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Crop residue-derived dissolved organic matter accelerates the decomposition of native soil organic carbon in a temperate agricultural ecosystem

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ABSTRACT

Crop residue-derived dissolved organic matter (DOM) plays an important role in soil carbon (C) cycling. To investigate the effects of maize residue-derived DOM and urea additions on the native soil organic carbon (SOC) decomposition and soil net C balance a pot experiment was carried out during the winter wheat growing season in the North China Plain (NCP). The results showed that adding maize residue-derived DOM alone (RDOM) or together with urea (RDOM + N) accelerated the decomposition of native SOC and resulted in a net SOC loss. The net loss of SOC was 3.90 ± 0.61 and 3.53 ± 0.48 g C m^{-2} in RDOM and RDOM + N treatments, respectively. The stimulatory effect of per unit DOM-C addition on the native SOC decomposition was 0.25 ± 0.05 and 0.45 ± 0.07 for the RDOM and RDOM + N treatments, respectively. Increases in the microbial biomass and the activity of β-glucosidase, invertase and cellobiohydrolase as well as soil mineral N content were responsible for a more intense priming effect in DOM-amended soils. The positive relationship between primed soil C and soil available N ($R = 0.76$, $P < 0.05$) suggested that the stimulation of decomposition of native SOC by DOM addition would be enhanced by nitrogen fertilizer application.

1. Introduction

Soil carbon pool as the largest terrestrial carbon pool is twice as large as that in the atmosphere, and three times as large as that in the biotic pool. Therefore, even a small change in the turnover intensity of soil organic carbon (SOC) can lead to a large change of CO_2 concentration in the atmosphere and have potential feedbacks to climate [1,2]. Inputs of exogenous organic matter may accelerate or retard the mineralization of native SOC through a priming effect, and thus have a potential to change SOC dynamics [3,4]. Many previous studies on the priming effect are conducted under laboratory conditions and simple organic substrates (e.g. glucose and sucrose) are often used [5–7], whereas little information is available about the effect of plant-derived DOM (a common and representative input to soils) on the priming effect under field conditions. Numerous studies have observed that global warming and elevated atmospheric CO_2 can increase DOM input to soil through litter decomposition and root exudates [8–10], therefore, it is very important to investigate the impact of plant-derived DOM on the decomposition of native SOC in the field.

Plant-derived DOM is more complex than simple organic substrates and composed of a rapidly and slowly decomposable fraction [11]. Thus, apart from the priming effect induced by labile DOM, a fraction of the recalcitrant DOM may remain in the soil and offset the SOC loss induced by priming. Therefore, we cannot state that DOM input is not beneficial for soil C sequestration just according to a positive priming effect, since primed C is only a part of soil C budget [6]. We should take the primed C and the added C remaining in the soil into account. Many priming studies have focused on the native SOC loss induced by labile organic matter input, but less attention has been paid to the net SOC balance between the primed C and the added C remained in the soil [6,12]. Therefore, apart from the priming effect induced by plant-derived DOM, it is necessary to investigate its net effect on soil C balance.

In general, the priming effect is induced by the exogenous organic carbon but its intensity is controlled by soil nutrient availability [7,13]. However, previous studies have much controversy about soil N availability on the decomposition of native SOC [7,12,14]. Two opposite theories have been proposed. One is called "stoichiometric decomposition" theory, which means if the C and N inputs with substrate meet the demands of microorganisms, the microbial activity and the decomposition rate will increase, that is, a higher soil N availability is likely to accelerate the decomposition of native SOC [7]. In contrast, the 'microbial nitrogen mining' theory

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hypothesizes that low-N availability stimulates SOC decomposition, since in N-poor condition the microbes need decompose recalcitrant SOC to acquire N to meet their demands [7]. Although priming effects have been extensively studied after the supply of labile organic substrate, few studies have focused on the interactive effect of N availability and labile organic substrates on the priming effect. Therefore, a combination of DOM and N application in the field with crop growth is favorable for us to understand their interactive effects on the decomposition of native SOC, and helps us to improve fertility management and increase soil C sequestration in agricultural soils.

Soil organic matter is composed of different organic substrates with different forms and degradability [7]. Its decomposition can occur through several processes initiated by extracellular hydrolysis. There is evidence hydrolytic enzyme activity such as β-glucosidase, invertase and cellobiohydrolase responses sensitively during the decomposition process of SOC [15,16], and extracellular enzyme activities reflect well the functions of decomposer communities, depending on metabolic requirements and nutrient availability [7,17]. Therefore, the activities of extracellular enzymes may change significantly during the decomposition of native SOC and exogenous labile organic substrates. The linkage between extracellular enzyme activities and the priming effect during the decomposition of organic substrates may be important to clarify the underlying mechanisms of the priming effect.

In China, approximately 70 million tons of crop residues are produced each year [18]. A winter wheat-summer maize is a general cropping system in the North China Plain (NCP). Incorporation of crop residues directly into soils is a traditional practice in the NCP when crops are being harvested by machine [19]. A large quantity of grinding crop residue input will increase the release and production of crop residue-derived DOM. Therefore, we hypothesized that: (1) the input of crop residue-derived DOM will stimulate the decomposition of native SOC through a positive priming effect; (2) addition of N into DOM-amended soils will enhance the intensity of the priming effect induced by DOM addition; and (3) the stimulation of decomposition of native SOC by addition of DOM will be supported by increases in microbial biomass and enzyme activity. The purposes of this study were (1) to investigate the effects of maize residue-derived DOM input on the decomposition of native SOC and soil net C balance during the wheat growing season; (2) to determine the N availability on the priming effect induced by DOM addition; and (3) to link microbial biomass and extracellular enzyme activities with the observed priming effect.

2. Materials and methods

2.1. Study site

This study was carried out at Yucheng Agricultural Experiment Station of Chinese Academy of Sciences (36°50′N, 116°34′E), which is located in the North China Plain with a mean annual temperature and precipitation of 13.1 °C and 593 mm, respectively. About 70% of the annual precipitation falls between June and September. The soil samples were collected from the upper 20 cm layer of soil which has been practiced for a rotation of summer maize and winter wheat for at least about 30 years in this experimental station. Soils were air-dried and sieved (<2 mm) and then thoroughly mixed. The total C and N concentrations in the soil were 15.16 and 0.89 g kg^{-1}, respectively. Soil was described as calcaric fluvisols with a pH of 7.90. The sand, silt, and clay contents in the soil were 12%, 66% and 22%, respectively.

2.2. ^{13}C-labeled DOM

A pulse labeling method was used to label maize leaves [20]. Briefly, maize leaves were exposed to 99.9% atom % $^{13}CO_2$ for 5 days, and the daily labeling lasted for 3 h from 9:00 to 12:00 am. The maize leaf was enveloped with an airtight transparent plastic tent which was 90 cm in length and 30 cm in width. The air in the plastic tent was evacuated, taking care not to physically damage the maize plants and leaves, prior to labeling. At the end of labeling, maize seedlings were harvested, dried, and ground. Thereafter, the finely ground maize residues were put into an 80 L barrel and extracted for one month in 50 L of deionized water at 25 °C. Following extraction, the supernatant solution containing the ^{13}C-labeled DOM was filtered through 0.45 μm membrane filters, stored at −20 °C, and thawed before application to soil. Samples were immediately analyzed for initial DOC, total nitrogen (TN) and δ^{13}C-DOC.

2.3. Experimental design

A pot experiment with winter wheat cropping system was set up on 10 October 2014. The pot experiment included four treatments: soil

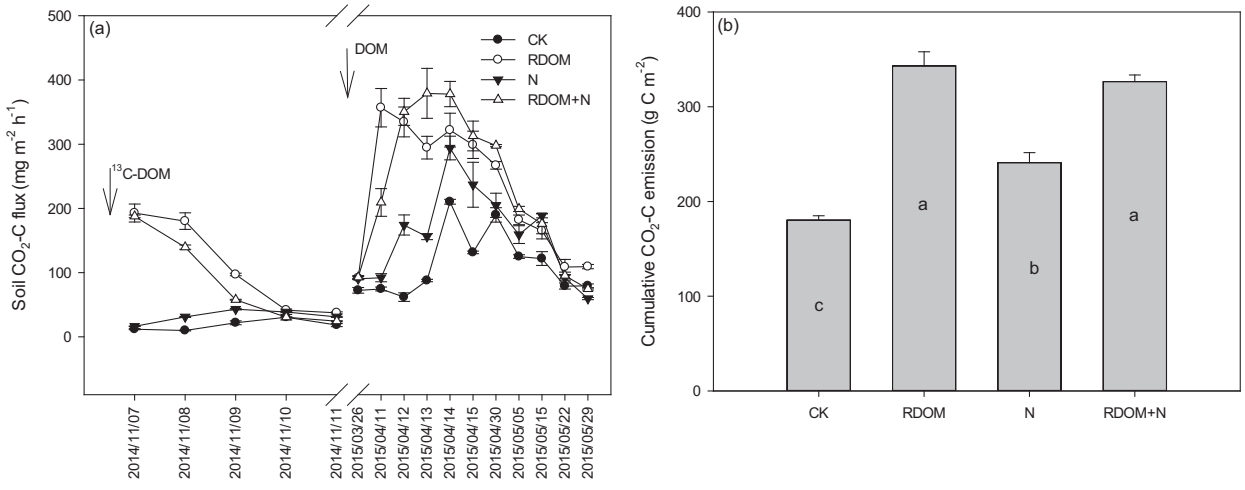

Fig. 1. Effects of maize residue-derived DOM and urea additions on (a) soil CO_2 flux and (b) cumulative soil CO_2 emission. CK represents soil amended with deionized water (●), RDOM means soil amended with maize residue-derived DOM (○), N means soil amended with urea (▼), RDOM + N represents soil amended with maize residue-derived DOM and urea (△). Bars indicate standard error of the mean ($n = 3$). Arrows indicate the dates of the inputs of maize residue-derived DOM and urea. Different letters in the same column represent significant difference at $P < 0.05$.

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Table 1
Summary of *p*-values of two-way ANOVA with effects of DOM, urea and their interaction on soil total CO_2 emission, priming effect, MBC, MBN and the activity of β-glucosidase, invertase and cellobiohydrolase.

	DOM	N	DOM × N
Total soil CO_2 emission	<0.01	<0.05	<0.01
Native SOC-derived CO_2	<0.01	<0.05	<0.01
MBC	<0.05	<0.01	<0.05
MBN	<0.01	<0.01	<0.01
β-Glucosidase	<0.01	ns	ns
Cellobiohydrolase	<0.01	ns	<0.01
Invertase	<0.01	ns	<0.01
DOC	ns	<0.01	ns
Mineral N	<0.01	<0.01	<0.01

Note: ns, not significant.

without substrate addition (CK), soil amended with ^{13}C-labeled maize residue-derived DOM (RDOM, δ^{13}C-DOC = 154.00‰), soil amended with urea (N), and soil amended with ^{13}C-labeled maize residue-derived DOM and urea (RDOM + N). Three replications of each treatment were laid out in a completely randomized block design. The pots (30 cm in height and 40 cm in diameter) were filled with 40 kg of soil samples at a density of 1.3 g cm^{-3}. To simulate field conditions, pots were buried into the field and equilibrated under field conditions for two weeks before seeds were sowed. The initial soil moisture was adjusted to 70% of water holding capacity, and the water holding capacity of the soil is 21%. Winter wheat grown in each pot was evenly sown in two rows with a 15 cm row spacing on 14 October 2014, and harvested on 8 June 2015. Each pot grew 80 wheat plants and thinned to 60 plants after seedlings appeared.

In the wheat growing season, all treatments (except the CK) received the same total amount of N application at a rate of 200 kg N ha^{-1}, which is the recommended N application rate in the NCP. The RDOM treatment received 200 kg DOM-N ha^{-1}, and the N treatment received 200 kg urea-N ha^{-1}, whereas the RDOM + N treatment received 100 kg DOM-N ha^{-1} and 100 kg urea-N ha^{-1}. Both DOM and urea were split applied at two growth stages: wheat seedlings appeared (6 November 2014) and boot stage (10 April 2015). To achieve the same soil moisture, both DOM and urea were applied as solution and the control was applied the same amount of deionized water.

To differentiate the priming effect induced by the application of DOM, the ^{13}C-labeled DOM was only applied in the first stage, whereas in the second stage we added the DOM without labeled. The unlabeled DOM was extracted from the unlabeled maize which grew in the same condition as the above mentioned labeled maize. The total amount of DOM-C applied to soil associated with the addition of DOM was 320 and 160 kg DOM-C ha^{-1} in the RDOM and RDOM + N treatments, respectively. Therefore, we only calculated the priming effect induced by the first application of ^{13}C-labeled DOM, which lasted for about 154 d after the addition of ^{13}C-labeled DOM.

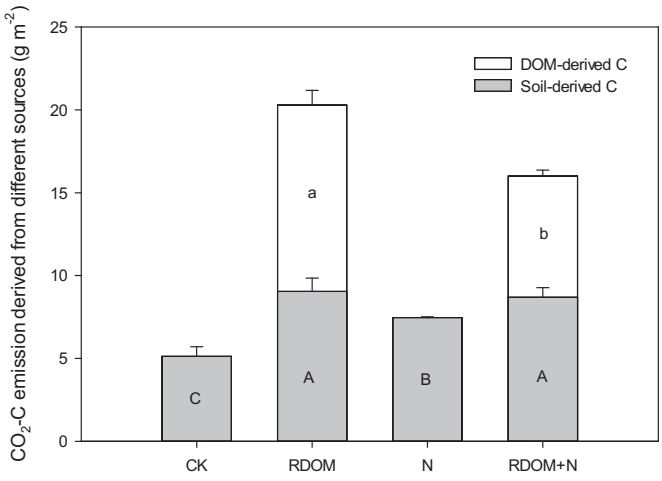

Fig. 3. Contributions of DOM- and SOC-derived C to the cumulative CO_2 emissions over 154 days following the addition of ^{13}C-labled DOM. CK represents soil amended with deionized water, RDOM means soil amended with maize residue-derived DOM, N means soil amended with urea, RDOM + N represents soil amended with maize residue-derived DOM and urea. Different letters (lower case letters for DOM-derived C, upper case letters for SOC-derived C) indicate significant difference at $P < 0.05$. Bars indicate standard error of the mean ($n = 3$).

2.4. Gas sampling and analysis

Gas samples for CO_2 analyses were measured daily in the first week after the addition of DOM and urea, and then sampled weekly in the following measurement, except when the soil was covered by snow in winter (from December to February). Gas samples were taken from closed static-chamber (25 cm in height and 10 cm in diameter). The chambers were inserted directly into the soil to a 5 cm depth in November 2014 after wheat was germinated. Care was taken to minimize disruption to the soil, particularly to that inside of the chamber, during

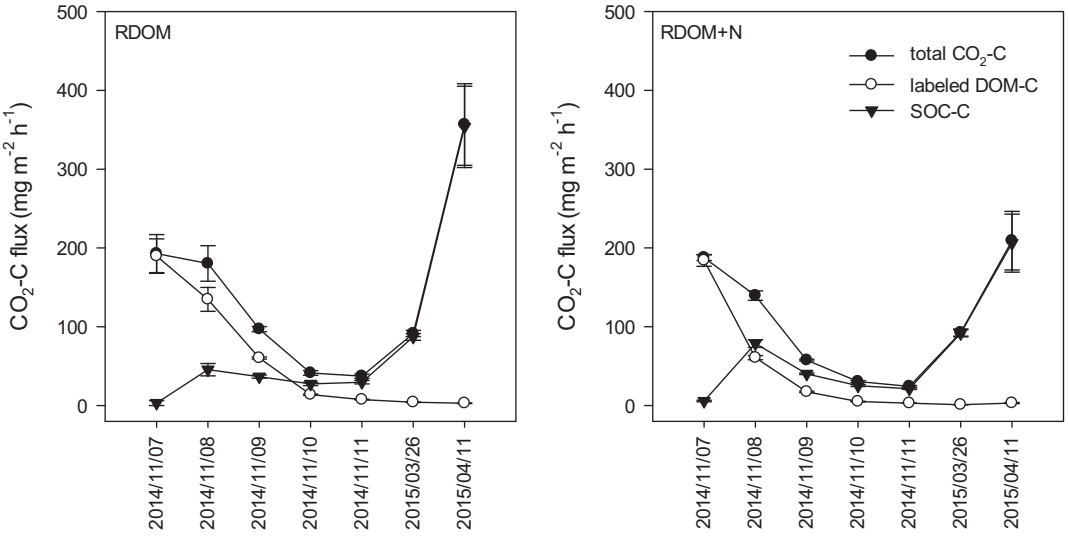

Fig. 2. Dynamics of the CO_2 emission rate from labeled DOM-C and native SOC during the first 154 days after the addition of ^{13}C-labeled DOM. Values represent means ± SE, $n = 3$.

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Table 2
Effects of ^{13}C-labeled DOM and N additions on the soil C balance (g C m^{-2}, means ± SE).

	RDOM	N	RDOM + N
① DOM-C input	16a	0c	8b
② DOM-C lost as CO_2-C	11.25 ± 0.52a	0c	7.32 ± 0.21b
③ DOM-C remained in soil	0.02 ± 0.05a	0a	0.03 ± 0.01a
④ Native SOC lost as CO_2-C via positive PE	3.92 ± 0.47a	2.34 ± 0.03b	3.56 ± 0.33a
Soil carbon balance(③–④)	−3.90 ± 0.61a	−2.34 ± 0.03b	−3.53 ± 0.48a

Note: Different letters in the same row indicate significant difference at $P < 0.05$.

insertion. To minimize any effects of diurnal variations in CO_2 emissions, gas samples were taken between 9:00 am and 11:00 am at each sampling time.

Gas samples were taken 4 times at a sampling interval of 5 min (i.e., 0, 5, 10, and 15 min) with 25 mL syringes after closing the chamber. In addition, the air temperature inside the chamber and the soil temperature and moisture at 5 cm depth were recorded at the time of gas sampling. The concentrations of CO_2 were determined using a gas chromatograph equipped with FID and ECD detectors (Agilent GC 4890, Kyoto, Japan).

The CO_2 samples for δ^{13}C-CO_2 analysis were collected after the application of ^{13}C-labeled DOM. Approximately 300 mL of headspace gas was collected after closing the chamber for about 4 h. After that the isotopic gas was injected into a pre-evacuated exetainer. The isotopic signature of the gas was measured with isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher Scientific, Inc., USA). The cumulative gas emissions were calculated by a linear interpolation between the measured daily fluxes.

The amounts of DOM- and SOC-derived C were calculated as the following equations [13]:

$$C_{RDOM} = C_t \times \frac{(\delta_t - \delta_{ck})}{(\delta_{RDOM} - \delta_{ck})} \quad (1)$$

$$C_s = C_t \times \frac{(\delta_{RDOM} - \delta_t)}{(\delta_{RDOM} - \delta_{ck})} \quad (2)$$

where C_t ($C_t = C_{RDOM} + C_s$) is the total CO_2-C emission and δ_t is the corresponding δ^{13}C value of the CO_2-C evolved from soil amended with ^{13}C-labeled DOM. C_{RDOM} is the amount of C originated from the labeled DOM-C and δ_{RDOM} is the δ^{13}C value of the labeled DOM-C ($\delta^{13}C$ = 154.00‰). C_s is the amount of C originated from native SOC and δ_{ck} is the δ^{13}C value of the CO_2-C evolved from soil without substrate addition.

The priming effect (PE) during the wheat growing season (from day 1 to day 154 after the addition of ^{13}C-labeled DOM) was calculated as the following equations [13]:

$$PE = [CO_2 - C]_{treatment} - [CO_2 - C]_{ck} \quad (3)$$

where $[CO_2\text{-}C]_{treatment}$ is the amount of CO_2-C derived from native SOC in the treatments with the addition of ^{13}C-labeled DOM and/or N; $[CO_2\text{-}C]_{ck}$ is the amount of CO_2-C derived from soil without substrate addition. In the present study, we assumed that the CO_2-C derived from urea breakdown was completely released during that period. Therefore, the urea-derived CO_2-C emission was subtracted from the total CO_2-C emission (4.28 and 2.14 g C m^{-2} for the N and RDOM + N treatments, respectively).

Soil net C balance was calculated as the difference between the amount of ^{13}C-DOM remaining in soil and the amount of soil-derived C lost because of the priming effect.

2.5. Soil chemical analysis

Soil samples (0–20 cm) were collected after the gas sampling and in an interval of every two weeks, except when the soil was covered by snow in winter (from December to February). Soils were sampled at a distance of 5 cm away from the wheat with a 4.9-cm diameter soil core sampler for chemical analysis.

Microbial biomass was determined by the chloroform fumigation extraction method [21]. The microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined by the difference between fumigated and non-fumigated soils using a corrective K factor of 0.38 and 0.45, respectively [22].

The β-glucosidase activity was assayed by the modified method of Eivazi and Tabatabai [23], using 2 g of fresh soil and incubated with 8 mL MUB buffer (pH 6.0) and 2 mL *p*-nitrophenyl b-D-glucopyranoside (PNGP) as the substrate for 1 h at 37 °C. Thereafter, the suspension was added with 2 mL of 0.5 M $CaCl_2$ and 16 mL of 0.1 M Tri-hydroxymethyl adjusted to pH 12.0 with NaOH. The suspension was filtered and the cleavage of the glycosyl moiety of PNGP was determined by measuring the release of *p*-nitrophenolate at 400 nm by colorimetric analysis. Values were corrected for a blank (substrate added immediately after the addition of $CaCl_2$ and Tri-hydroxymethyl buffer).

Invertase activity was determined as followed: 5 g of fresh soil sample was incubated with 15 mL of 1.2% sucrose solution, which was

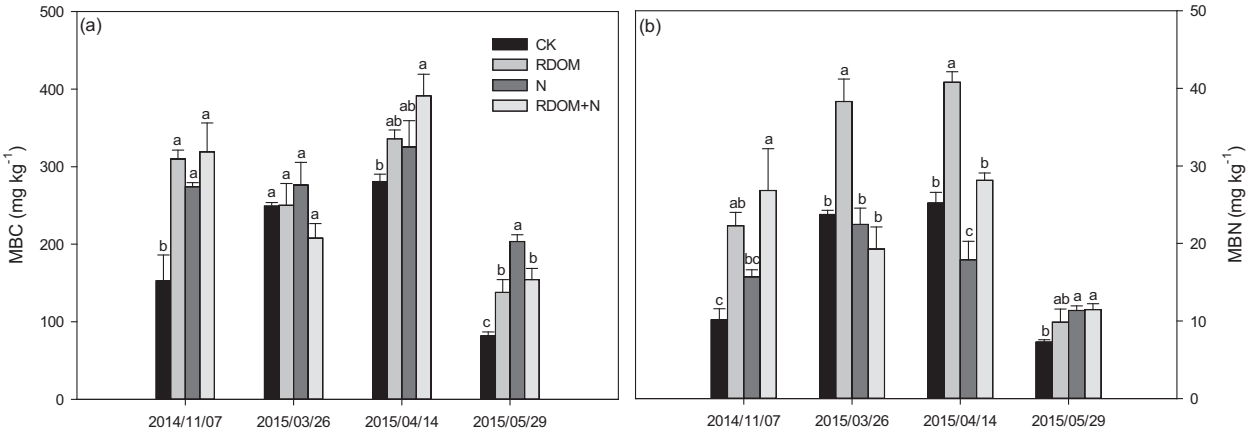

Fig. 4. Effects of maize residue-derived DOM and urea additions on (a) soil MBC content and (b) soil MBN content. CK represents soil amended with deionized water (■), RDOM means soil amended with maize residue-derived DOM (■), N means soil amended with urea (■), RDOM + N represents soil amended with maize residue-derived DOM and urea (■). Bars indicate standard error of the mean ($n = 3$). Different letters in the same column represent significant difference at $P < 0.05$.

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dissolved with 0.2 M acetic acid buffer solution adjusted to pH 5.5 with natrium aceticum. The sucrose solution was incubated at 50 °C for 3 h in the dark. Immediately after incubation, the soil suspension was filtered and 0.5 mL filtrate was used to measure the amount of glucose produced using 3,5-dinitrosalicylic acid colorimetry method at 540 nm by colorimetric analysis. Blanks were incubated without substrate.

The cellobiohydrolase activity was determined as followed: 5 g of fresh soil sample was incubated with 15 mL of 0.7% carboxymethyl cellulose solution, which was dissolved with 0.2 M acetic acid buffer solution (pH 5.5). The carboxymethyl cellulose solution was incubated at 50 °C for 24 h in the dark. Immediately after incubation, the soil suspension was filtered and 0.5 mL filtrate was measured using 3,5-dinitrosalicylic acid colorimetry method at 540 nm by colorimetric analysis.

Soil available N (NH_4^+-N and NO_3^--N) was extracted with 1 M NaCl (soil: solution = 1:5) by shaking for 30 min [24]. The dissolved NH_4^+-N and NO_3^--N in the extracts were analyzed by flow injection autoanalyzer (FIA) (SEAL Analytical, AA3, Germany).

The $\delta^{13}C$ of soil were measured by isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher Scientific, Inc., USA).

2.6. Statistical analysis

Data were confirmed with the Kolmogorov-Smirnov test for normality and with the Levene's test for homogeneity of variance prior to statistical analysis. All statistical analyses were performed using SPSS 16.0 (SPSS, Chicago, IL, USA). Repeated measures ANOVA with LSD test were performed to analyze the effects of maize residue-derived DOM and urea additions on soil CO_2 fluxes. Two-way ANOVA with LSD test was performed to analyze the difference in the cumulative CO_2, the SOC-derived CO_2-C emission, microbial biomass, enzyme activity and soil available N under different treatments. Independent-Samples *t*-test was used to compare the difference in the DOM-derived CO_2-C emission from soil amended with DOM alone and together with urea. Correlations between soil CO_2 emission and soil biochemical factors were measured by the Pearson correlation method. The significant difference was considered at $P < 0.05$. Graphs were performed using the SigmaPlot 12.5 software (Systat Software Inc., California, USA).

3. Results

3.1. Soil CO_2 emissions

Application of DOM alone or together with urea accelerated the emissions of total soil CO_2 (Fig. 1). The largest soil CO_2 fluxes were observed just after 1–5 days of the addition of DOM and/or urea, with maximum fluxes amounting to 404 mg CO_2-C m^{-2} h^{-1} (Fig. 1a). Total soil CO_2 emissions were significantly affected by the addition of DOM, urea and their interactive effects (Table 1). As compared with CK, the cumulative CO_2 emission increased by 47 ± 4%, 25 ± 6%, and 45 ± 2% in the RDOM, N and RDOM + N treatments, respectively (Fig. 1b).

3.2. Priming effect induced by the addition of DOM

Most of the CO_2 emissions were derived from the decomposition of added ^{13}C-labeled DOM just after the application of DOM (Fig. 2). The contribution of DOM-C to the total CO_2 flux decreased from 98% after the first day of DOM addition to 12–20% after 5 days of DOM addition and further decreased to 1–2% after 154 days of addition. In contrast, the contribution of SOC-derived C to the total CO_2 flux increased gradually (Fig. 2). This indicated that a shift in C substrate contributing to the CO_2 flux occurred during that period. In that period (from day 1 to day 154 following the first addition of ^{13}C-labeled DOM), about 56 ± 1% and 46 ± 1% of the cumulative CO_2 emissions were derived from the added ^{13}C-labeled DOM in the RDOM and RDOM + N treatments, respectively (Fig. 3).

The decomposition of native SOC was affected by the DOM, N and their interactive effect (Table 1). The cumulative priming effect in RDOM, N and RDOM + N was 3.92 ± 0.47, 2.34 ± 0.03, and 3.56 ± 0.33 g C m^{-2}, respectively (Fig. 3 and Table 2). The stimulatory effect of per unit of added DOM-C on the decomposition of native SOC was 0.45 ± 0.07 and 0.25 ± 0.05 for the RDOM + N and RDOM treatments, respectively. This indicated that the priming effect induced by DOM was enhanced by the addition of urea. According to the primed C and the ^{13}C-DOM remained in the soil we calculated the soil C input-output balance. The result showed that the supply of DOM alone or together with

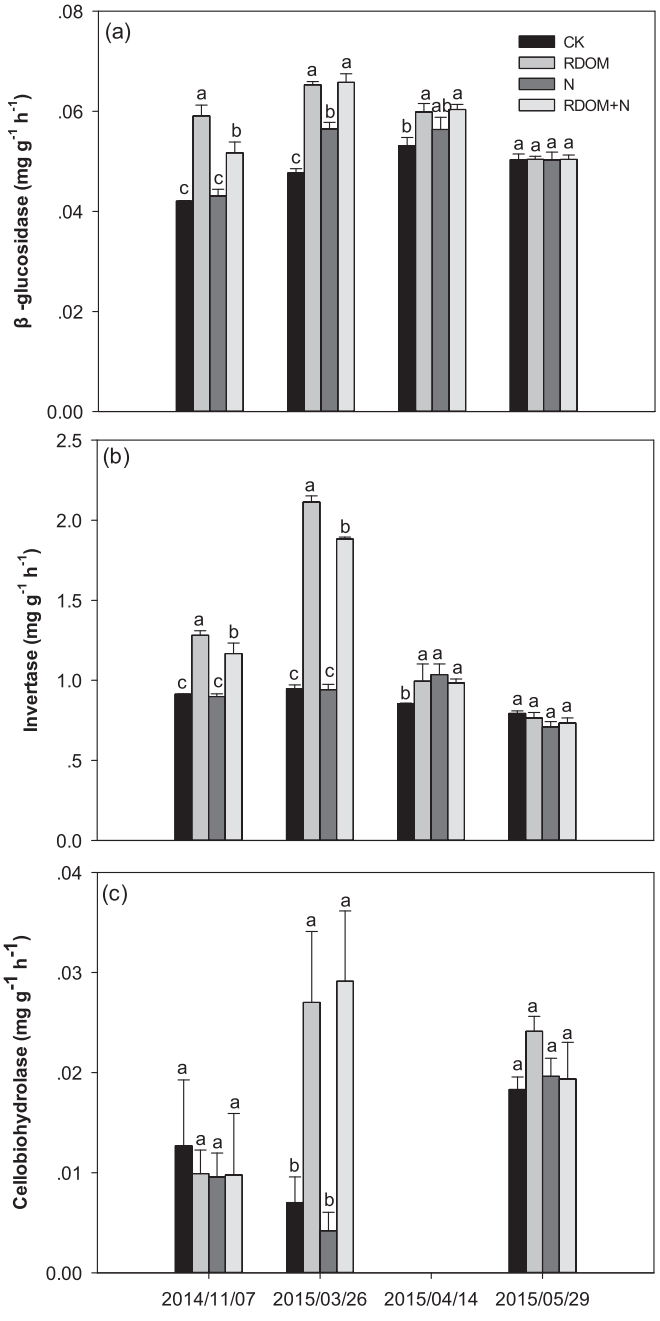

Fig. 5. Effects of maize residue-derived DOM and urea additions on the activity of (a) β-glucosidase, (b) invertase, (c) cellobiohydrolase and (d) urease. CK represents soil amended with deionized water (■), RDOM means soil amended with maize residue-derived DOM (■), N means soil amended with urea (■), RDOM + N represents soil amended with maize residue-derived DOM and urea (■). Bars indicate standard error of the mean ($n = 3$). Different letters in the same column represent significant difference at $P < 0.05$.

urea accelerated the decomposition of native SOC and resulted in a negative soil C balance (Table 2).

3.3. Soil microbial biomass

The MBC and MBN contents were affected by the application of DOM, urea and sampling time (Fig. 4). The MBC content increased rapidly just after the first application of DOM and urea, and then it decreased gradually. Throughout the entire experiment, the MBC contents in the RDOM, RDOM + N and N treatments were comparable, but significantly higher than the CK. The highest MBN was observed in the RDOM treatment, followed by the RDOM + N and N treatments. The responses of MBC and MBN to the addition of DOM and urea suggested that the tested soil could be co-limited by C and N.

3.4. Microbial enzyme activity

The activity of β-glucosidase, invertase and cellobiohydrolase increased significantly after the first and second applications of DOM and urea (Fig. 5). This effect was more pronounced in the RDOM and RDOM + N treatments than that in the N treatment. However, the positive effect of DOM addition on the activity of β-glucosidase, invertase and cellobiohydrolase decreased gradually over time. Overall, enzyme activities directed toward acquisition of labile carbon increased after the addition of DOM.

3.5. Soil DOC and available N contents

Throughout the entire wheat growing season, soil DOC content was not significantly affected by the application of DOM (Table 1 and Fig. 6a), but its content decreased significantly in the N treatment. This could be ascribed to a higher microbial biomass and enzyme activity in the RDOM, N and RDOM + N treatments in comparison with CK (Figs. 4 and 5), which led to a faster decomposition of the DOC in those treatments. Soil mineral N contents in the RDOM, N and RDOM + N treatments were comparable but significantly higher than the CK. This indicated that the maize residue-derived DOM was easily available by microbes, and thus led to a rapid release of inorganic N. Another explanation could be that adding DOM accelerated the mineralization of native SOC, and thus increased the available N content in the soil.

3.6. Impacts of soil biochemical parameters on SOC-derived CO_2

SOC-derived CO_2 emission could account for 88% of the variation in total soil CO2 emission (Table 3). The native SOC-derived CO_2 emission was closed related with MBC, MBN, β-glucosidase, invertase and soil mineral N content (Table 3), and MBC, β-glucosidase, and soil mineral N content played a more important role than other parameters in determining the mineralization of SOC. This suggested that a more intense priming in the DOM-amended soil was attributed to increases in microbial biomass, microbial activity and soil N availability after the addition of DOM.

4. Discussion

4.1. DOM addition on the native SOC decomposition and soil C balance

Adding DOM alone or in combination with urea resulted in a more intensive priming effect as compared with adding N alone (Fig. 3). This was because the supply of easily available C increased microbial biomass, enzyme activity and soil N availability (Figs. 4–6), and these factors were positively correlated with the emission of native SOC-derived CO_2 (Table 3). Such a positive effect of easily available C addition on the turnover of SOM supported our first hypothesis and this result was consistent with previous studies obtained in laboratories [7,25–27]. Increases in microbial biomass and the activity of β-glucosidase and invertase were associated with a higher primed C emission in DOM-amended soils indicated that adding DOM mainly stimulated the decomposition of labile SOC fractions. Similar results were also observed by Derrien et al. [25] and Kuzyakov and Bol [28], who found that the labile organic carbon input could active microbial activity, and thus decomposed different soil organic matter pools according to their degradability.

The present study showed that adding DOM alone or in combination with urea accelerated the decomposition of native SOC, but previous laboratory study demonstrated that combined application of DOM and urea indeed accelerated the decomposition of native SOC, whereas single application of DOM had no significant effect on the decomposition of native SOC [20]. The divergent results might be due to the difference in the ratio of DOM-C to the MBC [29]. Under field conditions, the ratio of DOM-C to MBC was 32.9% and 16.4% in the RDOM and RDOM + N treatments, respectively. The ratio, however, in the corresponding treatment was 66.6% and 33.3% under laboratory conditions, respectively. It has been proposed that when the ratio of the added easily available organic C to MBC is higher than 50%, an exponential decrease of the priming effect or even a switch to negative values is often observed [29]. Therefore, different results obtained from laboratory and field studies indicate that the intensity of priming effect is determined by the energy input to soil microorganisms.

In this study, about 70–91% of the labeled DOM was emitted as CO_2 over 154 days and very few ^{13}C-DOM remained in the soil (Table 2). Therefore, we supposed that the rest of ^{13}C-labled DOM might be incorporated into the microbial biomass as other studies have reported [12,17,30]. From this experiment, the soil C input-out balance was negative in DOM-amended soils (Table 2), suggesting that increased DOM-C input decreased soil C sequestration. This result was also supported by our rough estimation of the second times application of unlabeled DOM. The negative relationship between the amount of C input and soil C sequestration indicated that a greater C input could be partly offset by a faster SOC turnover. This was why many long-term field observations showed that plant litter was

Table 3
The Pearson correlations between soil CO_2 emission and soil biochemical parameters during the wheat growing seasons ($n = 12$).

	Total CO_2 emission	SOC-derived CO_2 emission	MBC	MBN	β-Glucosidase	Invertase	Cellobiohydrolase	DOC	Mineral N
Total CO_2 emission	1	**0.94****	**0.71****	**0.79****	**0.86****	0.56	**0.67***	−0.11	**0.68***
SOC-derived CO_2 emission		1	**0.76****	**0.70***	**0.77****	**0.64***	0.56	−0.26	**0.76****
MBC			1	0.35	**0.59***	0.52	0.24	**−0.60***	**0.85****
MBN				1	**0.81****	**0.66****	**0.71***	0.38	0.48
β-Glucosidase					1	0.47	**0.58***	−0.10	**0.71****
Invertase						1	0.22	0.13	0.56
Cellobiohydrolase							1	0.24	0.35
DOC								1	−0.49
Mineral N									1

Note: **and * indicate significant correlations at $P < 0.01$ and $P < 0.05$, respectively.

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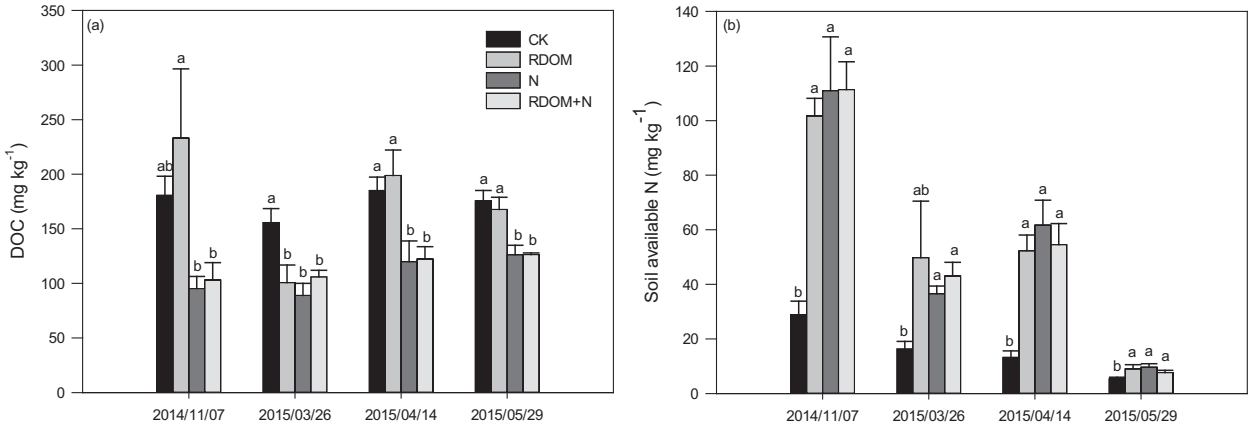

Fig. 6. Effects of maize residue-derived DOM and urea additions on (a) soil DOC content and (b) soil available N. CK represents soil amended with deionized water (■), RDOM means soil amended with maize residue-derived DOM (■), N means soil amended with urea (■), RDOM + N represents soil amended with maize residue-derived DOM and urea (■). Bars indicate standard error of the mean ($n = 3$). Different letters in the same column represent significant difference at $P < 0.05$.

incorporated to soil in large quantities, but this was not associated with an increase in soil C content [3]. Thus, a greater C input to soil may be compensated for a larger CO_2 release due to the priming effect induced by the C input.

4.2. N supply on the decomposition of native SOC

In the present study, the stimulatory effect of per unit of DOM-C addition on the decomposition of native SOC in the RDOM + N treatment was significantly higher than the RDOM treatment, suggesting that there was a significant positive interactive effect of DOM and urea on the mineralization of SOC. This result was consistent with the studies conducted by Chen et al. [7] and Conde et al. [14]. They reported that combined application of sucrose/glucose and N induced a greater priming effect compared to single application of sucrose/glucose.

The significantly positive relationship between the decomposition of native SOC and soil available N content (Table 3) suggested that the decomposition could be N limited. This result supported the "stoichiometric decomposition" theory. Namely, if the soil is rich in available C, an increase in soil N availability will increase the microbial activity, and thus facilitates the SOC decomposition [7]. Previous studies also showed that N addition accelerated the decomposition of light soil carbon fractions [31,32]. We are uncertain whether such a positive effect induced by N addition in the present study can last for a long time, since this experiment is short-term. A few N deposition studies have demonstrated that N deposition will be beneficial for soil C sequestration in the long term, even though it accelerates the decomposition of rapidly decomposing soil carbon fractions in the early stage [33,34]. Therefore, the long-term effect of N addition on the mineralization of SOC in our study site should be further investigated. Moreover, in the context of an increase in anthropogenic N deposition and/or mineral N fertilizer application [35], as well as increases in the plant-derived DOC input to soils due to warming and elevated atmospheric CO_2 [8], the interactive effect of soil C and N availability on the decomposition of native SOC should be incorporated into the model of soil organic matter dynamics.

4.3. Implications of crop residues-derived DOM on the SOC decomposition in the NCP

Previous studies have reported that the priming effect is short-lived. However, recent studies have demonstrated that the priming effect can last for a long time even though the added substrates are completely exhausted [12]. Therefore, it is important to consider it since pulse inputs of labile organic carbon frequently occur in plant-soil systems. Continuous or occasional input of easily available carbon associated with plant residues and root exudates can act as an external inducer and accelerate or retard the decomposition of native SOC [25,36].

In the North China Plain, the crop residue production is about 10,000 kg DM ha^{-1} in the maize growing season [19,37]. If all the maize residues are returned into the soil and the DOM-C accounts for 5%–15% of the crop residue-derived C input [11,38], there is about 250–750 kg DOM-C ha^{-1} of maize residue-derived DOM input to the soil in the wheat growing seasons. The MBC contents in the surface soil (0–20 cm) vary from 135 to 300 mg kg^{-1} [39–41], which are equivalent to 351–780 kg MBC ha^{-1} (assume the soil with a bulk density of 1.3 g cm^{-3}). Therefore, the ratios of DOM-C to MBC range from 32% to 214% in the wheat growing season. These ratios are higher or lower or comparable in comparison with the critical threshold value of 50% proposed by Blagodatskaya and Kuzyakov [29]. This indicates that positive and neutral as well as negative priming effects may occur due to the input of crop residue-derived DOM in the wheat growing season. The direction and magnitude of the priming effect may depend on the amount of DOM-C input via the decomposition of crop residues and the microbial biomass at that time. Therefore, in order to increase soil C sequestration, controlling both the amount of crop residues input to soil and the time crop residues incorporated into soil are very important in the farming practices.

5. Conclusions

Application of maize residue-derived DOM accelerated the decomposition of native SOC and resulted in a net SOC loss. The net loss of SOC from RDOM and RDOM + N was about 3.90 ± 0.61 and 3.53 ± 0.48 g C m^{-2}, respectively. Moreover, the addition of urea further enhanced the stimulatory effect of per unit of DOM-C added on the decomposition of native SOC. The corresponding value was 0.25 ± 0.05 and 0.45 ± 0.07 for the RDOM and RDOM + N treatments, respectively. Increases in microbial biomass and enzyme activity as well as soil N availability were responsible for the stimulation of native SOC mineralization in DOM-amended soils. In the context of an increase in the DOM input to soils due to the production of more litter and root exudates under global warming and elevated atmospheric CO_2 levels as well as increases in anthropogenic N deposition and/or mineral N fertilizer application, a more intense priming effect may occur in the temperate agricultural ecosystem. As only one type of soil was tested in this study, whether the results were suitable for other soil types remain unclear. Studies carried out in tropical and subtropical soils have demonstrated that the labile carbon retention can compensate for the CO_2 released by priming, and thus increases C sequestration [6]. Therefore, further investigation should not only pay attention to the priming effect

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induced by C and N inputs, but also focus on their net effects on soil C balance, and these effects may be soil-specific.

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