig. S4). All the biomes had similar negative responses of the microbial C:N and F:B to N addition (Figs. 2d and e), and positive response of the GP:GN except for deserts (Fig. 2f).

3.3.2. N addition regimes

The relationships between the RRs of microbial communities

Table 1
Effects of N addition on soil properties, including soil pH, available N, dissolved organic C (DOC), organic C (SOC), and total N.

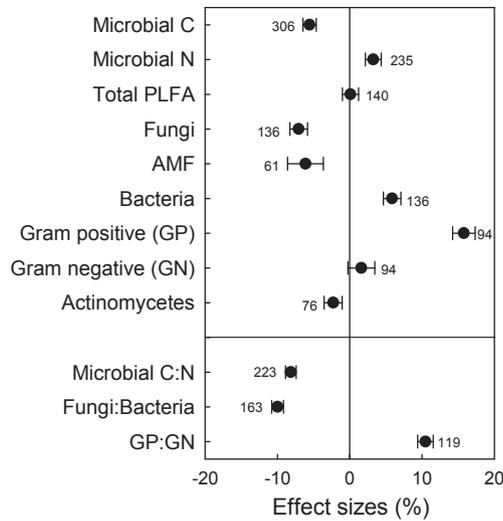| Soil properties	Sample size	Effect size (%)	95% CI (%)
pH	238	−2.9	−3.1, −2.8
Available N	170	92.8	90.7, 95.0
DOC	80	12.4	10.7, 14.2
SOC	251	4.8	4.2, 5.5
Total N	220	8.6	8.0, 9.2

Fig. 1. Effect sizes and 95% confidence intervals (CIs) of N addition on microbial biomass and community composition. The vertical line is drawn at effect size of zero. Sample size for each variable is shown next to the CI bar. AMF, arbuscular mycorrhizal fungi.

biomass of fungi was increased by the lowest rate of N addition (<50 kg N ha^{-1} year^{-1}), but was inhibited otherwise, which was enhanced across N addition rates (Fig. S6). The lowest rate of N addition had no significant effect on microbial C:N, F:B, and GP:GN, but the highest rates of N addition (>200 kg N ha^{-1} year^{-1}) caused the highest response percentages of changes in C:N, F:B, and GP:GN (Figs. 3d−f).

The effect of N addition on microbial biomass was enhanced by study duration ($P < 0.01$; Fig. S5). Moreover, the RR of the F:B significantly decreased as the study duration increased; and the RR of the GP:GN was positively correlated with the study duration ($P < 0.01$; Fig. S5).

3.3.3. Soil factors

The RR of the microbial biomass was not significantly correlated with the RR of soil pH or soil available N ($P > 0.05$; Figs. 4a, d). However, the RRs of the F:B and GP:GN were negatively and positively correlated with the RR of the soil available N, respectively ($P < 0.01$; Figs. 4e and f). No significant relationship was found between the RR of soil pH and the RR of the F:B or GP:GN ($P > 0.05$; Figs. 4b and c).

3.3.4. Climatic factors

The RR of the F:B was positively correlated with MAT ($P < 0.01$) and MAP ($P = 0.01$) but negatively correlated with latitude ($P < 0.01$; Fig. S7). However, the RR of the microbial biomass was not correlated with latitude, MAT, or MAP (Fig. S7).
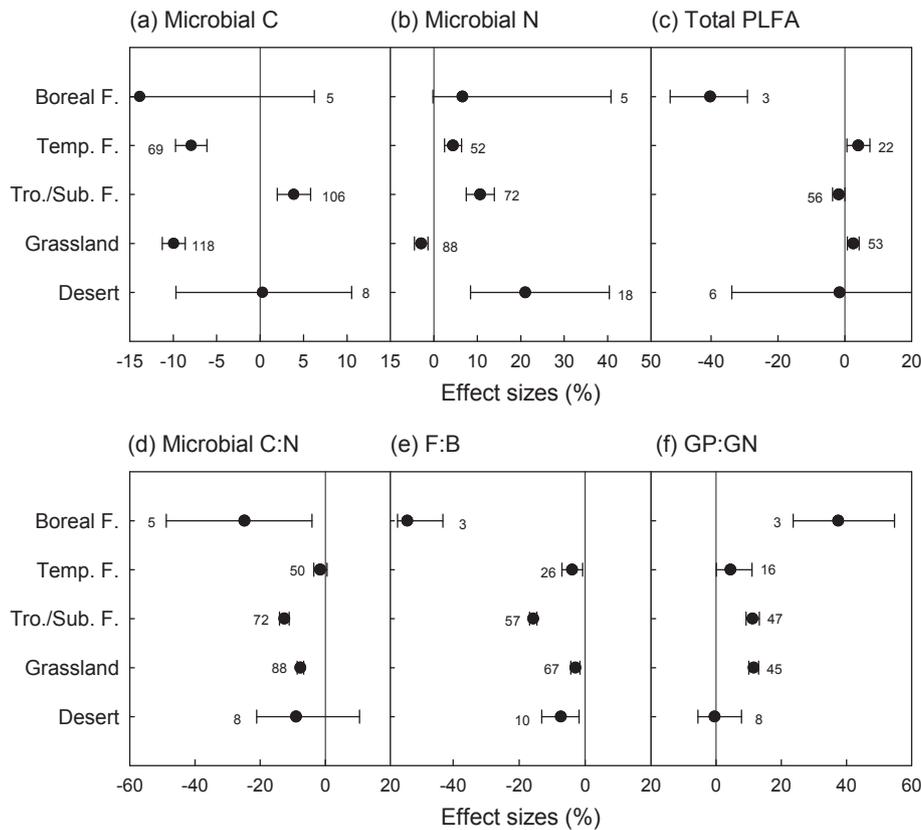
and N-addition rates were non-significant ($P > 0.05$; Fig. S5). Nitrogen addition stimulated the MBC and total PLFA when the rates were lower than 100 kg N ha^{-1} year^{-1}, but reduced them when the rates were greater than 100 kg N ha^{-1} year^{-1} (Figs. 3a, c). The

Fig. 2. Effect sizes and 95% confidence intervals (CIs) of N addition on microbial biomass and community composition (fungi:bacteria (F:B) and Gram positive: Gram negative bacteria ratio (GP:GN)) by biomes. The vertical line is drawn at effect size of zero. Sample size for each variable is shown next to the CI bar. Boreal F., boreal forests; Temp. F., temperate forests; Tro./Sub. F., tropical/subtropical forests.
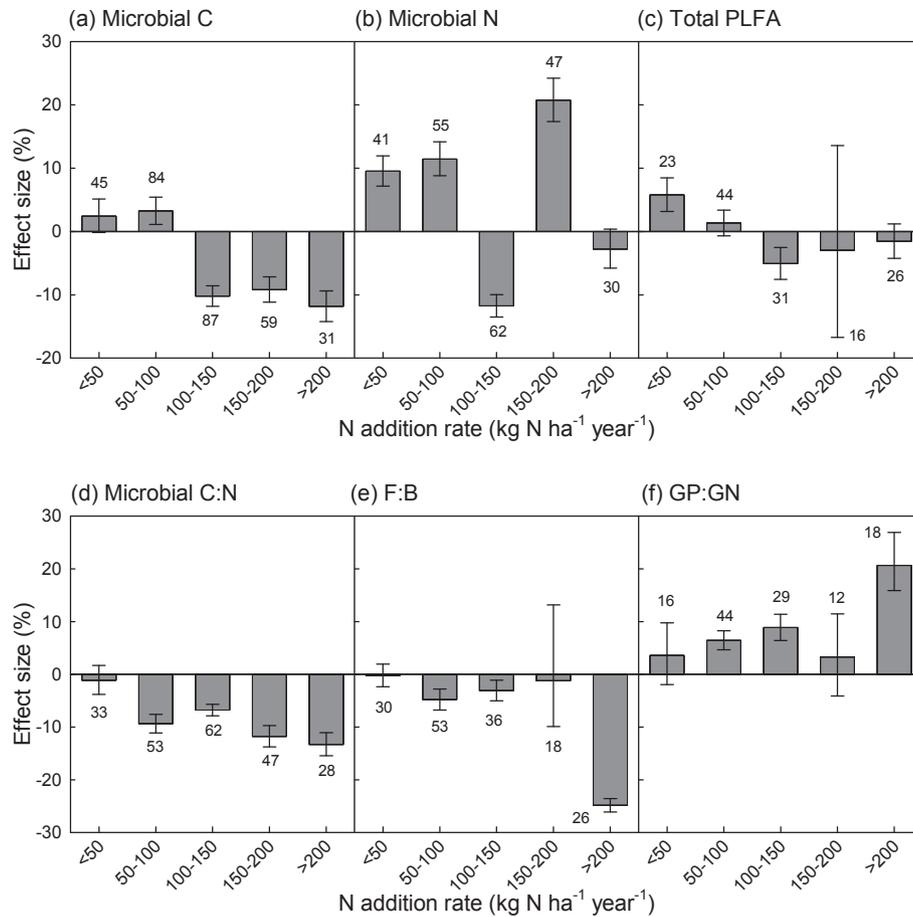
Fig. 3. Effect sizes and 95% confidence intervals (CIs) of N addition on microbial biomass and community composition (fungi:bacteria (F:B) and Gram positive: Gram negative bacteria ratio (GP:GN)) by N-addition rates. Sample size for each variable is shown next to the CI bar.

3.3.5. Principal component analyses

Principal component analyses (PCA) were employed to evaluate the multivariate effects of N addition regimes, environmental conditions, soil properties, and their interaction on microbial communities and their interaction. For microbial biomass, the PC 1 showed strong positive loadings for MAP and MAT, but negative loadings for latitude. The PC 2 had positive loadings for N-addition rate and the RR of the available N, but negative loadings for the RR of pH (Fig. 5a). The RR of microbial biomass had higher loading in PC1 (0.31) than PC2 (−0.10) despite that both loadings were relatively weak (Fig. 5a). Different from the microbial biomass, the RRs of the F:B and GP:GN had strong loadings in PC 1 (associated with climate factors, study duration, and the RR of available N), but were perpendicular to PC 2 (associated the RR of pH and N-addition rates) (Figs. 5b and c). These results were consistent with the results of the regression analyses (Fig. 4; Fig. S7).

4. Discussion

4.1. Consistent effects of N addition on microbial community composition

Our meta-analysis revealed that N addition consistently decreased microbial C:N and F:B across all the biomes and N-addition rates (Figs. 2d, e and 3d, e). Similar responses between the F:B and microbial C:N to N addition were mainly caused by significantly higher biomass C:N of the fungal communities than bacterial communities (Strickland and Rousk, 2010; Zhou et al.,

2017a). This result has important implications. First, the microbial C:N provides an indication for the relative prevalence of fungi over bacteria, and vice versa (Nannipieri et al., 2003; Strickland and Rousk, 2010; Zechmeister-Boltenstern et al., 2015). Second, extracellular enzymes are the proximate agents of SOM decompositions, and their activities can be used as indicators of microbial nutrient demand, soil nutrient cycling, and soil respiration (Zechmeister-Boltenstern et al., 2015; Jian et al., 2016). Extracellular enzymes are produced largely depending upon the community composition. For example, phenol oxidase and peroxidase are produced primarily by fungal communities (especially by white rot fungi), while hydrolase is produced primarily by bacterial communities (Cusack et al., 2011). Therefore, the decrease in F:B induced by N addition provides a potential explanation for the findings of the recent meta-analysis (Jian et al., 2016) that N addition stimulates the hydrolase activities but inhibits the activities of phenol oxidase and peroxidase.

Nitrogen addition also shifted the GP:GN (Figs. 1 and 2f). Indeed, the Gram stain separates bacteria according to cell wall characteristics. In the realm of microorganisms, GP bacteria have strong, thick, and interlinked peptidoglycan cell wall, while GN bacteria have single-layer cell wall and outer membrane (Schimel et al., 2007). Nevertheless, previous studies did find ecological and physiological distinctions between GP and GN bacteria. For example, the majority of microbes responsible for the breakdown of litter and soil organic matter are GP bacteria and fungi. However, many microorganisms carrying out "specialized" or "narrow" functions in soil are GN bacteria (Schimel, 1995; Schimel et al.,
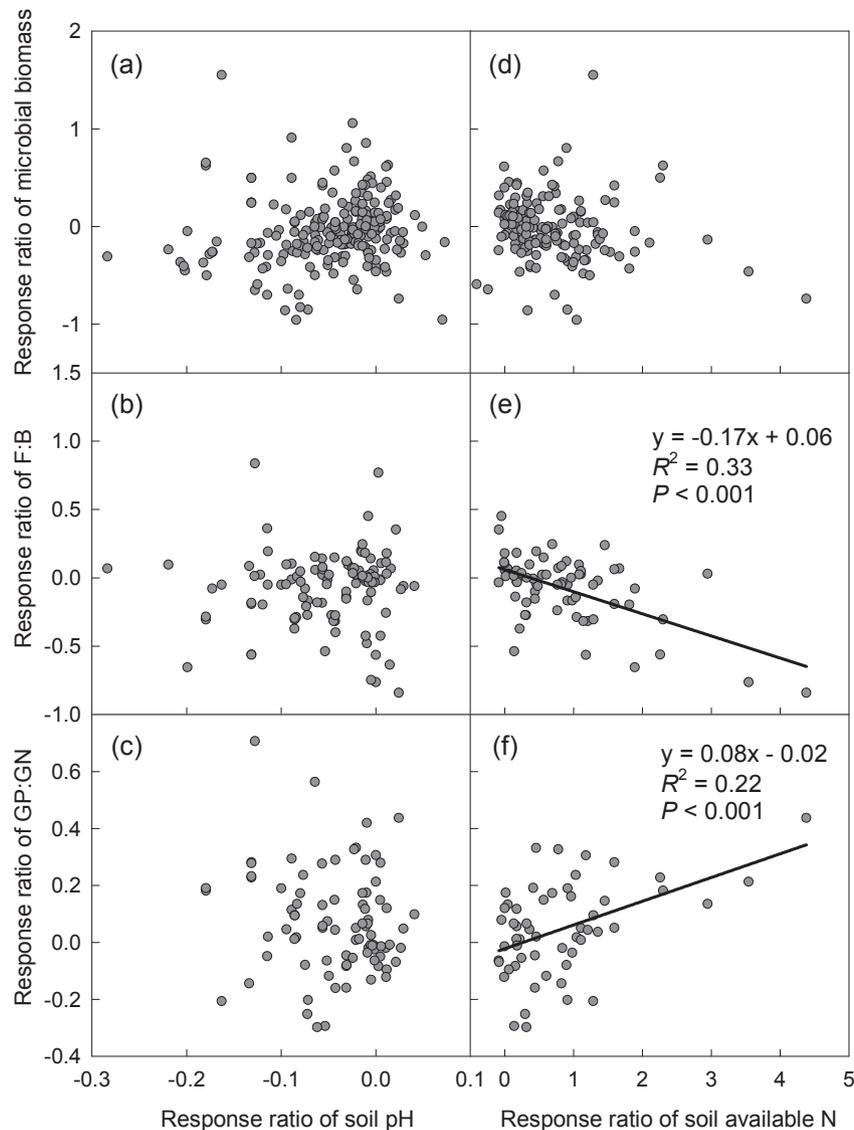
Fig. 4. Relationships between response ratios of microbial communities and response ratios of soil available N or pH. F:B, fungi:bacteria ratio; GP:GN, Gram positive: Gram negative bacteria ratio.

2007). The GP and fungi are thought to be more inherently resistant to environmental stress than the GN (Schimel, 1995; Schimel et al., 2007; Zechmeister-Boltenstern et al., 2015). In addition, GN bacteria are favored in soils with high nutrient content, while GP bacteria are oligotrophic communities and use recalcitrant C fractions with low nutrient availability like fungi (Fierer et al., 2003; Kramer and Gleixner, 2008; Fanin et al., 2013; Zechmeister-Boltenstern et al., 2015; Zhou et al., 2017a). Nevertheless, the increase in GP:GN seems to contradict the soil available N enhanced by the N addition (Table 1). A possible explanation is that soil microbes may be co-limited by other elements rather than N. For example, Fanin et al. (2013) found that the P-driven stoichiometric control is important for understanding of the spatiotemporal heterogeneity of the GP:GN.

Moreover, the increased GP:GN induced by N addition may have important ecological implications. Specifically, the positive response of the GP:GN may be conducive to the decomposition of recalcitrant SOM and C acquisition by the microbial communities. A recent meta-analysis found that N addition led to significant increases in lignin content in plant and litter (Liu et al., 2016).

Moreover, high N availability induced by N addition could be attributable to a progressive inhibition of growth or ligninase activity of white rot fungi, and consequently lead to the accumulation of recalcitrant compounds in soils (Treseder, 2008; Liu et al., 2016; Tian et al., 2017). Therefore, the decreases in fungal biomass (Fig. 1) and the increases in recalcitrant C (Liu et al., 2016) probably have negative effects on recalcitrant SOM decomposition and microbial C acquisition (i.e., result in C limitation; Janssens et al., 2010). However, since the redundancy of species and functions is believed to be typical in soils, a reduction in any group of species may have little effect on overall soil processes due to other microorganisms' substitution (Nannipieri et al., 2003). Therefore, the GP bacteria likely play an important role in the C acquisition by microbial communities and the decomposition of recalcitrant SOM especially when the fungal biomass decreased under external N input.

4.2. Effects of N addition on microbial growth

Effects of N deposition on microbial biomass are confounding in field studies, probably because N addition directly or/and indirectly
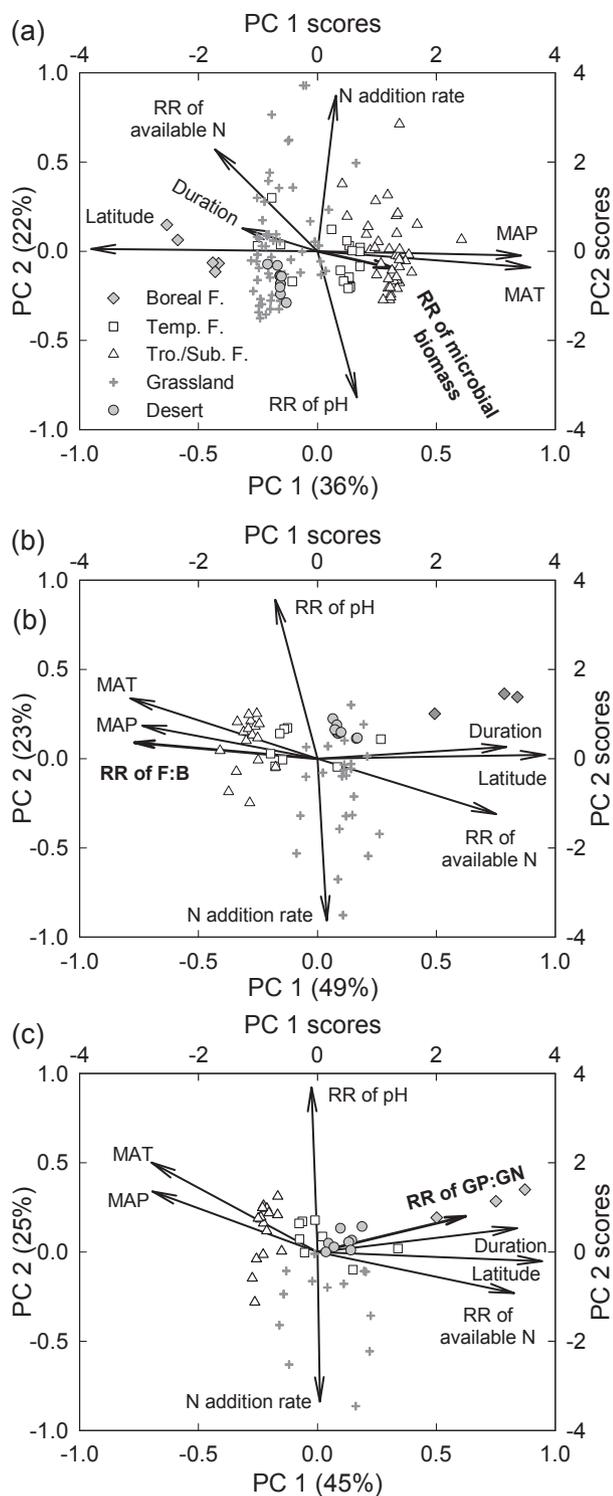
Fig. 5. Principal component analyses (PCA) for multivariate effects of soil properties (response ratios (RRs) of soil pH and soil available N), climate factors (latitude, mean annual temperature (MAT), and mean annual precipitation (MAP)), and N addition regimes (N addition rates and study duration) on RRs of microbial biomass and community composition (fungi:bacteria (F:B) and Gram positive: Gram negative bacteria ratio (GP:GN)). Boreal F., boreal forests; Temp. F., temperate forests; Tro./Sub. F., tropical/subtropical forests.

increases microbial C and N resources that may stimulate microbial growth, and also induces soil acidification that may inhibit microbial growth (Table 1; Treseder, 2008; Chen et al., 2015). Thus, it is

understandable for previous studies that reported divergent effects of N addition on microbial biomass. For example, several meta-analyses reported the reduction of MBC after N addition (Janssens et al., 2010; Liu and Greaver, 2010), and highlighted the pathway of soil acidification and C limitation. However, these studies ignored the effects of biomes types, methodologies, and N addition rates. In the present study, N addition suppressed the MBC in temperate forests and grasslands, but significantly stimulated the MBC in tropical/subtropical forests (Fig. 2a). In addition, methodologies might introduce uncertainty, i.e., the responses of the MBC estimated by the chloroform fumigation–extraction and total PLFA were opposite at both global and biome scales (Figs. 1 and 2a, c). Previous meta-analyses, based on the data set of MBC, suggested that external N inputs suppressed soil microbial growth (Janssens et al., 2010; Liu and Greaver, 2010). However, Treseder (2008) also found that N addition reduced MBC but not total PLFA at the global scale. In addition, N addition tended to increase the MBC, MBN, and total PLFA when the N-addition rate was less than 100 kg N ha^{-1} year^{-1} (Fig. 3a–c). These results, together with the positive response of MBN (Fig. 1), imply that soil microbes, coupled with high N concentrations in tissues, high growth rates, fast turnover, and the production of N rich extracellular enzymes, would be widespread in the N-limited habitats like plant communities (Vitousek and Howarth, 1991; LeBauer and Treseder, 2008; Xia and Wan, 2008; Farrer and Suding, 2016).

Our meta-analysis revealed that N addition affected microbial communities mainly through enhanced resources rather than soil acidification. First, both regression and principal component analyses showed that soil acidification had no significant effect on microbial biomass (Figs. 4a and 5a). Second, although N addition resulted in the soil acidification (Table 1), the biomass of the total bacteria and GP bacteria showed positive responses across all the experiments (Fig. 1). Third, the PCA showed that the vectors of the RR of the F:B and GP:GN were almost parallel to the vector of the RR of the available N but perpendicular to the vector of the RR of pH (Fig. 5b and c). The above phenomena, together with the regression analysis (Fig. 4), suggest that N addition regulate the F:B and GP:GN due to the increased N availability. The plausible interpretation is that copiotrophic bacteria require more N per unit biomass C accumulation than oligotrophic fungi (Fierer et al., 2007; Manzoni et al., 2012; Waring et al., 2013; Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015). Therefore, our results suggest that the changes in microbial communities after N addition mainly result from the increase in N availability rather than soil acidification. This finding is consistent with recent studies reporting that resource availability controlled the responses of the plant-soil system to N enrichment (Geisseler and Scow, 2014; Farrer and Suding, 2016; Geisseler et al., 2016).

Reviews of previous global syntheses indicate that the global-response magnitude of the MBC to N addition tends to decrease with increased sizes of the dataset. Specifically, the MBC reduction caused by N addition was 18% reported by Treseder (2008; 24 studies), 20% by Liu and Greaver (2010; 57 studies), 7% by Lu et al. (2011b; 179 studies), non-significant by Yue et al. (2016; 176 studies), and minor effect in our study (5.6%, 306 studies; Fig. 2a). Two recent studies also found a positive response of microbial biomass in global agricultural systems (Geisseler and Scow, 2014; Geisseler et al., 2016). Divergent responses between MBC and MBN are partly attributed to the remarkable flexibility of microbial C:N stoichiometric ratios across microbial groups. Bacteria and fungi are the dominant decomposers in soils with the mean biomass C:N of 5 and 15, respectively (Strickland and Rousk, 2010). Nitrogen addition reduced MBC but stimulated MBN, verified by recent laboratory incubation studies (Yang and Zhu, 2015; Zhou et al., 2017b), may result from the negative response of F:B to N

addition (Fig. 1). Therefore, species shifts might accentuate or mitigate the ecosystem-level effects of alterations in biomass, depending on the functional roles of individual taxa (Treseder, 2008). Moreover, N addition decreased F:B, implying that the turnover rate of whole microbial communities increases (Six et al., 2006; Schimel et al., 2007; Waring et al., 2013). Therefore, the decreased microbial biomass does not always mean that N addition inhibits microbial growth.

Although the N deposition rate of 10 kg N ha^{-1} year^{-1} was regarded as the critical threshold (Dentener et al., 2006; Bobbink et al., 2010), simulated N deposition experiments generally applied very high N addition rates (LeBauer and Treseder, 2008; Xia and Wan, 2008; Janssens et al., 2010; Peng et al., 2017; Fig. S2). Therefore, current simulated N deposition experiments may limit our understanding of the effects of the actual N deposition. Despite this fact, when the N addition rate is lower than 100 kg N ha^{-1} year^{-1}, ten times higher than of global normal rate, the positive response of microbial growth was reported in our analysis (Fig. 3). Again, such results fundamentally differ from previous notion that global N deposition inhibits microbial growth (Treseder, 2008; Janssens et al., 2010; Liu and Greaver, 2010).

In summary, the present meta-analysis using a novel global data set across various biomes and methodologies revised the previous notion that N addition inhibits microbial biomass. Meanwhile, divergent responses of the MBC, MBN, and total PLFA were partly explained by the changes in microbial community composition. Nitrogen addition affected the microbial communities mainly through resources enhancement rather than soil acidification. Resource competition-dependent microbial community assembly (shifts in fungi, GP, and GN bacteria) is critical for microbial functions, like SOM decomposition and soil C cycling, under N deposition scenarios.

Acknowledgements

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.09.015.

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