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Effects of nitrogen addition on soil organic carbon mineralization after maize stalk addition

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ABSTRACT

Nitrogen (N) fertilization or increasing N deposition could significantly increase soil N availability, which could alter soil carbon cycling. Our understanding of the effects of N addition on the priming effect (PE) of carbon inputs on soil organic matter (SOM) mineralization (i.e. change of SOM mineralization after the addition of exogenous substrate) is still limited. Here we compared the effects of ammonium nitrate (NH_4NO_3) and urea ($CO(NH_2)_2$) on the PE of maize stalk on SOM mineralization. Results showed that during the 209-d incubation, maize stalk addition induced a SOM priming of 135 mg C kg^{-1} soil. Both NH_4NO_3 and urea addition significantly decreased SOM mineralization when there was no maize stalk addition. Compared to control, treatments of NH_4NO_3 + stalk and urea + stalk increased the 209-d cumulative SOM mineralization by 8.4% and 30.2%, respectively. The 55.0% lower positive PE under NH_4NO_3 + stalk treatment than stalk alone treatment hinted that N-mining mechanism prevailed under low N availability, while the 63.0% higher cumulative PE under urea + stalk treatment might be attributed to the function of co-metabolism mechanism. Nevertheless, net C sequestration (stalk-C incorporation into the soil minus primed soil C) among different stalk + N treatments was not statistically different because new C incorporation under urea + stalk treatment tended to be higher than that under NH_4NO_3 + stalk treatment. Our results suggested that NH_4NO_3 and urea had contrasting effects on SOM priming when maize stalk was added, which might complicate the selection of N fertilizers during straw returning practice in field conditions.

1. Introduction

Elevated nitrogen (N) input to ecosystems via atmospheric deposition or fertilizer application is an important global change driver with strong potential to modify biogeochemical processes of terrestrial ecosystems [1,2]. Understanding of how N addition and different N forms affect soil biogeochemical cycling is essential to evaluate the ecological impacts of elevated and component-complicated N input [3,4]. Owing to the important role of soil organic carbon (C) pool in mediating climate change and maintaining food security, numerous studies have assessed how N addition could affect soil C cycling, including C input (i.e. litter fall) [5,6] and output (i.e. soil organic matter (SOM) mineralization) [7,8]. However, our understanding about the effects of different forms of N on the interactions between C inputs and outputs, such as the priming effect (PE) [9,10], which is the change in SOM mineralization induced by the addition of exogenous substrates [11], is still limited.

PE could influence soil C sequestration [12], and could be affected by many substrate properties (such as substrate quantity and quality) and soil properties (such as nutrient status and microbial community composition) [13–15]. However, whether different chemical forms of N addition affect PE to the same degree is still not clear. In croplands, straw returning is widely recommended as an important management practice to raise soil fertility, increase soil C sequestration, and reduce soil erosion. In addition, urea and NH_4NO_3 are two types of frequently used N fertilizers in farmlands and their interactive effects with straws on SOM mineralization are hypothesized to be different. Based on the preferential substrate unitization theory, microbes would use added N because they are easier to be used [16], causing negative PE. When exogenous N and straw are added simultaneously, based on the "N

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mining" theory, which believes positive PE is caused due to microbial demand and utilization of N from SOM [17,18], PE is expected be less positive or even negative compared to straw alone addition because microbes would use exogenous N instead. In addition, ammonium nitrate addition could cause ammonium poisoning to soil microbes or lower soil pH, reducing microbial activities [19,20] and causing negative PE. However, the situation of urea addition would be more complicated because microbes need to produce urease first, which might enhance microbial activity during the early period of hydrolysis [21]. More research is needed to study the interactive effects of N addition with straw addition on PE on SOM mineralization.

Here, we investigated the effects of NH_4NO_3 addition and urea addition on stalk decomposition and SOM mineralization of a farmland soil in a lab incubation study. ^{13}C-labelled maize (*Zea mays* L.) stalk was used to track C flow and to quantify PE on SOM mineralization. We hypothesized that compared to maize stalk alone treatment, the addition of NH_4NO_3 + stalk would induce weaker positive PE, while the negative effects of urea addition on stalk-primed SOM mineralization would be less significant considering that urea hydrolysis is accompanied by enhanced microbial activities [21]. The objectives of this study were: (i) to determine how chemical forms of N additions (NH_4NO_3 vs. urea) affect SOM mineralization and PE; and (ii) to investigate whether the chemical forms of N additions modify net C sequestration (stalk-derived C incorporation into the soil minus primed soil C).

2. Materials and methods

2.1. Soils and ^{13}C-labelled stalk

Soil (0–20 cm) was collected from a maize (*Zea mays* L.) field in the National Field Observation and Research Station of Shenyang Agroecosystems, Liaoning Province, China (41°31′N, 123°24′E). The soil had been used for maize cropping since 2006 and was classified as an Alfisol, with a silty clay loam texture (14.8% sand, 66.3% silt and 18.9% clay), pH 6.37, soil organic C of 13.9 g kg^{-1} soil, total N of 1.3 g kg^{-1} soil and $\delta^{13}C$ value of −22.4‰. For detailed descriptions of the study site, see Lü et al. [22]. Ten soil clods (15 cm × 15 cm, 0–20 cm) were randomly collected using a shovel in this field on April 20th, 2014 and then bulked together. Soil samples were air-dried, passed through a 2 mm sieve and visible plant residues were removed prior to use.

Maize stalk (i.e. stem without leaves) was from the ^{13}C-labelled maize grown under field conditions at the USDA/ARS Rice Research Unit in Beaumont, Texas. Briefly, several plants at the grain-filling portion of the life cycle were covered with a transparent chamber (3 m × 0.9 m × 2.4 m, L × W × H), and labelled with 50 L of 99.3 atom % $^{13}CO_2$ delivered continuously for 1600 h, for detailed description of the labeling procedure see Wang et al. [23]. Stalks were dried at 50 °C and milled to pass through a 2 mm sieve prior to incubation. Maize stalks have a C content of 44.8%, N content of 1.0%, Klason-lignin content of 11.4%, and a $\delta^{13}C$ value of +132.6‰.

2.2. Laboratory incubation

A series of 1 L Mason jars, each containing 250 g dry soil, were prepared. Six treatments with four replicates were set up: N0L0 (control, neither N nor maize stalk was added), N1L0 (NH_4NO_3 addition, no stalk), N2L0 (urea addition, no stalk), N0L1 (stalk addition alone, no N), N1L1 (both NH_4NO_3 and stalk addition), N2L1 (both urea and stalk addition). Maize stalk (< 2 mm) was mixed with soils, and mineral N was added in the form of aqueous solution. The N and maize stalk addition rates to the soil were 154 mg N kg^{-1} soil and 3.44 g C kg^{-1} soil, respectively, corresponding to twice of local fertilization rates and actual maize straw yields to a soil depth of 20 cm. Soils were pre-incubated for 10 days before treatment and incubated at 25 °C and 60% of water-holding capacity for 209 days after treatment. Deionized water was added as necessary to maintain soil water content.

2.3. Samplings and measurements

During the incubation, the jars were sealed with Parafilm® M for most of the time to minimize evaporation without affecting gas exchange. The air accumulation method was used to collect gas samples every 2–4 days. Briefly, jars were first flushed with CO_2-free air for 1 h to reduce headspace CO_2 concentration to < 10 ppm, then tightly sealed for certain hours (3 h for treatments with stalk addition, while 6 h for treatments without stalk addition) to accumulate respired CO_2. After that, 150 ml gas sample was taken from each jar with a syringe. For detailed description of the air accumulation method see Paterson and Sim [24] or Wang et al. [14]. Gas samples were analyzed for CO_2 concentration and $\delta^{13}CO_2$ signatures within 24 h using a CO_2 isotope analyzer (CCIA-36d-EP, LGR, USA). After 209 days incubation, all soils in the jars were recovered to measure dissolved organic C (DOC) and microbial biomass C (MBC) using the chloroform fumigation-K_2SO_4 extraction method [25]. The total organic carbon (TOC) concentration and $\delta^{13}C$ value of K_2SO_4 extracts were analyzed by a Picarro iTOC-CRDS Isotopic Carbon Analyzer (Picarro-OI, USA) [14]. To quantify C sequestration (stalk-derived C incorporation into the soil matrix), soils and the remaining stalks were recovered separately using the water-washing method (i.e. add deionized water to the soil-stalk-mixture, shaken for 30 min at 200 rpm, and then recover the floated stalk and the soil sinking to the bottom respectively). The recovery rate of this method was 92.6% (n = 4) at the beginning of the experiment. After recording the mass loss of stalks, a Thermo Finnigan (DELTA Plus XP) stable isotope ratio mass spectrometer was used to determine total C content and $\delta^{13}C$ value of soils and the remaining stalks.

2.4. Calculations

To calculate the contribution of added maize stalk to CO_2 respiration, a two end-member mixing model was used:

$$P_{stalk} = (\delta^{13}C_t - \delta^{13}C_0) / (\delta^{13}C_{stalk} - \delta^{13}C_{soil}) \quad (1)$$

where P_{stalk} is the proportion of stalk-derived CO_2; $\delta^{13}C_t$ and $\delta^{13}C_0$ are the $\delta^{13}C$ values (‰) of respired CO_2 in the "stalk + soil" treatments and control, respectively; $\delta^{13}C_{stalk}$ and $\delta^{13}C_{soil}$ are the $\delta^{13}C$ value (‰) of maize stalk and initial soil, respectively [26]. For urea treatments (N2L0 and N2L1), considering the similar $\delta^{13}C$ signature of urea (−20.2‰) with soil (−22.4‰), both urea-derived CO_2 and SOM-derived CO_2 were grouped to SOM-derived CO_2.

The contribution of stalk-derived C to TOC in fumigated (F) and non-fumigated (NF) soils could be calculated following Equation (1). Stalk-derived MBC was calculated as Paterson and Sim [24]:

$$MBC_{Stalk} = [(P_{Stalk} \times TOC)_F - (P_{Stalk} \times TOC)_{NF}] / K_{EC} \ (K_{EC} = 0.45) \quad (2)$$

PE was calculated by comparing the amount of SOM-derived CO_2 in stalk-addition or N-addition treatments to the amount of CO_2 produced in control [11]. 209-d cumulative CO_2 production, stalk decomposition, SOM mineralization, and PE were estimated by integrating the corresponding respiration rate over time. For urea treatments (N2L0 and N2L1), urea-derived CO_2 was deducted from SOM-derived CO_2 during the calculation of cumulative CO_2 production, cumulative SOM mineralization, and cumulative PE considering the similar $\delta^{13}C$ signature of urea (−20.2‰) and soil (−22.4‰). Here, we assumed all urea-C (66 mg C kg^{-1} soil) was respired considering its rapid hydrolysis and little microbial assimilation. For example, Marsh et al. [27] found that after 29 days incubation, the recovery of urea-^{14}C as CO_2 could reach 85%. Another study on black soil in China showed that after 6 weeks of incubation, the residual urea-^{13}C was less than 1% [28].

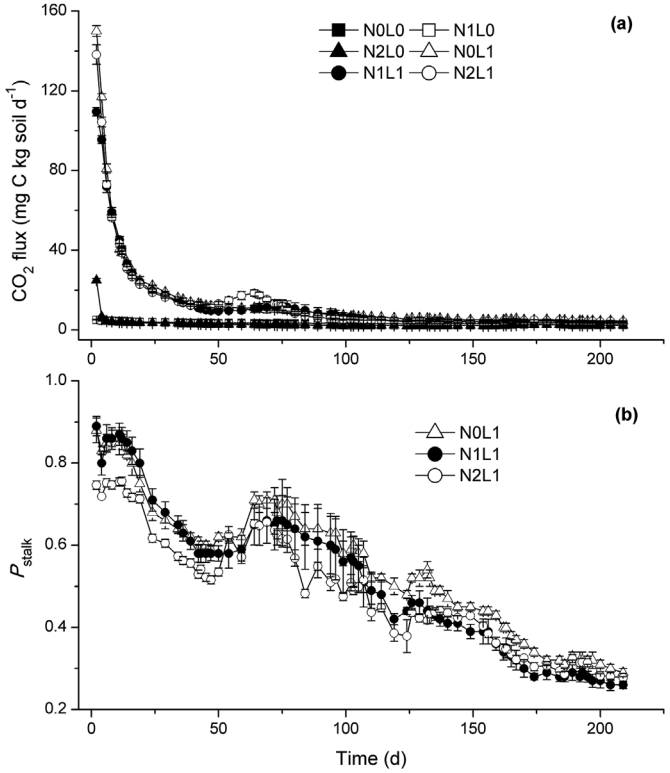

Fig. 1. Temporal variations of CO_2 emission rate (a) and the contribution of added maize stalk to CO_2 respiration (P_{stalk}) (b) under different treatments. Results are means ± SD (n = 4) on every single time-point. N0L0 (control, neither N nor maize stalk was added), N1L0 (NH_4NO_3 addition alone), N2L0 (urea addition alone), N0L1 (stalk addition alone), N1L1 (both NH_4NO_3 and stalk addition), N2L1 (both urea and stalk addition).

2.5. Statistical analysis

Statistical analysis was carried out with the SPSS 18.0 package (SPSS, Chicago, IL). Normality and homogeneity of variance of data were tested prior to analyses. Two-way and one-way ANOVAs were used to analyze the effects of stalk addition and/or N addition on the cumulative CO_2 respiration, SOM mineralization, stalk decomposition, and PE, respectively. Tukey HSD tests were used to examine the differences in the mean values among treatments. A $P < 0.05$ was chosen to indicate statistical significance.

3. Results

3.1. CO_2 production

Compared to soils without N addition (N0L0 and N0L1), NH_4NO_3 and urea supressed CO_2 flux (Fig. 1) and averagely decreased the 209-d cumulative CO_2 production by 16.5% and 17.9%, respectively (Fig. 2, Table 1); compared to soils without stalk application (N0L0, N1L0 and N2L0), stalk addition averagely induced a 3.3 times increase in the 209-d cumulative CO_2 production ($p < 0.05$) (Fig. 2, Table 1). NH_4NO_3 (N1L1) and urea addition (N2L1) depressed the 209-d cumulative stalk decomposition (1912 mg C kg^{-1} soil) by 12.1% and 16.4%, respectively. Compared to control (N0L0) (729 mg C kg^{-1} soil), NH_4NO_3 addition (N1L0) and urea addition (N2L0) decreased 209-d cumulative SOM mineralization by 22.0% and 27.6%, respectively in soils without stalk addition. However, in stalk-amended soils, NH_4NO_3 addition (N1L1) and urea addition (N2L1) stimulated 209-d cumulative SOM mineralization by 8.4% and 30.2%, respectively, compared to N0L0 (Fig. 2).

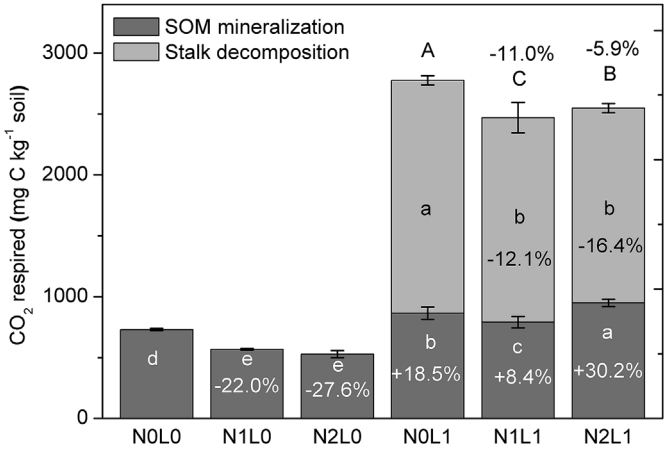

Fig. 2. Cumulative stalk decomposition (light gray bar), cumulative soil organic matter (SOM) mineralization (dark gray bar) and total CO_2 respiration after the 209 d incubation. Results are means ± SD (n = 4). Different letters above the bar (capital letters), in light gray bar (lowercase letters in black color) and in dark gray bar (lowercase letters in white color) denote significant differences ($p < 0.05$) in the total CO_2 respiration, cumulative stalk decomposition and cumulative SOM mineralization respectively among treatments based on Tukey HSD tests. Data above the bar, in light gray bar, and in dark gray bar represent percentage change of cumulative CO_2 respiration compared to N0L1, cumulative stalk decomposition compared to N0L1 and cumulative SOM mineralization compared to N0L0 respectively.

Table 1
F-value of two-way ANOVA (stalk addition × N form) on total CO_2 respiration and SOM mineralization.

	Total CO_2 respiration	SOM mineralization
Stalk[a]	9582.7**	372.0**
N form[b]	45.1**	25.4**
Stalk × N form	4.87*	39.8**

*The mean difference is significant at the 0.05 level; ** the mean difference is significant at the 0.01 level.

[a] "Stalk" represents 2 levels of stalk addition (with or without stalk addition).

[b] "N form" represents 3 patterns of N addition (NH_4NO_3, urea or no N addition).

3.2. Priming effect and soil C sequestration

During the 209-d incubation, NH_4NO_3 alone and urea alone induced a negative SOM priming of 161 mg C kg^{-1} soil and 201 mg C kg^{-1} soil, respectively. For stalk alone treatment (N0L1), PE on the 209-d cumulative SOM mineralization was 135 mg C kg^{-1} soil (Fig. 3). When NH_4NO_3 was added (N1L1), the combined PE of NH_4NO_3 + stalk addition was only 61 mg C kg^{-1} soil (Fig. 3). When urea was added (N2L1), the combined PE of urea + stalk addition was 220 mg C kg^{-1} soil, which was significantly higher than that of N0L1 and N1L1. However, there was no difference ($p > 0.05$) in gross C sequestration (stalk-derived C incorporation into the soil) and net C sequestration (stalk-derived C incorporation into the soil minus primed soil C) among the three stalk addition treatments (Fig. 4).

3.3. Soil DOC and MBC

There were no significant differences ($p > 0.05$) in soil DOC among different treatments after the 209-d incubation (Table 2). For stalk-amended treatments (N0L1, N1L1 and N2L1), stalk-derived DOC and SOM-derived DOC were also no significant differences (Fig. 5a). Total MBC in stalk-amended soils were averagely 1.1 times higher than that in soils without stalk addition (averagely 115 mg C kg^{-1} soil) ($p < 0.05$) (Table 2). For stalk-amended soils, stalk-derived MBC

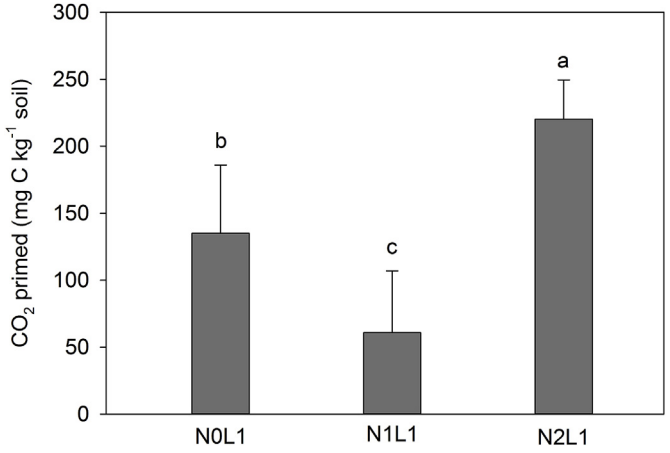

Fig. 3. Cumulative priming effect (PE) after the 209 d incubation. Different letters above the bar denote significant differences ($p < 0.05$) among treatments based on Tukey HSD tests (n = 4).

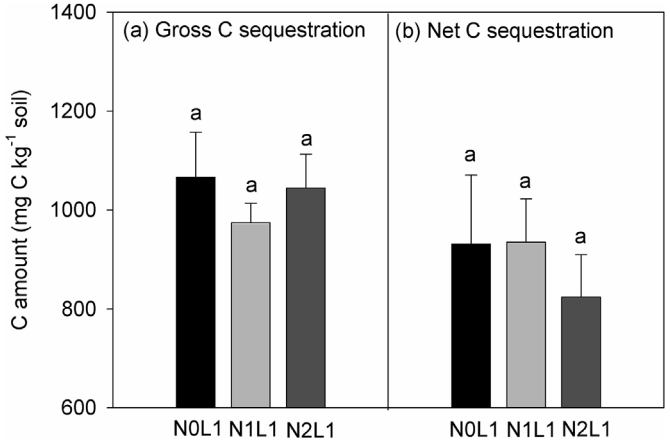

Fig. 4. (a) Gross carbon (C) sequestration (stalk-derived C incorporation into the soil) and (b) net C sequestration (stalk-derived C incorporation into the soil minus primed soil C) in three stalk treatments after the 209 d incubation. The same letter above the bars means no significant differences ($p > 0.05$) among treatments.

Table 2
Dissolved organic carbon (DOC), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and MBC/MBN in different treatments after the 209 d incubation. Mean values (SD) are shown (n = 4).

Treatment	DOC (mg C kg^{-1} soil)	MBC (mg C kg^{-1} soil)	MBN (mg N kg^{-1} soil)	MBC/MBN
N0L0	81.5(4.3)	137(18)	33.8(4.2)	4.1(1.0)
N1L0	82.0(1.5)	104(9)	67.6(20.6)	1.6(0.3)
N2L0	74.9(7.7)	105 (14)	69.6(19.0)	1.6(0.4)
N0L1	78.3(1.0)	240 (22)	22.4(3.3)	10.9(1.6)
N1L1	77.4(2.2)	230 (35)	74.6(12.6)	3.1(0.6)
N2L1	78.2(1.2)	248 (19)	70.1(8.3)	3.6(0.5)
Results of two-way ANOVA (p value)				
Stalk	0.35	0.00	0.81	0.00
N form	0.17	0.14	0.00	0.00
Stalk × N form	0.11	0.20	0.39	0.00

decreased by 18.4% when NH_4NO_3 was added ($p < 0.05$), while it had little change when urea was added (Fig. 5b). Total MBN in N-added soils (N1L0, N2L0, N1L1 and N2L1) were averagely 1.5 times higher than that in soils without N addition (Table 2). However, there were no significant differences in MBC, MBN and MBC/MBN between NH_4NO_3 treatments and urea treatments regardless of stalk addition (all $p > 0.05$) (Table 2).

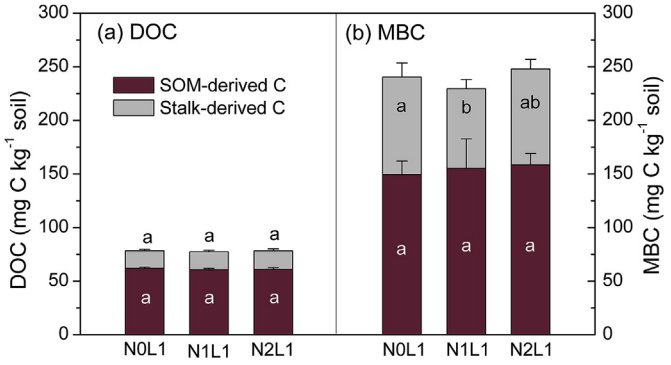

Fig. 5. Stalk- and SOM-derived C in (a) DOC and (b) MBC of 3 stalk treatments after the 209 d incubation. The same letter inside or above the bars means no significant differences ($p > 0.05$) among treatments.

3.4. Fate of added stalk

After the 209-d incubation, the total recovery of stalk-C in CO_2, DOC, soil matrix (DOC was excluded) and the remaining stalk was 98.9% for N0L1, 95.7% for N1L1 and 99.2% for N2L1, respectively (Table 3). The proportion of the remaining stalk-C in N1L1 or N2L1 was 6.3% and 10.0% higher than in N0L1, respectively, which was consistent with the less stalk-derived CO_2 in N1L1 or N2L1 and similar gross C sequestration (stalk-C incorporation into the soil) among the three treatments (Table 3, Figs. 2 and 4a).

4. Discussion

4.1. SOM priming of N fertilization and stalk addition

We found that the effects of N addition on SOM mineralization was similar between the two types of N fertilizer when applied alone. In the absence of stalk addition, NH_4NO_3 (N1L0) and urea addition (N2L0) significantly reduced SOM mineralization by 22.0% and 27.6% compared to control ($p < 0.05$), respectively (Fig. 2), which supported the hypothesis that microbes preferentially used exogenous N and induced less mineralization of SOM. In addition, both NH_4NO_3 and urea addition could increase NH_4^+ concentration rapidly and cause ammonium poisoning to soil microbes [19,20], which is disadvantageous to SOM mineralization. The reduction of MBC in N1L0, N2L0 compared to control we observed (MBC of N0L0, N1L0 and N2L0 after the 209-day incubation were 137, 104 and 105 mg C kg^{-1} soil, respectively) further supported this explanation (Table 2). Our results were consistent with the findings of Ding et al. [29] and Grandy et al. [30] who also found negative effects of N fertilization on SOM mineralization in agricultural soils.

For stalk alone treatment without N addition (N0L1), stalk application increased the 209-d cumulative SOM mineralization by 135 mg C kg^{-1} soil (compared to N0L0) (Fig. 2). Many mechanisms, e.g. preferential substrate utilization, nutrient mining theory and co-metabolism theory, could function together to induce negative/positive

Table 3
Recovery rate (%) of added stalk in different C pools at the end of the 209 d incubation. Mean values (SD) are shown (n = 4).

Treatment	CO_2%	DOC %	Soil matrix %	Remaining stalk %	Total recovery %
N0L1	55.6(1.1)	0.5(0.04)	30.5(2.6)	12.3(3.5)	98.9(2.9)
N1L1	48.8(3.6)	0.5(0.04)	27.8(1.1)	18.6(1.8)	95.7(3.4)
N2L1	46.5(1.1)	0.5(0.06)	29.9(2.0)	22.3(2.5)	99.2(1.6)

PE on SOM mineralization [9,23]. Previous studies suggested that PE mechanisms were closely related to residue decomposition stage [23,31,32]. Preferential substrate utilization is a response of microbes to high substrate C availability, which could lead to low or even negative PE but might only last for initial several days [23,31]. Nutrient mining mechanism proposes that C addition could increase N-mining from SOM as a response of soil microbial growth to available C sources but limiting nutrients [17,18], which prevails during the slow residue decomposition phase with low nutrient availability [23]. Co-metabolism mechanism proposes that the extracellular enzymes produced by stalk-stimulated microbes are capable of degrading recalcitrant SOM, causing positive PE [11], which depends on microbial activity varying with residue decomposition stage [31]. Our result of 209-d cumulative PE (135 mg C kg^{-1} soil) under N0L1 reflected a combined effect of all these mechanisms.

4.2. The interactive PE of stalk and N fertilization

In the presence of stalk addition, the inhibition effect of N addition on SOM mineralization was not only counter-balanced, but also reversed. When NH_4NO_3 was added (N1L1), NH_4NO_3 + stalk addition increased SOM mineralization by 8.4% (61 mg C kg^{-1} soil), indicating a significant lower positive PE than stalk alone treatment (N0L1) (Fig. 3). Our result supported previous findings that mineral N (particularly NH_4^+) addition suppressed the PE on SOM mineralization [33] and hinted that N-mining mechanism prevailed under stalk alone treatment (N0L1). NH_4NO_3 + stalk (N1L1) addition increased N availability to soil microbes (Fig. 6), inhibiting the utilization of SOM as N sources. Therefore, although NH_4NO_3 + stalk treatment (N1L1) still had positive PE, the effect was much lower than stalk alone addition (N0L1). Alternatively, although MBC and SOM-derived MBC were not statistically different among different N addition levels when stalk was present (Table 2, Fig. 5), stalk-derived MBC had the tendency to be lower under NH_4NO_3 + stalk treatment (N1L1) compared to stalk alone addition (N0L1) (Fig. 5), suggesting potentially less “co-metabolism” of SOM by soil microbes.

On the contrary, the PE of urea + stalk addition (N2L1) was 220 mg C kg^{-1} soil, which was higher than the PE by stalk alone (N0L1, 135 mg C kg^{-1} soil) (Fig. 3). Therefore, when stalk was present, urea addition did not inhibit SOM mineralization as NH_4NO_3 did. Both the “N-mining” and the “co-metabolism” mechanism probably exist during the urea hydrolysis process. While NH_4^+ was directly used by microbes under NH_4NO_3 + stalk treatment (N1L1), urea needs to be hydrolyzed by soil urease first. While soil microbial activity could not be effectively enhanced under low substrate C availability (e.g. N2L0) due to C limitation, microbial activity could be enhanced under high substrate C availability (e.g. N2L1). In order to synthesize urease, microbes need to get more N from SOM, different from the mechanism under NH_4NO_3 addition when they could use N directly from NH_4NO_3. In addition, the synthesis of urease may be accompanied by the synthesis of other extracellular enzymes, which were capable of degrading recalcitrant SOM with the amended stalk as the energy source, and caused higher PE under N2L1 than under N0L1 or N1L1 (Fig. 3) (co-metabolism theory). The stimulation of microbial growth by stalk addition (as indicated by higher MBC compared to control experiment without stalk addition in Table 2) and the tendency of higher stalk-derived MBC under urea + stalk treatment (N2L1) than NH_4NO_3 + stalk treatment (N1L1) (Fig. 5) further supported this statement. Therefore, no matter which mechanism of PE existed, the effects of urea and stalk addition on SOM mineralization were different from the effects of NH_4NO_3 and stalk addition. For better understanding of PE mechanisms, further studies should pay more attention to the change of microbial biomass and extracellular enzyme activities over time.

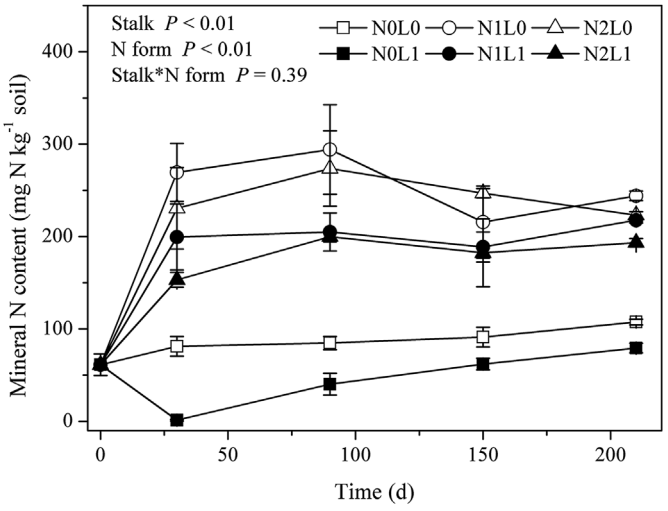

Fig. 6. Dynamics of mineral N (NH_4^+ + NO_3^-) content (mg N kg^{-1} soil) under different treatments during the 209 d incubation. Results are means ± SD (n = 4) on every single time-point.

4.3. C budget of stalk-C sequestration and SOM priming

It should be noted that stronger positive PE does not necessarily mean greater net C loss because maize stalk incorporation could compensate primed SOM mineralization. In fact, we found that after 209 days incubation, there was no significant difference in gross C sequestration (total amount of stalk-derived C incorporation into the soil) or net C sequestration (total amount of stalk-derived C incorporation into the soil minus primed soil C) among different stalk + N treatments (Fig. 4). Therefore, considering its higher N use efficiency by plants compared to NH_4NO_3, urea is still recommended as N fertilizer although the combination of urea fertilizer and stalk amendment caused higher PE on SOM mineralization. Moreover, in natural ecosystems, in the context of increasing litterfall caused by elevated CO_2, elevated reactive organic N (including urea) deposition might stimulate soil respiration [4,34], different from previously reported suppression of soil respiration under mineral N addition alone [35,36]. Thus, to verify our results, further studies should pay more attention on this different N form effects on C cycling and other ecological processes.

5. Conclusions

Our results suggested that different chemical forms of N had distinct effects on the PE of stalk on SOM mineralization. When there was no stalk addition, both NH_4NO_3 and urea addition significantly decreased SOM mineralization. Stalk addition caused positive PE and increased SOM mineralization regardless of N addition or not, and the positive PE under urea + stalk treatment was much higher than that under NH_4NO_3 + stalk treatment. However, net C sequestration (stalk-C incorporation into the soil minus primed soil C) among different stalk + N treatments was not statistically different because new C incorporation under urea + stalk treatment tended to be higher than that under NH_4NO_3 + stalk treatment. Our lab incubation experiment only provided evidence of different effects of NH_4NO_3 and urea on soil C cycling, and more *in situ* work is needed to test the generality of our findings and to guide agricultural practices.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2018.10.002.

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