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Effects of exogenous organic/inorganic nitrogen addition on carbon pool distribution and transformation in grassland soil

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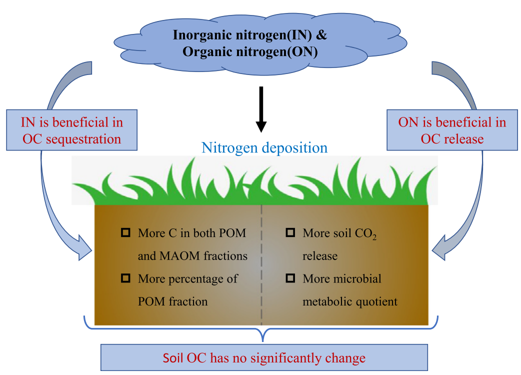
HIGHLIGHTS

- We studied the effects of N addition in different forms on soil carbon in grassland.
- Relative to the control, N addition did not significantly affect the soil organic C concentrations.
- Inorganic N addition affected carbon distribution in particulate and mineral associated fractions.
- Organic N addition reduced the inorganic N-induced increase in soil organic C.
- N addition reduced soil organic C mineralization by 26 % compared with the control in topsoil.

GRAPHICAL ABSTRACT



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ABSTRACT

Aims: Increases in nitrogen (N) deposition may significantly affect the organic carbon (OC) cycle in soil. The inconsistent findings of the influence of added N on soil OC pools highlight the need of quantifying responses of the OC pool distribution to N addition. Moreover, the influence of N addition with a mixture of organic and inorganic N on OC pool distribution and stabilization in grassland soil remains unclear.
Methods: We carried out a five-year field experiment with adding N to examine the effects of different types of N addition on soil OC pool distribution and transformation in a meadow steppe in Inner Mongolia. We applied N in the ratios of inorganic N (IN) and organic N (ON) at 10:0 (N1), 7:3 (N2), 5:5 (N3), 3:7 (N4), 0:10 (N5), and 0:0 (CK), respectively. We measured OC content in bulk soil, particulate organic matter (POM), and mineral-associated organic matter (MAOM) fractions. Additionally, a short-term soil incubation was conducted to assess potential OC mineralization.
Results: Our study showed no significant effect on soil organic carbon content of different ratios of IN/ON addition. N addition reduced microbial biomass C/N ratio, the fraction of mineral-associated organic matter, cumulative CO_2 emission, and microbial metabolic quotient. Compared with ON addition alone, IN addition alone showed a stronger effect on the C in different soil fractions and soil OC mineralization. The particulate organic matter (POM) fraction was more sensitive to N addition than the mineral-associated organic matter (MAOM) fraction.
Conclusions: Our results suggest that the contribution of N in organic and inorganic forms affecting OC pool distribution with different turnover rates should be considered when assessing the effects of N addition types on soil OC processes in grassland.

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1. Introduction

In terrestrial ecosystems, soil stores the largest organic carbon (OC) pool comprising over three times that of the atmosphere [1]. Small changes in the decomposition rate of soil OC may significantly impact the atmospheric CO_2 concentration [2]. Grassland ecosystems play a particularly important role in the global carbon (C) cycle [3], with its C storage accounting for one-third of the global terrestrial ecosystem [4]. An improved understanding of the soil C cycle in grasslands is therefore essential for accurately assessing its contribution to the global C budget.

Atmospheric nitrogen (N) deposition in terrestrial ecosystems has increased three times over the past century and continues to increase [5]. N deposition comprises both organic nitrogen (ON) and inorganic nitrogen (IN) deposition. Although IN is the main component of atmospheric N deposition, increasing evidence demonstrates the large proportion of atmospheric N deposition accounted for by ON [6,7]. For instance, on a global scale, ON deposition accounted for about 30 % of total atmospheric N deposition [6]. Zhang et al. [7] monitored atmospheric N deposition at 32 study sites over five years in China and, found that ON accounts for between 28 % and 50 % of the total N content. However, few studies have focused on the effects of ON versus IN deposition on soil OC in grassland ecosystems.

Nitrogen deposition can affect plant diversity, plant biomass, soil C mineralization rate, and soil microbial activity, thus impacting C sequestration [8], which has been shown to increase the decomposition of easily mineralizable OC and promote the stability of refractory C [9,10]. In addition, increases in N deposition can significantly increase plant C assimilation and soil C sequestration [1]. While many studies have suggested that N deposition could increase, have no effect on, or decrease the decomposition of soil OC [9–12]. These inconsistent findings have caused uncertainty surrounding the soil C cycle in response to N deposition. This discrepancy may be due to the different N forms studied in the experiments. For instance, Riggs et al. [13] reported that ON addition decreased microbial respiration of unoccluded OM by as much as 29 % relative to control plots. Chen et al. [14] demonstrated that ON addition promoted C sequestration in temperate grassland soil. Chen et al. [15] found that particulate organic carbon is more vulnerable to IN addition than mineral-associated organic carbon in a soil of an alpine meadow. Therefore, the forms in which N is added to the soil should not be neglected in examining the effects of N deposition on OC.

Soil organic matter is extremely complex; its formation involves a series of physical, chemical, and biological processes. Based on different turnover times and persistence mechanisms, soil organic matter can be divided into particulate organic matter (POM; being predominantly of plant origin) and mineral-associated organic matter (MAOM; derived from microbial residues with chemical bonding to minerals and physical protection in small aggregates fractions [16,17]. Generally, OC in the POM has a high turnover rate, which is readily affected by environmental changes [18]; however, OC in the MAOM is more stable and has a slow turnover. Numerous studies have explored the effects of N addition on the change of relative OC content and the change of absolute OC content, but the conclusions are inconsistent [15,19]. For example, Chen et al. [19] found that N addition significantly decreased OC in the MAOM, while Chen et al. [15] found that OC in the MAOM showed no significant trend with increasing N addition. These inconsistent findings highlight the need—in light of increasing global N deposition—for quantifying responses of the OC pool distribution to N addition. In the past, most studies on the effects of N addition to soil OC in grassland focused on one single type of N addition, i.e., either IN or ON [20–22]. IN or ON additions may show different effects on soil carbon processes. For example, IN deposition inhibited soil organic C decomposition and enzymatic activities. By contrast, ON addition resulted in the accelerated transformation of recalcitrant compounds into labile compounds and increased CO_2 emission in forest soil [9]. However, the effects of different proportions of IN and ON on the soil OC pool distribution and stabilization in grassland remain largely unclear.

Our objectives were to examine the effects of different types of N (IN/ON) addition on (i) the soil OC pool distribution, and (ii) mechanisms controlling its distribution in grassland. We tested the following hypotheses: (1) the OC content in soil and two contrasting fractions is significantly changed under the addition of different N forms [15,20], and (2) IN deposition exerts a higher suppression effect on OC decomposition than that of ON deposition [9]. We carried out N addition experiments in the Erguna meadow steppe in Inner Mongolia over five years. We used ammonium nitrate as the IN source and an equal proportion of urea and glycine as the ON source.

2. Materials and methods

2.1. Site description and experiment design

The experiment was established in a temperate steppe (N 50°10′; E 119°22′) at Erguna Forest-Steppe Ecotone Research Station of the Institute of Applied Ecology, Chinese Academy of Sciences. The site has a cold temperate continental climate, with a mean annual temperature of −2.4 °C and mean annual precipitation of 360 mm (1950–2017), which falls mostly from June to August. The soil type is chernozem, and the dominant plant species are *Leymus chinensis* and *Stipa baicalensis*. We measured the basic chemical properties of the 0–10 cm soil in the control treatment in 2019 as follows: total N 2.23 $g \cdot kg^{-1}$, available phosphorus 32.5 $mg \cdot kg^{-1}$, available potassium 215.3 $mg \cdot kg^{-1}$, pH 5.66; for 10–20 cm soil, total N 1.96 $g \cdot kg^{-1}$, available phosphorus 33.9 $mg \cdot kg^{-1}$, available potassium 127.5 $mg \cdot kg^{-1}$, pH 5.75.

In May 2014, we established 18 field plots (6 m × 6 m) with six treatments with different inorganic versus organic nitrogen ratios (IN:ON): the control (hereafter "CK"), IN:ON = 10:0 (hereafter "N1"), IN:ON = 7:3 (hereafter "N2"), IN:ON = 5:5 (hereafter "N3"), IN:ON = 3:7 (hereafter "N4") and IN:ON = 0:10 (hereafter "N5") at the experimental site, each randomly assigned in triplicate (randomized block design). Buffer zones 1 m wide separated the plots. We used NH_4NO_3 as the IN source, and urea and glycine mixed in equal proportions, based on their N concentrations, as the ON source. To the control plots (CK) we added only water. During the field experiments, we fertilized each plot every May with mixed N solutions to a total amount of 10 g N m^{-2} yr^{-1}. For example, the N2 (IN: ON = 7:3) treatment plots were sprayed with 20 L mixed N solution comprising 720 g NH_4NO_3 (the equivalent of 7 g IN m^{-2} yr^{-1}), 116 g urea (the equivalent of 1.5 g ON m^{-2} yr^{-1}) and 289 g glycine (the equivalent of 1.5 g ON m^{-2} yr^{-1}). N was applied once per year. It should be noted that these total N addition rates exceed the natural deposition rates. The N additions of the N2 treatment were largely in line with the average contribution of ON to total natural atmospheric N deposition both in China (28 %) and globally (30 %) [6]. The field site inorganic N deposition rates ranged from 4.53 kg N ha^{-1} to 12.21 kg N ha^{-1} with a mean value of 8.07 kg N ha^{-1} during the entire growing season [23].

2.2. Sample collections

Soil samples were collected in mid-August 2019. In each of the 18 plots, three soil cores were taken randomly at the depths of 0–10 cm and 10–20 cm (after removing surface litter) by a 5 cm diameter soil auger. These three cores were mixed as a composite soil sample for the plot. The soil samples were kept in sealed bags and immediately taken back to the laboratory for analysis. We carefully removed gravel and visible plant material from the composite soil samples by passing them through a 2.0 mm sieve in the laboratory. We divided the fresh soil samples into two parts, storing one part at 4 °C and air-drying the other part. We determined soil pH using a pH meter at a 1:5 (soil: water) ratio of soil and $CaCl_2$ solution. We determined soil microbial biomass carbon (MBC) and nitrogen (MBN) using the chloroform–fumigation–extraction method [24]. We measured both total C and total N contents in soil and soil organic matter fractions (POM, MAOM, and MBC) using a C/N analyzer (Elementar Vario MACRO, Germany). The total soil carbon was close to soil organic carbon because the carbonate content exceeded the detection limit.

2.3. Soil fractions separation

We fractionated soil organic matter according to the approach by Lavallee et al. [25]. Briefly, we dispersed a 10 g air-dried sample by submerging it into 30 mL of 5 g·L^{-1} sodium hexametaphosphate solution and shaking it on a shaking incubator for 15 h at 180 rpm min^{-1}. After dispersion, we sieved the samples and rinsed them several times with deionized water to pass through a 0.53 mm sieve. We then obtained two soil fractions: POM >53 μm and MAOM <53 μm. All fractions were dried in the oven at 55 °C and recorded the weight of each. We determined the OC content in the two fractions with a C/N analyzer (Elementar Vario MACRO, Germany).

2.4. Soil incubations

We conducted a short-term incubation to determine the potential of OC mineralization. Briefly, we adjusted 25 g of dry soil to 60 % of the soil water holding capacity with deionized water and placed it in a 125 mL bottle. At the same time, we set three bottles without soil as the control. Before incubation, we pre-incubated the soils at 25 °C in the dark for 7 days to establish optimal conditions for microbial activity. We monitored respiration on days 1, 3, 7, 14, and 21 of incubation and added water to the soil to a constant weight each time. We determined CO_2 emissions by gas chromatography (Glarus 680 GC, PerkinElner).

2.5. Soil analysis and statistics

We calculated the percentage of POM and MAOM by the following formula:

$$\text{Percentage}_{\text{POM}} = \text{M}_{\text{POM}}/\text{M}_{\text{soil}} \times 100\% \tag{1}$$

$$\text{Percentage}_{\text{MAOM}} = \text{M}_{\text{MAOM}}/\text{M}_{\text{soil}} \times 100\% \tag{2}$$

where M_{POM} (g) is the mass of POM fraction after fractionation, M_{MAOM} (g) is the mass of MAOM fraction after fractionation, and M_{soil} (g) is the bulk soil mass before fractionation. The two fractions together constituted total soil OC.

According to the OC content in the bulk soil (g·kg^{-1} soil) and the percentage of POM and MAOM (%), we calculated the C in each fraction (g·kg^{-1} soil).

$$\text{C in POM} = \text{Percentage}_{\text{POM}} \times \text{Soil OC content}/100\% \tag{3}$$

$$\text{C in MAOM} = \text{Percentage}_{\text{MAOM}} \times \text{Soil OC content}/100\% \tag{4}$$

In this study, we used the first-order kinetic equation to fit the soil OC mineralization from the incubation experiment by SigmaPlot 14.0 [26]:

$$C_t = C_0\left(1 - e^{-kt}\right) \tag{5}$$

where C_t is the cumulative OC mineralization of soil at time t (g·kg^{-1}); C_0 represents the amount of total potentially mineralisable C (g·kg^{-1}), and k is the mineralization rate constant (day^{-1}). To avoid correlation between model parameters, we used a fixed $k = 0.025$ d^{-1} for all treatments and estimated C_0 for each treatment by curve fitting.

The sensitivity index (SI) related to N addition treatments for soil OC fractions was calculated as follows [27]:

$$\text{SI} = \frac{\text{C}_\text{N} - \text{C}_\text{K}}{\text{C}_\text{K}} \times 100 \tag{6}$$

where: C_N is OC content in different fractions under five N addition treatments, and C_K is OC content in different fractions in the control treatment.

We define microbial respiration per unit of microbial biomass as the microbial metabolic quotient [28].

One-way ANOVA and the Duncan post hoc test were used to analyze all data. In all cases, we considered differences to be statistically significant at $P < 0.05$. All results are expressed as the mean of three replicates. All data and statistical analyses were performed using SPSS 23 for Windows, SigmaPlot software, or Microsoft Office.

3. Results

3.1. OC contents in the soil and isolated fractions

Compared with the control, neither ON nor IN addition significantly affected the soil OC content ($p > 0.05$) (Fig. 1a). Different ON/IN additions did not significantly affect the OC contents of the two fractions (Fig. 1c, d). Compared with the control, the OC content had an increasing tendency in the 0–20 cm of POM fraction and 10–20 cm of MAOM fraction (Fig. 1c; Fig. 1d), while the OC content had a decreasing tendency in the 0–10 cm of MAOM fraction with increasing ON/IN ratios (Fig. 1d).

3.2. Soil microbial biomass carbon

Nitrogen addition significantly affected MBC in the 10–20 cm ($p < 0.05$) (Fig. 1b). The N3 treatment significantly increased the 10–20 cm MBC compared with other N addition treatments ($p < 0.05$). Although N addition did not significantly affect MBC in the 0–10 cm, the MBC of the N1 treatment was 53 % higher than the control.

3.3. C/N ratios

Nitrogen addition did not significantly affect soil C/N ratios, POM, and MAOM ($p < 0.05$) (Table 1). However, it significantly affected the C/N ratio of microbial biomass for both soil layers ($p < 0.05$). For the 0–10 cm, the C/N ratio of microbial biomass in the N1, N2, N3, and N5 treatments was significantly lower than that of the control ($p < 0.05$). For the 10–20 cm, except for the N3 treatment that had a significantly higher C/N ratio of microbial biomass ($p < 0.05$), the treatments decreased the C/N ratio of microbial biomass compared to the control as N2 > N5 > N1 > N4.

3.4. OC percentage of fractions

For the 0–10 cm, N1 and N2 treatments significantly increased the percentage of POM and decreased the percentage of MAOM compared with the control ($p < 0.05$) (Fig. 2). For the 10–20 cm, ON/IN addition did not significantly affect the POM and MAOM fractions.

3.5. The C in two fractions

Nitrogen addition significantly increased the C in both POM and MAOM fractions compared with the control ($p < 0.05$). For the 0–10 cm, the C of POM fraction was significantly higher under treatments N1, N2, N3, and N5; and the N1, N3, and N5 treatments significantly increased the C of the MAOM fraction compared with the control ($p < 0.05$). For the 10–20 cm, the N1 treatment significantly increased the C of POM, and the N1, N2, N3, and N5 treatments significantly increased the C of MAOM compared with the control. Generally, the N1 treatment had the strongest effect on C of both the POM and the MAOM fractions, irrespective of soil depth and N addition.

3.6. Sensitivity of OC fractions

The POM fraction was more sensitive to changes in the proportion of ON/IN than the MAOM fraction in both soil layers (Table 2). Compared with other N treatments, the N1 treatment had the highest impact for both fractions in the 0–10 cm. However, for the 10–20 cm, the N5 treatment had the highest impact on the POM fraction and the N2 treatment had the highest impact on the MAOM fraction.

3.7. Soil OC mineralization

Nitrogen addition reduced soil OC mineralization by 26 % on average compared with the control in the 0–10 cm layer (Fig. 4a), and the N1 treatment showed the strongest decrease, with an average

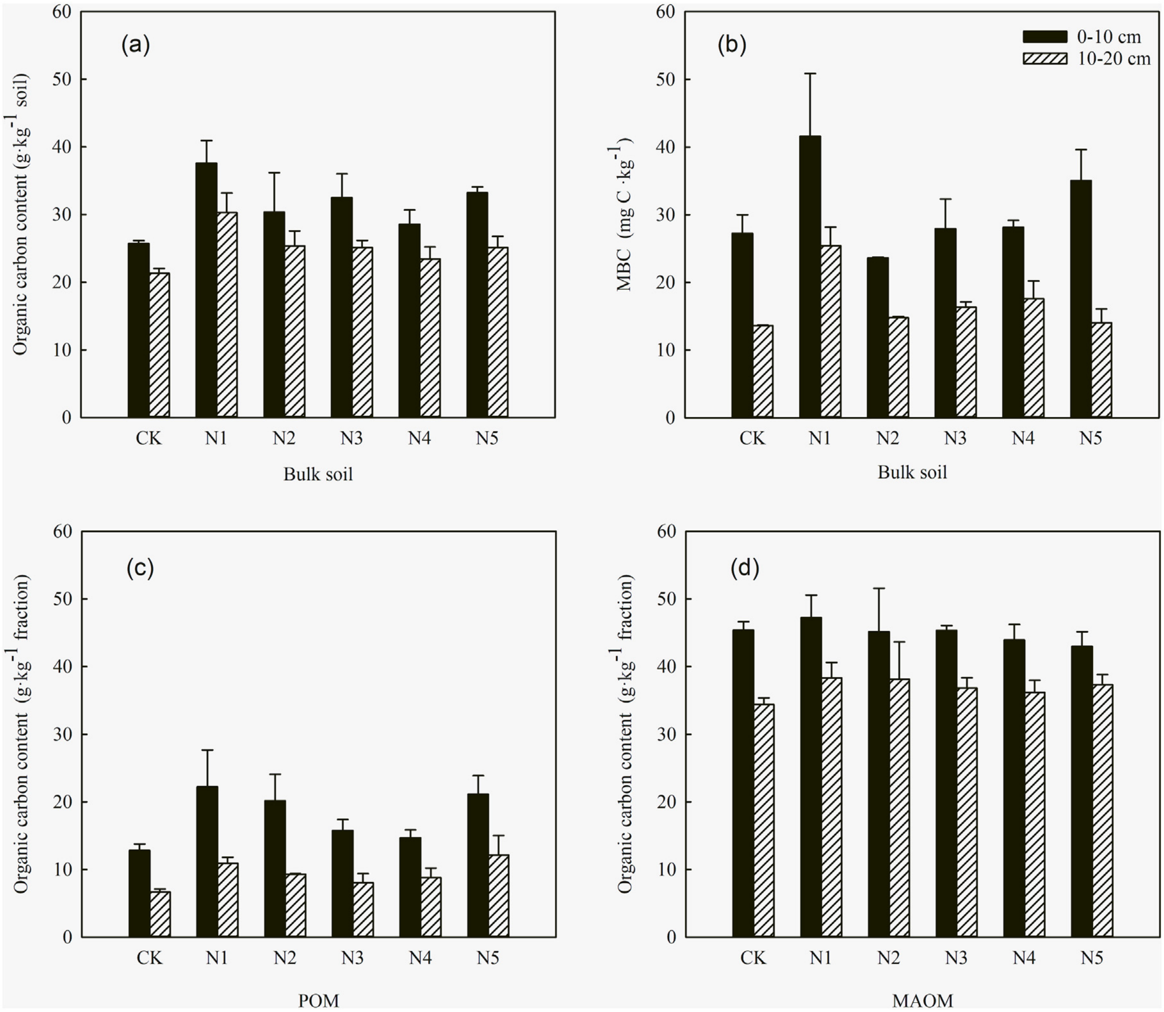

Fig. 1. Effects of different ON/IN addition treatments on SOC, MBC, organic carbon content of POM and MAOM. SOC: soil organic carbon content; MBC: microbial biomass carbon; POM: particulate organic matter; MAOM: mineral-associated organic matter. CK, control; N1, IN:ON = 10:0; N2, 7:3; N3, 5:5; N4, 3:7; N5, 0:10. The absence of letters indicates that there is no significant difference between treatments in the same soil depth.

reduction of 40 % compared with the control. For the 10–20 cm layer, soil OC mineralization was also reduced compared with that of the control (Fig. 4b). Cumulative CO_2 release decreased as N4 > N3 > N5 > N1 > N2. Moreover, the soil OC mineralization rate decreased with depth.

3.8. Soil OC mineralization potential

In this study, we used a first-order kinetic equation to fit the soil OC mineralization (Table 3). In the case of all treatments, the model values of R^2 were above 0.99 and very similar ($p < 0.01$). For the 0–10 cm, N1, N3,

Table 1
Carbon/nitrogen ratios in soil, POM (particulate organic matter), MAOM (mineral-associated organic matter) and soil microbial biomass under different organic/inorganic nitrogen addition treatments. Values are means (SE) (n = 3); CK, control; N1, IN:ON = 10:0; N2, 7:3; N3, 5:5; N4, 3:7; N5, 0:10; Different lower case letters indicate significant difference among different treatments in the same layer ($P < 0.05$); mean ± SE.

Depth (cm)	Treatments	C/N ratios			
		Soil	POM	MAOM	Microbial biomass
0–10	CK	11.59 ± 0.60^{a}	14.11 ± 0.60^{a}	9.48 ± 0.13^{a}	21.93 ± 3.48^{a}
	N1	11.07 ± 0.50^{a}	14.70 ± 1.95^{a}	9.10 ± 0.06^{a}	11.37 ± 1.92^{c}
	N2	12.13 ± 0.14^{a}	14.39 ± 0.62^{a}	9.00 ± 0.18^{a}	8.35 ± 1.90^{c}
	N3	11.90 ± 0.29^{a}	13.54 ± 0.64^{a}	9.12 ± 0.42^{a}	13.12 ± 0.82^{bc}
	N4	10.63 ± 0.55^{a}	13.79 ± 0.25^{a}	8.91 ± 0.41^{a}	20.23 ± 4.47^{ab}
	N5	11.85 ± 0.41^{a}	15.11 ± 0.32^{a}	9.10 ± 0.20^{a}	7.74 ± 0.17^{c}
10–20	CK	11.16 ± 1.10^{a}	14.58 ± 1.68^{a}	9.25 ± 0.17^{a}	14.21 ± 2.35^{b}
	N1	10.93 ± 0.71^{a}	16.66 ± 1.65^{a}	9.04 ± 0.24^{a}	11.52 ± 1.26^{bc}
	N2	11.15 ± 0.44^{a}	14.07 ± 1.58^{a}	9.05 ± 0.33^{a}	7.29 ± 1.12^{c}
	N3	11.75 ± 0.57^{a}	13.76 ± 1.69^{a}	9.30 ± 0.58^{a}	21.62 ± 0.76^{a}
	N4	11.12 ± 0.53^{a}	15.26 ± 0.84^{a}	9.13 ± 0.25^{a}	13.62 ± 1.41^{b}
	N5	11.57 ± 0.08^{a}	13.74 ± 0.61^{a}	9.34 ± 0.13^{a}	8.74 ± 1.35^{c}

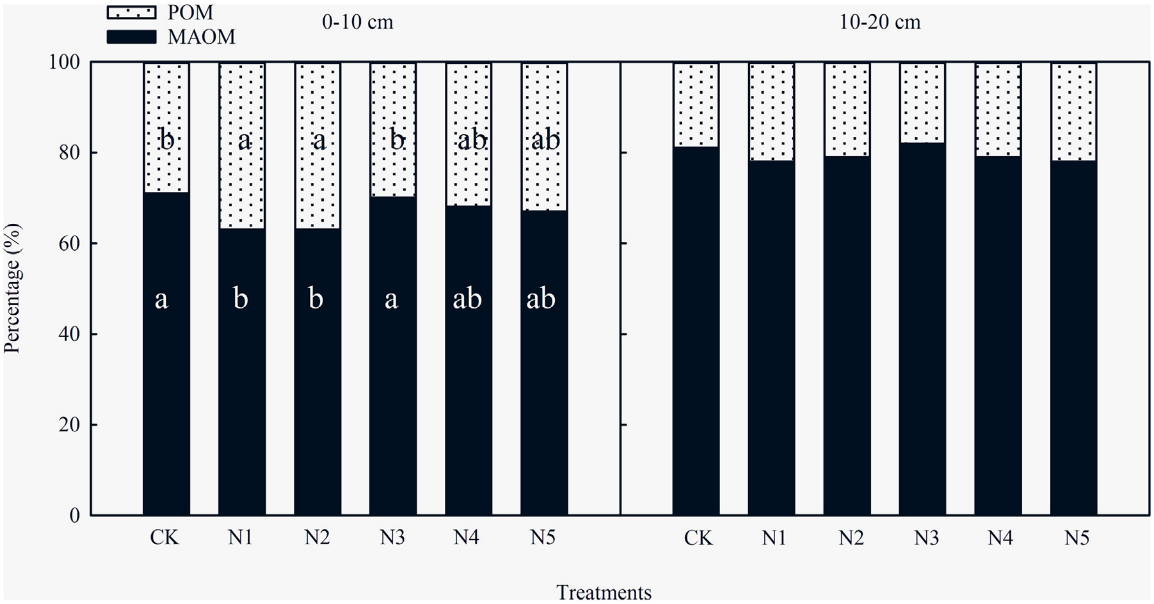

Fig. 2. Effects of different ON/IN addition treatments on the percentage of organic carbon fractions. POM and MAOM represent particulate organic matter and mineral-associated organic matter; CK, control; N1, IN:ON = 10:0; N2, 7:3; N3, 5:5; N4, 3:7; N5, 0:10. Error bars represent ± standard error of mean (n = 3). Means with different letters are significantly different. The significance threshold is α = 0.05.

N4, and N5 treatments significantly decreased the C_0 compared with CK (p < 0.05), and N1 had the lowest C_0. For the 10–20 cm soil, C_0 of all N addition treatments was significantly lower than that of the control (p < 0.05). On average, the C_0 in the 0–10 cm soil was higher than that in the 10–20 cm layer.

3.9. Microbial metabolic quotient

Soil microbial metabolic quotients under different N addition treatments were generally lower than that in the control (Fig. 5), and this was significant for the 10–20 cm. For the 0–10 cm, the microbial metabolic quotient decreased as: CK > N2 > N3 > N5 > N4 > N1. For the 10–20 cm, all ON/IN addition treatments significantly decreased the soil microbial metabolic quotient (p < 0.05), which decreased as: CK > N3 > N5 > N4 > N2 > N1. Overall, the N1 treatment had the lowest microbial metabolic quotient at both soil depths.

4. Discussion

4.1. Effects of different ON/IN addition treatments on soil OC pools

In this study, OC contents in soil and its fractions (POM and MAOM) did not significantly differ among treatments despite yearly N addition with different proportions of N (Fig. 1a, c, d). The addition of N with different proportions and forms in this study did not significantly alter annual net primary productivity [29] and the mineralization of soil OC and this may explain the lacking effect on OC contents (Fig. 4), which contradicted our first hypothesis. Previous studies have reported inconsistent effects of N addition on OC, with N addition increasing, decreasing, or having no effect on soil OC contents [20,30,31]. These inconsistent results might be due to the difference between ecosystems, the levels of N application, and the duration of N application [32]. In terrestrial ecosystems, plant roots and litter are the main sources of soil OC [33], and N addition can potentially increase plant aboveground biomass and decrease root competition for available nutrients, affecting soil C inputs [34]. A meta-analysis showed that N stimulation of C storage occurred primarily in plant pools but little in soil pools [35], which may explain why soil OC content was not affected by N addition in our study.

4.2. Effects of different ON/IN addition treatments on mass distribution of soil fractions

Our results showed that N addition increased the percentage of POM and N1 and N2 treatments had the strongest impact on the POM percentage increase (Fig. 2). MAOM fraction tended to give way to the POM fraction in the 0–10 cm and N1 addition more strongly promoted the C of both fractions (Figs. 2 and 3), which aligns with several other N addition studies [15,19,36]. Recently, Ye et al. [37] proposed a new conceptual framework that integrates plant, microbial and geochemical mechanisms to reconcile

Table 2
Sensitivity index (%) of OC fractions in 0–10 cm and 10–20 cm layers under different organic/inorganic N addition treatments. N1, IN:ON = 10:0; N2, 7:3; N3, 5:5; N4, 3:7; N5, 0:10.

Depth (cm)	Fraction	Treatments				
		N1	N2	N3	N4	N5
0–10	POM	75.5	61.6	24.6	16.2	64.7
	MAOM	4.4	0.4	0.2	−3.3	−5.2
10–20	POM	63.1	40.4	22.6	33.3	88.2
	MAOM	11.2	11.4	7.5	5.1	8.5

Table 3
Mineralization potential of soil organic carbon under different N additions. C_0 represents the amount of total potentially mineralizable C; k is the mineralization rate *constant; CK, control; N1, IN:ON = 10:0; N2, 7:3; N3, 5:5; N4, 3:7; N5, 0:10.

Depth (cm)	Treatments	C_0 (g·kg^{-1} SOC)	k (d^{-1})	R^2	P
0–10	CK	13.57 ± 0.30^{a}	0.025	0.9997	<0.0001
	N1	8.42 ± 0.41^{d}	0.025	0.9991	<0.0001
	N2	13.19 ± 0.45^{a}	0.025	0.9987	<0.0001
	N3	9.01 ± 0.39^{cd}	0.025	0.9992	<0.0001
	N4	9.63 ± 0.40^{c}	0.025	0.9987	<0.0001
	N5	11.05 ± 0.16^{b}	0.025	0.9996	<0.0001
10–20	CK	11.05 ± 0.25^{a}	0.025	0.9996	<0.0001
	N1	6.14 ± 0.61^{cd}	0.025	0.9999	<0.0001
	N2	5.90 ± 0.61^{d}	0.025	0.9998	<0.0001
	N3	7.68 ± 0.52^{b}	0.025	0.9993	<0.0001
	N4	7.62 ± 0.41^{bc}	0.025	0.9995	<0.0001
	N5	7.29 ± 0.13^{bcd}	0.025	0.9991	<0.0001

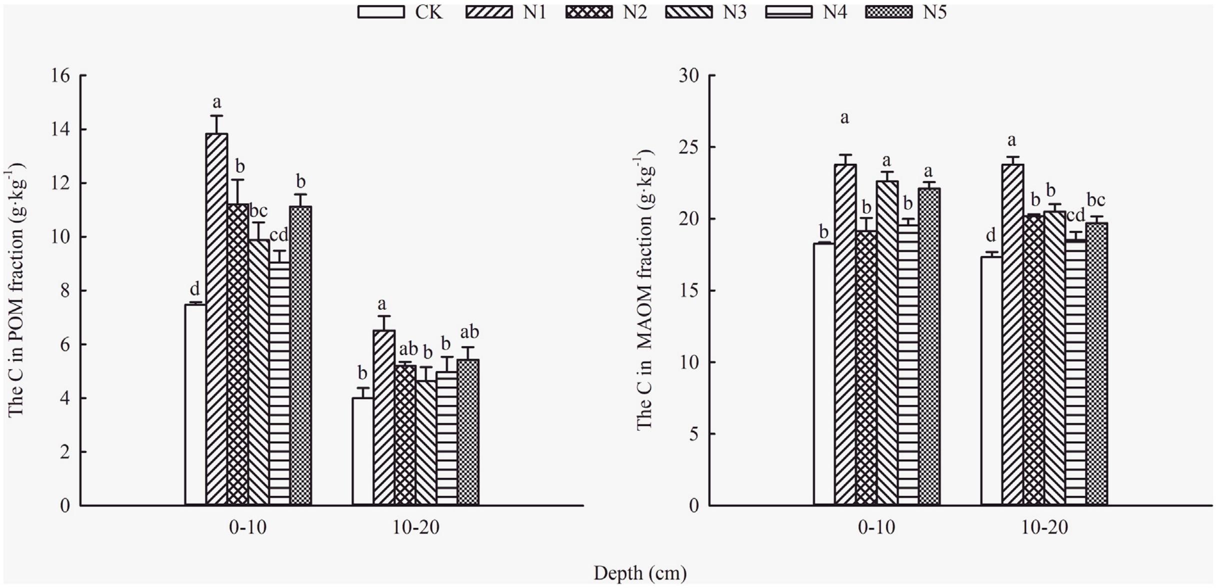

Fig. 3. Effects of different ON/IN addition treatments on organic carbon fractions. POM and MAOM represent particulate organic matter and mineral-associated organic matter; CK, control; N1, IN:ON = 10:0; N2, 7:3; N3, 5:5; N4, 3:7; N5, 0:10. Error bars represent ± standard error of mean (n = 3). Means with different letters are significantly different. The significance threshold is α = 0.05.

diverse and contrasting impacts of N on soil C, showing that the C in POM is primarily controlled by plant input, while the C in MAOM is determined by microbial biomass/necromass and interactions between the organic C and soil minerals. MAOM was the dominant fraction, accounting for 67 % and 80 % in the 0–10 cm and 10–20 cm soil respectively (Fig. 2), which agrees with previous studies showing that a large portion of the OC in soil was associated with soil minerals [36,38]. There are two possible reasons for these results: Firstly, N addition stimulated the aboveground and litter biomass [29], and N1 has the strongest effect in all treatments and is significantly higher than CK. The increased POM fraction under IN addition is likely due to the stimulation of aboveground plant C input. Secondly, IN addition can alter the relative changes in proportions of POM and MAOM [13, 37].

Our results showed that OC in POM was more sensitive to N addition than that in MAOM (Table 2), which was in line with results from previous studies [16,19,39] showing that the POM fraction was more vulnerable to environmental changes, such as N deposition. In addition, the N1 treatment had the strongest impact on OC in both POM and MOAM fractions compared with other N addition treatments, indicating that mineral N addition increases C of both fractions (Fig. 1 and Fig. 4). For the 10–20 cm, the sensitivity differed for the two fractions (Table 2), which may result from less impact of the deeper soil layer from plant responses to N addition.

4.3. Soil OC mineralization

Previous studies have shown that N addition can lead to soil acidification, reduce microbial growth, and reduce soil respiration [19,40]. In this study, the inhibitory effect of N addition on soil respiration was observed (Fig. 5). Two reasons may explain the results. Firstly, the relative availability of C and N affects the ratio of fungi to bacteria, which probably exhibits systematic differences in growth rate, biomass turnover, and C utilization efficiency [41,42]. Secondly, soil pH decreased significantly under N1 treatment in our experiment [29]. Soil pH has an opposing effect on the growth of fungi and bacteria [43], which may lead to the inhibition of microbial respiration. In addition, Chen et al. [44] found that soil acidification after adding IN exerts a greater control on soil respiration than soil N

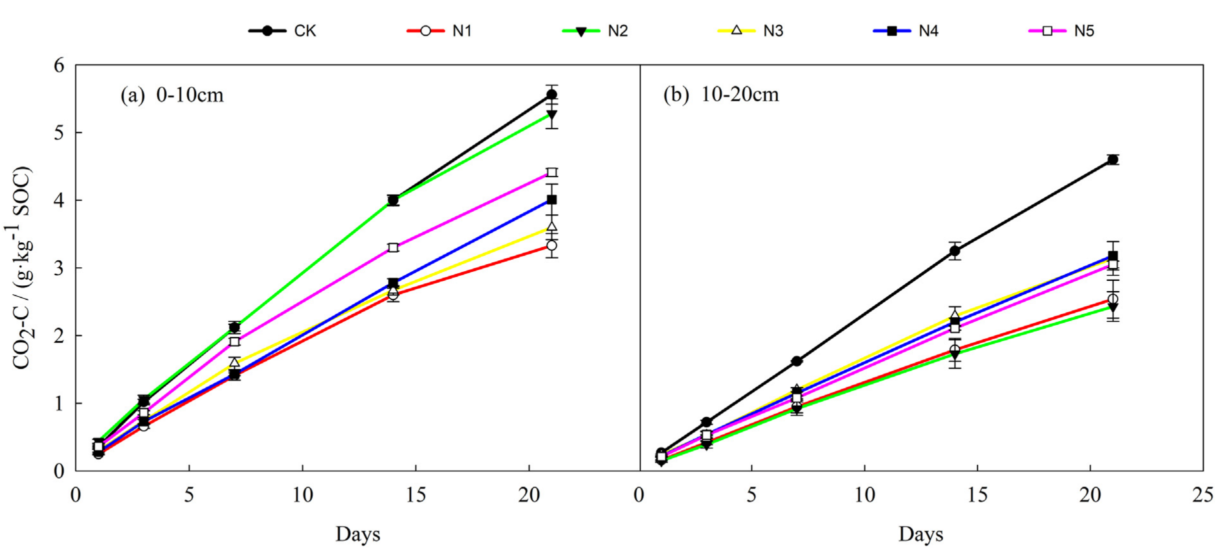

Fig. 4. Effects of different ON/IN addition treatments on the cumulative CO_2 emissions. CK, control; N1, IN:ON = 10:0; N2, 7:3; N3, 5:5; N4, 3:7; N5, 0:10.

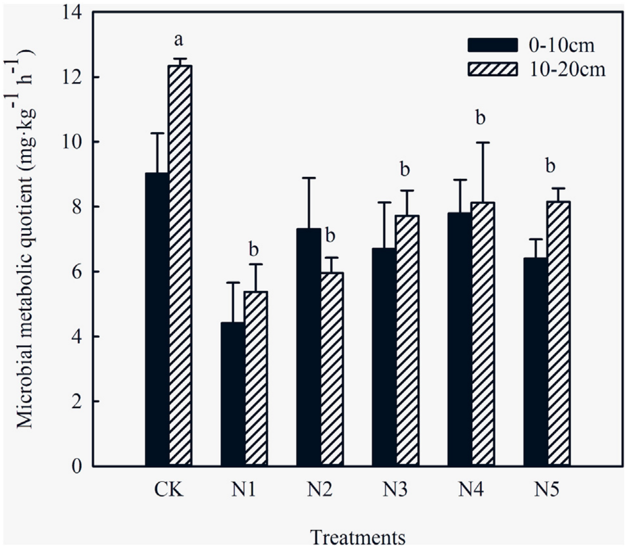

Fig. 5. Effects of different ON/IN addition treatments on the soil microbial metabolic quotient. CK, control; N1, IN:ON = 10:0; N2, 7:3; N3, 5:5; N4, 3:7; N5, 0:10. Error bars represent ± standard error of mean (n = 3). Means with different letters are significantly different. The significance threshold is α = 0.05.

availability when subjected to long-term N enrichment. The close relationship between soil pH and soil respiration can also explain why the topsoil C_0 under N1 treatment was the lowest in our study (Table 3), and the inhibitory effect of IN on soil respiration was higher than that of ON (Fig. 4).

The microbial metabolic quotient is the ratio of basal respiration to microbial biomass, which is an indicator of the activity level of the microbial community [45]. With a higher microbial metabolic quotient, the carbon dioxide emission per unit of biomass will be higher and the carbon use efficiency will be lower [46]. Our results indirectly indicated that N addition decreased the microbial utilization efficiency of soil OC (Fig. 5). Compared with other treatments, the N1 treatment had the lowest microbial metabolic quotient in both soil depths (Fig. 5), indicating that IN addition would be beneficial to soil OC sequestration. These results support our findings that the cumulative soil respiration was reduced with IN addition (Fig. 4), because IN may inhibit soil microbial activities.

In general, our mineralization-related results showed that IN addition inhibited soil OC mineralization and was conducive to grassland soil carbon sequestration. This may be attributed to promoted soil acidification, which could directly or indirectly affect soil carbon use efficiency and community composition under continuous N addition [37,47]. Other reports conclude that IN addition may inhibit the activity of enzymes related to organic decomposition [9,48,49], which may also explain the inhibition of soil OC mineralization by the addition of IN.

The results imply that although the added different types of N did not significantly alter soil OC contents, long-term nitrogen (N) addition can affect soil organic carbon pool within different soil fractions with different turnover rates and reduce OC decomposition, which further affects OC stabilization in grassland soil. For understanding the microbial mechanisms of the influence of added N on the soil OC pool distribution and stabilization, future research should include an in-depth study of the effects of N addition on soil enzyme activity and microbial community.

5. Conclusions

Soil OC contents were not significantly affected by different forms of N addition in our experiment. For different soil C fractions, IN addition significantly increased the C in both POM and MAOM fractions compared with the control and the IN addition alone had a stronger effect. Moreover, N addition reduced the microbial metabolic quotient and soil OC mineralization. Overall, although the short-term (5-years) N addition had no significant effect on soil OC, the C distribution of soil fractions and OC mineralization was strongly affected by IN addition. Our results suggest that instead of considering a single source of N (organic or inorganic), both N types should be considered when assessing the responses of grassland soil C cycling to globally increasing N deposition in a long-term perspective.

CRediT authorship contribution statement

Menghan Wang: Experiment implement, Data curation, Writing original draft.
Fucui Li: Methodology, Reviewing and Editing.
Lili Dong: Conceptualization, Experiment design.
Xiang Wang: Methodology, Reviewing and Editing.
Liebao Han: Methodology and Reviewing.
Jørgen E. Olesen: Reviewing and Editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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