



Consistent effects of canopy vs. understory nitrogen addition on the soil exchangeable cations and microbial community in two contrasting forests



Leilei Shi ^{a,b}, Hongzhi Zhang ^{a,b}, Tao Liu ^{a,b}, Weixin Zhang ^a, Yuanhu Shao ^a, Denglong Ha ^c, Yuanqiu Li ^d, Chuangmao Zhang ^d, Xi-an Cai ^a, Xingquan Rao ^a, Yongbiao Lin ^a, Lixia Zhou ^a, Ping Zhao ^a, Qing Ye ^a, Xiaoming Zou ^e, Shenglei Fu ^{a,*}

^a Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

^b University of Chinese Academy of Sciences, Beijing 100049, China

^c Jigongshan National Natural Reserve, Xinyang, Henan 464000, China

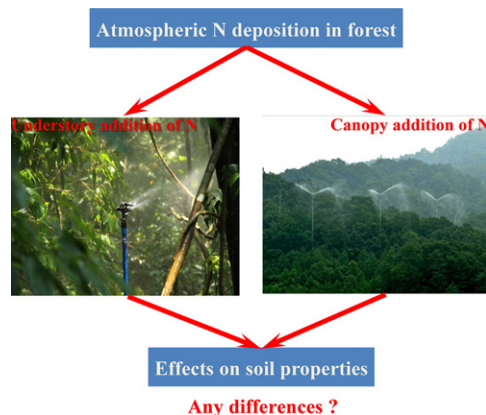
^d Shimentai National Natural Reserve, Yingde, Guangdong 513000, China

^e Institute for Tropical Ecosystem Studies, University of Puerto Rico, San Juan, PR 00931-1910, USA

HIGHLIGHTS

- A novel field N manipulation experiment with both UAN and CAN was established.
- N addition reduced pH, BS and Ca and increased Al at temperate forest.
- N addition reduced biomasses of most soil microbial groups at subtropical forest.
- Effects of CAN on forest soils were not significantly different from that of UAN.
- No interactions between N treatment approach and study site or N addition rate.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 26 November 2015

Received in revised form 15 February 2016

Accepted 15 February 2016

Available online 27 February 2016

Keywords:

N deposition

Canopy addition of N

Understory addition of N

ABSTRACT

Anthropogenic N deposition has been well documented to cause substantial impacts on the chemical and biological properties of forest soils. In most studies, however, atmospheric N deposition has been simulated by directly adding N to the forest floor. Such studies thus ignored the potentially significant effect of some key processes occurring in forest canopy (i.e., nitrogen retention) and may therefore have incorrectly assessed the effects of N deposition on soils. Here, we conducted an experiment that included both understory addition of N (UAN) and canopy addition of N (CAN) in two contrasting forests (temperate deciduous forest vs. subtropical evergreen forest). The goal was to determine whether the effects on soil exchangeable cations and microbial biomass differed between CAN and UAN. We found that N addition reduced pH, BS (base saturation) and exchangeable Ca and increased exchangeable Al significantly only at the temperate JGS site, and reduced the biomass of most soil microbial groups only at the subtropical SMT site. Except for soil exchangeable Mn, however, effects on soil chemical

* Corresponding author at: 723 Xingke Road, Tianhe District, Guangzhou 510650, China.

E-mail address: sfu@scbg.ac.cn (S. Fu).

Soil microbial community
Soil exchangeable cations

properties and soil microbial community did not significantly differ between CAN and UAN. Although biotic and abiotic soil characteristics differ significantly and the responses of both soil exchangeable cations and microbial biomass were different between the two study sites, we found no significant interactive effects between study site and N treatment approach on almost all soil properties involved in this study. In addition, N addition rate (25 vs. 50 kg N ha⁻¹ yr⁻¹) did not show different effects on soil properties under both N addition approaches. These findings did not support previous prediction which expected that, by bypassing canopy effects (i.e., canopy retention and foliage fertilization), understory addition of N would overestimate the effects of N deposition on forest soil properties, at least for short time scale.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

There is a growing consensus that increased atmospheric nitrogen (N) deposition due to human activities has emerged as one most serious global change problem (Vitousek et al., 1997; Galloway et al., 2008; Schlesinger, 2009; Pardo et al., 2011; Penuelas et al., 2013). At the global scale, the average rate of atmospheric N deposition has recently been estimated to be 105 Tg N yr⁻¹ (Galloway et al., 2008), and this rate is predicted to continually increase in the future (Dentener et al., 2006; Stocker et al., 2013). Furthermore, N deposition is especially serious in some “hot spots” such as central and southeastern China (Liu et al., 2013; Jia et al., 2014). Enhanced N deposition has been well documented to cause a series of detrimental effects, such as decline in plant diversity (Bobbink et al., 2010) and soil acidification (Tian and Niu, 2015), on terrestrial ecosystems.

Nitrogen deposition exerts profound effects on soil abiotic properties (Lucas et al., 2011; Tian and Niu, 2015) and biotic properties (Frey et al., 2004; Treseder, 2008). Many studies have indicated that soil chemical processes, such as soil acidification, are sensitive to N deposition (Gundersen and Rasmussen, 1990; Högberg et al., 2006; Lieb et al., 2011; Lucas et al., 2011; Lu et al., 2014; Tian and Niu, 2015). An excessive input of N to soils often significantly changes soil exchangeable cations and their exchange capacity (Bowman et al., 2008; Gundersen et al., 2006; Lucas et al., 2011); high N input, for example, gradually depletes nutrient base cations (i.e., Ca²⁺, Mg²⁺, K⁺, and Na⁺) and causes toxic metal ions (i.e., Al³⁺, Fe³⁺, and Mn²⁺) to accumulate in soils (Lucas et al., 2011). These N-induced negative effects on soil chemical processes are an important threat to ecosystem functioning, such as decreasing soil buffering capacity, decline of plant productivity (Stevens et al., 2010; Chen et al., 2013), and inhibition of soil biological processes (Kuperman and Edwards, 1997). In addition, N deposition also has a significant effect on soil microbial biomass and community structure (Frey et al., 2004; Waldrop et al., 2004). At global scale, a meta-analysis showed that anthropogenic N additions have been estimated to induce a reduction of soil microbial biomass by 15% (Treseder, 2008).

Our current understanding of how and the extent to which N deposition affects forest soil chemical properties and microbial communities, however, is largely derived from field experiments with understory addition of N (UAN; N is directly added to the forest floor). Apparently, forest canopy processes have been overlooked in most field N manipulation experiments. In fact, most N deposited from the atmosphere does not reach the forest floor directly but instead first contacts and passes through the canopy layer (Gaige et al., 2007; Wortman et al., 2012; Zhang et al., 2015). In this process, the forest canopy can retain a substantial proportion of the deposited N (Gaige et al., 2007) which could be taken up by canopy tree leaves and/or transformed into other forms (Adriaenssens et al., 2012), thus changing the quality and quantity of N deposited onto forest soils (Houle et al., 2015). A recent study suggested that, in failing to consider the effect of the forest canopy, previous N addition experiments may have overestimated the effects of N deposition on forest soils and related processes, and pose a great challenge for understanding the patterns and dynamics of forest ecosystems under N deposition in the future (Zhang et al., 2015).

However, no experimental studies have yet been performed to assess whether the effects of N deposition on forest soils differ depending on whether N is directly added to the forest floor or is added above the forest canopy.

In the present study, we conducted a field experiment that included both understory addition of N (UAN) and canopy addition of N (CAN) treatments in two contrasting forests (a temperate deciduous forest and a subtropical evergreen forest) in central and southern China (Zhang et al., 2015). The objective of this study was to determine if these two approaches of N addition (CAN vs. UAN) have different effects on forest soil abiotic (exchangeable cations) and biotic (microbial biomass) properties. As previous studies demonstrated, ecosystem background and the level of N addition often influence the responses of soil properties to N deposition (Treseder, 2008; Lu et al., 2011; Tian and Niu, 2015). Therefore, we also examine if forest type and N addition rate would alter the effects of N treatment approach (CAN vs. UAN). We hypothesized that the effects on both soil exchangeable cations and microbial biomass are weaker for CAN than for UAN, as predicted by Zhang et al. (2015). We also hypothesized that the effects of N treatment approach (CAN vs. UAN) would be affected by both forest type and N addition rate.

2. Materials and methods

2.1. Study sites

The experiment was conducted in two contrasting forest sites both located in climate transitional zones: the Jigongshan (JGS) site and the Shimentai (SMT) site. The JGS site was located in the Jigongshan National Nature Reserve (31°46′–31°52′ N, 114°01′–114°06′ E), Henan Province, Central China, which is in a climate transitional zone from subtropical to warm temperate. The mean annual temperature at the JGS Reserve is 15.2 °C, and the mean annual rainfall is 1119 mm. The background rate of N deposition in precipitation is about 19.6 kg N ha⁻¹ yr⁻¹ in this region (Zhang et al., 2015). The dominant vegetation at the JGS site was a deciduous temperate forest, which was 45 years old at the time of the study. The dominant canopy tree species at the JGS site were *Quercus acutissima* Carruth., *Quercus variabilis* Bl., and *Liquidambar formosana* Hance. The region has a yellow-brown sandy-loam soil (Zhang et al., 2015).

The SMT study site was in the Shimentai National Nature Reserve (24°22′–24°31′ N, 113°05′–113°31′ E), Guangdong Province, southern China, which is dominated by a subtropical monsoon climate with alternating wet and dry seasons. The mean annual temperature is 20.8 °C, and the mean annual rainfall is 1700 mm. The rate of N deposition in precipitation is about 34.1 kg N ha⁻¹ yr⁻¹ (Zhang et al., 2015), with equal quantities of oxidized and reduced forms of N (Fang et al., 2011; Huang et al., 2012). The dominant vegetation at the SMT site was a broadleaved evergreen forest, which was 50 years old at the time of the study. The dominant canopy tree species at the SMT site were *Cryptocarya concinna*, *Schima superba*, *Machilus chinensis*, *Castanea henryi* (Skan) Rehd., and *Engelhardtia roxburghiana* (Zhang et al., 2015). The site has a latosolic red clay-loam soil (Zhang et al., 2015).

2.2. Experimental design

At both study sites, N was added in two approaches: canopy addition of N (CAN) and understory addition of N (UAN). CAN was designed to realistically simulate atmospheric N deposition in forest ecosystems. UAN represents the conventional method of N addition that has been used in most N deposition experiments over the past two decades. The same five treatments were used at each site: 1) CAN at 25 kg N ha⁻¹ yr⁻¹ (CAN25); 2) CAN at 50 kg N ha⁻¹ yr⁻¹ (CAN50); 3) UAN at 25 kg N ha⁻¹ yr⁻¹ (UAN25); 4) UAN at 50 kg N ha⁻¹ yr⁻¹ (UAN50); and 5) a control (CK, without N addition). At each site, the five treatments were randomly assigned to five plots in each of four blocks in a completely randomized block design. Each plot was circular and had an area of 907 m².

N was added to each treated plot as NH₄NO₃ solutions of targeted concentrations. For each treatment except the control, the amount of NO₃⁻-N used in the NH₄NO₃ solution was equal to the amount of NH₄⁺-N. For each application of N, the NH₄NO₃ solution was added in a volume equivalent to 3 mm of precipitation. Treatments were applied monthly from April to October (seven times in 1 year), and treatment dates corresponded to the phenology of the JGS forest, i.e., the first treatment was applied 1 week before all buds had opened (mid-April), and the last was applied as leaf litter began to fall (mid-October). The total solution applied per year to each plot was equivalent to 21 mm of precipitation, which was <1–2% of the total annual precipitation at JGS or SMT, respectively. Therefore, the confounding effect caused by water addition was negligible.

UAN treatments were applied with an automatic irrigation system that sprinkled the N solutions onto the forest floor from a height of 1.5 m. CAN treatments were applied with a forest canopy spraying system built in the center of the corresponding plots. The working principles and validity of the CAN system were described in detail by Zhang et al. (2015). Briefly, the N solutions were pumped to a height of 35 m (which was about 5 m above the forest canopy) in PVC pipes (10 cm diameter) that were attached to a 35-m high supporting tower. The N solutions were evenly sprayed onto the canopies by two crane sprinklers that could freely turn 360°. Sprays were applied in the morning or evening on days with minimal sunshine and when the wind speed was <1 m/s. The first treatment event was performed in the middle of April 2013 at both study sites.

2.3. Field soil sampling and laboratory analysis

Soil samples from each plot at both sites were collected in October 2013 (after the 7th treatment) and in August 2014 (after the 11th treatment). Each plot was evenly divided into four quadrants and two soil samples (0–10 cm depth) were randomly taken with a soil core (3 cm × 10 cm) from each quadrant. The eight soil cores from each plot were thoroughly mixed and combined into one composite sample. The fresh soil samples were passed through a 2-mm sieve immediately after sampling. One subsample was freeze-dried for extraction of soil microbial phospholipid fatty acids (PLFAs). The remaining soil samples were air-dried for soil chemical analysis.

Soil pH was measured in a soil/deionized water suspension (1:2.5) using a pH meter (Mettler Toledo, Shanghai, China). Soil exchangeable cations were measured using the methods described by Hendershot et al. (2007) and Bowman et al. (2008). The air-dried soil samples were extracted in 0.1 M BaCl₂ solutions, and concentrations of exchangeable Ca, Mg, K, Na, Al, Fe, and Mn ions in extracts were determined with an inductively coupled plasma optical emission spectrometer (ICP-OES, Perkin Elmer, Waltham, MA, USA).

The soil microbial community was investigated using PLFA analysis as described by Bossio and Scow (1998). The lipids in each freeze-dried soil sample were extracted in a single-phase mixture of chloroform:methanol:phosphate buffer (1:2:0.8 by vol.; pH 7.4), and the extracts were analysed with a gas chromatograph equipped with a

flame-ionisation detector (Agilent 6890, Agilent Technologies, Palo Alto, CA, USA). The abundance of individual PLFAs was expressed as nmol PLFAs g⁻¹ dry soil (nmol g⁻¹), and the sum of all individual PLFAs was used as a measure of viable total microbial biomass (TB) (Frostegård and Bååth, 1996). Different groups of soil microorganisms were indicated by specific PLFA markers. Gram-positive bacteria (G⁺) were indicated by the branched and saturated PLFAs i-15:0, a-15:0, i-16:0, i-17:0, and a-17:0. Gram-negative bacteria (G⁻) were indicated by the monounsaturated and cyclopropyl PLFAs 16:1Ω7c, 18:1Ω7c, cy17:0, and cy19:0 (Frostegård and Bååth, 1996; Zogg et al., 1997; Bossio and Scow, 1998; Zelles, 1999; Cusack et al., 2011; Fanin et al., 2013). Saprotrophic fungi (F) were indicated by the PLFAs 18:1Ω9 and 18:2Ω6 (Frostegård and Bååth, 1996; Cusack et al., 2011; Fanin et al., 2013), whereas the fatty acid 16:1Ω5 was used as an indicator of arbuscular mycorrhizal fungi (AM) (Nordby et al., 1981; Olsson, 1999; van Diepen et al., 2010; Cusack et al., 2011). The methyl (Me) branched fatty acids 16:0 10Me and 18:0 10Me were used as indicators of actinomycetes (A) (van Diepen et al., 2010; Cusack et al., 2011). Two structural parameters were used as indicators of general soil microbial community structure: G⁺:G⁻ biomass ratio and F:B biomass ratio (where B presents bacteria, here equals to G⁺ plus G⁻) (Frostegård and Bååth, 1996; Cusack et al., 2011; Fanin et al., 2013).

2.4. Data analyses

Soil cation exchange capacity (CEC) was calculated as the sum of the seven measured exchangeable cations (Ca, Mg, K, Na, Al, Fe, and Mn) on an equivalent basis (m mol (+) kg⁻¹ dry soil). Soil base saturation (BS, %) was calculated as that percentage of the CEC represented by base cations (Ca, Mg, K, and Na). The seven exchangeable cations were expressed as the percentage of the CEC represented by each of the seven exchangeable cations (%). Prior to statistical analyses, all data were tested for normality using the Shapiro-Wilk test and for homoscedasticity using the Levene test. We used repeated measures analysis of variance (ANOVA) to test for the effects of N treatment type (CAN vs. UAN), N addition rate, study site, sampling time, and their interactions on soil pH, CEC, BS, exchangeable cations, and soil microbial parameters. For each study site, the effect of treatments on soil CEC, BS, exchangeable cations, and soil microbial parameters was assessed using one-way ANOVA, and multiple comparisons were conducted with a post hoc Tukey HSD test. If there were no effects of N addition rate and treatment type, *t*-tests were used to determine the differences in soil pH, CEC, BS, exchangeable cations, and soil microbial parameters between the control and N addition treatments (all four treatments with N addition were considered together) for each study site. Differences were considered significant at the 0.05 level. All statistical analyses were performed with SPSS 18.0.

3. Results

3.1. Effects of canopy vs. understory N additions on soil pH, CEC, BS, and exchangeable cations

The two study sites (JGS and SMT) differed significantly in their soil buffering systems (Table 1; Fig. 1A, C–F, H–J) except for the soil CEC and exchangeable Na (Table 1; Fig. 1B, G). At the SMT forest, the soil had a pH of 3.55 (Fig. 1A), exchangeable Al³⁺ accounted for 93% of the CEC (Fig. 1H), and the base cations (especially Ca²⁺ and Mg²⁺) were largely depleted (Fig. 1C–F). At the JGS forest, the soil had a pH of 4.40 (Fig. 1A); base cations represented >60% of the exchangeable cations (Fig. 1C); and exchangeable Ca²⁺ alone accounted for 51% of the CEC (Fig. 1D); in addition, exchangeable Al³⁺ accounted for 34% of the CEC at the JGS forest (Fig. 1H).

The repeated-measures ANOVA showed that N treatment approach (CAN vs. UAN) significantly affected soil exchangeable Mn (Table 1; Fig. 1J) but did not affect soil pH, CEC, BS, and other exchangeable

Table 1
Effects of N treatment approach (CAN vs. UAN), N addition rate (25 vs. 50 kg N ha⁻¹ yr⁻¹), study site (JGS vs. SMT), sampling time (year 2013 and 2014), and two way interactions between N treatment approach and N addition rate, study site, and sampling time on soil pH, ECE, BS, and exchangeable cations.

Variable	NTP		NDR		Site		Time		NTP × NDR		NTP × site		NTP × time	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
pH	0.01	0.94	0.52	0.48	239.20	<0.001	164.29	<0.001	0.16	0.70	0.57	0.47	2.43	0.10
CEC	0.01	0.91	0.05	0.82	0.00	0.98	120.21	<0.001	0.96	0.34	0.03	0.97	1.63	0.21
BS	0.16	0.70	2.18	0.15	441.05	<0.001	2.31	0.14	0.59	0.45	0.54	0.44	0.07	0.79
Ca	0.29	0.60	2.61	0.12	342.92	<0.001	1.27	0.27	0.17	0.68	3.14	0.06	0.29	0.60
Mg	0.27	0.61	0.03	0.86	461.60	<0.001	0.22	0.64	1.54	0.22	1.77	0.19	1.17	0.29
K	0.56	0.46	0.06	0.81	47.48	<0.001	8.34	0.01	0.75	0.39	1.30	0.29	2.74	0.10
Na	0.04	0.84	0.59	0.45	0.20	0.65	30.91	<0.001	6.11	0.002	1.69	0.20	0.15	0.70
Al	0.08	0.78	2.09	0.16	457.72	<0.001	0.03	0.86	0.76	0.39	0.66	0.42	0.06	0.80
Fe	0.66	0.42	5.86	0.03	185.84	<0.001	280.33	<0.001	0.37	0.55	0.41	0.67	1.18	0.29
Mn	4.70	0.04	0.79	0.38	360.34	<0.001	99.47	<0.001	2.81	0.10	3.92	0.03	0.08	0.78

NTP, N treatment approach; NDR, N addition rate; Site, study site; Time, sampling time; NTP × NDR, interactions between N treatment approach and N addition rate; NTP × Site, interactions between N treatment approach and study site; NTP × Time, interactions between N treatment approach and sampling time; CEC, cation exchange capacity; BS, base saturation.

cations (Table 1; Fig. 1A–I). The interactive effects between N treatment approach (CAN vs. UAN) and site were also only significant for soil exchangeable Mn (Table 1). N addition rate did not significantly affect soil pH, CEC, BS, and exchangeable cations except for exchangeable Fe (Table 1); and the interactions between N treatment approach (CAN vs. UAN) and N addition rate were only significant for soil exchangeable Na (Table 1). Sampling time significantly affected soil pH, CEC, exchangeable K, Na, Fe, and Mn, but the interaction between N treatment approach (CAN vs. UAN) and sampling time was not significant for all these soil properties (Table 1).

Based on one-way ANOVA, low rates of N addition (CAN25 and UAN25) significantly decreased base saturation (BS) and exchangeable Ca²⁺ (Fig. 1C, D), and significantly increased exchangeable Al³⁺ (Fig. 1H) at the JGS site. In addition, N addition (pooling of all treatments with N addition) also significantly decreased soil pH (*t*-test, *P* = 0.007; Fig. 1A) at the JGS site. However, soil pH, BS, CEC, and all the exchangeable cations at the SMT site were not affected by any of N addition treatments (CAN or UAN at 25 and 50 kg N ha⁻¹ yr⁻¹).

3.2. Effects of canopy vs. understory N additions on soil microbial community

Soil microbial biomasses (total and five major groups) were always higher at the SMT forest site than at the JGS forest site (Fig. 2A, D–I), but the F:B biomass ratio and G⁺:G⁻ ratio did not significantly differ between the two study sites (Table 1). The repeated-measures ANOVA showed that total microbial biomass, the biomasses of five major microbial groups, and microbial structure parameters such as F:B biomass ratio and G⁺:G⁻ biomass ratio were not significantly affected by N treatment approach (CAN vs. UAN) (Table 2; Fig. 2A–H). Sampling time significantly affected all soil microbial properties except for the F:B biomass ratio (Table 2). There were no significant interactions between N treatment approach (CAN vs. UAN) and N addition rate, study site, and sampling time for any of the soil microbial properties (Table 2). Relative to the control treatment, N addition (pooling of all treatments with N addition) significantly decreased the biomasses of soil Gram-negative bacteria (*t*-test, *P* = 0.047; Fig. 2E), fungi (*t*-test, *P* = 0.047; Fig. 2F), and AM fungi (*t*-test, *P* = 0.028; Fig. 2G) at the SMT forest site. However, N addition did not affect the soil microbial biomass at the JGS forest site.

4. Discussion

In the present study, we determined whether the changes in forest soil exchangeable cations and microbial biomass caused by N addition differed when N was added to the canopy (in the CAN treatment) vs. to the forest floor (in the UAN treatment). N addition was found to reduce pH, BS and exchangeable Ca and to increase exchangeable Al significantly at the JGS site, but it reduced the biomasses of most groups

of the soil microbial community at the SMT site. Contrary to our initial hypothesis and to the suggestion of Zhang et al. (2015), CAN and UAN treatments had similar effects on all soil chemical properties and soil microbial properties except for exchangeable Mn.

The observed consistent effects of CAN and UAN treatments on soil properties in the present study may have several explanations. Theoretically, the most direct explanation would be that little or no N was retained by the forest canopy and that the quantity of N entering the soil was therefore similar for CAN and UAN treatments. We find this explanation to be unsatisfactory because the agents that are likely to move N through the canopy to the soil, i.e., rain and wind, were absent during the experiment. More importantly, recent studies have clearly shown that the canopies of several kinds of forests retain a substantial portion of the deposited atmospheric N (Gaije et al., 2007; Dail et al., 2009; Fenn et al., 2013; Houle et al., 2015) through N adsorption and uptake by tree leaves, branches, twigs, and associated lichens and bryophytes (Sparks, 2009; Adriaenssens et al., 2012; Eichert and Fernández, 2012). Another possible explanation for the lack of difference between the CAN and UAN treatments in our study may be that the N retention capacities of the forest canopies were saturated because of the high background rate of N deposition at both study sites (19.6 kg N ha⁻¹ yr⁻¹ at the JGS site and 34.1 kg N ha⁻¹ yr⁻¹ at the SMT site). With saturation of the canopy's capacity to retain N, the experimentally added N would not be adsorbed by the canopy and would pass through the canopy to the forest floor. However, we have no direct evidence to support this explanation for our results.

Yet another possible explanation for the similar effects of the CAN and UAN treatments is that even though the quantity of N reaching the soil may have been significantly reduced by the CAN treatment, the soil properties at both study sites may have been relatively insensitive to differences in N input because past, natural inputs had been high enough. Both study sites are located in "hot spots" of N deposition in China (Liu et al., 2013; Zhu et al., 2015). With very high background rates of N deposition and the quantities of N we experimentally added, perhaps the effects of N on soil parameters had already reached a plateau (i.e., <25 kg N ha⁻¹ yr⁻¹) at both sites, such that the response to the CAN and UAN treatments would be relatively similar. This explanation is consistent with another finding in our study, which was that the responses of almost all soil properties to two rates of N addition (25 and 50 kg N ha⁻¹ yr⁻¹) did not significantly differ at either site.

A final possible explanation for the consistent effects of CAN and UAN treatments may be the relatively short duration of our experiment. We expected CAN and UAN treatments to have different effects on soil properties in part because of the foliage fertilization induced by addition of N to the canopy. Such foliage fertilization can alter the physiology of the canopy plants (Sparks, 2009; Adriaenssens et al., 2012; Eichert and Fernández, 2012) and thereby change the quality of resources (i.e., the C:N ratio of litter and root exudates) that ultimately enter the soil (Bardgett and Wardle, 2010; Wortman et al., 2012). This effect of

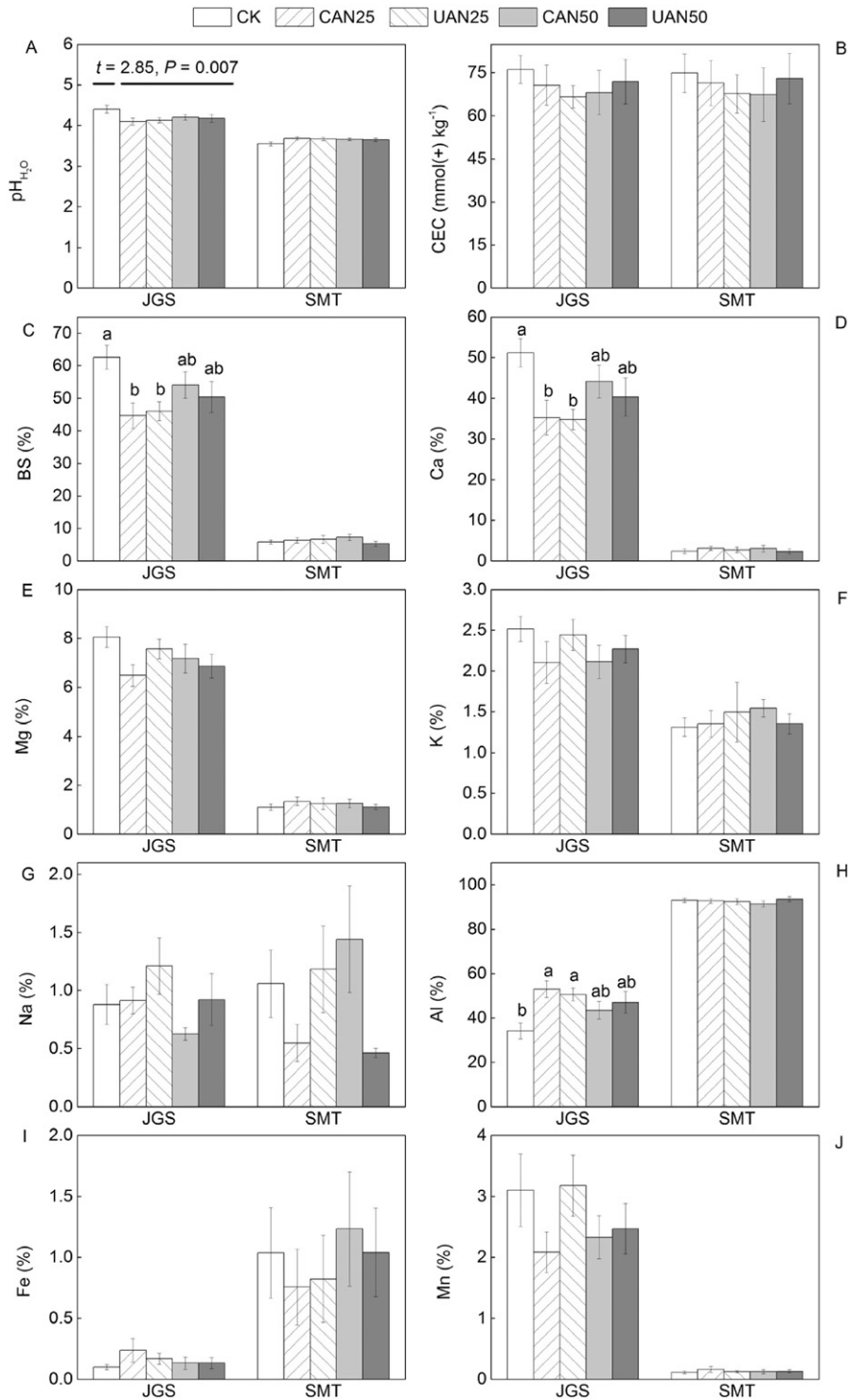


Fig. 1. Soil pH (A), cation exchange capacity (CEC) (B), base saturation (BS) (C), and percentage of CEC accounted for by seven cations (% on an equivalent basis) (D–J) at 0–10 cm depth as affected by five N deposition treatments at Jigongshan (JGS) and Shimentai (SMT) forest sites in China. Values are means \pm standard error (SE), $n = 8$. Within each group of five bars, values with different letters are significantly different ($P < 0.05$). CK, control and without N addition; CAN25, canopy addition of N with $25 kg N ha^{-1} yr^{-1}$; UAN25, understory addition of N with $25 kg N ha^{-1} yr^{-1}$; CAN50, canopy addition of N with $50 kg N ha^{-1} yr^{-1}$; UAN50, understory addition of N with $50 kg N ha^{-1} yr^{-1}$.

foliar fertilization, however, requires time (Bardgett and Wardle, 2010), perhaps more time than the 2 years of our experiment. Therefore, it is hard to distinguish the CAN and UAN effects on forest soils at relatively short time scales.

The two experimental sites in our study differed greatly in their exchangeable cation composition and microbial biomass because of differences in climate, vegetation, and soil (Zhang et al., 2015). The SMT experimental site, which was located in a humid subtropical forest,

had a very low soil pH (3.6), very low soil BS (5.9%), and mostly exchangeable Al^{3+} in its soil cation pool (93%). These characteristics are common in tropical and subtropical humid forests worldwide (Vogt et al., 2006; Brady and Weil, 2010; Quesada et al., 2010; Lu et al., 2014) because high rainfall and temperature favor the rapid dissolution and leaching of easily weatherable base cations and the subsequent release of and dominance by Al^{3+} in the soil (von Uexkull and Mutert, 1995; Quesada et al., 2010). In the soil of the JGS experimental site

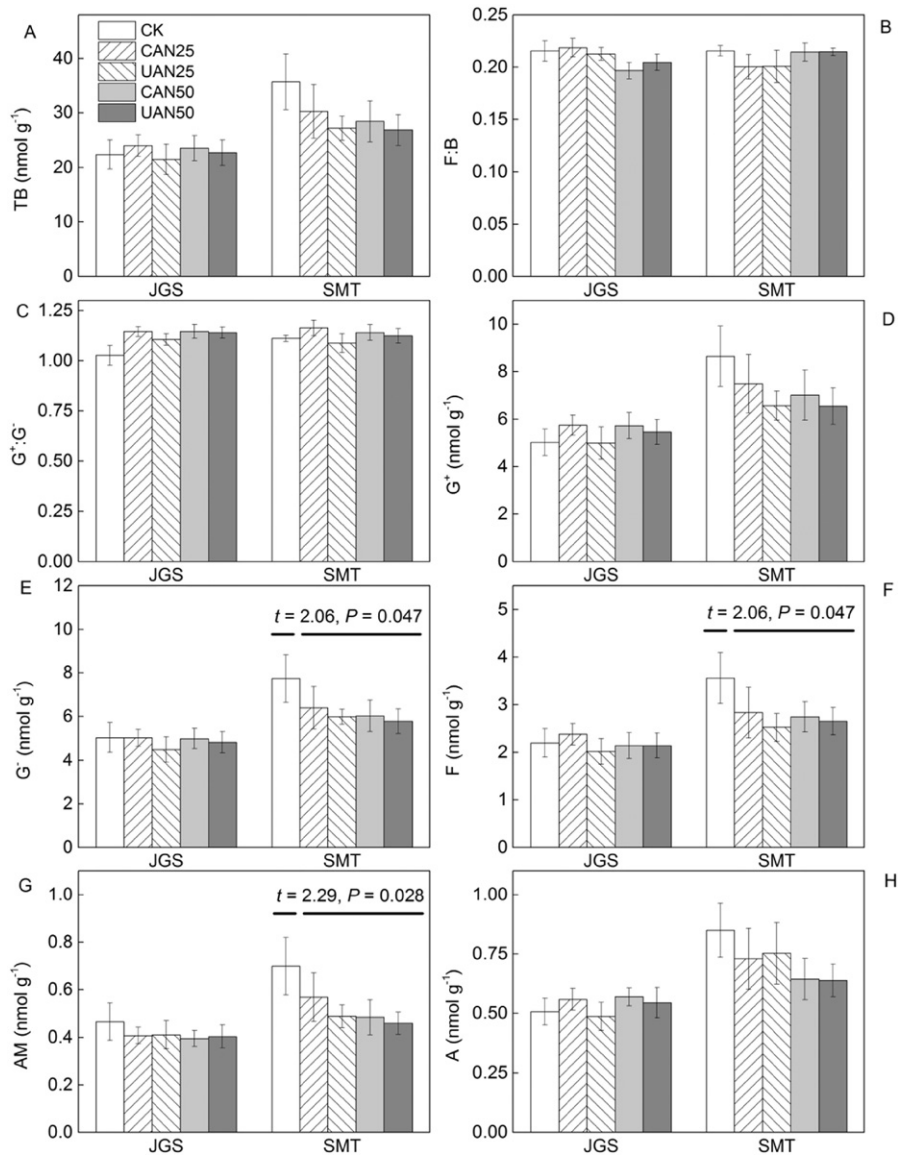


Fig. 2. Responses of soil microbial biomass and community structure to N deposition treatments at 0–10 cm soil depth at Jigongshan (JGS) and Shimentai (SMT) sites in China. Values are means \pm standard error (SE), $n = 8$. TB, total microbial biomass; F:B, ratio of fungal to bacterial biomass; G^+ , biomass of Gram-positive bacteria; G^- , biomass of Gram-negative bacteria; $G^+ : G^-$, ratio of Gram-positive to Gram-negative bacterial biomass; F, biomass of saprotrophic fungi; AM, biomass of arbuscular mycorrhizal fungi; A, biomass of actinomycetes; CK, control and without N addition; CAN25, canopy addition of N with 25 kg N ha⁻¹ yr⁻¹; UAN25, understory addition of N with 25 kg N ha⁻¹ yr⁻¹; CAN50, canopy addition of N with 50 kg N ha⁻¹ yr⁻¹; UAN50, understory addition of N with 50 kg N ha⁻¹ yr⁻¹.

Table 2
Effects of N treatment approach (CAN vs. UAN), N addition rate (25 vs. 50 kg N ha⁻¹ yr⁻¹), study site (JGS vs. SMT), sampling time (year 2013 and 2014), and two way interactions between N treatment approach and N addition rate, study site, and sampling time on soil microbial biomasses and community structure parameters.

Variable	NTP		NDR		Site		Time		NTP \times NDR		NTP \times Site		NTP \times Time	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
TB	1.55	0.22	0.03	0.86	28.10	<0.001	58.96	<0.001	0.24	0.63	2.75	0.08	0.02	0.88
G^+	2.15	0.15	0.00	0.99	32.06	<0.001	65.17	<0.001	0.33	0.57	3.16	0.06	0.04	0.83
G^-	1.02	0.32	0.03	0.87	28.99	<0.001	44.66	<0.001	0.16	0.69	2.09	0.14	0.05	0.83
A	0.24	0.63	0.39	0.54	26.97	<0.001	44.34	<0.001	0.02	0.89	2.30	0.12	0.01	0.94
F	1.08	0.31	0.02	0.90	21.71	<0.001	50.50	<0.001	0.63	0.43	2.19	0.13	0.01	0.93
AM	0.38	0.54	0.74	0.40	17.14	<0.001	47.04	<0.001	0.16	0.69	1.86	0.17	0.35	0.56
F:B	0.00	0.96	0.05	0.83	0.00	0.96	0.52	0.48	0.24	0.63	0.00	1.00	1.22	0.28
$G^+ : G^-$	2.06	0.16	0.28	0.60	1.25	0.27	21.27	<0.001	0.93	0.34	1.66	0.21	0.54	0.47

NTP, N treatment approach; NDR, N addition rate; Site, study site; Time, sampling time; NTP \times NDR, interactions between N treatment approach and N addition rate; NTP \times Site, interactions between N treatment approach and study site; NTP \times Time, interactions between N treatment approach and sampling time; TB, total microbial biomass; F:B, ratio of fungal to bacterial biomass; G^+ , biomass of Gram-positive bacteria; G^- , biomass of Gram-negative bacteria; $G^+ : G^-$, ratio of Gram-positive to Gram-negative bacterial biomass; F, biomass of saprotrophic fungi; AM, biomass of arbuscular mycorrhizal fungi; A, biomass of actinomycetes.

(located in a deciduous forest in a transitional zone between a warm temperate and subtropical climate), however, both exchangeable base cations (especially Ca^{2+}) and Al^{3+} were abundant. According to soil acidification theory (van Breemen et al., 1983), the soil at the JGS forest site, which had a pH of 4.4, was in a buffering transitional state, i.e., it was changing from being buffered by base cations to being buffered by Al^{3+} . In addition, soil microbial biomass was significantly higher at the SMT site than at the JGS site, possibly because of the high primary productivity and favorable environmental conditions in the subtropical forest (Zhang et al., 2015) because both resource availability and site conditions can greatly affect microbial growth (Bardgett, 2005). For example, microbial growth could be stimulated by the relatively high soil organic matter (SOM) in subtropical forests because SOM is an essential source of energy, carbon, and nutrients for soil microorganisms (Coleman et al., 2004).

Many previous studies have indicated that ecosystem background affects the responses of soil properties to N deposition (Janssens et al., 2010; Lucas et al., 2011; Lu et al., 2014). In accordance with these studies, we found that both the soil exchangeable cations and microbial biomass showed contrasting responses to N deposition between the two study sites. For soil exchangeable cations, experimental N deposition significantly decreased soil base saturation and exchangeable Ca^{2+} and markedly increased the exchangeable Al^{3+} at the JGS forest site, but N addition did not significantly affect any soil exchangeable cations at the SMT forest site. The different response of soil exchangeable cations to N deposition at the two study sites may be attributed to the differences in the dominant cations in these soils. Because nutrient base cations (especially exchangeable Ca^{2+}) with high mobility are highly sensitive to N induced acidification (Tomlinson, 2003; Gundersen et al., 2006; Boxman et al., 2008; Gruba and Mulder, 2008; Lucas et al., 2011; Perakis et al., 2013) and are easily depleted (Gundersen and Rasmussen, 1990; Chadwick and Chorover, 2001; Tian and Niu, 2015), the base cations dominating JGS soil were sensitive to N deposition. As a result, experimental N inputs at the JGS site greatly accelerated the shift from soil buffering by base cations to buffering by exchangeable Al^{3+} . The high valence exchangeable Al^{3+} , however, has a high potential to buffer against acidification (Tian and Niu, 2015), and has a high affinity for soil colloidal particles (Tomlinson, 2003; Lu et al., 2014); as a consequence, the exchangeable Al^{3+} dominated SMT soil was less sensitive than the JGS soil to N deposition. For microbial biomass, N deposition significantly decreased the biomasses of soil Gram-negative bacteria, fungi, and AM fungi at the subtropical SMT forest, but did not affect microbial biomass at the JGS forest site. These different responses at the two sites may also be attributed to the different characteristics of the two soils. For example, inorganic N that is added to the soil can react with the high content of soil organic matter and metal cations (i.e., Al^{3+}) at the subtropical SMT forest site and form recalcitrant complexes that may be unavailable to soil microorganisms and may therefore cause a decline in microbial biomass (Soderstrom et al., 1983; Fog, 1988; Treseder, 2008; Janssens et al., 2010).

We expected that the effects of CAN vs. UAN treatments on soil properties would depend on site conditions. For example, we expected that the difference between CAN and UAN treatments at the temperate JGS site would be significant because of the dominance of highly mobile soil base cations (i.e., Ca), which are sensitive to N addition as discussed earlier and in previous studies (Lucas et al., 2011; Tian and Niu, 2015). Contrary to this expectation, however, we found that the effects of CAN on soil abiotic and biotic properties were similar to the effects of UAN at both sites. In addition, we did not find a significant interaction between study site and N addition approaches. These results suggest that our explanations for the consistent effects of CAN and UAN treatments on soil properties may apply to different kinds of forests.

Previous studies have indicated that the degree of soil response to N addition could depend on the quantity of N added (Tian and Niu, 2015). In our study, however, the effects did not significantly differ between the N rates of 25 and 50 kg N ha⁻¹ yr⁻¹). This is possibly because the

relatively low concentration of N addition and because of relatively short duration of N treatment in our study compared with other studies (Lu et al., 2014), thus did not reach the threshold of ecosystem to persist N perturbations. In line with this result, the CAN effects on soil exchangeable cations and microbial biomass were similar to UAN effects at both N addition rates. Therefore, these results may indicate that our conclusion about the lacking of differences between CAN and UAN effects on forest soils could persist across different levels of N addition.

5. Conclusion

Based on the field N manipulative experiments with two approaches of N treatment (CAN vs. UAN) at two contrasting forest ecosystems (temperate deciduous forest vs. evergreen subtropical forest), we demonstrated that although soil characteristics differed significantly and the responses of both soil exchangeable cations and microbial biomass were different in these two study sites, the effects of canopy addition of N on soil exchangeable cations and microbial biomass were not significantly different from the effects of understory addition of N. These results did not support our hypothesis and other previous prediction (Zhang et al., 2015) which expected that understory addition of N in most N manipulation experiments would overestimate or incorrectly capture the N deposition effects on soil properties in forest ecosystems due to canopy retention and foliage fertilization, at least for short time scale. Whether some soil variables would respond to different N addition approaches in different ways await longer time monitoring. However, relatively short time scale of our experiment is a potential limitation of our study. While considering the importance of canopy processes in the forest N deposition studies and the lack of experimental studies in this area, we think our short time-span experimental study and effort is essential in order to: 1) enhance the understanding of the role of canopy processes in the responses of soil properties to atmospheric N deposition in forests; and 2) stimulate more comprehensive researches in this area in the future.

Acknowledgments

This research was supported by the National Basic Research Program of China (2011CB403204), the National Natural Science Foundation (31470559), the National Natural Science Foundation of Major International (Regional) Joint Research Project (31210103920), and the NSFC-Guangdong Joint Project (U1131001).

References

- Adriaenssens, S., Staelens, J., Wuyts, K., Samson, R., Verheyen, K., Boeckx, P., 2012. Retention of dissolved inorganic nitrogen by foliage and twigs of four temperate tree species. *Ecosystems* 15, 1093–1107. <http://dx.doi.org/10.1007/s10021-012-9568-5>.
- Bardgett, R.D., 2005. *The Biology of Soil: A Community and Ecosystem Approach*. Oxford University Press, Oxford, UK.
- Bardgett, R.D., Wardle, D.A., 2010. *Aboveground-belowground Linkages: Biotic Interactions, Ecosystem Processes, and Global Change*. Oxford University Press, Oxford, UK.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Ciederby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J.W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L., De Vries, W., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol. Appl.* 20, 30–59. <http://dx.doi.org/10.1890/08-1140.1>.
- Bossio, D.A., Scow, K.M., 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. *Microb. Ecol.* 35, 265–278. <http://dx.doi.org/10.1007/s002489900082>.
- Bowman, W.D., Cleveland, C.C., Halada, L., Hřeško, J., Baron, J.S., 2008. Negative impact of nitrogen deposition on soil buffering capacity. *Nat. Geosci.* 1, 767–770. <http://dx.doi.org/10.1038/ngeo339>.
- Boxman, A.W., Peters, R.C., Roelofs, J.G., 2008. Long term changes in atmospheric N and S through fall deposition and effects on soil solution chemistry in a Scots pine forest in the Netherlands. *Environ. Pollut.* 156, 1252–1259. <http://dx.doi.org/10.1016/j.envpol.2008.03.017>.
- Brady, N.C., Weil, R.R., 2010. *Elements of the Nature and Properties of Soils*. third ed. Prentice Hall Press, Upper Saddle River, NJ, USA.
- Chadwick, O.A., Chorover, J., 2001. The chemistry of pedogenic thresholds. *Geoderma* 100, 321–353. [http://dx.doi.org/10.1016/S0016-7061\(01\)00027-1](http://dx.doi.org/10.1016/S0016-7061(01)00027-1).

- Chen, D., Lan, Z., Bai, X., Grace, J.B., Bai, Y., 2013. 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. Ecol.* 101, 1322–1334. <http://dx.doi.org/10.1111/1365-2745.12119>.
- Coleman, D.C., Crossley, D.A., Hendrix, P.F., 2004. *Fundamentals of soil ecology*. Academic press, London, UK.
- Cusack, D.F., Silver, W.L., Torn, M.S., Burton, S.D., Firestone, M.K., 2011. Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. *Ecology* 92, 621–632. <http://dx.doi.org/10.1890/10-0459.1>.
- Dail, D.B., Hollinger, D.Y., Davidson, E.A., Fernandez, I., Sievering, H.C., Scott, N.A., Gaige, E., 2009. Distribution of nitrogen-15 tracers applied to the canopy of a mature spruce-hemlock stand, Howland, Maine, USA. *Oecologia* 160, 589–599. <http://dx.doi.org/10.1007/s00442-009-1325-x>.
- Dentener, F., Drevet, J., Lamarque, J.F., Bey, I., Eickhout, B., Fiore, A.M., Hauglustaine, D., Horowitz, L.W., Krol, M., Kulshreshtha, U.C., Lawrence, M., Galy-Lacaux, C., Rast, S., Shindell, D., Stevenson, D., Van Noije, T., Atherton, C., Bell, N., Bergman, D., Butler, T., Cofala, J., Collins, B., Doherty, R., Ellingsen, K., Galloway, J., Gauss, M., Montanaro, V., Müller, J.F., Pitari, G., Rodriguez, J., Sanderson, M., Solomon, F., Strahan, S., Schultz, M., Sudo, K., Szopa, S., Wild, O., 2006. Nitrogen and sulfur deposition on regional and global scales: a multimodel evaluation. *Global Biogeochem. Cy.* 20, GB4003. <http://dx.doi.org/10.1029/2005GB002672>.
- Eichert, T., Fernández, V., 2012. Uptake and release of elements by leaves and other aerial plant parts. In: Marschner, P. (Ed.), *Marschner's Mineral Nutrition of Higher Plants*. Academic Press, London, UK, pp. 71–84.
- Fang, Y., Gundersen, P., Vogt, R.D., Koba, K., Chen, F., Chen, X., Yoh, M., 2011. Atmospheric deposition and leaching of nitrogen in Chinese forest ecosystems. *J. For. Res.* 16, 341–350. <http://dx.doi.org/10.1007/s10310-011-0267-4>.
- Fanin, N., Fomin, N., Buatois, B., Hättenschwiler, S., 2013. An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant litter-microbe system. *Ecol. Lett.* 16, 764–772. <http://dx.doi.org/10.1111/ele.12108>.
- Fenn, M.E., Ross, C.S., Schilling, S.L., Baccus, W.D., Larrabee, M.A., Lofgren, R.A., 2013. Atmospheric deposition of nitrogen and sulfur and preferential canopy consumption of nitrate in forests of the Pacific Northwest, USA. *Forest Ecol. Manag.* 302, 240–253. <http://dx.doi.org/10.1016/j.foreco.2013.03.042>.
- Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. *Biol. Rev.* 63, 433–462. <http://dx.doi.org/10.1111/j.1469-185X.1988.tb00725.x>.
- Frey, S.D., Knorr, M., Parrent, J.L., Simpson, R.T., 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *For. Ecol. Manag.* 196, 159–171. <http://dx.doi.org/10.1016/j.foreco.2004.03.018>.
- Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol. Fertil. Soils* 22, 59–65. <http://dx.doi.org/10.1007/BF00384433>.
- Gaige, E., Dail, D.B., Hollinger, D.Y., Davidson, E.A., Fernandez, I.J., Sievering, H., White, A., Halteman, W., 2007. Changes in canopy processes following whole-forest canopy nitrogen fertilization of a mature spruce-hemlock forest. *Ecosystems* 10, 1133–1147. <http://dx.doi.org/10.1007/s10021-007-9081-4>.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320, 889–892. <http://dx.doi.org/10.1126/science.1136674>.
- Gruba, P., Mulder, J., 2008. Relationship between aluminum in soils and soil water in mineral horizons of a range of acid forest soils. *Soil Sci. Soc. Am. J.* 72, 1150–1157. <http://dx.doi.org/10.2136/sssaj2007.0041>.
- Gundersen, P., Rasmussen, L., 1990. Nitrification in forest soils: effects from nitrogen deposition on soil acidification and aluminum release. *Rev. Environ. Contam. T.* 13, 1–45.
- Gundersen, P., Schmidt, I.K., Raulund-Rasmussen, K., 2006. Leaching of nitrate from temperate forests—effects of air pollution and forest management. *Environ. Rev.* 14, 1–57. <http://dx.doi.org/10.1139/A05-015>.
- Hendershot, W.H., Lalonde, H., Duquette, M., 2007. Ion exchange and exchangeable cations. In: Carter, M.R., Gregorich, E.G. (Eds.), *Soil Sampling and Methods of Analysis, second ed.* CRC Press, Boca Raton, FL, USA, pp. 197–206.
- Högberg, P., Fan, H., Quist, M., Binkley, D., Tamm, C.O., 2006. Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. *Glob. Chang. Biol.* 12, 489–499. <http://dx.doi.org/10.1111/j.1365-2486.2005.01102.x>.
- Houle, D., Marty, C., Duchesne, L., 2015. Response of canopy nitrogen uptake to a rapid decrease in bulk nitrate deposition in two eastern Canadian boreal forests. *Oecologia* 177, 29–37. <http://dx.doi.org/10.1007/s00442-014-3118-0>.
- Huang, L., Zhu, W., Ren, H., Chen, H., Wang, J., 2012. Impact of atmospheric nitrogen deposition on soil properties and herb-layer diversity in remnant forests along an urban–rural gradient in Guangzhou, southern China. *Plant Ecol.* 213, 1187–1202. <http://dx.doi.org/10.1007/s11258-012-0080-y>.
- Janssens, I.A., Dieleman, W., Luysaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.-D., Tang, J., Law, B.E., 2010. Reduction of forest soil respiration in response to nitrogen deposition. *Nat. Geosci.* 3, 315–322. <http://dx.doi.org/10.1038/ngeo844>.
- Jia, Y., Yu, G., He, N., Zhan, X., Fang, H., Sheng, W., Zuo, Y., Zhang, D., Wang, Q., 2014. Spatial and decadal variations in inorganic nitrogen wet deposition in China induced by human activity. *Sci. Rep.* 4, 3763. <http://dx.doi.org/10.1038/srep03763>.
- Kuperman, R.G., Edwards, C.A., 1997. Effects of acidic deposition on soil invertebrates and microorganisms. *Rev. Environ. Contam. T.* 148, 35–137.
- Lieb, A.M., Darrouzet-Nardi, A., Bowman, W.D., 2011. Nitrogen deposition decreases acid buffering capacity of alpine soils in the southern Rocky Mountains. *Geoderma* 164, 220–224. <http://dx.doi.org/10.1016/j.geoderma.2011.06.013>.
- Liu, X., Zhang, Y., Han, W., Tang, A., Shen, J., Cui, Z., Vitousek, P., Erisman, J.W., Goulding, K., Christie, P., Fangmeier, A., Zhang, F., 2013. Enhanced nitrogen deposition over China. *Nature* 494, 459–462. <http://dx.doi.org/10.1038/nature11917>.
- Lu, M., Yang, Y., Luo, Y., Fang, C., Zhou, X., Chen, J., Yang, X., Li, B., 2011. Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. *New Phytol.* 189, 1040–1050. <http://dx.doi.org/10.1111/j.1469-8137.2010.03563.x>.
- Lu, X., Mao, Q., Gilliam, F.S., Luo, Y., Mo, J., 2014. Nitrogen deposition contributes to soil acidification in tropical ecosystems. *Global Change Biol.* 20, 3790–3801. <http://dx.doi.org/10.1111/gcb.12665>.
- Lucas, R.W., Klaminder, J., Futter, M.N., Bishop, K.H., Egnell, G., Laudon, H., Högberg, P., 2011. A meta-analysis of the effects of nitrogen additions on base cations: implications for plants, soils, and streams. *For. Ecol. Manag.* 262, 95–104. <http://dx.doi.org/10.1016/j.foreco.2011.03.018>.
- Nordby, H.E., Nemeček, S., Nagy, S., 1981. Fatty acids and sterols associated with citrus root mycorrhizae. *J. Agric. Food Chem.* 29, 396–401. <http://dx.doi.org/10.1021/jf00104a043>.
- Olsson, P.A., 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiol. Ecol.* 29, 303–310. <http://dx.doi.org/10.1111/j.1574-6941.1999.tb00621.x>.
- Pardo, L.H., Fenn, M.E., Goodale, C.L., Geiser, L.H., Driscoll, C.T., Allen, E.B., Baron, J.S., Bobbink, R., Bowman, W.D., Clark, C.M., Emmett, B., Gilliam, F.S., Greaver, T.L., Hall, S.J., Lilleskov, E.A., Liu, L., Lynch, J.A., Nadelhoffer, K.J., Perakis, S.S., Robin-Abbott, M.J., Stoddard, J.L., Weathers, K.C., Dennis, R.L., 2011. Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. *Ecol. Appl.* 21, 3049–3082. <http://dx.doi.org/10.1890/10-2341.1>.
- Penuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., Boucher, O., Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M., Janssens, I.A., 2013. Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. *Nat. Commun.* 4, 2934. <http://dx.doi.org/10.1038/ncomms3934>.
- Perakis, S.S., Sinkhorn, E.R., Catricala, C.E., Bullen, T.D., Fitzpatrick, J.A., Hynick, J.D., Cromack Jr., K., 2013. Forest calcium depletion and biotic retention along a soil nitrogen gradient. *Ecol. Appl.* 23, 1947–1961. <http://dx.doi.org/10.1890/12-2204.1>.
- Quesada, C.A., Lloyd, J., Schwarz, M., Patiño, S., Baker, T.R., Czimczik, C., Fyllas, N.M., Martinelli, L., Nardoto, G.B., Schmerler, J., Santos, A.J.B., Hodnett, M.G., Herrera, R., Luizão, F.J., Arneeth, A., Lloyd, G., Dezaeo, N., Hilke, I., Kuhlmann, I., Raessler, M., Brand, W.A., Geilmann, H., Moraes Filho, J.O., Carvalho, F.P., Araujo Filho, R.N., Chaves, J.E., Cruz Junior, O.F., Pimentel, T.P., Paiva, R., 2010. Variations in chemical and physical properties of Amazon forest soils in relation to their genesis. *Biogeosciences* 7, 1515–1541. <http://dx.doi.org/10.5194/bg-7-1515-2010>.
- Schlesinger, W.H., 2009. On the fate of anthropogenic nitrogen. *P. Natl. Acad. Sci. USA* 106, 203–208. <http://dx.doi.org/10.1073/pnas.0810193105>.
- Soderstrom, B., Baath, E., Lundgren, B., 1983. Decrease in soil microbial activity and biomass owing to nitrogen amendments. *Can. J. Microbiol.* 29, 1500–1506.
- Sparks, J.P., 2009. Ecological ramifications of the direct foliar uptake of nitrogen. *Oecologia* 159, 1–13. <http://dx.doi.org/10.1007/s00442-008-1188-6>.
- Stevens, C.J., Thompson, K., Grime, J.P., Long, C.J., Gowing, D.J.G., 2010. Contribution of acidification and eutrophication to declines in species richness of calcifuge grasslands along a gradient of atmospheric nitrogen deposition. *Funct. Ecol.* 24, 478–484. <http://dx.doi.org/10.1111/j.1365-2435.2009.01663.x>.
- Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), 2013. *IPCC: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK (1535 pp).
- Tian, D., Niu, S., 2015. A global analysis of soil acidification caused by nitrogen addition. *Environ. Res. Lett.* 10, 024019. <http://dx.doi.org/10.1088/1748-9326/10/2/024019>.
- Tomlinson, G.H., 2003. Acidic deposition, nutrient leaching and forest growth. *Biogeochemistry* 65, 51–81. <http://dx.doi.org/10.1023/A:1026069927380>.
- Treseder, K.K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecol. Lett.* 11, 1111–1120. <http://dx.doi.org/10.1111/j.1461-0248.2008.01230.x>.
- van Breemen, N., Mulder, J., Driscoll, C.T., 1983. Acidification and alkalization of soils. *Plant Soil* 75, 283–308. <http://dx.doi.org/10.1111/j.1461-0248.2008.01230.x>.
- van Diepen, L.T., Lilleskov, E.A., Pregitzer, K.S., Miller, R.M., 2010. Simulated nitrogen deposition causes a decline of intra- and extraradical abundance of arbuscular mycorrhizal fungi and changes in microbial community structure in northern hardwood forests. *Ecosystems* 13, 683–695. <http://dx.doi.org/10.1007/s10021-010-9347-0>.
- Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecol. Appl.* 7, 737–750. <http://dx.doi.org/10.2307/2269431>.
- Vogt, R.D., Seip, H.M., Larssen, T., Zhao, D., Xiang, R., Xiao, J., Luo, J., Zhao, Y., 2006. Potential acidifying capacity of deposition experiences from regions with high NH₄⁺ and dry deposition in China. *Sci. Total Environ.* 367, 394–404.
- von Uexküll, H.R., Mutert, E., 1995. Global extent, development and economic impact of acid soils. *Plant Soil* 171, 1–15.
- Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. *Soil Biol. Biochem.* 36, 1443–1451. <http://dx.doi.org/10.1016/j.soilbio.2004.04.023>.
- Wortman, E., Tomaszewski, T., Waldner, P., Schleppei, P., Thimonier, A., Eugster, W., Buchmann, N., Sievering, H., 2012. Atmospheric nitrogen deposition and canopy retention influences on photosynthetic performance at two high nitrogen deposition Swiss forests. *Tellus B* 64, 17216.
- Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biol. Fertil. Soils* 29, 111–129. <http://dx.doi.org/10.1007/s003740050533>.

- Zhang, W., Shen, W., Zhu, S., Wan, S., Luo, Y., Yan, J., Wang, K., Liu, L., Dai, H., Li, P., Dai, K., Zhang, W.X., Liu, Z., Wang, F., Kuang, Y., Li, Z., Lin, Y., Rao, X., Li, J., Zou, B., Cai, X., Mo, J., Zhao, P., Ye, Q., Huang, J., Fu, S., 2015. CAN canopy addition of nitrogen better illustrate the effect of atmospheric nitrogen deposition on forest ecosystem? *Sci. Rep.* 5, 11245. <http://dx.doi.org/10.1038/srep11245>.
- Zhu, J., He, N., Wang, Q., Yuan, G., Wen, D., Yu, G., Jia, Y., 2015. The composition, spatial patterns, and influencing factors of atmospheric wet nitrogen deposition in Chinese terrestrial ecosystems. *Sci. Total Environ.* 511, 777–785. <http://dx.doi.org/10.1016/j.scitotenv.2014.12.038>.
- Zogg, G.P., Zak, D.R., Ringelberg, D.B., MacDonald, N.W., Pregitzer, K.S., White, D.C., 1997. Compositional and functional shifts in microbial communities due to soil warming. *Soil Sci. Soc. Am. J.* 61, 475–481. <http://dx.doi.org/10.2136/sssaj1997.03615995006100020015x>.