Effects of nitrogen deposition on soil microbial communities in temperate and subtropical forests in China

Tian, Di ; Jiang, Lai ; Ma, Suhui ; Fang, Wenjing ; Schmid, Bernhard ; Xu, Longchao ; Zhu, Jianxiao ; Li, Peng ; Losapio, Gianalberto ; Jing, Xin ; Zheng, Chengyang ; Shen, Haihua ; Xu, Xiaoniu ; Zhu, Biao ; Fang, Jingyun

Abstract: Increasing nitrogen (N) deposition has aroused large concerns because of its potential negative effects on forest ecosystems. Although microorganisms play a vital role in ecosystem carbon (C) and nutrient cycling, the effect of N deposition on soil microbiota still remains unclear. In this study, we investigated the responses of microbial biomass C (MBC) and N (MBN) and microbial community composition to 4–5 years of experimentally simulated N deposition in temperate needle-leaf forests and subtropical evergreen broadleaf forests in eastern China, using chloroform fumigation extraction and phospholipid fatty acid (PLFA) methods. We found idiosyncratic effects of N addition on microbial biomass in these two types of forest ecosystems. In the subtropical forests, N addition showed a significant negative effect on microbial biomass and community composition, while the effect of N addition was not significant in the temperate forests. The N addition decreased MBC, MBN, arbuscular mycorrhizal fungi, and the F/B ratio (ratio of fungi to bacteria biomass) in the subtropical forests, likely due to a decreased soil pH and changes in the plant community composition. These results showed that microbial biomass and community composition in subtropical forests, compared with the temperate forests, were sensitive to N deposition. Our findings suggest that N deposition may have negative influence on soil microorganisms and potentially alter carbon and nutrient cycling in subtropical forests, rather than in temperate forests.

DOI: https://doi.org/10.1016/j.scitotenv.2017.06.057

Effects of nitrogen deposition on soil microbial communities in temperate and subtropical forests in China

Di Tian[1], Lai Jiang[1], Suhui Ma[1], Wenjing Fang[1], Bernhard Schmid[2], Longchao Xu[3], Jianxiao Zhu[1], Peng Li[1], Gianalberto Losapio[2], Xin Jing[1], Chengyang Zheng[1], Haihua Shen[3], Biao Zhu[1], Jingyun Fang[1]*

[1]*Department of Ecology, College of Urban and Environmental Sciences, and Key Laboratory for Earth Surface Processes of the Ministry of Education, Peking University, Beijing 100871, China;*

[2]*Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland;*

[3]*State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China*

*Correspondence author:
Dr. Jingyun Fang
Department of Ecology, Peking University
Beijing 100871, China
E-mail: jyfang@ urban.pku.edu.cn

Abstract:

Increasing nitrogen (N) deposition in China has aroused concerns because of its potential negative effects on forest ecosystems. Although microorganisms play a vital role in ecosystem carbon (C) and nutrient cycling, the effects of N deposition on soil microbiota still remain unclear. We investigated the responses of microbial biomass C (MBC) and N (MBN) and microbial community structure to 4-5 years of experimentally simulated N deposition in temperate and subtropical forests in eastern China using chloroform fumigation extraction and phospholipid fatty acid (PLFA) methods. We found idiosyncratic effects of N addition on microbial biomass in these two forest ecosystems. In the subtropical forests, N addition showed relatively significant effects on microbial biomass and community structure. Conversely, in temperate forests the effect was weaker. Specifically, the MBC, MBN, arbuscular mycorrhizal fungi and the fungi to bacteria ratio in N fertilized plots in the subtropical forests showed a general decreasing trend. These shifts in microbial biomass and community structure might have been caused by a decreased soil pH and changes in the vegetation composition during the 4-5 years of N addition in the subtropical forests. Our findings suggest that microbial biomass and community structure in subtropical forests with acidic soil is extremely sensitive to N deposition. Generally, N deposition may exhibit an overall negative influence on microorganisms, especially in forests which are already close to N saturation, such as the subtropical forests in our study.

Keywords: forest ecosystem; microbial biomass; microbial community; N deposition; PLFA

1. Introduction

With the rapid growth of the human population, global anthropogenic nitrogen (N) production has increased approximately threefold during 1850-2010 (Galloway et al., 2014). High rates of reactive N emission have led to an increase in atmospheric N deposition in China (Liu et al., 2013), which further arouses concerns about its potentially negative effects on various ecosystems (Liu et al., 2011), including forests. In these ecosystems, soil microbiota are essential for ecosystem biodiversity, productivity and energy dynamics (van der Heijden et al., 2008). Different taxonomic groups of microbes play specific roles in soil nutrient cycling, hence changes in microbial biomass and community structure induced by N deposition may lead to changed sequestration of C and N in ecosystems and affect greenhouse gas production at global levels (Compton et al., 2004; Janssens et al., 2010). Although many studies have documented effects of N deposition on plants (Bobbink et al., 2010), major uncertainties still remain about the effects of N deposition on soil microbiota and soil nutrient cycling (Sutton et al., 2014).

A previous meta-analysis of 82 field studies generalized that N deposition has negative effects on microbial biomass in boreal forests (Treseder 2008). Another study about the effects of N fertilization on microbial communities across 28 temperate grasslands also found generally negative effects (Leff et al., 2015). However, many studies also recognized that the effects of N deposition on microbial biomass and community structure varied with forest types. For example, in temperate and boreal forests, which are mostly limited by N, either positive or not-significant responses of microbial biomass to moderate N addition were found (DeForest et al., 2004; Allison et al., 2008; Allison et al., 2009; Contosta et al., 2015). Moreover, with repeated N fertilization over prolonged time and higher dosage, negative effects on microbes seem to be more common (Wallenstein et al., 2006; Turlapati et al., 2013; Frey et al., 2014). However, discrepancies still remain among the few reports about the effects of N deposition on microbial biomass and community structure in subtropical and tropical forests (Balser 2001; Li et al., 2015; Liu et al., 2015). As most previous investigations and observations only focused on a single site or a single forest type, mainly performed in temperate and boreal forests, multi-site N-fertilization experiments carried out across latitudinal gradients and different forest types are

needed to improve our understanding of microbial responses to N deposition.

We set up such a multi-site N-fertilization experiment in China in 2010 (Network of Nutrient Enrichment Experiments in China's Forests NEECF, Du et al., 2013). In the present paper we reported how biomass carbon (C) and N and the community structure of soil microbiota responded to the simulated N deposition in temperate and subtropical forests after 4-5 years of treatment. Microbial biomass was extracted from soil using the method of chloroform fumigation and microbial community structure was characterized using phospholipid fatty acid (PLFA) analysis. Specifically, we tested the following hypotheses: 1) N deposition enhances microbial biomass in N-limited temperate forests, but decreases microbial biomass in subtropical forest that are closer to N saturation (i.e., N availability in the forest ecosystem exceeded the demand of plant and microbes, see Aber et al., 1989); and 2) N deposition leads to shifts in microbial community structures both in temperate and subtropical forests.

2. Materials and Methods

2.1 Study sites

Our experiments were conducted at four sites belonging to two typical forest types in the context of the NEECF project (Du et al., 2013). We chose the sites of *Genhe* (GH, Inner Mongolia Autonomous Region) and *Wuying* (WY, Heilongjiang province), located near the Great Khingan Mountains in north-eastern China, to represent natural temperate forests. In *Genhe,* the dominant tree species is *Larix gmelini* with averaged DBH (diameter at breast height) of 17.1 ± 2.1 cm and height of 15.6 ± 1.4 m. The stand basal area is 54.4 ± 8.1 m^2 ha^{-1}. The other species included the trees *Betula platyphylla*, *Populus davidiana* and understory species in the genera of *Ledum*, *Vaccinium*, *Rhododendron* and *Betula*. In *Wuying*, the dominant tree species is *Pinus koraiensis* with averaged DBH of 24.9 ± 5.8 cm and height of 17.4 ± 1.6 m. The stand basal area is 7.3 ± 1.8 m^2 ha^{-1}. The other species included trees in the genera of *Tilia, Abies, Acer, Fraxinus and Betula.* The plant communities in these temperate forests represent the typical low species diversity and distinct two-layer vertical structure in northern temperate regions in China. Simultaneously, we chose the sites of *Guniujiang* (GNJ, Anhui province) and *Wuyishan* (WYS, Fujian province), located in southern China, to represent natural subtropical forests. In *Guniujiang* and *Wuyishan*,

the dominant tree species are *Castanopsis eyrie* and *C. carlesii* with averaged *DBH* of 20.2 ± 0.8 cm and 16.2 ± 1.3 cm and height of 12.4 ± 0.2 m and 18.4 ± 0.4 m, respectively (Tian et al., 2017). The values of stand basal area are 25.9 ± 11.7 m^2 ha^{-1} and 45.3 ± 7.6 m^2 ha^{-1}, respectively. The understory species are mainly in the genera of *Castanopsis*, *Cunninghamia, Dendropanax, Rhododendron* and *Daphniphyllum*. These two subtropical forests have three-layer vertical structures which are representative of typical subtropical evergreen forests. All these forests in our NEECF project were well-protected from human activities. Detailed information about the soil properties, climatic conditions and background N deposition at each site have been reported in Du et al. (2013) (see also Table 1).

123

124 **Table 1**

125 General information about the four forests sites in the NEECF project used in the present study.

126

Site	Location	Altitude (m)	AMT (℃)	AMP (mm)	Growing season (months)	Soil type	Dominant species	Soil C (mg g^{-1})	Soil N (mg g^{-1})	Soil P (mg g^{-1})	pH	Since	N deposition
GH	50°56'N, 121°30'E	825	-5.4	481	6-8	Brown	*Larix gmelini*	323.2(17.3)	12.7(0.9)	0.8(0.0)	5.9 (0.1)	May 2010	5.5
WY	48°07'N, 129°11'E	350	-0.5	654	5-9	Dark brown	*Pinus koraiensis*	104.8(1.3)	6.0(1.0)	1.2(0.1)	5.5 (0.1)	May2010	7
GNJ	30°01'N, 117°21'E	375	9.2	1650	1-12	Yellow brown	*Castanopsis eyrei*	50.2(7.7)	3.7(0.3)	0.5(0.0)	4.2 (0.1)	March2011	10.6
WYS	27°39'N, 117°57'E	700	18	1889	1-12	Yellow red	*Castanopsis carlesii*	36.4(6.9)	2.3(0.4)	0.3(0.0)	4.6 (0.1)	June 2011	16

127

128 MAT: mean annual temperature (°C); MAP: mean annual precipitation (mm); Soil C: soil total C content of 0-10 cm soil, values in the bracket
129 indicate standard error (SE) (mg g^{-1}); Soil N: soil total N content of 0-10 cm soil, values in the bracket indicate standard error (SE) (mg g^{-1}); Soil
130 P: soil total P content of 0-10 cm soil, values in the bracket indicate standard error (SE) (mg g^{-1}). N deposition: ambient annual amount of N
131 deposition (kg N ha^{-1} yr^{-1}).

2.2 Experimental design

Ammonium nitrate (NH_4NO_3) has been applied to simulate N deposition since 2010 and 2011(Table 1). At each site, we applied NH_4NO_3 at the level of 50 (N50) or 100 kg N ha^{-1} $year^{-1}$ (N100). Control plots (CK) received no fertilizer. Natural levels of N deposition at the study sites were much lower (Table 1), so we did not take these amounts into consideration when evaluating the effects of N treatments on microbial biomass and community structures. At each site, three blocks with three fully randomized plots of 20 m × 20 m with similar plant community and soil conditions were established (Du et al., 2013). In total, there are 36 plots across the four sites, i.e. twelve for each treatment and the control.

2.3 Soil sampling

At the end of July 2015, 4-5 years after the start of the fertilizer application, we took soil samples from 3 depths of the mineral soil layer (after moving the surface litter layer) in nine plots at each site. First, each plot was partitioned into 5 subplots along a diagonal direction. Soil cores were taken within the subplots along three depths of 0-10 cm, 10-20 cm and 20-40 cm, respectively. Then, the soil cores at the same depth in the same plot were mixed in situ to form one pooled sample per depth per plot. The samples were transported to the laboratory in -4℃ coolers and sieved through a 2-mm mesh after removal of stones, roots and litter. Second, each sample was separated into four subsamples to measure soil moisture, contents of carbon, nitrogen, phosphorus and pH, microbial biomass and community structure, respectively.

2.4 Measurements of soil moisture, contents of carbon, nitrogen, phosphorus and soil pH

A first subsample of 20 g was dried at 105℃ to determine soil moisture. A second subsample of 30 g was air-dried and then used to determine soil nutrient content, including soil total C, total N, total P, and pH. Soil total C and N concentration were determined by a CHN analyzer using Dumas combustion (Elementar vario EL III, Elementar, Hanau, Germany). Soil total P concentration was measured by a molybdate/ascorbic acid method after H_2SO_4-$HCLO_4$ digestion. Soil pH was

166 measured in water suspension with a 1:2.5 soil:water ratio.
167
168 **2.5 Measurements of microbial biomass and community structure**
169
170 A third subsample of 50 g was stored at -4℃ and later used for the determination of
171 microbial biomass C and N by the method of chloroform fumigation extraction
172 (Margesin & Schinner, 2005). During chloroform fumigation extraction, the time of
173 soil incubation in $CHCl_3$ atmosphere was extended from 24 h to 48 h in our
174 experiment to fully kill all soil microorganisms. Then, the extracting solutions in
175 0.5mol/L K_2SO_4 of soil samples after chloroform fumigation extraction were detected
176 through oven oxidation by Multi N/C 3100 (Analytik Jena, Jena, Germany). The
177 factors in the transformations from the detected soil total organic carbon and nitrogen
178 to microbial biomass carbon and nitrogen were 0.45 and 0.54, respectively (Margesin
179 & Schinner, 2005).
180
181 A fourth subsample was used to determine microbial community structure through
182 phospholipid fatty acid (PLFA) analysis (detailed methods explained in Bossio &
183 Scow (1998) and McGenity et al. (2017)). The separation, quantification and
184 identification of the resultant fatty acid methyl esters were done using the same
185 procedures and equipment as in Chen et al. (2015). Concentrations of individual
186 PLFAs were calculated based on the 19:0 internal standard concentrations and
187 abundances were expressed as *nmol* per gram dry soil. All the fatty acids were used to
188 describe the structure of microbial communities. Considering the nonuniformity of the
189 PLFA-markers used in previous studies, we combined universally used fatty acid
190 markers to indicate specific taxonomic groups (see supporting Table S1). We used the
191 ratio of the sum of fatty acids in each specific taxonomic group and the sum of total
192 fatty acids in each sample to indicate the relative abundance of specific taxonomic
193 groups. We determined the fungi to bacteria ratio (F:B) by dividing the sum of
194 fungal biomarkers by the sum of bacterial biomarkers (Frostegård and Bååth 1996).
195
196 **2.6 Statistical analysis**
197
198 Considering our experimental design and our focus on the differences of microbial
199 biomass and community structure between two types of forest ecosystems without or

with N fertilization, we used linear mixed models to test the effects of forest type (fixed effect) and N fertilization (fixed effect) on microbial biomass (response). In the model, the four experiment sites nested in the two forest types were used as a random-effects variable. Tests were performed using Residual Maximum Likelihood (REML). Furthermore, to compare microbial biomass C and N and the relative abundance of specific taxonomic microbial groups across soil depths and N treatments in each forest type, we used one-way ANOVA analysis. When the results of one-way ANOVA showed a significant difference, we used the Tukey's honest significant difference (HSD) tests to conduct the multi-comparisons among three N treatments at each soil depth. We finally used linear regression to investigate the relationship between microbial biomass (response) and measured soil nutrient contents (predictor).

To test for significant differences in overall microbial community composition or specific taxonomic groups between subtropical and temperate forests or between N fertilization treatments, we used permutational multivariate ANOVA (PERMANOVA). In the PERMANOVA model, Bray-Curtis dissimilarity matrices were used to represent microbial community composition. Forest types (subtropical or temperate) and N treatments (CK, N50, N100) were used as predictor variables and the factor "soil depth" was used as "strata" to restrict permutation within the same soil depths, according with Leff et al. (2015). Then, the redundancy analysis (RDA) was used to visualize the shifts of microbial community structure between the N fertilization treatments. To explore which environmental variables explained most of the variation in microbial community structure, we included soil depth, soil moisture, pH, soil C content, soil N content, soil P content, microbial biomass C and N as explanatory variables.

All statistical analyses were performed with the statistical software R (version 3.2.2., R Core Team, 2015) using the package *asreml* (Gilmour et al., 2009) and the package *vegan* (Oksanen et al. 2015) with the Adonis and envfit functions.

3. Results

3.1 Effects of N fertilization on soil C, N, P content and pH in subtropical and temperate forests

Soil C, N, P and pH differed strongly among different soil depths between subtropical

and temperate forests (Table 1 & Fig. S2). Soil C and N content and pH in temperate forests are significantly higher than that in subtropical forests, while soil P showed the converse pattern. N fertilization showed no significant effect on soil C, N, P content and C:N ratio (Table 1& Fig. S2, $p > 0.05$ in all cases). However, N fertilization generally decreased soil pH (Table 1 & Fig.1, $p = 0.04$), especially at the 0-10 cm soil depth in subtropical forests where the value of soil pH were below 5 (Fig.1a-b, for WYS 0-10 cm soil, $p = 0.07$).

Table 1 Effects of predictors (i.e. soil depth, forest type, N treatment and their interactions) on soil nutrient contents and pH values tested with linear mixed-effects models. Table entries are *F*-values with significance levels indicated by asterisks ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). Treat: N-fertilization treatments of CK, N50 and N100. Depth: soil depths of 0-10 cm, 10-20 cm and 20-40 cm. Forest: the forest types in this study including the subtropical and temperate forests.

Predictor	Df	C (mg g^{-1})	N (mg g^{-1})	C:N ratio	P (mg g^{-1})	pH
Depth	2	89.5^{***}	79.3^{***}	83.8^{***}	24.3^{***}	22.0^{***}
Forest	1	1.5	1.8	1.5	31.2^{***}	7.4^{**}
Depth:Forest	2	37.2^{***}	23.3^{***}	1.3	7.4*	4.0
Treat	2	0.3	1.2	2.7	4.6	6.4^{*}
Depth : Treat	4	2.1	2.1	5.8	1.3	3.6
Forest : Treat	2	0.1	0.6	2.8	1.1	1.7

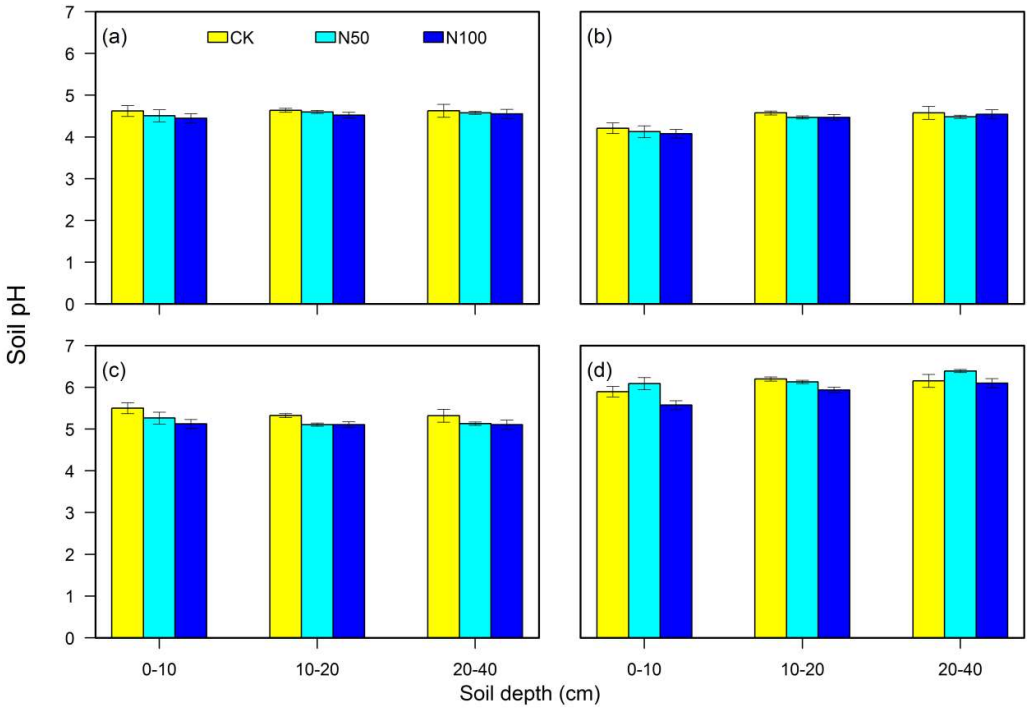


Fig.1. Effects of N fertilization (CK: control; N50: 50 kg N ha^{-1} $year^{-1}$; N100: 100 kg N ha^{-1} $year^{-1}$) on soil pH in subtropical forests of (a) GNJ and (b) WYS (see Methods) and temperate forests of (c) WY and (d) GH among three soil depth levels.

3.2 Effects of N fertilization on microbial biomass C and N and PLFAs

Overall, we did not find strong effects of N fertilization on microbial biomass C (MBC) and N (MBN) and MBC to MBN ratios detected by the method of chloroform fumigation extraction in the two forest types (Table 2). However, the results of one-way ANOVA conducted on each forest type showed significant decreases of microbial biomass C and N at 0-10 cm soil depth in N fertilized plots in the subtropical forests (Fig.2 & Table S2, for the effects of N fertilization on MBC and MBN at 0-10 cm in subtropical forests, p = 0.009 and p = 0.01, respectively). Furthermore, N fertilization showed no significant effect on microbial biomass C and N and MBC:MBN ratios at the other soil depths (Fig.2 &Table S2).

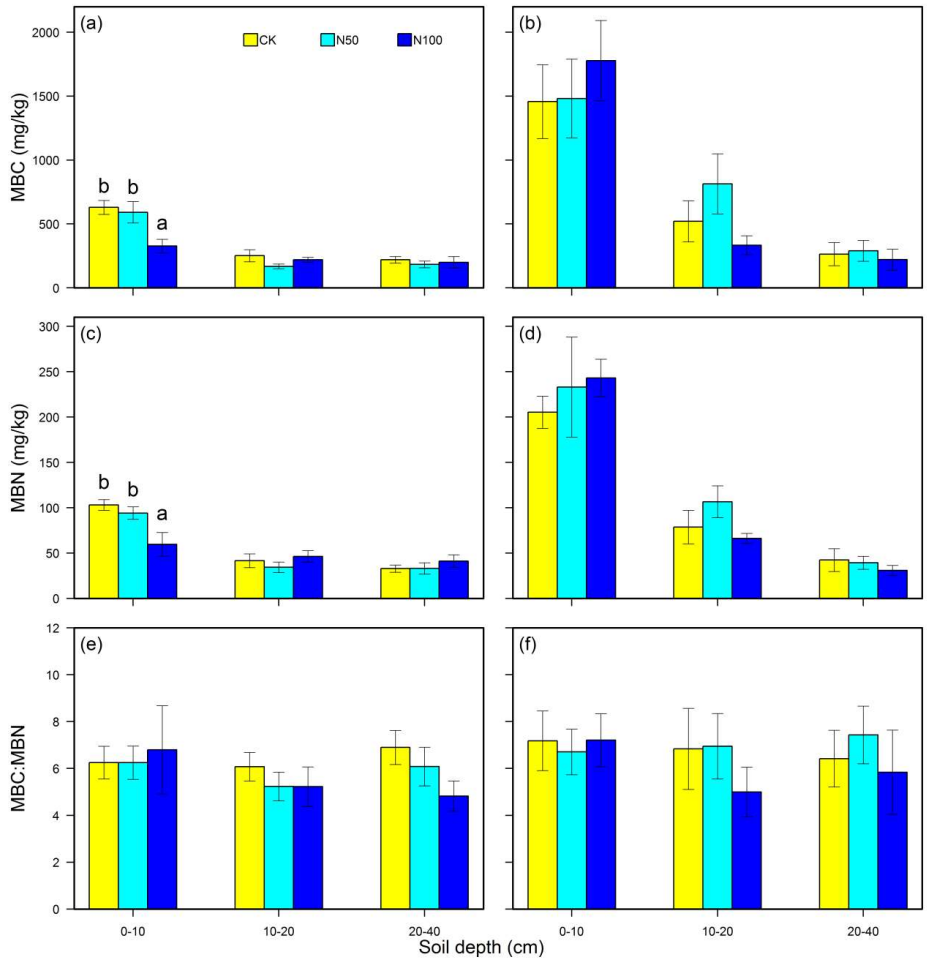


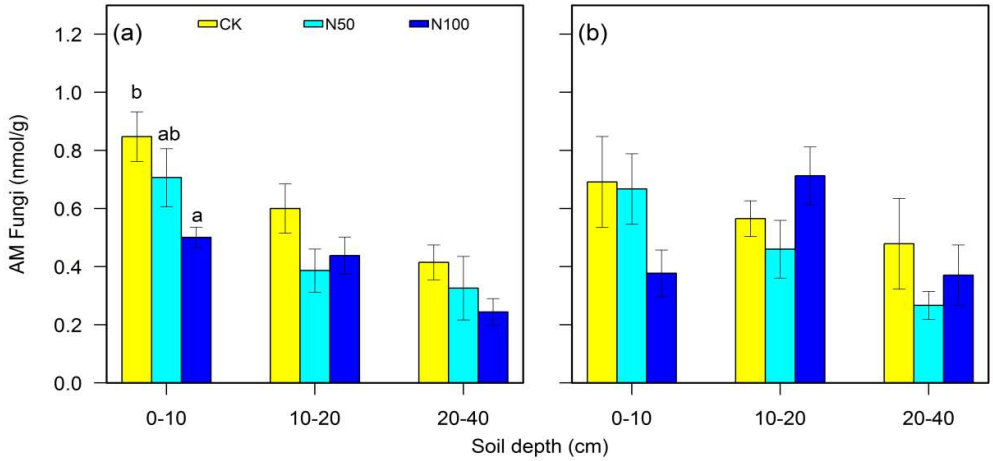
Fig.2. Effects of N fertilization on microbial biomass C (a-b), microbial biomass N (c-d) and MBC to MBN ratios (e-f) in two forest types. (a), (c) and (e) indicate the subtropical forests; (b), (d) and (f) indicate the temperate forests. Different letters indicate significant differences among N fertilization treatments at the same soil depth (one-way ANOVA, $p < 0.05$).

We also observed a generally negative effect of N fertilization on microbial biomass indicated by PLFAs (Table 2 & Fig. S2, $F = 7.5$, $p = 0.02$), especially for bacteria (Table 2, $F = 7.0$, $p = 0.03$) and fungi (Table 2, $F = 6.2$, $p = 0.05$). Although no strong effect of N fertilization was detected on total PLFAs, considering specific PLFAs bacteria groups, the responses of fungi and actinomycetes were significantly different among soil depths and between forest types (Table S3 & Fig. S2). Moreover, soil arbuscular mycorrhizal fungi content significantly decreased with the increasing of N fertilization at 0-10 cm soil depth in the subtropical forests (Fig.3, $p = 0.02$).

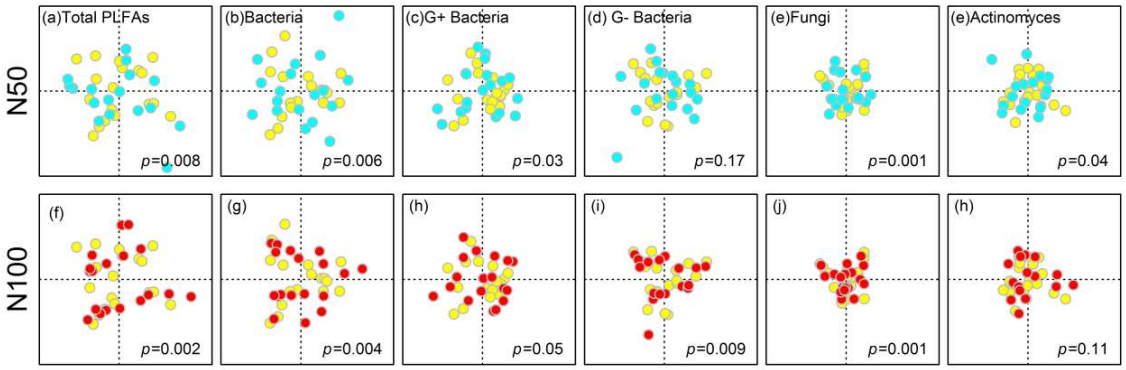
Table 2 Effects of fixed factors on soil microbial biomass and microbial PLFAs analyzed by linear mixed-effects models. Table entries are *F*-values with significance levels indicated by asterisks ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). Treat: N-fertilization treatment (factor with three levels); STC: total carbon content per gram dry soil (mg g^{-1}); STN: total nitrogen content per gram dry soil (mg g^{-1}); STP: total phosphorus content per gram dry soil (mg g^{-1}).

Source	A	B	C	D	E	F	G	H	I	J
Latitude	67.8***	11.5***	0.5	0.2	0.1	0.0	0.1	0.4	1.1	0.0
Depth	167.2***	184.4***	3.8	35.5***	32.9***	36.0***	34.5***	57.5***	28.5***	48.0***
Latitude : Depth	64.4***	55.3***	0.0	21.2***	21.6***	5.2	22.6***	22.1***	15.8***	9.3**
Forest type	15.7***	0.6	5.2*	0.4	0.5	0.4	0.3	0.5	0.1	0.1
Treat	1.3	1.0	3.7	7.5*	7.0*	13.4**	3.7	6.2*	4.6	14.6***
Latitude : Treat	2.0	1.6	0.9	2.0	1.8	2.5	2.0	3.8	3.1	2.5
Depth : Treat	2.3	0.5	4.6	13.8**	13.1*	13.7**	14.1**	14.8**	13.6**	18.2**
Forest : Treat	0.3	0.4	1.4	2.1	1.8	2.1	1.4	0.7	3.2	0.2
pH	0.0	0.1	5.6*	2.8	3.1	5.3*	2.3	0.2	2.6	4.3*
STC	30.5***	5.3*	0.0	10.8**	13.0***	28.1***	5.6*	1.9	0.6	8.3**
STN	1.5	2.6	0.1	7.3**	9.2**	6.1*	9.0**	2.7	3.5	6.6*
STP	0.3	8.5**	1.1	12.2***	11.3***	11.3***	11.5***	15.2***	11.3***	14.8***

Note: The uppercase letters in the table indicate: A: Soil microbial biomass carbon (mg kg^{-1}) (MBC); B: Soil microbial biomass nitrogen (mg kg^{-1}) (MBN); C: Soil microbial biomass carbon: microbial biomass nitrogen ratio (MBC:MBN); D: Total PLFAs content (nmol g^{-1}); E: Bacterial PLFAs content (nmol g^{-1}); F: Gram-positive Bacterial PLFAs content (nmol g^{-1}); G: Gram-negative Bacterial PLFAs content (nmol g^{-1}); H: Fungal PLFAs content (nmol g^{-1}); I: Actinomycetic PLFAs content (nmol g^{-1}); J: Arbuscular Mycorrhizal Fungal PLFAs content (nmol g^{-1}).



292 **Fig.3.** Effects of N fertilization on the content of arbuscular mycorrhizal fungi
293 indicated by PLFA maker (16:1ω5c) in subtropical (a) and temperate (b) forests.
294 Different letters indicate significant differences among N fertilization treatments at the
295 same soil depth (one-way ANOVA, $p < 0.05$).
296
297 **3.3 Effects of N fertilization on microbial community structure**
298 The results of redundancy analysis (RDA) showed that the overall microbial
299 community structure and specific taxonomic groups of bacteria, fungi and
300 actinomycetes in subtropical forests responded significantly to both N50 and N100
301 fertilization (Fig.4, all cases except (d) and (h) with $p < 0.05$). Considering the
302 temperate forests, microbial community structure was relatively stable across the
303 different N fertilization treatments (Fig.5, for all cases $p > 0.05$).



304 **Fig.4.** Redundancy Analysis (RDA) showing differences of six microbial taxonomic
305 groups between unfertilized plots (yellow points) and N50 fertilized (blue points) or

N100 fertilized (red points) plots in subtropical forests. Points represent individual samples. *P*-values refer to permutational multivariate ANOVA results.

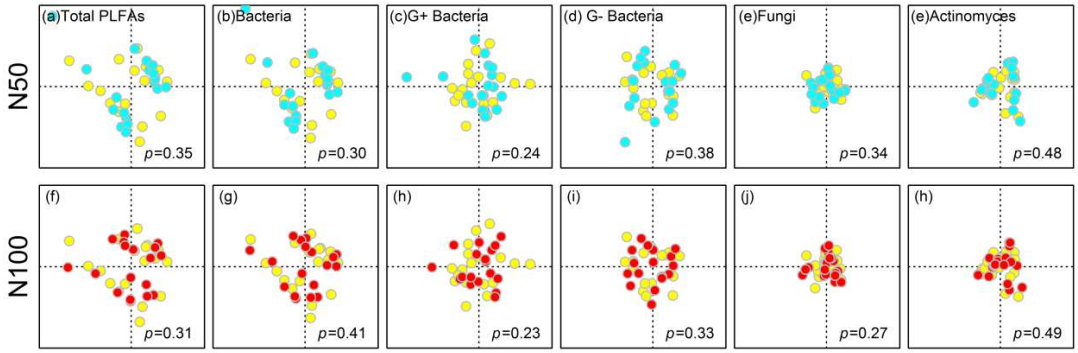


Fig.5. Redundancy Analysis (RDA) showing differences of six microbial taxonomic groups between unfertilized plots (yellow points) and N50 fertilized (blue points) or N100 fertilized (red points) plots in temperate forests. Points represent individual samples. *P*-values refer to permutational multivariate ANOVA results.

Furthermore, by investigating the specific microbial taxonomic groups indicated by PLFA makers in Table S1, we observed a consistent decreasing pattern of the relative abundance of fungi with the increasing level of fertilizer application in subtropical forests at 0-10 cm and 10-20 cm soil depths (Table S3 & Fig.6a, $p < 0.05$). Remarkably, the N100 treatment led to a particularly strong decline in the relative abundance of arbuscular mycorrhizal fungi at all three soil depths in subtropical forests (Table S3 & Fig.6b, $p < 0.05$ in all cases). Similarly, the F:B ratio at 0-10 cm and 10-20 cm soil depths in N fertilized treatments showed a declining tendency in subtropical forests (Table 3, $p = 0.005$). In sharp contrast, neither of the relative abundances of specific microbial groups in the temperate forests was significantly shifted by N fertilization (Table S3, $p > 0.05$ in all cases).

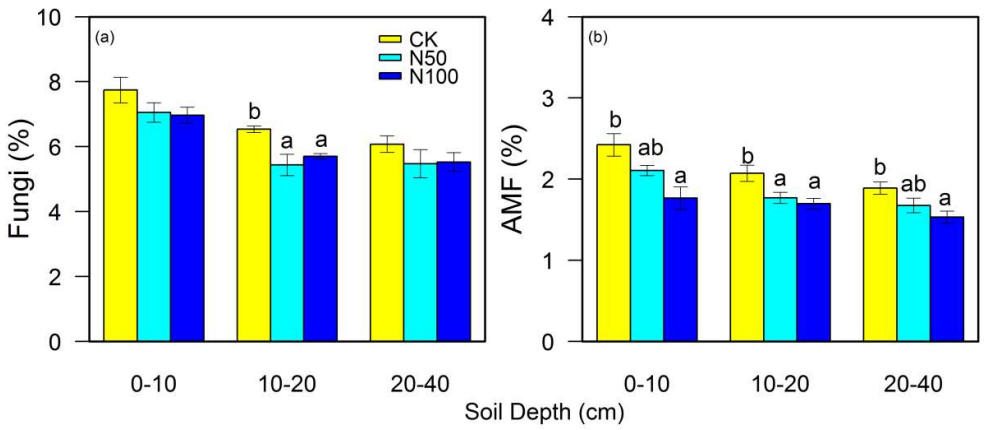


Fig.6. Effects of N fertilization on the relative abundance of soil fungi (%) and arbuscular mycorrhizal fungi (AMF, %) at three soil depths in subtropical forests. Different letters indicate significant differences between N treatments (one-way ANOVA analysis: $p < 0.05$).

4. Discussion

Overall, microbial biomass C and N showed weak response to 4-5 years' N fertilization across different forest types in our current study. Specifically, no significant effect of N fertilization on microbial biomass and community structure emerged in temperate forests, while substantial reduction of microbial biomass and shift of microbial community structure appeared in subtropical forests. Furthermore, N fertilization decreased the relative abundances of specific taxonomic groups, especially arbuscular mycorrhizal fungi in subtropical forests.

In forest ecosystems, soil microbes are influenced by both biotic and abiotic factors (Fierer & Jackson, 2006), such as climatic and edaphic conditions, vegetation, resource availability, production and decomposition of litter (Prescott & Grayston, 2013; Contosta et al., 2015). Particularly important for soil microorganisms is soil nutrient availability. Traditionally, previous studies considered that soil C and N were crucial elements influencing microbial biomass (Demoling et al., 2008; Liu et al., 2012). Therefore, the enhancement of labile C through litter decomposition and the alleviation of N-deficiency with the effects of N deposition would result in increased microbial biomass (Treseder 2008; Cusack et al., 2011). However, many other studies have reported negative or in some cases no effect of N deposition on microbial

biomass although the mechanisms underlying the effects are still unclear (Frey et al., 2004; Wu et al., 2013; Liu et al., 2015). In our study, we indeed found positive correlations between microbial biomass C and N concentrations and soil C content (see Table 2 & Fig.S3). Hence, we hypothesize that the overall weak effect of N fertilization on microbial biomass could be mainly attributed to the stable content of soil C and N. Moreover, we found that seven categories of extracellular enzymes closely tied to C, N and P cycling processes (including β-1, 4-glucosidase, β-D-cellobiohydrolase, phenol oxidase, peroxidase, β-1, 4-N-acetyl-glucosaminnidase, leucine aminopeptidase and acid phosphatase) did not show significant response to N fertilization in both forest types (Jing et al., *in review*). The consistent weak responses of soil microbial biomass and extracellular enzymes to N fertilization in our study indicate that soil microorganisms (in terms of biomass and activity) in these forests have not been severely impacted by 4-5 years of moderate N fertilization.

Studies focused on the ecological effects of N deposition have proposed that continuous N deposition may lead to N saturation of forest ecosystems, which suggests that the N availability exceeded the demand of plant and microbes (Aber et al., 1989; Lu et al., 2014). As a consequence of N saturation, negative effects of N addition on plants and microbes are more likely to appear in forests that had experienced high cumulative dose effects of long-term N deposition or fertilization (Fisk & Fahey, 2001; Wallenstein et al., 2006; van Diepen et al., 2011). For example, Allison et al. (2008) reported that microbial community composition shifted after 8 years of N fertilization in a boreal forest in Alaska. Boot et al. (2016) found that microbial biomass and fungal C in subalpine forest ecosystem were reduced remarkably after 17 years' N fertilization. However, our results are consistent with some other short-term and long-term previous studies which supported the hypothesis that microbial community could be resistant to N fertilization for years to decades (Frey et al., 2004; Frey et al., 2014; Liu et al., 2015; Contosta et al., 2015). Hence, the short-term duration of N fertilization may be one reason determining the microbes' robustness to changes due to N fertilization in our temperate forests.

Nevertheless, the duration of chronic N deposition may be one factor regulating below-ground microbial process, but clearly not all explaining the contrasting responses of different forest ecosystems (Treseder 2008; Geisseler & Scow, 2014).

Converse to the temperate forests, microbial biomass and community structure in our subtropical forests did significantly changed with N fertilization. It is generally recognized that subtropical and tropical forests are more phosphorus (P) limited and less N limited than temperate and boreal forests (Vitousek & Howarth 1991; Matson et al., 1999; Fanin et al., 2015). Adding further N to these forests may lead to a high risk of N saturation and aggravated P limitation, which might further negatively affect the survival of microbial communities. This effect might be detected even in a relatively short term study (Hall et al., 2003; Lu et al., 2014). Indeed, we could find a general decreasing pattern of soil P content with the increasing of N fertilization in the subtropical forests (Figure S1). Moreover, we previously found that some other indicators of P limitation were related to a decline in tree growth rate in our subtropical forests (Tian et al., 2017). Hence, we suggest that the potential P limitation in subtropical forests could be accelerated by N fertilization and consequently suppress the microbial biomass. In summary, the contrasting responses of microbial biomass and community structures to N fertilization between subtropical and temperate forests revealed the specific effects of N deposition on forests ecosystems.

In addition to soil nutrient availability, the responses of microbial communities to N fertilization likely depend on multiple factors that differ among forest ecosystems (Van Diepen et al., 2011). Among various soil properties influencing soil microbial communities, pH has widely been recognized as a critical factor, especially in humid regions with acidic soils, because most microorganisms are inhibited universally when the pH is below 4.5 (Högberg et al., 2007; Rousk et al., 2011; Chen et al., 2013). Soil acidification aggravated by N deposition would lead to serious consequences, including exhausted base cations, elevated exchangeable H^+ and Al^{3+} mobilization and exert pronounced toxicity to soil biota (Högberg et al., 2006; Lu et al., 2014). A systematic study across a large pH gradient ranging from 3.7 to 8.3 has also observed a pH-related stress to microbial biomass and PLFAs when the soil pH was below 5 (Rousk et al., 2009). In our study, microbial biomass and the relative abundance of fungi, especially arbuscular mycorrhizal fungi, declined sharply in subtropical forests after 5 years' N fertilization. Simultaneously, soil pH showed a mild decrease of about 0.1-0.4 units with an average value of 4.3 in N fertilized plots. On the contrary, microbial biomass and community structure remained relatively stable in the

418 temperate forests, where the average soil pH was 5.7. Furthermore, we found that pH
419 together with other soil properties (soil N and C content, soil moisture, etc.; Fig.S4)
420 could jointly explain 47%, 41% and 33% of the variances of microbial community
421 compositions at 0-10 cm, 10-20 cm and 20-40 cm soil depths, respectively. Even
422 though pH alone explained a smaller part of the variances, small differences in soil pH
423 variation may be responsible for the contrast changes of microbial biomass and
424 community structure between subtropical and temperate forests with the same dosage
425 of N fertilization. Furthermore, our results coincide with previous conclusion that soil
426 pH of 5 in many ecosystems seems to be a critical threshold determining the effect of
427 N deposition on microorganisms (Geisseler & Scow, 2014).
428
429 Across several main microbial groups indicated by PLFA makers, fungi in subtropical
430 forests were relatively more sensitive to N fertilization. Our results of the negative
431 effects of N fertilization on fungi, especially arbuscular mycorrhizal fungi, and fungi
432 to bacteria ratios in subtropical forests were in consistent with previous laboratory
433 experiments and studies conducted in boreal, temperate and tropical forests (Högberg
434 et al., 2003; DeForest et al., 2004; Allison et al., 2009; Van Diepen et al., 2011; Wu et
435 al., 2013). Generally, the reduction of fungi resulted from less nutrients allocated to
436 root and arbuscular mycorrhizal fungi due to the enhanced nutrient availability and
437 plant nutrient acquisition by N deposition (Johnson et al., 2003; Treseder 2008;
438 Hasselquist et al., 2016). Although we did not find significant effect of N fertilization
439 on soil C, N and P content, our results highlighted an important direction that changes
440 of fungal taxonomic groups in humid subtropical forests should be considered as well
441 as the shift of the overall microbial community structure. This is also relevant for
442 ecosystem functioning as arbuscular mycorrhizal fungi plays a pivotal role in
443 plant-soil nutrient cycling (Van Diepen et al., 2010) and aboveground plant diversity
444 (Dean et al., 2014).
445
446 Due to the symbiotic interactions between plants and microorganisms (Sundqvist et
447 al., 2014), the dominant tree species and the changes of plant communities in the
448 subtropical and temperate forests might also influence microbial biomass and
449 community structure by producing different litter and root exudates in the rhizosphere
450 (Urbanová et al., 2015). Considering the mechanisms underlying the different
451 responses of microbial groups, plant diversity in our study sites could offer an

452 explanation for the shift of microbial community structure. Indeed, understory
453 saplings, shrubs and seedlings drastically decreased and groundcover ferns nearly
454 disappeared after experiencing 4 years of N fertilization in subtropical forests (Tian et
455 al., 2017). Conversely, the dominant herbaceous plant *Deyeuxia angustifolia* showed
456 an expanded coverage in the understory plant community in temperate forests (Du,
457 2017). In light of plants and soil microbes interactions (Leff et al., 2015), some
458 microbial species could have lost their hosts during the extinction of understory plant
459 species and the change of plant community structure in the fertilized forest
460 ecosystems (Thoms et al, 2010; Fu et al., 2015). Therefore, the shifts of aboveground
461 plant community and belowground microbial community structure in subtropical
462 forests consistently revealed a unique sensitive plant-soil feedback to N deposition.
463
464 **5. Conclusions**
465
466 Soil microbial biomass and community structure differed clearly between subtropical
467 and temperate forests. Overall, microbial biomass C and N showed weak response to
468 4-5 years' N fertilization across different forest types. Specifically, we found
469 idiosyncratic effects of N addition on microbial biomass in these two forest
470 ecosystems. No significant effect of N fertilization on microbial biomass and
471 community structure emerged in temperate forests, while substantial reduction of
472 microbial biomass and shift of microbial community structure appeared in subtropical
473 forests. Furthermore, we observed a decline of microbes in specific taxonomic groups,
474 especially arbuscular mycorrhizal fungi, in N fertilized plots in subtropical forests.
475 Changes of soil pH and plant community composition during 4-5 years of N
476 fertilization in subtropical forests might have played an important role in causing
477 these microbial community shifts. Our findings suggest that microbial community
478 structure in subtropical forests with acidic soil is more sensitive to N fertilization than
479 that in temperate forests. As the magnitude and direction of the effects of N
480 deposition can vary among different forest types, it still remains difficult to predict the
481 specific effects of N deposition on individual forest ecosystems. Nevertheless, our
482 findings support the general conclusion that N deposition exerts an overall negative
483 influence on forest ecosystems, especially in subtropical forests.
484
485

Funding: This study was funded by the National Natural Science Foundation of China (31321061 and 31330012). Gianalberto Losapio was financially supported by the Swiss National Science Foundation (PZ00P3_148261).

Acknowledgements: We wish to thank Bing Han, Yongchang Zhu, Jingjing Wang and Zefu Wang for their great help in field soil sampling and experiment. We appreciate helpful suggestions on the statistical analysis from Peter Schmid, Shan Luo, Xiao Chen, Qinggang Wang and Yaoqi Li. We also thank Wei Wang, Zhiheng Wang and Zhiyao Tang for their helpful suggestions on the manuscript.

References

Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM. Nitrogen saturation in northern forest ecosystems. BioScience 1989; 39: 378-386.

Allison SD, Czimczik CI, Treseder KK. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Global Change Biol 2008; 14: 1156-1168.

Allison, SD, Lebauer DS, Ofrecio MR, Reyes R, Ta AM, Tran TM. Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. Soil Biol Biochem 2009; 41:293-302.

Balser TC. The impact of long-term nitrogen addition on microbial community composition in three Hawaiian forest soils. In Optimizing Nitrogen Management in Food and Energy Production and Environmental Protection: Proceedings of the 2nd International Nitrogen Conference on Science and Policy. Scientific World 2001; S2: 500-504.

Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, et al. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecol Appl 2010; 20: 30-59.

Boot CM, Hall EK, Denef K, Baron JS. Long-term reactive nitrogen loading alters soil carbon and microbial community properties in a subalpine forest ecosystem. Soil Biol Biochem 2016; 92: 211-220.

Bossio DA, Scow KM. Impacts of Carbon and Flooding on Soil Microbial Communities: Phospholipid Fatty Acid Profiles and Substrate Utilization Patterns. Microb Ecol 1998; 35: 265-278.

Chen DM, Lan ZC, Bai X, Grace JB, Bai YF. Evidence that acidification-induced declines in plant diversity and productivity are mediated by changes in below-ground communities and soil properties in a semi-arid steppe. J Ecology 2013; 101: 1322-1334.

Chen DM, Mi J, Chu PF, Cheng JH, Zhang LX, Pan QM, et al. Patterns and drivers of soil microbial communities along a precipitation gradient on the Mongolian Plateau. Landscape Ecol 2015; 30: 1669-1682.

Compton JE, Watrud LS, Porteous LA, DeGrood S. Response of soil microbial biomass and community composition to chronic nitrogen additions at Harvard forest. Forest Ecol Manag 2004; 196: 143-158.

529 Contosta, AR, Frey SD, Cooper AB. Soil microbial communities vary as much over
530 time as with chronic warming and nitrogen additions. Soil Biol Biochem 2015;
531 88: 19-24.
532 Cusack DF, Silver WL, Torn MS, Burton SD, Firestone MK. Changes in microbial
533 community characteristics and soil organic matter with nitrogen additions in two
534 tropical forests. Ecology 2011; 92(3): 621-632.
535 Dean SL, Farrer EC, Taylor DL, Porras-Alfaro A, Suding KN, Sinsabaugh RL.
536 Nitrogen deposition alters plant-fungal relationships: linking belowground
537 dynamics to aboveground vegetation change. Mol Ecol 2014; 23: 1364-1378.
538 DeForest JL, Zak DR, Pregitzer KS, Burton AJ. Atmospheric nitrate deposition and
539 the microbial degradation of cellobiose and vanillin in a northern hardwood
540 forest. Soil Biol Biochem 2004; 36: 965-971.
541 Demoling F, Nilsson LO, Bååth E. Bacterial and fungal response to nitrogen
542 fertilization in three coniferous forest soils. Soil Biol Biochem 2008; 40:
543 370-379.
544 Du EZ, Zhou Z, Li P, Hu XY, Ma YC, Wang W, et al. NEECF: a project of nutrient
545 enrichment experiments in China's forests. J Plant Ecol 2013; 6: 428-435.
546 Du EZ. Integrating species composition and leaf nitrogen content to indicate effects of
547 nitrogen deposition. Environ Pollut 2017; 221: 392-397.
548 Fanin N, Hättenschwiler S, Schimann H, Fromin N. Interactive effects of C, N and P
549 fertilization on soil microbial community structure and function in an Amazonian
550 rain forest. Funct Ecol 2015; 29: 140-150.
551 Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities.
552 Proc Natl Acad Sci USA 2006; 103: 626-631.
553 Fisk MC, Fahey TJ. Microbial biomass and nitrogen cycling responses to fertilization
554 and litter removal in young northern hardwood forests. Biogeochemistry 2001;
555 53: 201-223.
556 Frey SD, Knorr M, Parrent JL, Simpson RT. Chronic nitrogen enrichment affects the
557 structure and function of the soil microbial community in temperate hardwood
558 and pine forests. Forest Ecol Manag 2004; 196: 159-171.
559 Frey SD, Ollinger S, Nadelhoffer K, Bowden R, Brzostek E. Chronic nitrogen
560 additions suppress decomposition and sequester soil carbon in temperate forests.
561 Biogeochemistry 2014; 121: 305-316.
562 Frostegård A, Bååth E. The use of phospholipid fatty acid analysis to estimate

563 bacterial and fungal biomass in soil. Biol Fert Soils 1996; 22: 59-65.
564 Fu X, Yang F, Wang J, Di Y, Dai X, Zhang X, et al. Understory vegetation leads to
565 changes in soil acidity and in microbial communities 27 years after reforestation.
566 Sci Total Environ 2015; 502: 280-286.
567 Galloway JN, Winiwarter W, Leip A, Leach AM, Bleeker A, Erisman JW. Nitrogen
568 footprints: past, present and future. Environ Res Lett 2014; 9: 115003.
569 Geisseler D, Scow KM. Long-term effects of mineral fertilizers on soil
570 microorganisms-A review. Soil Biol Biochem 2014; 75: 54-63.
571 Gilmour AR, Gogel BJ, Cullis BR. Thompson R, Butler D. ASReml user guide
572 release 3.0. VSN International Ltd, Hemel Hempstead, UK 2009.
573 Hall SJ, Matson PA. Nutrient status of tropical rain forests influences soil N dynamics
574 after N additions. Ecol Monogr 2003; 73: 107-129.
575 Hasselquist NJ, Metcalfe DB, Inselsbacher E, Stangl Z, Oren R, Näsholm T, et al.
576 Greater carbon allocation to mycorrhizal fungi reduces tree nitrogen uptake in a
577 boreal forest. Ecology 2016; 97: 1012-1022.
578 Högberg MN, Bååth E, Nordgren A, Arnebrant K, Högberg P. Contrasting effects of
579 nitrogen availability on plant carbon supply to mycorrhizal fungi and
580 saprotrophs–a hypothesis based on field observations in boreal forest. New
581 Phytol 2003; 160: 225-238.
582 Högberg MN, Högberg P, Myrold DD. Is microbial community composition in boreal
583 forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia
584 2007; 150: 590-601.
585 Högberg P, Fan HB, Quist M, Binkley D, Tamm CO. Tree growth and soil
586 acidification in response to 30 years of experimental nitrogen loading on boreal
587 forest. Global Change Biol 2006; 12: 489-499.
588 Janssens IA, Dieleman W, Luyssaert S, Subke JA, Reichstein M, Ceulemans R, et al.
589 Reduction of forest soil respiration in response to nitrogen deposition. Nature
590 Geosci 2010; 3: 315-322.
591 Jing X, Chen X, Jiang L, Li P, Ma SH, Tian D, et al. Nitrogen deposition has minor
592 effect on soil extracellular enzyme activities in six Chinese forests. Sci Total
593 Environ 2017; *in submission*.
594 Johnson NC, Rowland DL, Corkidi L, Egerton-Warburton LM, Allen EB. Nitrogen
595 enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands.
596 Ecology 2003; 84(7): 1895-1908.

597 Leff JW, Jones SE, ProberSM, Barberán A, Borer ET, Firn JL, et al. Consistent
598 responses of soil microbial communities to elevated nutrient inputs in grasslands
599 across the globe. Proc Natl Acad Sci USA 2015; 112: 10967-10972.
600 Li J, Li ZA, Wang FM, Zou B, Chen Y, Zhao J, et al. Effects of nitrogen and
601 phosphorus addition on soil microbial community in a secondary tropical forest
602 of China. Biol Fert Soils 2015; 52: 207-215.
603 Liu L, Gundersen P, Zhang T, Mo JM. Effects of phosphorus addition on soil
604 microbial biomass and community composition in three forest types in tropical
605 China. Soil Biol Biochem 2012; 44: 31-38.
606 Liu XJ, Zhang Y, Han WX, Tang AH, Shen JL, Cui ZL, et al. Enhanced nitrogen
607 deposition over China. Nature 2013; 494: 459-463.
608 Liu XJ, Duan L, Mo JM, Du EZ, Shen JL, Lu XK, et al. Nitrogen deposition and its
609 ecological impact in China: an overview. Environ Pollut 2011; 159: 2251-2264.
610 Liu L, Gundersen P, Zhang W, Zhang T, Chen H, Mo JM. Effects of nitrogen and
611 phosphorus additions on soil microbial biomass and community structure in two
612 reforested tropical forests. Sci Rep 2015; 5:14378. doi: 10.1038/srep14378.
613 Lu XK, Mao QG, Gilliam FS, Luo YQ, Mo JM. Nitrogen deposition contributes to
614 soil acidification in tropical ecosystems. Global Change Biol 2014; 20:
615 3790-3801.
616 Margesin R, Schinner F. Monitoring and Assessing Soil Bioremediation. Springer
617 Berlin Heidelberg 2005.
618 Matson, PA, McDowell WH, Townsend AR, Vitousek PM. The globalization of N
619 deposition: ecosystem consequences in tropical environments. Biogeochemistry
620 1999; 46: 67-83.
621 McGenity TJ, Timmis KN, Nogales B. Hydrocarbon and lipid microbiology protocols,
622 Springer protocols handbooks, 2017; 65-76, doi: 10.1007/8623_2016_213.
623 Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, et al. *Vegan:*
624 *Community Ecology Package*. 2015; R package version 2.0.3.
625 Prescot CE, Grayston SJ. Tree species influence on microbial communities in litter
626 and soil: Current knowledge and research needs. Forest Ecol Manag 2013; 309:
627 19-27.
628 R Development Core Team. *R: A Language and Environment for Statistical*
629 *Computing*. 2015; R Foundation for Statistic Computing, Vienna.
630 Rousk J, Brookes PC, Bååth E. Contrasting Soil pH Effects on Fungal and Bacterial

631 Growth Suggest Functional Redundancy in Carbon Mineralization. Appl Environ
632 Microb 2009; 75: 1589-1596.
633 Rousk J, Brookes PC, Bååth E. Fungal and bacterial growth responses to N
634 fertilization and pH in the 150-year 'Park Grass' UK grassland experiment.
635 FEMS Microb Ecol 2011; 76: 89-99.
636 Sundqvist MK, Liu Z, Giesler R, Wardle DA. Plant and microbial responses to
637 nitrogen and phosphorus addition across an elevational gradient in subarctic
638 tundra. Ecology 2014; 95: 1819-1835.
639 Sutton MA, et al. (eds.), Nitrogen Deposition, Critical Loads and Biodiversity.
640 Springer Science + Business Media Dordrecht 2014; doi: 10.1007/978-94
641 -007-7939-6_28.
642 Tian D, Li P, Fang WJ, Xu J, Luo YK, Yan ZB, Zhu B, Wang JJ, Xu XN, Fang JY.
643 Contrasting growth responses among plant growth forms to nitrogen fertilization
644 in a subtropical forest in China. Biogeosciences Discussion 2017; doi:
645 10.5194/bg-2016-416.
646 Thoms C, Gattinger A, Jacob M, Thomas FM, Gleixner G. Direct and indirect effects
647 of tree diversity drive soil microbial diversity in temperate deciduous forest. Soil
648 Biol Biochem 2010; 42: 1558-1565.
649 Treseder KK. Nitrogen additions and microbial biomass: A meta‐analysis of
650 ecosystem studies. Ecol Lett 2008; 11: 1111-1120.
651 Turlapati SA, Minocha R, Bhiravarasa PS, Tisa LS, Thomas WK, Minocha SC.
652 Chronic N-amended soils exhibit an altered bacterial community structure in
653 Harvard Forest, MA, USA. FEMS Microb Ecol 2013; 83: 478-493.
654 Urbanová M, Šnajdr J, Baldrian P. Composition of fungal and bacterial communities
655 in forest litter and soil is largely determined by dominant trees. Soil Biol
656 Biochem 2015; 84: 53–64.
657 Van Der Heijden, MG, Bardgett RD, Van Straalen NM. The unseen majority: soil
658 microbes as drivers of plant diversity and productivity in terrestrial ecosystems.
659 Ecol Lett 2008; 11: 296-310.
660 Van Diepen LTA, Lilleskov EA, Pregitzer KS, Miller RM. Simulated nitrogen
661 deposition causes a decline of intra- and extraradical abundance of arbuscular
662 mycorrhizal fungi and changes in microbial community structure in northern
663 hardwood forests. Ecosystem 2010; 13: 683-695.
664 Van Diepen LTA, Lilleskov EA, Pregitzer KS. Simulated nitrogen deposition affects

665 community structure of arbuscular mycorrhizal fungi in northern hardwood
666 forests. Mol Ecol 2011; 20: 799-811.
667 Vitousek PM, Howarth RW. Nitrogen limitation on land and in the sea: how can it
668 occur? Biogeochemistry 1991; 13: 87-115.
669 Wallenstein MN, McNulty S, Fernandez IJ, Boggs J, Schlesinger WH. Nitrogen
670 fertilization decreases forest soil fungal and bacterial biomass in three long-term
671 experiments. Forest Ecol Manag 2006; 222: 459-468.
672 Wu JP, Liu WF, Fan HB, Huang GM, Wan SZ, Yuan YH, et al. Asynchronous
673 responses of soil microbial community and understory plant community to
674 simulated nitrogen deposition in a subtropical forest. Ecol Evol 2013; 3(11):
675 3895-3905.