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Inhibition effects of N deposition on soil organic carbon decomposition was mediated by N types and soil nematode in a temperate forest

Qingkui Wang[a,b,*], Peng Tian[a,c], Shengen Liu[a,c], Tao Sun[a]

[a] Huitong Experimental Station of Forest Ecology, CAS Key Laboratory of Forest Ecology and Management, Institute of Applied Ecology, Shenyang 110016, China
[b] National Research Station of Huitong Forest Ecosystem, Huitong 418307, China
[c] University of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Increasing nitrogen (N) deposition may alter soil organic carbon (SOC) decomposition, thereby strongly affecting SOC storage in terrestrial ecosystems. Its specific influence may depend on the different types of N deposition and soil nematodes. However, little is known about how N deposition and soil nematodes affect the SOC cycle process. To address this issue, we evaluated the effects of different types of N deposition on SOC decomposition under the conditions of applying nematocide or not in a temperate forest. Soils collected from the simulated N deposition forest for 5 years were incubated in the presence and absence of soil nematocide at 15 °C for 150 days. N deposition suppressed soil C cycle processes, such as SOC decomposition and soil enzyme activities, and caused the accumulation of labile SOC, which depended on N types. A mixture of inorganic and organic N (MN) deposition had the highest suppression of SOC decomposition at 31.5%, followed by organic N (ON) deposition (24.4%) and inorganic N (IN) deposition (19.8%), thereby suggesting that inhibition effects of N deposition on SOC decomposition based on a single IN or ON source are underestimated. Nematocide application stimulated SOC decomposition, with the highest in MN (19.5%), followed by IN (13.5%), ON (11.2%), and control treatment (4.6%). The stimulation effect of SOC decomposition by soil nematode exclusion also depended on N types. N deposition and soil nematode exclusion had no interactive effect on SOC decomposition. These results imply that atmospheric N deposition favors the increase of C stocks in soil by reducing the SOC loss, and that N types should be considered during assessment of N deposition effects on soil C cycle processes.

1. Introduction

Globally, anthropogenically reactive nitrogen (N) deposition in the terrestrial ecosystem have increased by more than threefold in the past century (Galloway et al., 2008) and will continue to increase, particularly in China (Liu et al., 2013). Increased reactive N input has caused widespread effects on terrestrial ecosystems, such as soil biodiversity loss and alteration of soil organic carbon (SOC) decomposition (e.g., Mo et al., 2008; Tu et al., 2013; Liu et al., 2016). The SOC amount in terrestrial ecosystems is more than thrice the amount of atmospheric C (Schlesinger and Andrews, 2000). Accordingly, a small change in SOC decomposition rate could have a large effect on SOC sequestration and dynamics and atmospheric CO_2 concentration (Davidson and Janssens, 2006). In terrestrial ecosystems, therefore, how N deposition affects the dynamics of SOC and their functions involving the C balance and global C cycle needs to be elucidated. Although numerous efforts have been made to investigate the effects of N deposition on SOC decomposition, consistent conclusions are lacking (e.g., Janssens et al., 2010; Tu et al., 2013; Wang et al., 2017). Inconsistencies increase the uncertainty in predicting the response of soil C cycle and dynamics to global environment change.

An important source of the inconsistencies in the effects of N deposition on SOC decomposition may be the differences in the type of N used in variable experiments. For instance, Du et al. (2014) found that addition of inorganic N (IN) inhibited soil respiration in a temperate forest, whereas the opposite result was obtained with organic N (ON) addition. Furthermore, most previous studies using IN or ON as lone sources of N deposition may not accurately reflect the effect of atmospheric N deposition on SOC decomposition in terrestrial ecosystems because atmospheric N deposition contains both IN and ON components (Cornell, 2011). Globally, ON accounts for about 30% of the total N deposition (Jickells et al., 2013), and this proportion increases with organic manure application. However, a significant knowledge gap remains in our understanding of how different types of N deposition

* Corresponding author at: Huitong Experimental Station of Forest Ecology, CAS Key Laboratory of Forest Ecology and Management, Institute of Applied Ecology, Shenyang, 110016, China.
E-mail address: wqkui@163.com (Q. Wang).

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influence SOC sequestration. Therefore, identification of the effects of different types of N deposition on SOC decomposition is necessary to accurately assess the actual effect of atmospheric N deposition on SOC cycle processes.

Reactive N inputs in forest soils potentially affect SOC decomposition by altering soil microorganisms, i.e., soil microbial community composition, and enzyme activity (Mo et al., 2008; Du et al., 2014; Liu et al., 2016). Recently, Du et al. (2014) demonstrated that IN addition in a temperate forest repressed cellulase and polyphenol oxidase activities, but ON addition promoted enzyme function. Therefore, we assumed that different effects of IN and ON on soil microbial community composition and activity might result in different responses of SOC decomposition to different types of N deposition, which needed further elaboration. Furthermore, a shift in soil nematode community after N deposition may significantly affect SOC decomposition because soil nematodes can contribute up to 40% of SOC decomposition (De Ruiter et al., 1993; Sinsabaugh et al., 2002; Zhou et al., 2013). Although N deposition generally decreases the soil total nematode abundance and diversity, this response varies among trophic groups (Murray et al., 2006; Wei et al., 2012; Sun et al., 2013). However, information on whether the responses of soil nematode community to IN and ON is different remains highly uncertain. It is important to resolve the SOC decomposition by soil microorganisms.

The present study examines the potential effects of N types, soil nematodes, and their interaction on SOC decomposition in a temperate forest ecosystem that has been subjected to long-term simulated N deposition. Therefore, soils from long-term simulated N deposition experiment with different N types were used to investigate soil respiration, microbial community composition and activity, and labile and recalcitrant pools of SOC. This study aims to address how different types of N deposition (IN and ON) affect SOC decomposition and to determine whether this effect depends on soil nematode community. This work specifically differs from previous studies because the we consider the different types of exogenous N and its combination with the soil nematode community. In this study, we proposed the following hypotheses: (1) the ON deposition had a higher suppression effect on SOC decomposition than IN deposition because the C content in ON could decrease microbial mining of soil organic C; and (2) the soil nematode community would modify the effects of N deposition on SOC decomposition.

2. Material and methods

2.1. Site description and experiment design

The present study was conducted in a typical temperate forest located at Laoshan Forest Research Station in Heilongjiang Province, China (127°34′E, 45°20′N). The site has a continental temperate monsoon climate, characterized by a strong monsoon and windy spring, a warm and humid summer, and a dry and cold winter. Annual precipitation ranges from 600 mm to 800 mm and mostly falls in July and August. The mean annual air temperature is 2.7 °C, and the average air temperatures in January and July are −19.6 °C and 20.9 °C, respectively. The parent material at the site is granite bedrock, and the soil is a well-drained Hap-Boric Luvisol (dark brown forest soil in the Chinese Soil Taxonomic System) with high organic matter content.

A forest where *Larix gmelinii* is the dominant species was chosen as the sample site. Twelve 10 × 20 m^2 plots with a buffer zone of 15 m between any two plots were randomly established in April 2010. Afterward, each plot was treated with various N solutions. This experiment included four N deposition treatments with triplicates. In this work, NH_4NO_3 was chosen as the IN source; urea and glycine were selected as the main organic components of atmospheric N deposition and mixed equally as ON sources (Cornell, 2011). A mixture of IN and ON (MN) was used at a ratio of 7:3, which was almost equal to the average ratio of atmospheric N deposit. The control treatment (CT) involved only the addition of water. During the field experiment, each plot was sprayed with N solutions at a rate of 10 g N m^{-2} yr^{-1} from 2010. N was applied monthly in six equal applications of 1.667 g N from May to October. In each application, the fertilizer was dissolved in 50 L of water, and the solution was sprayed to each plot using a portable sprayer at the early month. In the CT, 50 L of fertilizer-free water was simultaneously sprayed.

2.2. Soil sampling and analysis

In this experiment, soils from the surface mineral layers with 10 cm depth in each plot were sampled in the autumn of 2015. Soils received totally 56.7 g N m^{-2} before sampling. After removing the litter layer, eight soil cores were randomly collected in each plot using a metal corer and mixed as a composite sample. Fresh soil samples were stored in sealed bags, transported immediately to the laboratory, and passed through a 2-mm mesh to eliminate plant residues such as decomposed leaves and root. An aliquot of each soil sample was used to determine SOC decomposition, and the rest was used to determine chemical properties, microbial community composition, and enzymatic activities.

SOC and total N concentrations in soil samples were measured using a C/N analyzer (Elementar, Germany). Soil total P was directly measured through colorimetry, whereas soil mineral N (sum of ammonium and nitrate N) was extracted using 2 M KCl solution and determined by colorimetry. Soil-available P was analyzed through a colorimetric molybdate blue method after extraction with 1 M NH_4F solution. Soil pH was determined using a pH meter at 1:2.5 (weight:volume) ratio of soil and deionized water. Table 1 lists the basic chemical properties of soils under long-term N deposition. Labile organic C (LOC) contents were determined through $KMnO_4$ oxidation, as described by Blair et al. (1995). The recalcitrant organic C (ROC) contents were calculated as the difference between total SOC and LOC. Enzymatic activities involved in C cycles (β-glucosidase, cellulase, and lignin peroxidase) were assayed using a UV spectrophotometer. Table S1 lists the enzyme assays and relevant international unit definitions.

Soil microbial community composition was assessed using phospholipid fatty acid (PLFA) analysis. Total PLFA biomarkers in a soil sample represent all living cells because phospholipids are essential membrane components of all living microbes (Bossio and Scow, 1998). In addition, different microbial groups produce specific or signature types of PLFA biomarkers, which quantify important microbial groups and provide direct information about the active microbial community structure (Bossio and Scow, 1998). PLFAs were extracted from the soil according to the protocol (White and Ringelberg, 1998). Qualitative

Table 1
Soil chemical properties under different types of N deposition in a *Larix gmelinii* forest.

Treatment	SOC (g kg^{-1})	Total N (g kg^{-1})	NH_4-N (mg kg^{-1})	NO_3-N (mg kg^{-1})	Available P (mg kg^{-1})	pH
CT	61.2 ± 8.1a	5.02 ± 1.10a	5.25 ± 1.09c	49.2 ± 6.6c	28.5 ± 2.5a	4.47 ± 0.18a
IN	69.0 ± 7.4a	5.69 ± 1.21a	22.52 ± 3.91a	79.4 ± 5.7b	26.3 ± 4.0a	4.54 ± 0.13a
ON	67.3 ± 7.6a	5.56 ± 1.02a	7.03 ± 1.50bc	82.7 ± 12.7ab	25.3 ± 3.0a	4.61 ± 0.21a
MN	61.6 ± 9.1a	5.48 ± 0.76a	10.94 ± 2.20b	103.2 ± 13.7a	23.5 ± 4.1a	4.48 ± 0.06a

CT, ON, IN and MN represent control, organic N, inorganic N and the mixture of organic and inorganic N deposition treatments, respectively.

and quantitative fatty acids were analyzed with an Agilent 6890 GC and the MIDI Sherlock Microbial Identification System. Fatty acids were quantified by calibration against standard solutions of FAME 19:0. Total bacterial content was derived as the sum of i15:0, a15:0, 15:0, i16:0, 16:1ω7c, 16:1ω9c, 16:0, a17:0, i17:0, cy17:0, 17:0, 18:0, cy19:0, and 20:0 PLFAs (Hill et al., 2000). The i15:0, a15:0, i16:0, i17:0, and a17:0 PLFAs served as markers for Gram-positive bacteria, whereas 16:1ω7c, 16:1ω9c, cy17:0, and cy19:0 PLFAs were markers for Gram-negative bacteria. The 18:1ω9(c,t), 18:1ω7c, and 18:2ω9,12c PLFAs functioned as markers for fungi.

SOC decomposition was quantified by measuring CO_2 release from soils over a 150-day incubation period. To investigate the effect of soil nematode on SOC decomposition, nematocide was added to one part of each fresh soil to exclude the soil nematodes at 15 days before incubation. The inhibition of soil nematode community by the nematocide was well and reached an average of 90% (Table S2). The nematocide affected the soil microbial community (Table S3). Microcosms were constructed by placing 100–120 g (equal to 80 g dry soil) fresh soil from each plot into 500 mL amber jars with airtight lids. A total of 24 microcosms (4 treatments × 2 soil nematodes × 3 replicates) were constructed. Microcosms were incubated at 15 °C with a precision of ± 0.2 °C from the set point. In this study, soil moisture was adjusted to 60% of the water-holding capacity and maintained with the addition of distilled water at regular intervals (7 days). CO_2 release was measured using a LI-COR 820 infrared gas analyzer.

2.3. Calculation and statistical analysis

Soil nematode effect on SOC decomposition was calculated using the equation: soil nematode effect (%) = (1 − NA/NP) × 100%, where NA and NP were the amounts of CO_2 respired in the absence and presence of soil nematode, respectively.

One-way ANOVA was performed to analyze the effects of N deposition on LOC, ROC, soil nematode, microbial community, and enzyme activities. Two-way ANOVA was used to analyze the effects of N type, soil nematode, and their interaction on SOC decomposition and N effect. One-way ANOVA was also employed to examine the effects of N deposition on soil nematodes. Type-III sums of squares were used to assess the significance of main effects and interactions between N types and soil nematode. Significant differences among treatment methods were investigated by using the least significant difference multiple-comparison post hoc test (LSD). All statistical analyses were performed using the SPSS software (version 18.0; SPSS Inc., Chicago, Illinois, USA), and significant differences were accepted at $P < 0.05$.

3. Results

IN and ON deposition increased the LOC ($P < 0.05$; Fig. 1a) but did not affect ROC contents ($P > 0.05$; Fig. 1b). A significant increase in LOC/ROC ratio occurred in ON deposition treatments ($P < 0.05$; Fig. 1c). IN and MN deposition significantly reduced the total soil nematode abundance ($P < 0.05$; Table 2). Among the four trophic groups, MN deposition decreased the abundance of bacterivores and fungivores ($P < 0.05$), and IN deposition reduced the abundance of plant-parasites and omnivorous predators ($P < 0.05$), while increased fungivore abundance ($P < 0.05$). MN deposition mainly altered the soil microbial community (Table 3) by significantly decreasing the concentrations of fungal, arbuscular mycorrhizal fungal, and Gram-negative bacterial PLFAs ($P < 0.05$). However, the Gram-positive:-Gram-negative bacteria ratio ($P < 0.05$) was raised. IN deposition increased the bacteria:fungi ratio. Soils under IN and MN had lower β-glucosidase activity ($P < 0.05$; Fig. 2). All types of N deposition significantly decreased the cellulase and lignin peroxidase activities by 67.1%–83.4% of the CT measurement ($P < 0.05$).

N deposition significantly suppressed SOC decomposition in terms of soil CO_2 release regardless of soil nematodes (Fig. 3A), although CO_2 release had similar temporal patterns among different treatments. The amount of cumulative CO_2 release was 19.8%, 24.4%, and 31.5% lower in IN, ON, and MN treatments than in CT after incubating for 150 days, respectively, suggesting that MN had the highest suppression effect on SOC decomposition than IN or ON alone. Soil nematode exclusion slightly increased the cumulative CO_2 release from native SOC (Fig. 3B), although the difference was not statistically significant ($P = 0.052$). The effect of soil nematode exclusion on SOC decomposition had an average of 12.2% across all treatments (Fig. 4). MN deposition showed the highest soil nematode effect (19.52%), followed by IN (13.54%) and ON (11.25%). CT (4.56%) showed the lowest soil nematode effect.

The strength of the inhibition effect by N deposition on SOC decomposition was closely related to N types ($F_{2,12} = 26.5$, $P < 0.001$; Table 4) and soil nematode ($F_{1,12} = 26.4$, $P < 0.001$), but their interaction was insignificant ($P = 0.384$). When soil nematodes were present, the inhibition strength was 23.1%, 26.7%, and 36.1% in IN, ON, and MN soils, respectively (Fig. 5). The inhibition strength was 16.5%, 22.2%, and 26.9% in IN, ON, and MN soils in the absence of soil nematodes, respectively. Soil nematode exclusion decreased the suppression effect of N deposition on SOC decomposition, and the decrease was higher in IN (28.8%) and MN deposition (25.4%), and lower in ON deposition (16.9%), thereby suggesting that suppression responses of N deposition on SOC decomposition to soil nematode exclusion depended on N types. However, within a given type of N deposition, the inhibition effect of N deposition on SOC decomposition was slightly but not statistically significantly higher under soil nematode presence (28.6%) compared with that under soil nematode absence (21.9%).

4. Discussions

Our study illustrates the effects of different types of N deposition, soil nematode, and their interactions on SOC decomposition in a temperate forest ecosystem. As expected, N deposition significantly

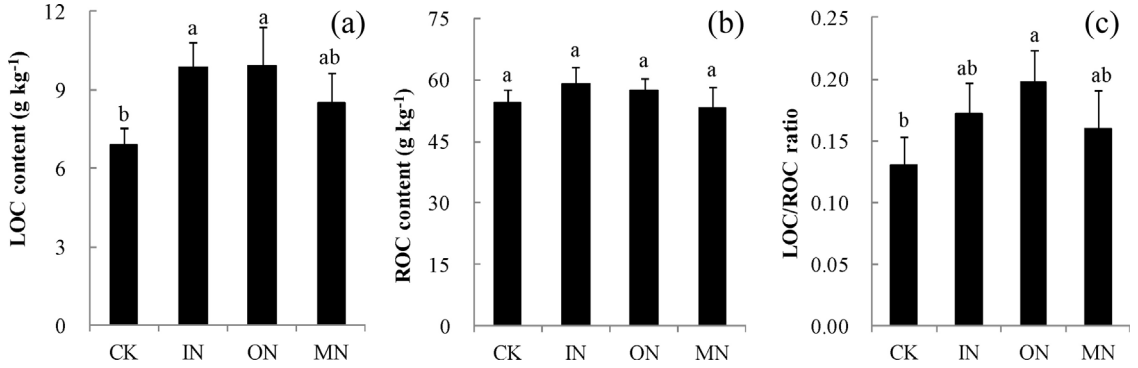

Fig. 1. Responses of labile organic C (LOC; a), recalcitrant organic C (ROC; b), and ratio of LOC/ROC (c) to various N fertilization treatments. Error bars indicate standard deviation.

Table 2
Effect of different N deposition types on the abundance of total nematode and trophic groups (individuals ·100 g^{-1} dry soil) in a temperate forest.

	Total Number	Bacterivores	Fungivores	Plant-parasites	Omnivores-predators
CT	1581.2 ± 122a	283.5 ± 34.1a	50.4 ± 8.9b	1127.6 ± 94.4a	119.7 ± 25.2ab
IN	1234.7 ± 108bc	258.3 ± 28.2a	107.1 ± 12.6a	812.6 ± 60.3b	56.7 ± 6.3c
ON	1461.5 ± 132ab	245.7 ± 31.5a	69.3 ± 10.6b	995.3 ± 144.9ab	151.2 ± 18.9a
MN	1157.8 ± 115c	81.9 ± 14.9b	17.6 ± 3.4c	963.8 ± 126.0ab	94.5 ± 25.2b

CT, ON, IN and MN represent control, organic N, inorganic N and the mixture of organic and inorganic N treatments, respectively.

Table 3
Effects of N deposition on soil microbial community (nmol g^{-1} soil) measured by PLFAs.

	Bacteria	Fungi	AMF	GP	GN	Bacteria:Fungi	GP:GN
CT	207.9 ± 41.7a	16.9 ± 2.2a	6.72 ± 1.58a	78.7 ± 8.5ab	99.1 ± 8.3a	12.3 ± 0.4b	0.79 ± 0.07b
IN	220.8 ± 53.1a	16.2 ± 2.5ab	6.56 ± 2.02ab	90.4 ± 7.8a	102.9 ± 11.4a	13.7 ± 0.2a	0.87 ± 0.11ab
ON	219.8 ± 68.5a	16.8 ± 2.3a	6.74 ± 2.30ab	91.8 ± 7.6a	99.8 ± 6.9a	13.0 ± 0.7ab	0.90 ± 0.09ab
MN	157.2 ± 19.7a	12.1 ± 1.3b	4.28 ± 0.50b	69.2 ± 7.7b	68.8 ± 6.6b	12.95 ± 0.2b	1.01 ± 0.12a

CT, ON, IN and MN represent control, organic N, inorganic N and the mixture of organic and inorganic N treatments, respectively. GP and GN denote gram-positive and gram-negative bacteria, respectively, and AFM denotes arbuscular mycorrhizal fungi.

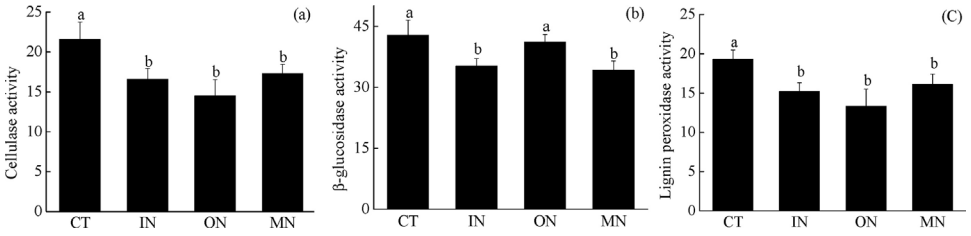

Fig. 2. Effects of N deposition on soil enzyme activities in this site. CT, ON, IN and MN represent control, organic N, inorganic N and the mixture of organic and inorganic N treatments, respectively.

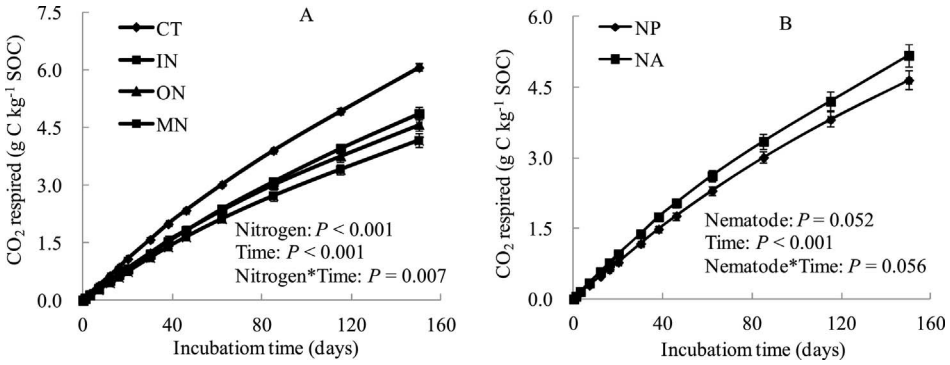

Fig. 3. Effects of nitrogen deposition (A, $n = 6$) and soil nematode (A, $n = 12$) on the cumulative CO_2 respired from soil organic carbon. IN, ON and MN represent inorganic N, organic N and the mixture of organic and inorganic N deposition treatments, respectively. NP and NA denote that soil nematodes are present and absent, respectively.

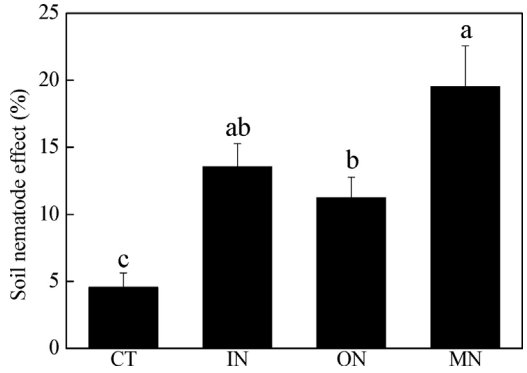

Fig. 4. Response of effects of N types on soil organic carbon decomposition to soil nematode calculated as the ratio of nematode absence to presence.

inhibited SOC decomposition, which generally agreed with previous observations that N addition decreased the heterotrophic respiration of soil organisms that decompose SOC in temperate forests (Janssens et al., 2010; Ramirez et al., 2010; Du et al., 2014), and other ecosystems (e.g., Tu et al., 2013; Wang et al., 2017). This indicates that the negative effects of N deposition on SOC decomposition are widespread in terrestrial ecosystems although this observation may be not universal. The suppression of SOC decomposition by N deposition partly arises from the significant increase of soil N availability induced by N deposition (Table 1). According to microbial mining N theory (Craine

Table 4
Results of the two-way analysis of variance for N deposition effect using N type, soil nematode, and their interaction as fixed factors.

Effects	*df*	SS	MS	*F*	*P*
Nitrogen (N)	2	415.0	207.5	26.5	< 0.001
Nematode (Ne)	1	206.8	206.8	26.4	< 0.001
N*Ne	2	16.3	8.1	1.0	0.384
Error	12	94.1			

Degree of freedom (*df*), type III sum of squares (SS), mean square (MS), *F* statistic, and statistical significance (*P*) are given for all the main effects, namely, N deposition, and soil nematode.

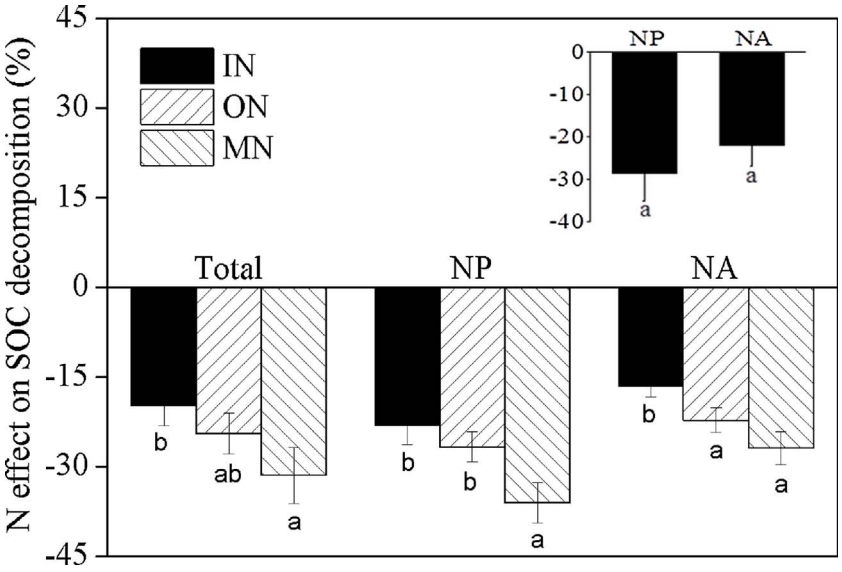

Fig. 5. Response of inhibition effect of N deposition on SOC decomposition to N types and soil nematode. ON, IN and MN represent organic N, inorganic N and the mixture of organic and inorganic N treatments, respectively. NP, NA represent the presence and absence of soil nematodes, respectively.

et al., 2007), the increase in soil N availability can decrease the microbial attack to SOC for acquiring N, thereby resulting in the reduction of SOC decomposition. Alternatively, changes in the composition of soil microbial community by N deposition (Table 3) might partly explain the reduction of SOC decomposition after N deposition (Demoling et al., 2008; Wang et al., 2014) because SOC decomposition is a function of microbial community and activity. An increase in Gram-positive to Gram-negative bacteria ratio suggested that SOC was more stable under N deposition because Gram-positive bacteria preferred to use the more recalcitrant SOC as C source. Finally, the reduction in SOC decomposition might also arise from the suppression of the synthesis of oxidative enzymes that decompose the recalcitrant fractions of SOC, such as lignin and cellulose (Carreiro et al., 2000; Waldrop et al., 2004). This speculation was supported by our data that cellulase and lignin-peroxidase activities in soils under N deposition were low (Fig. 2). The decline in oxidative enzyme activities may enhance the accumulation of recalcitrant SOC fractions (Liu et al., 2016), which is advantageous to soil C stability and consequently attenuates global climate change.

To our knowledge, our study is the first to evaluate the effects of different types of N deposition on SOC decomposition. As stated in our first hypothesis, we demonstrated that ON deposition had greater N effect on SOC decomposition than IN deposition, thereby suggesting that the effects of N deposition on SOC decomposition depended on the types of N. As indirect evidence in aspen and pine forests, Ramirez et al. (2010) found that the addition of urea decreased the soil respiration compared with inorganic N addition, but heterotrophic respiration was not distinguishable from total soil respiration. The strong inhibition effect of ON deposition on SOC decomposition may have remarkably resulted from an easy assimilation of ON than IN (Thirukkumaran and Parkinson, 2000). Moreover, additional available C provided by ON sources may repress the synthesis of oxidative and hydrolytic enzymes (cellulase and lignin peroxidase) (Fig. 2a and c) and allows soil microorganisms to metabolize excess N. The difference in soil NO_3^--N concentration among different N deposition treatments might partly justify the stronger inhibition effect of ON on SOC decomposition (Table 1) because of the significant negative correlation between SOC decomposition and soil NO_3^--N content. The higher relative abundance of NO_3^--N versus NH_4^+-N (Table 1) led to slightly lower activities of oxidative and hydrolytic enzymes in ON treatments than in IN treatments. As suggested by Cusack (2013), this relative abundance might be involved in driving the activities of oxidative enzymes that control SOC decomposition.

Furthermore, the suppression of SOC decomposition by MN was higher than that of any single IN or ON deposition (Fig. 5), which was beyond our expectation. This finding suggests that previous studies based on single form of N deposition underestimate the inhibition of SOC decomposition by atmospheric N deposition. Increasing atmospheric N deposition may play more roles during C sequestration in soils and attenuation of global climate change than previously predicted. The greater suppression of MN deposition may be due to the following reasons. MN deposition can simultaneously provide C and N sources for soil microorganisms to avoid any C and N limitation, thereby decreasing the microbial activity to gain C or N from SOC according to microbial stoichiometric decomposition theory (Chen et al., 2014). Our data, which showed Gram-negative bacterial and fungal PLFA concentrations being lower in soils under MN deposition than under a single type of N (Table 3), support this reason. The addition of a single type of N may also modify the balance of IN to ON for soil microorganisms (Sinsabaugh et al., 2002; Guo et al., 2011), thereby resulting in a higher stimulation effect on microbial functioning under a single type of N deposition than in MN deposition. Furthermore, the greatly declining number of bacterivores and fungivores in MN deposition treatments may be another possible mechanism for higher suppression of SOC decomposition by MN deposition over a single type of N deposition. In addition, other potential mechanisms regarding the influence of different types of N deposition on soil C cycle should be further investigated.

Our study is the first to evaluate the possibility of soil nematode mediating the effects of N deposition on SOC decomposition. We observed that soil nematode exclusion attenuates the suppression of SOC decomposition by N deposition (Fig. 5), thereby confirming our hypothesis that soil nematodes modify the N deposition effect on SOC decomposition. This observation was due to a higher increase in SOC decomposition following nematocide application in N deposition treatments than in CT (Fig. 3). Soil nematode exclusion may increase the soil microbial biomass and activity (Table S3) because of the feeding preference of soil nematodes (Rønn et al., 2002; Salinas et al., 2007). For instance, some studies have reported the negative effects of nematode grazers on microbial biomass (Djigal et al., 2004; Gebremikael et al., 2014), which positively correlates with SOC decomposition (Wang et al., 2017). Soil nematodes also modify the microbial community structure, particularly the bacterial community. Second, soil nematodes killed by a nematocide may provide additional C source for soil microorganisms, thereby causing a positive priming effect on native SOC (Wang et al., 2014) and consequently increasing the SOC decomposition.

Furthermore, we observed that the attenuation of nematode exclusion in the suppression of SOC decomposition by N deposition was

related to N types, thereby showing that IN had the greatest attenuation effect, whereas organic N had the lowest. The differences among N types might be attributed to the various effects of IN and ON on soil nematode and microbial communities (Rodríguez-Kíbana, 1986; Murray et al., 2006) because soil nematodes contribute up to 40% of the SOC decomposition (De Ruiter et al., 1993). In our study, IN and ON deposition had different effects on the abundance of fungivores and omnivorous predators (Table 2), thereby leading to a shift in the soil nematode community structure. Other underlying mechanisms should be further investigated in future experiments, and more work should be conducted.

In summary, we initially highlighted the effects of N types, soil nematode, and their interaction on SOC decomposition in a temperate forest. In general, N deposition suppressed SOC decomposition, but the magnitude of this suppression effect depended on N types and soil nematodes. A mixture of IN and ON deposition had the greatest suppression effect, implying that increasing atmospheric N deposition in temperate forest ecosystems can decrease more CO_2 emission levels from SOC decomposition than a single type of N deposition. Furthermore, N types should be considered when assessing the response of forest soil C cycling to globally increasing N deposition. A higher suppression of SOC decomposition by N deposition under the presence of soil nematodes indicates that soil nematodes mediate SOC decomposition by modifying the composition of the soil microbial community, and consequently affects the SOC storage. However, we did not identify the sources (labile and recalcitrant SOC) of the suppressed CO_2 in this experiment, although it is helpful to maintain the SOC storage if most of the suppressed CO_2 comes from recalcitrant SOC. In addition, the soils used in this experiment have been subjected to simulated N deposition only for 5 years, which may differ from the results of a long-term experiment. Therefore, future work is still necessary to investigate the long-term influence of different types of N deposition on the decomposition of labile and recalcitrant SOC and its underlying mechanism.

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References

Blair, G.J., Lefroy, R.D.B., Lisle, L., 1995. Soil carbon fractions based on their degree of oxidation and the development of a carbon management index for agricultural systems. Aust. J.Agric. Res. 46, 1459–1466.
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.
Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81, 2359–2365.
Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E., Kuzyakov, Y., 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Global Change Biol. 20, 2356–2367.
Cornell, S.E., 2011. Atmospheric nitrogen deposition: revisiting the question of the importance of the organic component. Environ. Pollut. 159, 2214–2222.
Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105–2113.
Cusack, D.F., 2013. Soil nitrogen levels are linked to decomposition enzyme activities along an urban-remote tropical forest gradient. Soil Biol. Biochem. 57, 192–203.
Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.
De Ruiter, P., Moore, J., Zwart, K., Bouwman, L., Hassink, J., Bloem, J., DeVos, J.A., Marinissen, J.C.Y., Didden, W.A.M., Lebrink, G., Brussaard, L., 1993. Simulation of nitrogen mineralization in the below-ground food webs of two winter wheat fields. J. Appl. Ecol. 30, 95–106.
Demoling, F., Nilsson, L.O., Bååth, E., 2008. Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. Soil Biol. Biochem. 40, 370–379.
Djigal, D., Brauman, A., Diop, T.A., Chotte, J.L., Villenave, C., 2004. Influence of bacterial-feeding nematodes (Cephalobidae) on soil microbial communities during maize growth. Soil Biol. Biochem. 36, 323–331.
Du, Y., Guo, P., Liu, J., Wang, C., Yang, N., Jiao, Z., 2014. Different types of nitrogen deposition show variable effects on the soil carbon cycle process of temperate forests. Global Change Biol. 20, 3222–3228.
Galloway, J.N., Townsend, A.R., Erisman, J.W., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320, 889–892.
Gebremikael, M.T., Buchan, D., De Neve, S., 2014. Quantifying the influences of free-living nematodes on soil nitrogen and microbial biomass dynamics in bare and planted microcosms. Soil Biol. Biochem. 70, 131–141.
Guo, P., Wang, C., Jia, Y., Wang, Q., Han, G., Tian, X., 2011. Responses of soil microbial biomass and enzymatic activities to fertilizations of mixed inorganic and organic nitrogen at a subtropical forest in east China. Plant Soil 338, 355–366.
Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., Maldonado-Ramirez, S., Lynch, S.T., Nelson, E.B., 2000. Methods for assessing the composition and diversity of soil microbial communities. Appl. Soil Ecol. 15, 25–36.
Janssens, I., Dieleman, W., Luyssaert, 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.
Jickells, T., Baker, A.R., Cape, J.N., Cornell, S.E., Nemitz, E., 2013. The cycling of organic nitrogen through the atmosphere. Philos. Trans. R. Soc. B 368, 20130115. http://dx.doi.org/10.1098/rstb.2013.0115.
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, 459–463.
Liu, J., Wu, N., Wang, H., Sun, J., Peng, B., Jiang, P., Bai, E., 2016. Nitrogen addition affects chemical composition of plant tissues, litter and soil organic matter. Ecology 97, 1796–1806.
Mo, J., Zhang, W., Zhu, W., Gundersen, P., Fang, Y., Li, D., Wang, H., 2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Global Change Biol. 14, 403–412.
Murray, P.J., Cook, R., Currie, A.F., Dawson, L.A., Gange, A.C., Grayston, S.J., Treonis, A.M., 2006. Interactions between fertilizer addition, plants and the soil environment: implications for soil faunal structure and diversity. Appl. Soil Ecol. 33, 199–207.
Rønn, R., McCaig, A.E., Griffiths, B.S., Prosser, J.I., 2002. Impact of protozoan grazing on bacterial community structure in soil microcosms. Appl. Environ. Microbiol. 68, 6094–6105.
Ramirez, K.S., Craine, J.M., Fierer, N., 2010. Nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied. Soil Biol. Biochem. 42, 2336–2338.
Rodríguez-Kíbana, R., 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. J. Nematol. 18 (2), 129–135.
Salinas, K.A., Edenborn, S.L., Sexstone, A.J., Kotcon, J.B., 2007. Bacterial preferences of the bacterivorous soil nematode *Cephalobus brevicauda* (Cephalobidae): effect of bacterial type and size. Pedobiologia 51, 55–64.
Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48, 7–20.
Sinsabaugh, R.L., Carreiro, M., Repert, D., 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60, 1–24.
Sun, X., Zhang, X., Zhang, S., Dai, G., Han, S., Liang, W., 2013. Soil nematode responses to increases in nitrogen deposition and precipitation in a temperate forest. PLoS One 8 (12). http://dx.doi.org/10.1371/journal.pone.0082468.
Thirukkumaran, C.M., Parkinson, D., 2000. Microbial respiration, biomass, metabolic quotient and litter decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorous fertilizers. Soil Biol. Biochem. 32, 59–66.
Tu, L., Hu, T., Zhang, J., Li, X., Hu, H., Liu, L., Xiao, Y., 2013. Nitrogen addition stimulates different components of soil respiration in a subtropical bamboo ecosystem. Soil Biol. Biochem. 58, 255–264.
Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., Gallo, M., Lauber, C., 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. Ecol. Appl. 14, 1172–1177.
Wang, Q., Wang, S., He, T., Liu, L., Wu, J., 2014. Response of organic carbon mineralization and microbial community to leaf litter and nutrient additions in subtropical forest soils. Soil Biol. Biochem. 71, 13–20.
Wang, Q., Zhang, W., Sun, T., Chen, L., Pang, X., Wang, Y., Xiao, F., 2017. N and P fertilization reduced soil autotrophic and heterotrophic respiration in a young *Cunninghamia lanceolata* forest. Agric. Forest Meteorol. 232, 66–73.
Wei, C., Zheng, H., Li, Q., Lü, X., Yu, Q., Zhang, H., Chen, Q., He, N., Kardol, P., Liang, W., Han, X., 2012. Nitrogen addition regulates soil nematode community composition through ammonium suppression. PLoS One 7 (8). http://dx.doi.org/10.1371/journal.pone.0043384.
White, D.C., Ringelberg, D.B., 1998. Signature lipid biomarker analysis. In: Burlage, R.S., Atlas, R., Stahl, D., Geesey, G., Sayler, G. (Eds.), Techniques in Microbial Ecology. Oxford University Press, New York, pp. 255–272.
Zhou, J., Sun, X., Jiao, J., Liu, M., Hu, F., Li, H., 2013. Dynamic changes of bacterial community under the influence of bacterial-feeding nematodes grazing in prometryne contaminated soil. Appl. Soil Ecol. 64, 70–76.