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published in
Biology and Fertility of Soils
2019

DOI (link to publisher)
10.1007/s00374-019-01357-8

document version
Publisher's PDF, also known as Version of record

Biology and Fertility of Soils (2019) 55:383–391
https://doi.org/10.1007/s00374-019-01357-8

ORIGINAL PAPER

Form of nitrogen deposition affects soil organic matter priming by glucose and cellulose

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Received: 8 December 2018 / Revised: 3 March 2019 / Accepted: 11 March 2019 / Published online: 25 March 2019

Abstract

To examine the interplay of C and N availability, glucose (high microbial availability) and cellulose (low microbial availability) were added to soils collected from a temperate forest that had received simulated N deposition for 6 years (organic and/or inorganic N). The priming effect was higher for glucose addition than for cellulose. N deposition decreased the priming effect of easily available glucose but increased the priming effect of cellulose. This confirmed an interactive effect of fresh organic matter (FOM) availability and N deposition on priming. Furthermore, the interactive effect was affected by the form of N deposition, with interaction mainly observed with organic N deposition. Qualitatively different patterns of priming were observed for the two FOM types and were accompanied by contrasting abundance of fungi and bacteria in the community, as determined by phospholipid fatty acid (PLFA) analysis. Organic N deposition increased bacterial biomass but decreased the intensity of priming. In contrast, a competitive advantage of fungi with respect to organic N sources may drive priming by cellulose. The results highlighted the importance of the availability of FOM in regulating the priming effect and showed that interactions between the form of N deposition and the availability of the FOM should be considered when predicting soil C cycling in scenarios of increased N deposition. Organic N deposition had a greater impact on priming effects than inorganic N deposition, and the influence of microbial availability of FOM largely depended on organic N deposition.

Keywords Priming effect · Fresh organic matter · Microbial availability · N deposition · Organic N

Introduction

Input of organic substances into soil often accelerates the mineralization of soil organic C (SOC), a phenomenon termed the priming effect (PE; Kuzyakov et al. 2000). The direction and magnitude of PE have been reported for a wide range of different fresh organic matter (FOM) additions, under various conditions (Aye et al. 2018; Di Lonardo et al. 2017; Kuzyakov and Bol 2006; Nottingham et al. 2009; Shahbaz et al. 2017), yet the underlying mechanisms remain unresolved. FOM chemical structure (Di Lonardo et al. 2017; Shahbaz et al. 2017), the amount of added FOM (Liu et al. 2017; Shahbaz et al. 2017), and the rates of FOM mineralization (Mason-Jones et al. 2018) have all been identified as important factors determining PE strength. These parameters all relate to the microbial availability of the added substrate, which here refers to its susceptibility to uptake and utilization by soil microorganisms (Chen et al. 2014). FOM can vary widely in microbial availability, with simple low-molecular-weight compounds generally highly available (e.g., sugars and amino acids), while high-molecular-weight polymers are less available (e.g., cellulose and lignin) (Aye et al. 2018; Koranda et al. 2014). Generally, the addition of FOM with high microbial availability stimulates more priming (Conde et al. 2005; Hamer and Marschner 2005a; Nottingham et al. 2009). This is consistent with the finding that priming was largely

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00374-019-01357-8) contains supplementary material, which is available to authorized users.

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determined by the type of FOM and was positively related to mineralization of the added substances (Mason-Jones et al. 2018). In addition, priming has also been related to the action of distinct microbial groups, which are responsible for transformation of FOM of differing microbial availability (Blagodatskaya and Kuzyakov 2008). It has been argued that fast-growing bacteria are more competitive toward easily utilizable FOM, whereas fungi dominate in the degradation of low-availability litter (Boer et al. 2005; Moore-Kucera and Dick 2008; Paterson et al. 2007; Poll et al. 2008). However, the proposed role of fungi in SOC priming (Fontaine et al. 2011) would then predict higher PE from low-availability FOM, rather than the observed trend. In addition, due to the distinct temporal patterns of microbial response to FOM (Aye et al. 2018; Nottingham et al. 2009), the duration of incubation may affect the observations. Sufficient incubation time is necessary to observe the full response of microbial communities to substrate additions.

Nitrogen availability has been identified as another important factor for priming (Fontaine et al. 2011). Previous studies have shown that N deposition generally decreases SOC priming by easily available substances due to alleviation of N limitation (Aye et al. 2018; Wang et al. 2014). The effect of N deposition on priming by high-molecular-weight polymers with low availabilities has seldom been reported. Polymeric C substrates benefit the growth of fungi under high N supply (Koranda et al. 2014), which might enhance the priming effect, considering the expected role of fungi in degrading high-molecular-weight polymers (Boer et al. 2005; Paterson et al. 2007). Therefore, N deposition might exert contrasting effects on priming, depending on the microbial availability of the added FOM. In addition, recent findings show that these C-N interactions cannot be explained by stoichiometry alone (Mason-Jones et al. 2018; Wild et al. 2017). Divergent observations of PE have been reported when the form of N deposition (organic and inorganic N) was different (Chen et al. 2017, 2018; Wang et al. 2014). For example, while mineral N can suppress SOC priming (Wang et al. 2014), organic N can stimulate strong PE (Hamer and Marschner 2005b; Wild et al. 2014). The effects of organic N deposition may therefore differ from those of more intensively studied inorganic N deposition. At a global scale, organic N accounts for 30–36% of the gross amount of N deposition (Cornell 2011). Furthermore, organic N deposition entails addition of C and N together and generally increases the labile organic C pool (Chen et al. 2018; Du et al. 2014). Since bacteria and fungi have different nutrient requirements, nutrient additions can alter the fungal/bacterial dominance in soils (Strickland and Rousk 2010; Nottingham et al. 2017). It is therefore plausible that nutrient availability and form have distinct effects on bacteria and fungi and, since these groups play different roles in priming, complex priming responses are induced by different nutrient manipulations.

Long-term N deposition experiments provide unique opportunities to investigate N effects on SOC dynamics. In particular, they integrate ecosystem responses to the N input that cannot be simulated under laboratory conditions. The influence of C and N inputs on PE could have important implications for the response of soil C stocks to global change. In this study, we sought to determine how PE is affected by FOM availability, N supply, and their interactions. We implemented a full-factorial experiment including FOM of contrasting availability (glucose, cellulose), and soils from a temperate forest research site with 6 years of contrasting long-term N deposition (none, organic, inorganic, or mixed N). We hypothesized that (1) cellulose would be mineralized more slowly than glucose, confirming its lower availability in this soil, and thus stimulate less priming; (2) N deposition would reduce priming effects of glucose but increase priming by cellulose; and (3) the effect of FOM availability on priming would differ with deposition of organic and/or inorganic N. The results could highlight the importance of FOM quality when studying PE and prove the occurrence of interactive effects between various forms of N deposition and FOM with contrasting availability. To test these hypotheses, we conducted 104-day incubations of temporal forest soils.

Materials and methods

Site description

We collected soils from a long-term N deposition simulation experiment at the Maoershan Forest Research Station of Northeast Forestry University in northeastern China (127°30′~127°34′E, 45°20′~45°25′N). The reserve has rolling mountainous terrain with elevations ranging from 300 m to 805 m. The site elevation is 340 m above sea level. The site has a continental temperate monsoon climate, with a strong monsoon windy spring, a warm and humid summer, and a dry and cold winter. Annual precipitation ranges from 600 to 800 mm, most of which falls in July and August. The background N deposition rate is about 1.92 g N m^{-2} a^{-1}, of which 22% is in the form of organic N. The mean annual air temperature is 2 .8°C, and average January and July air temperatures 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 Chinese Soil Taxonomic System; Gong et al. 1999). Soil texture is a silty clay with high organic matter and N content (Table 1).

Twelve plots (10 × 20 m) separated by 15 m were established in April 2010 in a *Larix gmelinii* plantation. Four treatments with various forms of N addition were randomly assigned to plots, with three replicates: no N addition (CT), inorganic N addition (IN), organic N addition (ON), and mixed addition of inorganic N and organic N (ION),

Table 1 Changes in main chemical properties in soil subjected to N deposition with various N forms

Treatment	CT	ON	IN	ION
pH	5.5 ± 0.1A	5. 5 ± 0.3A	5.5 ± 0.1A	5.4 ± 0.2A
Soil organic C ($g \cdot kg^{-1}$)	42.3 ± 2.2B	63.7 ± 4.3A	36.8 ± 3.4B	41.9 ± 3.0B
Dissolved organic C ($mg \cdot kg^{-1}$)	29.7 ± 3.7B	48.4 ± 4.0A	29.7 ± 4.0B	30.8 ± 3.0B
Labile organic C ($mg \cdot kg^{-1}$)	10.3 ± 1.0B	17.7 ± 1.5A	9.2 ± 1.3B	10.0 ± 0.8B
Water-soluble carbohydrates ($g \cdot kg^{-1}$)	101.0 ± 8.1B	215.5 ± 19.0A	104.7 ± 12.4B	115.4 ± 14.7B
Total nitrogen ($g \cdot kg^{-1}$)	3.7 ± 0.2B	4.4 ± 0.3A	3.3 ± 0.3B	3.8 ± 0.3B
C:N ratio	11.3 ± 0.3A	11.5 ± 0.2A	11.3 ± 0.3A	11.0 ± 0.5A
Available phosphorus ($mg \cdot kg^{-1}$)	18.4 ± 2.3A	21.9 ± 3.6A	22.4 ± 4.5A	22.1 ± 2.1A
NH_4^+-N ($mg \cdot kg^{-1}$)	5.5 ± 1.3A	6.8 ± 3.0A	4.3 ± 1.7A	6.8 ± 2.7A
NO_3^--N ($mg \cdot kg^{-1}$)	45.9 ± 3.4A	41.6 ± 4.9AB	41.1 ± 3.7AB	36.6 ± 4.3B

Data were means ± standard deviations. Different letters stand for difference at the 5% level of significance. CT, no N-added soil; ON, IN, and ION stand for soils subjected to organic, inorganic, and mixed N deposition, respectively

respectively. The mixture of inorganic and organic N was at an N ratio of 7:3, in accordance with the average ratio in atmospheric N deposition (Cornell 2011). Ammonium nitrate (NH_4NO_3) was used as inorganic N fertilizer, while an equal mixture of urea and glycine was used as organic N fertilizer (Cornell 2011). The fertilization rate was 10 g N m^{-2} a^{-1}. Fresh batches of fertilizer were dissolved in water and applied to the surface monthly in six equal parts from May to October each year (50 L solution per plot per application). The control plots received equal amounts of water. During experiment period, no disturbance except fertilization was produced in the experiment site.

Soil sampling and analysis

Soil samples were collected from the A horizon (0~10 cm) in August 2016, 25 days after the previous application. After removing the litter layer, 20 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 sieved to 2 mm after roots, and visible residues had been manually removed. Subsamples for determination of dissolved organic C (DOC), NH_4^+, and NO_3^- were stored field-moist at 4 °C until analysis. Other subsamples were air dried at room temperature for determining SOC, total N, and total P contents. The sand, silt, and clay contents in soils were 8.3%, 47.2%, and 44.5%, respectively. Soil bulk density was 1.38 g cm^{-3}.

Soil pH was measured in water extracts of 1:2.5 (*w*/*v*) of air-dried soil and deionized water. The SOC and total N contents were measured by dry combustion using a Vario Max CN elemental analyzer (Elementar, Germany). Soil exchangeable NH_4^+-N and NO_3^--N were determined by the indophenol blue colorimetric method and ultraviolet spectrophotometry, respectively (Kempers and Zweers 1986). The DOC content was extracted using a modified method from Ghani et al. (2003) with determination by elemental analyzer (Thermo Fisher Flash 2000, USA). Briefly, DOC was extracted from 10 g soil into 25 mL deionized water with agitation on an orbital shaker at 300 rpm for 30 min at room temperature. Separation was achieved by centrifugation at 2500×*g* for 20 min. Extracted DOC was filtered through a microfiltration membrane with 0.45 μm pore size and determined using a TOC-analyzer (Ghani et al. 2003). Soil-available P was extracted with a solution of 0.03 mol L^{-1} NH_4F-0.025 mol L^{-1} HCl (Bray P-1 method) at a sample/extractant ratio of 1:10 (*w*/*v*) and determined using molybdenum blue with spectrophotometric measurement at 700 nm (Murphy and Riley 1962). The labile organic C (LOC) was determined by the $KMNO_4$ oxidation method (Blair et al. 1995), and water-soluble carbohydrates (WSC) were determined by the anthrone-sulfuric acid method (Grandy et al. 2000). The concentrations of available P, LOC, and WSC were analyzed by spectrophotometric methods using a microplate reader (Thermo Fisher Multiskan FC, Germany).

Incubation experiment

Soils from each deposition treatment (CT, ON, IN, and ION) were used in this incubation experiment with three replications from each plot in the field. Each soil received one of three FOM additions: ^{13}C-labeled glucose or ^{13}C-labeled cellulose in the amount of 2% of SOC content (GA, CA), respectively, or no C addition (NA) as a reference. The $\delta^{13}C$ values of glucose and cellulose were 62.6‰ and 174.5‰, respectively.

Eighty grams of fresh soil (oven-dried weight) was placed into 500 mL Mason jars. Soil samples were pre-incubated for 24 h in the dark at 20 °C. Then, ^{13}C-labeled FOM in water was added homogeneously to the surface of the soil. Soil water content was adjusted to 60% of water-holding capacity by adding distilled water. Six additional Mason jars without soil

were incubated as blanks to account for CO_2 trapped when opening the jars. All the Mason jars were incubated in the dark at 20 °C for 104 days. Released CO_2 was trapped in alkali and measured at days 1, 3, 7, 14, 22, 30, 42, 56, 71, 87, and 104 after incubation. Briefly, a glass vial containing 20 mL of 0.2 M NaOH solution was placed in each Mason jar to trap evolved CO_2. Phosphoric acid (200 g/L) was added to 10 mL of NaOH solution to release the CO_2, and the $\delta^{13}C$ of CO_2 was measured using a spectroscopic stable isotope analyzer (Picarro G2131). The calibration of the analyzer was checked with standards each time before measuring samples, indicating a precision of 0.2 ‰ (average standard deviation). Sample $\delta^{13}C$ was always below 15‰. The remaining 10 mL of solution was used to determine the amount of released CO_2 by titration with 0.1 M HCl.

Partitioning sources of CO_2 and quantification of the PE

To calculate the amount of CO_2 derived from FOM and native SOC mineralization during the incubation period, the following equation was used:

$$C_F = C_T\ (\delta_T - \delta_S)/(\delta_F - \delta_S) \quad (1)$$

$$C_S = C_T - C_F \quad (2)$$

In the equations, C_T is the total amount of CO_2 during the considered time interval, and δ_T is the corresponding isotopic composition; C_F is the amount of C derived from FOM, and δ_F is the isotopic composition of the added FOM; C_S is the amount of C derived from SOC, and δ_S is the ^{13}C abundance of respired CO_2 from the control soil.

Cumulative PE (mg CO_2-C g^{-1} SOC) induced by the FOM addition was calculated as

$$PE = CO_{2f} - CO_{2nf} \quad (3)$$

where CO_{2f} is the cumulative amount of SOC-derived CO_2 in treatments with FOM addition, and CO_{2nf} is the amount of SOC-derived CO_2 with the same N treatment, but without labeled FOM addition.

Determination of main microbial groups

After incubation, soil samples were freeze dried at − 50 °C before analyzing main microbial groups by phospholipid fatty acid (PLFA) analysis (Crossman et al. 2004; Frostegård et al. 1991). Fatty acid methyl esters were separated and quantified on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an Ultra-2 column and flame ionization detector, and the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA). Fatty acids were quantified by calibration against standard solutions of FAME 19:0 (Matreya Inc. State College, PA, USA). For analyses of microbial group abundance, the following PLFA designations were used: bacteria i15:0, a15:0, i16:0, i17:0, a17:0, 16:1ω7c, cy17:0ω7c, 18:1ω7c, and cy19:0ω7c; fungi 18:1ω9c, and18:2ω6c (Moore-Kucera and Dick 2008).

Statistical analyses

We used one-way ANOVA to test for differences in soil properties among soils with various N deposition, followed by post hoc Tukey's test. Two-way ANOVA was applied to assess the effect of N deposition form, FOM form, and their interactions on cumulative PE and PLFA concentrations, followed by post hoc Tukey's test. Independent-samples *t* testing with unequal sample sizes was used to test the effect of N deposition on cumulative PE. Repeated-measures analysis of variance (ANOVA) was performed to test how N deposition form, FOM form, and their interactions affected the rate of FOM mineralization over time, followed by post hoc Tukey's test. All treatments were replicated three times. Finally, we used regression analyses to test for relationships between FOM mineralization rate and priming rate. All the statistical analyses were performed using SPSS version 22.0 for Windows (SPSS Inc. Chicago).

Results

Soil chemical properties

Changes in soil chemical properties largely depended on the form of long-term N deposition. The organic C pool was increased in soil receiving organic N deposition (ON; Table 1), with SOC, LOC, DOC, and WSC concentrations 1.5–2.1 times greater than in CT soil. Correspondingly, TN was also increased by organic N deposition. On the other hand, there was no significant difference of available P, C/N ratio, or NH_4^+-N contents among the various deposition treatments.

SOC, FOM mineralization, and priming effect

Both glucose and cellulose accelerated the cumulative CO_2 production from native SOC compared with no addition treatment, being approximately 50% higher in glucose treatment versus 25% higher in cellulose treatment (Fig. S1). Correspondingly, glucose addition induced 1.6–3.7 times higher cumulative PE than cellulose addition, irrespective of the form of N deposition ($P < 0.05$; Fig. 1). In addition, the response of priming to N deposition differed between GA and CA treatments. Overall, N deposition suppressed priming in the GA treatment, but

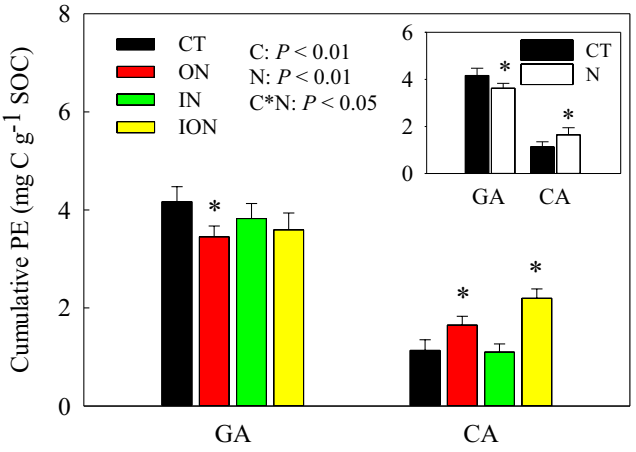

Fig. 1 Cumulative PE after glucose and cellulose addition to soils subjected to N deposition (incubation period 104 days). The vertical bars are standard deviations. CT, no N deposition amendment; N, N deposition amendment (means of organic N, inorganic N and mixed N deposition). GA, CA represent glucose and cellulose addition, respectively. C indicates FOM type, N indicates N deposition form. Asterisks stand for significant differences relative to controls

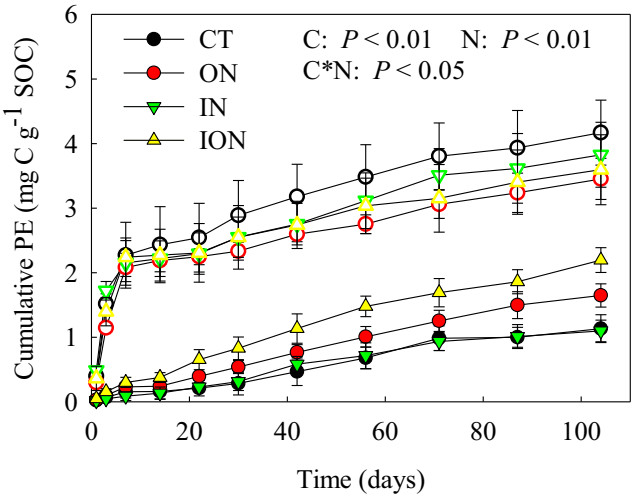

Fig. 2 Cumulative PE in soils subjected to N deposition with various N forms in glucose and cellulose addition treatments during the incubation (104 days). The vertical bars are standard deviations. C indicates microbial availability of FOM; N indicates N deposition form. CT, no N deposition amendment; ON, IN, and ION respectively organic N, inorganic N, and mixed N deposition amendment. Hollow symbols are for the glucose treatment; solid symbols for cellulose treatment

enhanced it in the CA treatment (Fig. 1, inset). However, the effect of N deposition on priming varied with the N forms. Specifically, organic N deposition followed the general pattern, with 17.2% lower cumulative PE in the GA treatment ($P < 0.05$) versus 45.3% higher PE in the CA treatment, relative to the control soil. In contrast, inorganic N deposition had no effect on PE in GA or CA treatments. Cumulative PE for the CA treatment was 93.5% higher in soil with ION deposition than in CT soil ($P < 0.05$). Different temporal patterns of priming were observed for glucose and cellulose (Fig. 2). Much of the priming by glucose occurred within the first few days, but an extended period of priming continued throughout the experiment. Priming by cellulose was relatively continuous during the incubation, with slight fluctuations.

Mineralization of glucose was faster and stronger than that of cellulose ($P < 0.01$; Fig. 3). Organic N deposition stimulated FOM mineralization in both GA and CA treatments ($P < 0.01$). Notably, glucose mineralization rate showed a positive linkage with priming rate in GA treatment (Fig. 4).

Main microbial groups

Microbial community composition was modified by N deposition and FOM additions. Significant increases of bacterial abundance in GA and CA treatments were detected ($P < 0.05$; Fig. 5a). Furthermore, a higher abundance of fungi was detected in CA than NA ($P < 0.05$; Fig. 5b). As a whole, fungi/bacteria ratios were increased by cellulose addition and reduced by glucose addition (Fig. 5c). Much higher concentrations of fungal PLFA in ON and ION soils were accompanied by elevated fungi/bacteria ratios in CA treatment ($P < 0.05$). In contrast, for GA treatment, organic N deposition increased the bacterial biomass, resulting in lower ratios of fungal to bacterial biomass.

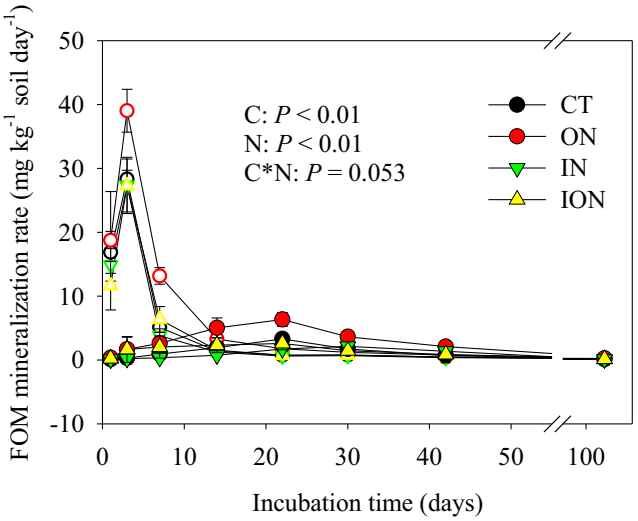

Fig. 3 Effects of N deposition form, FOM quality, and their interaction on the mineralization rate of fresh organic matter during the 104 days of incubation. C indicates FOM type; N indicates N deposition form. CT, no N deposition amendment; ON, IN, and ION respectively organic N, inorganic N, and mixed N deposition amendment. Hollow symbols indicate glucose mineralization rates; solid symbols indicate cellulose mineralization rates

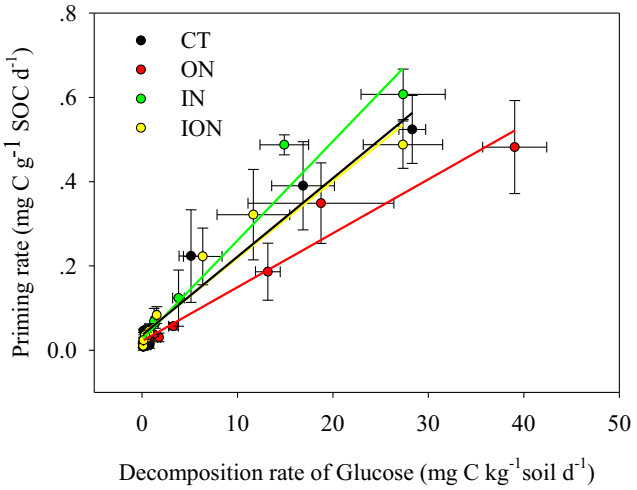

Fig. 4 Linear correlation between rates of C-induced PE and glucose mineralization rates. All the correlations are significant at a level of 0.05. The r^2 values for CT, ON, IN, and ION are 0.93, 0.92, 0.95, and 0.95, respectively. CT, no N deposition amendment; ON, IN, and ION respectively organic N, inorganic N, and mixed N deposition amendment

Discussion

FOM availability and its influence on PE

The mineralization of cellulose was lower than that of glucose (Fig. 3), which demonstrated that cellulose was less available. Correspondingly, cellulose induced lower PE than glucose, which was consistent with our hypothesis and previous observations (Conde et al. 2005; Hamer and Marschner 2005a; Nottingham et al. 2009). This PE was consistent with more available energy released by microbial metabolism (Blagodatskaya and Kuzyakov 2008; Dijkstra et al. 2013): with more energy available, more SOC is mineralized due to the enhanced activity of soil microorganisms. The absolute energy content in glucose and cellulose is nearly the same (Di Lonardo et al. 2017). Therefore, microbial availability of FOM determined the amount of FOM that could be utilized. Glucose was more easily utilized and thus provided more energy than cellulose, which facilitated the production of extracellular enzymes able to degrade native SOC (Aye et al. 2018).

Besides variations in mineralization and PE, the different FOM availabilities were accompanied by distinct changes in the composition of the microbial community (Kuzyakov 2010; Wang et al. 2015). Glucose was preferentially incorporated by bacteria (Fig. 5a), which respond rapidly to easily available substances and take a competitive advantage against fungi over the C source (Blagodatskaya et al. 2007; Chen et al. 2016). A positive relationship between glucose mineralization rate and priming rate (Fig. 4) was consistent with a mechanistic role of energy supply in priming by glucose. Thus, mineralization of glucose provided energy and C required for SOM mineralization. In contrast, there was no significant relationship between FOM mineralization and priming in the cellulose treatment (Fig. S2), suggesting a difference in the activated microbial groups (Guenet et al. 2010; Yin et al. 2016). PLFA was only measured at the end of the experiment, yet a considerable increase in fungal biomass was still evident (Fig. 5b). Since priming was occurring at this time, it likely arose from enhanced fungal activity, possibly due to the penetration of fungal hyphae into previously inaccessible microzones, allowing the degradation of complex insoluble SOC (Blagodatskaya et al. 2007; Fontaine et al. 2011). Compared with glucose, the lower mineralization rate of cellulose as well as the delayed response (Fig. 3) indicated weak preferential utilization of microbial community on FOM over SOC. This was completely different from the response to glucose, supporting a dominant role of fungi in cellulose degradation (Boer et al. 2005; Koranda et al. 2014; Poll et al. 2008).

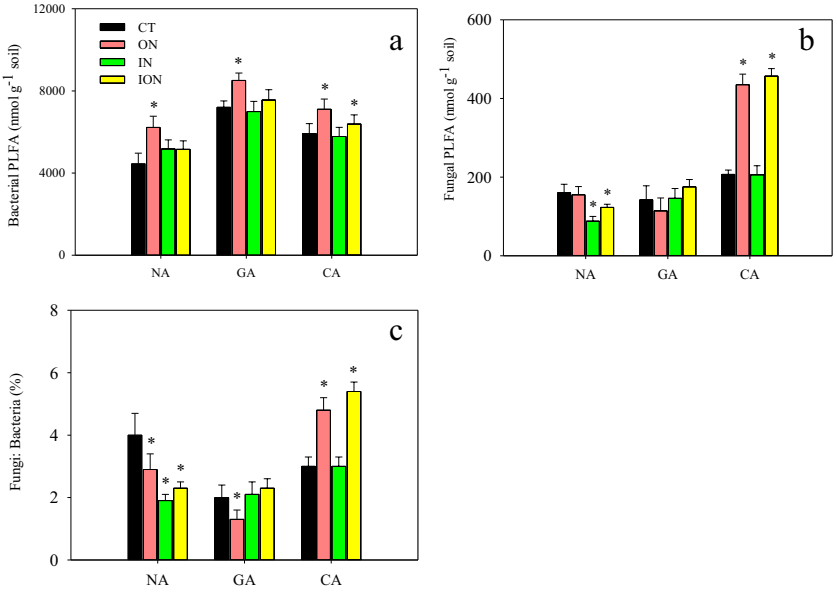

Fig. 5 **a** Bacterial and **b** fungal PLFA concentrations (nmol g^{-1} soil), and (**c**) fungi/bacteria ratios (%) of soils subjected to no N, organic N, inorganic N, and mixed N deposition in different FOM addition treatments, at the end of the incubation. Data are means ± standard deviations. Asterisks stand for significant differences relative to controls

Qualitatively different patterns of priming were observed for the two FOM types and were accompanied by contrasting patterns of fungal and bacterial growth.

Opposite effects of N deposition in glucose and cellulose treatments

For the first time, we found that N deposition has opposite impacts on priming by glucose and cellulose, supporting our second hypothesis. Specifically, these opposite effects were mainly attributed to organic N deposition. Notably, the microbial groups responded differently to N deposition (Fig. 5), which accounted for the different priming responses (Allison et al. 2008; Bowden et al. 2004; Ge et al. 2017). Organic N deposition increased bacterial biomass (Fig. 5a) and glucose mineralization, but reduced priming in the GA treatment, suggesting a bacterial community that was adapted to easily available C and N sources rather than the decomposition of complex SOC (Blagodatskaya et al. 2007; Chen et al. 2016). Application of organic N did not alter mineral N availability but elevated total N content, suggesting most added organic N was still stored in soil in organic form (Nannipieri and Paul 2009) and served as an available N and C source. Under conditions of C and N excess, the soil organic matter decomposing microorganisms switched to utilizing easily available FOM and microbial decomposition of SOC for nutrients was weakened, inducing a negative effect on priming (Blagodatskaya et al. 2007). This indicates a decreased priming intensity of bacterial community with increasing N supply. The lower PE might favor soil C sequestration in soil amended with organic N by reducing C loss. In the field, root exudates and rhizodeposits are mainly labile C (Bertin et al. 2003), and plant litter also contains easily available components. Therefore, the negative effect of organic N on SOC priming could partly explain the larger SOC pool in organic N soil after 6 years deposition amendment.

Higher priming effects induced by cellulose under organic N deposition were accompanied by a dramatic increase in fungal biomass as well as fungi/bacteria ratios (Fig. 5b, c). Cellulose incorporation into fungi was strongly dependent on N supply—in particular organic N—and this was associated with higher priming by cellulose (Fig. S3). Mineral N is lost quickly from soil by leaching, plant uptake, or microbial transformation to gaseous form (Tian et al. 2018). Frequent rainfalls during the period of fertilizer applications each year favor N leaching into deep soil. Organic N, in contrast, might be stored in soil for longer (Nannipieri and Paul 2009) and remain available for microbial utilization. Therefore, in contrast to previous results with short-term N manipulation (Koranda et al. 2014), only organic N promoted fungal dominance and increased the PE of cellulose. However, fungi remain uncompetitive against bacteria for glucose, even under organic N deposition. The presumed fungal contribution to priming requires both a competitive advantage over the C source (cellulose) and suitable N conditions (organic N). Therefore, our results indicate that explicit consideration of different microbial groups and their responses to C and N inputs is needed to untangle the C–N interactions of priming effects.

PE was not only related to the composition of main microbial groups, but also to the microbial biomass. Increases in microbial biomass, in particular of fungal biomass (Fig. 5b), due to organic N deposition could have driven the observed priming in the CA treatment (Blagodatsky et al. 2010; Kuzyakov and Bol 2006; Wang et al. 2015). This is consistent with a "co-metabolism" mechanism, as this mechanism suggests higher microbial biomass and activity would cause higher PE (Wang et al. 2015). Therefore, priming effects cannot be simply attributed to one or other microbial group but depend on the activities and metabolism of both groups, which in turn are determined by the C substrate as well as the N conditions.

Besides modifying biotic communities, organic N deposition also increased soil organic matter due to the organic content in deposition (Chen et al. 2017; Du et al. 2014). Available P was not correspondingly enriched, however, which could cause a relative limitation of P for microbial metabolism. Thus, it is possible that nutrient limitation due to increased organic C content also contributed to the decreased priming effect by GA for soil with organic N deposition.

Interactive effect of FOM availability and N deposition form on PE

The effect of N deposition on priming by different forms of FOM was regulated by N form of deposition, which indicated an interactive effect of N form and FOM availability and verified the third hypothesis. Organic N deposition had opposite effects on priming by glucose and cellulose, due to altered microbial community composition and changes in soil organic matter content. In contrast, inorganic N deposition exerted no effect on soil N content or priming effects in either treatment. Although an effect of inorganic N on priming has usually been found in lab experiments (Aye et al. 2018; Wang et al. 2014), this was not confirmed in this study. This is likely because the deposition experiment reflects the integrated effect of many complex N pathways under field conditions, including N leaching, plant uptake, or microbial transformation to gaseous form. Lastly, mixed N deposition did not change PE in GA, but increased PE in the CA treatment, further demonstrating the interactive effect of FOM availability and N deposition (Nottingham et al. 2017). The priming effect in the GA treatment remained stable, mainly because the major component of mixed deposition was inorganic N. Similarly, no changes in N availability were induced and the impact of inorganic N deposition was thus similar. In contrast, increased priming effects in the CA treatment under mixed N deposition were

accompanied by dramatic increases in fungal biomass and fungi/bacteria ratio, which were attributed to the organic N in mixed deposition (Koranda et al. 2014). Despite the lack of change in available N, organic N stored in soil might be the driver for the response of microorganisms, mainly fungi, and associated priming effects (Nannipieri and Paul 2009). In conclusion, glucose induces greater priming than cellulose, and N deposition with various N forms could modify the intensity of priming, even exerting contrasting effects on priming by FOMs with different microbial availabilities. Organic N in long-term deposition had a greater impact on priming effects than inorganic N deposition, and the influence of microbial availability of FOM largely depended on organic N deposition (Chen et al. 2018; Du et al. 2014). Therefore, organic N in deposition is particularly important, and the specific detection of organic N deposition is necessary to understand the likely impacts on receiving ecosystems.

Conclusion

Microbial availability of FOM regulates the intensity of PE: with less-available FOM, the priming effect is lower. In addition, N deposition was found for the first time to modulate the effect of FOM availability on priming. Organic N in deposition had greater effects on priming than inorganic N, and the influence of FOM availability on priming strongly depended on organic N deposition. The role of N deposition, and particularly the role of organic N, should be integrated into C cycling models that represent priming. Based on these results, we expect feedbacks between the deposition of N, altered litter quality, and the influence of these litter inputs on SOC stability.

Funding information This research was supported by the National Key R&D Program of China (grant no. 2016YFA0600801) and the National Natural Science Foundation of China (grant nos. 31570466 and 31830015).

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