Nitrogen input in different chemical forms and levels stimulates soil organic carbon decomposition in a coastal wetland

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ABSTRACT

Nitrogen (N) input significantly regulates soil organic carbon (SOC) storage in N-limited ecosystems. However, the regulatory direction, magnitude, and mechanisms of SOC decomposition under continuous N input in different chemical forms and levels in coastal wetlands are poorly understood. We investigated the impact of 6-year N input in a coastal wetland of the Yellow River Delta, examining the effects on plant growth and soil properties for different chemical forms (NH: ammonium, NO: nitrate, and NN: ammonium-nitrate) and levels (5 g N m^{-2} yr^{-1}, 10 g N m^{-2} yr^{-1}, and 20 g N m^{-2} yr^{-1}) at two depths (0–10 cm, 10–20 cm). We also set up an incubation experiment with N-treated soil to investigate SOC decomposition under different treatments. Six-year N input led to overall increases in the soil nutrients (i.e., total N, ammonium-N (NH$_4^+$-N), and nitrite-N (NO$_3^-$N)), stimulation of plant growth (i.e., plant biomass, height, and density), enhancement of soil C storage (i.e., total carbon, SOC, dissolved organic carbon, and microbial biomass carbon), and decreases in the electrical conductivity (EC). The incubation experiment revealed that N input-induced SOC decomposition stimulation is N-form dependent; NO input was the strongest stimulator of decomposition, when compared to NH and NN input. The stimulation differences among the dominant-form N inputs increased with increasing N supply levels. Structural equation modeling (SEM) analysis indicated that the stimulation of SOC decomposition by N input was associated with N-related changes in the soil nutrients, vegetation, soil C storage, and soil environment. Together, these results aid the evaluation of soil C cycling under future N deposition scenarios in coastal wetlands.

1. Introduction

Anthropogenic activities (e.g., agricultural fertilization and fossil fuel combustion) have remarkably accelerated reactive nitrogen (N) deposition over the last century (Galloway et al., 2008), which has affected the soil physicochemical properties and soil carbon (C) dynamics (Manning et al., 2008; Mo et al., 2008; Allison et al., 2009; Tao et al., 2013; Liu et al., 2016). Coastal wetlands are one of the most productive ecosystems on Earth, serving as natural "blue carbon" sinks due to their low rate of soil organic carbon (SOC) decomposition (Ferrati et al., 2005; Bridgham et al., 2006). The effects of N input on plant growth, microbial activities, and soil C transformation have been widely studied in various ecosystems, such as forests, croplands, and grasslands (Reay et al., 2008; Reich and Hobbie, 2013; Zhou et al., 2014; Wang et al., 2018). However, the effects of N input on SOC decomposition in coastal wetlands remain largely unexplored. Thus, experimental studies are required to investigate the underlying mechanisms of increasing N deposition and C dynamics in this ecologically important ecosystem.

The effects of N input on the terrestrial C cycle have been investigated in numerous field studies and laboratory experiments (Vitousek, 1982; Magill and Aber, 1998; Janssens et al., 2010; Neff

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Layout of quadrats setting

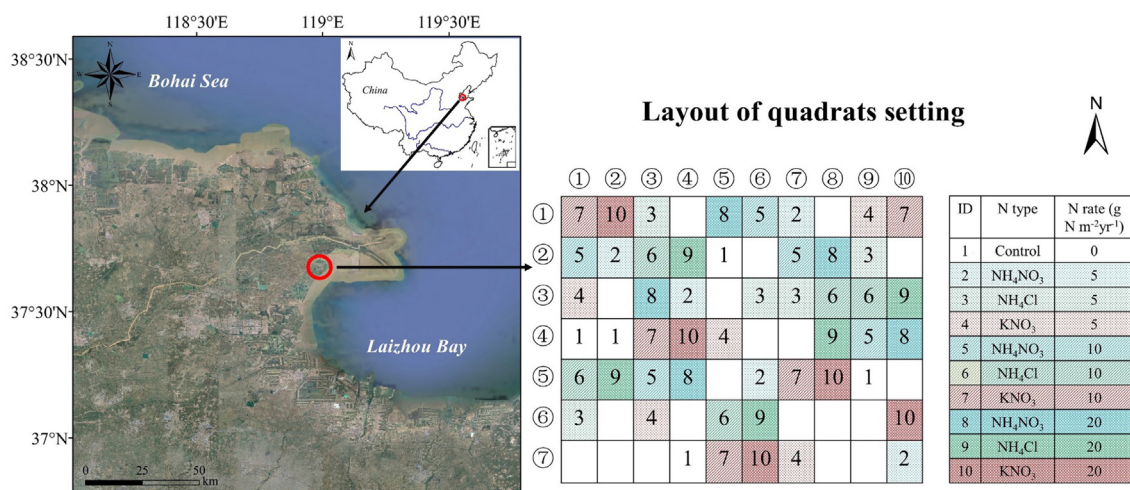| ID	N type	N rate (g N m⁻²yr⁻¹)
1	Control	0
2	NH_4NO_3	5
3	NH_4Cl	5
4	KNO_3	5
5	NH_4NO_3	10
6	NH_4Cl	10
7	KNO_3	10
8	NH_4NO_3	20
9	NH_4Cl	20
10	KNO_3	20

Fig. 1. Location and layout of the experimental plots used to simulate nitrogen deposition.

et al., 2002), yielding inconsistent results. Some studies have reported stimulating effects of N addition on SOC decomposition, primarily due to improved N availability and enhanced soil C content (Knorr et al., 2005; Tu et al., 2013). Suppressive effects of long-term N addition on SOC decomposition have also been reported, mainly due to lower pH and decreased microbial abundance, activity, and respiration (Mo et al., 2008; Wang et al., 2017). Ammonium-N and nitrite-N (henceforth, NH_4^+-N and NO_3^--N, respectively) are ecosystem-dependent inorganic N chemical forms in the soil. For example, NH_4^+-N levels are generally lower than NO_3^--N in forests, grasslands, and shrublands, but not in alpine meadows (Song et al., 2018). Fertilization increases the availability of soil nutrients but also shifts the chemical forms and levels of available N (Krupa, 2003; Neff et al., 2002). N input underlies variations in the soil microbial composition and available carbon (McKane et al., 2002; Song et al., 2018), so the impact on SOC decomposition can be explained by ecosystem processes and environmental factors affecting the quality of the organic matter (i.e., the lignin content, C:N ratio, soil pH, nutrient availability, and ecosystem types). For instance, in high-quality litter with a low C:N ratio, SOC decomposition is often accelerated by N input, due to higher microbial energy savings from increased N availability (Sjoberg et al., 2004; Knorr et al., 2005; Min et al., 2011). Previous research found that microbes prefer NH_4^+-N over NO_3^--N because of lower energy costs during assimilation (Puri and Ashman 1999). However, if N fertilization exceeds the ecosystem-dependent threshold, plant biomass productivity and/or plant species diversity will stabilize or even decline, due to changes in the soil properties (e.g., acidification) (Roem and Berendse, 2000; Evans et al., 2008). Increased acidification and osmotic pressure from long-term N input may also have negative impacts on the microbial activities of fungal groups (Treseder, 2008). Thus, N deposition is a driving force that can regulate plant growth, soil C storage, and microbial activities. To date, the feedback processes of N input, vegetation, and soil properties in coastal wetlands are unresolved, and the effects of continuous N input (in different chemical forms and levels) on SOC decomposition are unclear.

Ecosystem services contribute to human welfare, and the integrity of coastal ecosystems is crucial for coastal protection, erosion, flooding control, fisheries, and recreation (Costanza et al., 1997; Barbier et al., 2011). Coastal wetlands contain large carbon stocks, stabilize sediments, retain nutrients, and deposit particles, and have been thoroughly studied for the impacts of climate change and sea-level rise (Chambers et al., 2013; Meng et al., 2017). Evidence for the effects of N input on coastal wetlands, especially involving SOC dynamics, is limited, despite their vulnerability to environmental and anthropogenic changes. The coastal wetlands of the Yellow River Delta (YRD), located at the interface of the terrestrial and ocean ecosystems, contribute greatly to local C sequestration (Han et al., 2018). Under short-term N addition, NH_4^+-N and NO_3^--N accelerated SOC decomposition and stimulated carbon dioxide (CO_2) emissions in the coastal wetlands (Tao et al., 2013; Tao et al., 2018). The soil saline-alkaline stress of different ecosystems also affects the ecosystem's sensitivity to N input and plant diversity (Gao et al., 2019). However, the links between N input (in different chemical forms and levels) and the vegetation, soil C storage, soil environment, and SOC decomposition of coastal wetlands have received relatively little attention. Most studies have focused on the relationships between the different levels or chemical forms of N input and SOC decomposition, separately (Lugato et al., 2006; Gerdol et al., 2008; Poffenbarger et al., 2018). It is still unclear if increases in different chemical forms of N input affect SOC decomposition. We hypothesize that the responses of SOC decomposition to continuous N input in different chemical forms are regulated by the corresponding input levels, largely through N-related changes in the vegetation status, soil C storage, and soil environment. To test our hypothesis, we added NH_4^+-N and NO_3^--N in different forms and levels to experimental mesocosms in the YRD for 6 years, investigating the impacts of continuous N input on the vegetation and soil properties. We also set up a 40-day soil incubation experiment to quantify SOC decomposition by measuring CO_2 and methane (CH_4) under different N treatments. The main objectives were (i) to investigate changes in the vegetation and soil properties in a coastal wetland after 6 years of N input, (ii) to quantify the rate of SOC decomposition under various N addition treatments, and (iii) to understand how N input in different chemical forms accelerates SOC decomposition and the role of N input levels in regulating the stimulation effects.

2. Materials and methods

2.1. Nitrogen fertilization experiment

The experiment was established in the coastal wetland of the Yellow River Delta Ecological Research Station of Coastal Wetlands, Chinese Academy of Sciences (37°36′N, 118°57′E), in northeast Shandong Province, China (Fig. 1). In most coastal wetlands of the YRD, the groundwater level ranges from 1 to 3 m, with high salinity due to land–ocean interactions (Han et al., 2015). High evaporation transports soluble salt to the soil surface, extending saline-alkaline soils in the area (Zhang et al., 2011). The YRD coastal wetlands have a typical warm semi-humid continental monsoon climate, with a moderate temperature (annual average: 12.9 °C) and adequate precipitation (annual average: 560 mm) (Han et al., 2018), and the dominate plant types are

Phragmites australis and *Suaeda salsa* (Cui et al., 2009). The canopy height can reach 1.7 m in the growing season, with a canopy closure vegetation index ranging from 0.3 to 0.8 (Han et al., 2015). The N deposition rate in the YRD during the growing season (May to November) is nearly 2.26 g m^{-2}, and NO$_3$$^-$-N and NH$_4$$^+$-N are the main N chemical forms of dry and wet deposition, respectively (Guan et al., 2019).

The mesocosm experiment began in May 2012 with 50 quadrats (4 m × 6 m) in a *Phragmites*-dominated flat. To account for the horizontal movement and loss of additional N, the quadrats were approximately 1 m apart and separated from the surrounding vegetation. The experiment consisted of nine N input treatments (3 chemical forms × 3 levels) and a control group (CG) without any fertilizer addition. Each treatment was applied to 5 replicate quadrats (Fig. 1). The chemical N forms were ammonium-N (NH), nitrate-N (NO), and ammonium plus nitrate-N (NN) from ammonium chloride (NH$_4$Cl), potassium nitrate (KNO$_3$), and ammonium nitrate (NH$_4$NO$_3$), respectively. The N treatment levels were set at 5 g N m^{-2} yr^{-1} (low input, 5), 10 g N m^{-2} yr^{-1} (medium input, 10) and 20 g N m^{-2} yr^{-1} (high input, 20), to simulate natural and extreme levels of N deposition (Yu et al., 2014; Guan et al., 2019). The fertilizer was dissolved in 1.2 L of deionized water, with different concentrations for each N treatment (Table S1). We applied fertilizer to each quadrat evenly by a sprayer once per month for 66 months. The fertilizer was sprayed directly onto the soil or plant root to decrease interception by the plant branches and leaves. The method we applied is to simulate atmospheric nitrogen deposition, and low interception by branches and leaves could be reasonable (Tu et al., 2013, Wang et al., 2019a, 2019b).

2.2. Sampling and measurement of the plant traits

The vegetation and soil were sampled at the end of the growing season in October 2018. We randomly selected five sub-quadrats (1 m × 1 m) in each quadrat and collected all of the vegetation and soil. We recorded the density and height of *Phragmites australis* (common reed), the dominant plant in all sub-quadrats. Plant biomass was determined as the total above- (shoots) and below-ground (roots) biomass of *P. australis*. The plant samples were stored in hard envelopes and oven-dried at 80 °C for 48 h to quantify the biomass (g m^{-2}) in the laboratory.

2.3. Soil collection and analyses

Three unsubmerged soil samples from two soil layers (topsoil: 0–10 cm, subsoil: 10–20 cm) were randomly collected from each quadrat using a soil drill. The single-ring infiltration method (using a 7 cm diameter infiltrometer) was used to measure the maximum water-holding capacity. After removing the detritus, the soil samples were packed and sealed in soil bags until analysis in the laboratory. Partial soil samples from each N treatment were kept in a refrigerator (4 °C) until analysis of the soil microbial biomass carbon (MBC). After air-drying, the soil samples were sieved through a < 2 mm mesh for homogenization and stored at room temperature. The above-mentioned approach may have affected the microbial biomass and activity, but the impact of soil heterogeneity can be minimized (Qu et al., 2019).

The sample was air-dried, crushed, and divided into 2 subsamples. One subsample was sieved through a 2 mm mesh and tested for soil pH, electrical conductivity (EC), NH$_4$$^+$-N, and NO$_3$$^-$-N. The other subsample was crushed and sieved through a 0.15 mm mesh to measure the total C (TC), total N (TN), SOC, and soil dissolved organic carbon (DOC). TC and TN were directly analyzed on an elemental analyzer (Vario MACRO cube, Elementar Analysensysteme, Germany). For SOC, the soil samples were pretreated with hydrochloric acid (HCl, 1 mol/L) to remove total inorganic carbon, and then analyzed on the elemental analyzer. The soil pH was measured with a portable pH meter in a 1:5 (w/v) soil:water suspension potentiometrically, and EC was measured

via a portable conductivity meter. To determine the levels of NH$_4$$^+$-N and NO$_3$$^-$-N, 3 g of soil (dry mass) was extracted in 15 mL of 2 M potassium chloride (KCl) solution, followed by measurement on a continuous flow analyzer (AutoAnalyzer III, Seal, Germany) (Robertson et al., 1999). Before measuring the soil dissolved organic carbon (DOC), deionized water and incubated soil were mixed at a ratio of 1:5 and shaken for 1 h. The mixture was centrifuged at 6000 rpm for 25 min, followed by 0.45 μm vacuum filtration (Mavi and Marschner, 2012). Soil DOC was measured on a Shimadzu TOC analyzer (TOC-VCPH). MBC was measured by the chloroform-fumigation extraction method (Brookes et al., 1985), and the Shimadzu TOC analyzer (TOC-VCPH) was used for the MBC analyses. The characteristics of the soil samples, including TC, TN, SOC, DOC, MBC, pH, EC, NH$_4$$^+$, NO$_3$$^-$, and the C:N ratio, are shown in Tables S2 (topsoil) and S3 (subsoil).

2.4. Microcosm soil incubation for MBC analyses

We prepared sixteen 1 L jars per treatment combination for each soil layer, plus four additional blank controls (324 jars in total). We added 100 g of dry soil to each jar and adjusted the soil moisture to 60% of the maximum soil water-holding capacity by adding deionized water every other day. All 324 sample jars were incubated for 40 days at 25 ± 2 °C (Marton et al. 2012; Fang et al. 2019), using an air conditioning to control thermostat and applying air thermometer to monitor the lab temperature.

We divided the incubation period into 4 stages (10, 20, 30, and 40 days) to analyze the temporal patterns of microbial biomass. Four jars with soil and one blank were analyzed at each stage. We excluded MBC on day 1 because the initial water additions to stabilize the soil moisture may have caused an unstable priming effect.

2.5. CO$_2$ and CH$_4$ efflux and SOC decomposition in the microcosm incubated soil

The rates of CO$_2$ and CH$_4$ emission in the incubated jars were measured once every two days, and every jar was fitted with an airtight wooden cork with a long needle and a three-way-valve for 24 h before gas sampling. The collected gas was measured by gas chromatography (Agilent GC-7890, USA). Five empty jars were used as background levels to compare gas production (Liu et al., 2017).

We considered the total rates of CH$_4$-C and CO$_2$-C emissions as the rate of SOC decomposition (SOCD), calculated as:

$$\sum_{t=d1}^{d2} SOCD = \sum_{t=d1}^{d2} CH4\text{-}C + \sum_{t=d1}^{d2} CO2\text{-}C$$

where *d1* and *d2* represent the start and end days of the incubation phases, respectively.

2.6. Statistical analyses

Two-way and one-way analyses of variance (ANOVA) were conducted in IBM SPSS Statistics 23. We used a two-way ANOVA to assess differences in the soil properties, using the N levels, N chemical forms, and their interactions as the fixed effects. One-way ANOVA was applied to assess the statistical differences in the soil properties, plant biomass, height, and density, and SOC decomposition. The differences were significant when $p < 0.05$. Pearson correlation analysis was used to calculate the correlations between plant biomass, soil properties, and SOC decomposition.

Structural equation modeling (SEM) allows testing of the indirect links between N input and SOC decomposition. In this study, we assume that N input stimulates SOC decomposition via effects on the soil nutrients, vegetation, soil C storage, and soil environment. Pearson correlation analysis indicated that all of the variables were significantly correlated with SOC decomposition, so all were included in the SEM. To

Table 1

Repeated measures analysis of variance for properties of topsoil (0–10 cm) and subsoil (10–20 cm) using supply level, supply chemical forms and interactions as fixed effects.

	df	TN		TC		C/N		SOC		DOC	
		F	P	F	P	F	P	F	P	F	P
Topsoil											
Nitrogen levels (NL)	2	38.673	**< 0.001**	2.582	0.092	37.349	**< 0.001**	2.703	0.083	128.614	**< 0.001**
Nitrogen forms (NF)	2	15.004	**< 0.001**	55.089	**< 0.001**	13.541	**< 0.001**	14.575	**< 0.001**	10.957	**< 0.001**
NL × NF	4	7.515	**< 0.001**	23.375	**< 0.001**	4.632	**0.005**	8.743	**< 0.001**	34.065	**< 0.001**
Subsoil											
NL	2	22.779	**< 0.001**	78.295	**< 0.001**	120.353	**< 0.001**	103.634	**< 0.001**	4.494	**0.02**
NF	2	4.723	**0.016**	366.421	**< 0.001**	13.128	**< 0.001**	37.935	**< 0.001**	37.013	**< 0.001**
NL × NF	4	12.947	**< 0.001**	126.496	**< 0.001**	19.011	**< 0.001**	22.133	**< 0.001**	50.472	**< 0.001**

	df	MBC		EC		pH		NH_4^+-N		NO_3^--N	
		F	P	F	P	F	P	F	P	F	P
Topsoil											
NL	2	2.732	0.081	2.139	0.135	0.548	0.584	295.24	**< 0.001**	3003.989	**< 0.001**
NF	2	15.662	**< 0.001**	21.583	**< 0.001**	8.971	**< 0.001**	742.396	**< 0.001**	115.656	**< 0.001**
NL × NF	4	5.479	**0.002**	0.287	0.884	2.35	0.077	115.067	**< 0.001**	37.644	**< 0.001**
Subsoil											
NL	2	6.927	**0.003**	7.428	**0.002**	0.368	0.695	81.382	**< 0.001**	530.165	**< 0.001**
NF	2	0.167	0.846	18.193	**< 0.001**	26.845	**< 0.001**	168.525	**< 0.001**	0.277	0.76
NL × NF	4	2.23	0.089	5.172	**0.003**	11.197	**< 0.001**	44.162	**< 0.001**	10.009	**< 0.001**

TC: soil total nitrogen, TC: total carbon, C/N: C: N ratio, SOC: soil organic carbon, DOC: dissolved organic carbon, MBC: microbial biomass carbon, EC: electrical conductivity, NH_4^+-N: ammonium, NO_3^--N: nitrate. Significant P-values marked in bold ($p < 0.05$).

evaluate the SEM fit, we used the standardized root mean square residual (SRMR), the chi-square to degrees of freedom ratio (χ^2/df), and normed fit index (NFI) (Hair et al., 2017). The sample size was lower than 100, so the partial least square (PLS) method was used to predict complex predictive path models (Wong, 2013). The SEM was conducted in SmartPLS 3.2.8, a software for PLS-SEM (www.smartpls.com).

3. Results

3.1. Impacts of 6-year N input on soil properties and plant growth

The effects of the N levels, N chemical forms, and N levels × N chemical forms on the soil C pools (i.e., total C, SOC, and DOC), total N, NH_4^+-N, NO_3^--N, and EC were all highly significant ($p < 0.05$) (Table 1). The 6-year N input of different chemical forms and levels significantly increased the soil N contents, with higher soil total N (91.5%), NH_4^+-N (277.9%), and NO_3^--N (19.7%). N input also significantly enhanced the soil C pools, with 6.6%, 15.1%, and 228.8% increases in total C, SOC, and MBC, respectively. A lower C:N ratio (41.2%) and EC (21.1%) were observed in the topsoil and subsoil (Tables S2 and S3). Soil total C, SOC, and MBC in the NO treatments were generally higher than in the NH- and NN-treated soil.

Plant biomass, height, and density were used as indicators of the plant growth response to N input in different chemical forms and levels. The N fertilizer generally stimulated plant growth, with 17.6%, 20.9%, and 16.4% increases in plant biomass, height, and density, respectively (Fig. 2). For the NN and NO treatments, the stimulating effects on plant growth tended to increase with increasing N levels. However, NN input had a neutralizing effect on plant biomass and density. The differences in plant biomass among the dominant-forms of N increased with increasing N levels (NO > NH > NN).

3.2. Impacts of N input on CO_2 and CH_4 emissions and SOC decomposition

The cumulative CO_2 emissions over the 40-day incubation period were linear in all of the treatments (Fig. 3). In general, the CO_2 emissions from the topsoil were significantly higher than the subsoil

($p < 0.05$), and the highest emissions were observed in the NO treatments of both soil layers. There were no differences in the CH_4 emissions, and the cumulative CH_4 emissions tended to decrease during the incubation period (Fig. 4).

The SOC decomposition rate was determined by the total rates of CO_2 and CH_4 emissions in each treatment. During the 40-day incubation period, the N-treated soil had a higher rate of SOC decomposition, with an average enhancement of 41.6% and 37.7% in the topsoil and subsoil, respectively (Fig. 5). The NO-treated soil showed stronger stimulation of SOC decomposition relative to the NN- and NH-treated soils. The stimulation differences of SOC decomposition among 3-form N input generally increased with increasing input levels for two layers of incubated soils (NO > NH > NN).

3.3. Linking N-related soil properties to SOC decomposition

Pearson correlation analysis revealed significant correlations between the plant biomass, soil properties, and SOC decomposition (Table 2). For example, the rate of SOC decomposition was positively correlated with MBC, total C, SOC, total N, NO_3^--N, soil pH, and plant biomass, but negatively correlated with EC. MBC and SOC had significant positive correlations with total N, NO_3^-N, and plant biomass, and negative correlations with NH_4^+-N and EC ($p < 0.05$).

SEM analysis was used to explore the underlying mechanisms of N input, soil nutrients, vegetation, soil C storage, soil environment, and SOC decomposition (Fig. 6). The significance criteria of the fitted model were met, with SRMR, χ^2/df, and NFI of 0.077, 2.170, and 0.925, respectively. The final model explained 22.2% of the variance for soil nutrients, 61.1% for vegetation, 56.8% for soil C storage, 32.4% for soil environment, and 57.1% for SOC decomposition. The soil nutrients (total N, NH_4^+, and NO_3^-) and vegetation (plant biomass, height, and density) were positively driven by N input and positively associated with soil C storage (total C, SOC, DOC, and MBC), but negatively related to the soil environment (pH and EC). Soil C storage had a significant positive impact on SOC decomposition ($p < 0.001$), while the soil environment had a negative influence on SOC decomposition ($p < 0.05$).
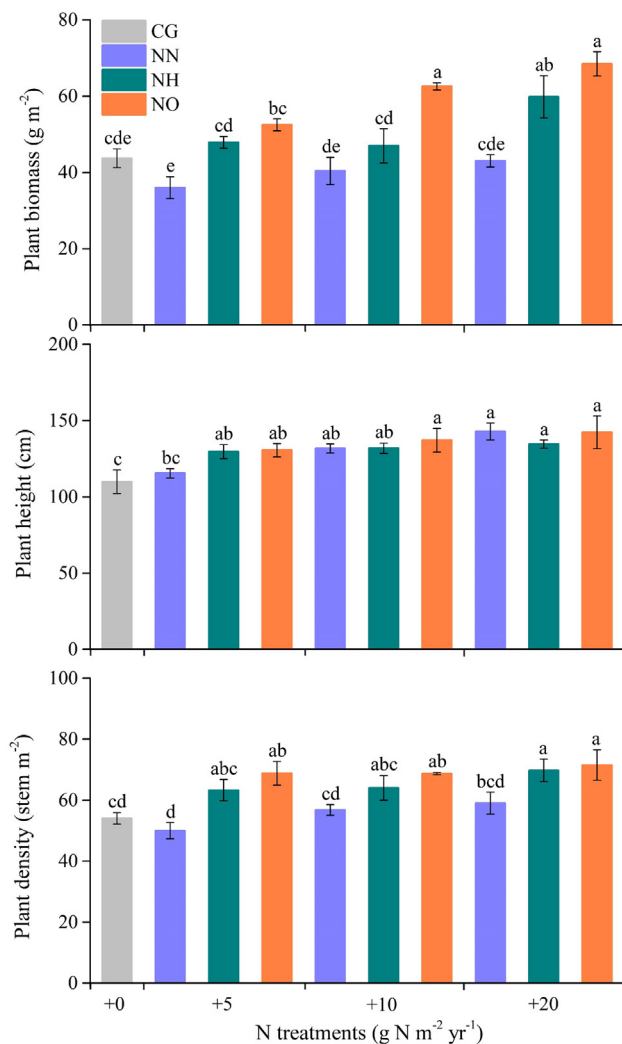
Fig. 2. Effects of nitrogen (N) input on the plant biomass, height and density of *Phragmites australis* in a coastal wetland of Yellow River Delta. Different lower case letters represent a highly significant difference ($p < 0.05$). CG, NO, NH and NN represent control group, adding KNO_3, NH_4Cl and NH_4NO_3, respectively. $+0$, $+5$, $+10$ and $+20$ represent no N input, adding 5, 10 and 20 g N m^{-2} yr^{-1}, respectively.

3.4. Linking MBC variation to SOC decomposition

The MBC for both soil layers tended to decline across the incubation period (days 10, 20, 30, and 40). The N-treated soil samples had a higher average MBC, compared to the CG samples, and the highest MBC was observed in NO-treated soil ($p < 0.05$) (Fig. 7). Significant positive correlations between SOC, MBC, and the rate of SOC decomposition were also observed, with an overall coefficient (r) of 0.65 and 0.69, respectively (Fig. 8).

4. Discussion

4.1. N-related changes in the soil properties and plant growth

Soil properties play a key role in soil C accumulation and transformation (Sims et al., 2012; Freschet et al., 2013). In this study, continuous N input in different chemical forms and levels significantly altered the soil properties (Tables S2 and S3). There was a negative correlation between EC and plant biomass (Table 2, $p < 0.05$), indicating that N-induced plant growth reduced the soil evaporation and increased the soil water content, indirectly decreasing soil EC (Qu et al.,

2019; Shaygan et al., 2018). Previous studies reported that long-term N input negatively affected soil microbial activities and biomass through increased acidification and osmotic pressure from the oxidation of NH_4^+ to NO_3^- (Evans et al., 2008; Treseder, 2008). We observed stable pH under N input in the topsoil, higher pH in the subsoil with the NO and NH treatments, and lower pH in the NN soil ($p < 0.05$). The significant positive correlation between pH and MBC (Table 2) may explain the MBC differences in the N-treated soils, which is consistent with previous studies reporting positive effects of increased soil pH on the microorganisms (i.e., ammonia-oxidizing bacteria) (Nicol et al., 2008; Ying et al., 2017). Our study also demonstrated that short-term N input cannot accurately predict the effects of long-term N deposition on soil microbial biomass and the soil environment (Wang et al., 2019a, 2019b). Evidence suggests that N input alleviates the N limitations of soil microorganisms and stimulates soil enzyme (urease) activity in the coastal wetlands of the YRD (Guan et al., 2019). In this study, N input generally increased the microbial biomass; we observed significant positive correlations between microbial biomass and soil total N, NO_3^--N, and MBC (Table 2). Therefore, soil salinization and nutrient limitations in the YRD coastal wetlands may be alleviated by N input, which influences the soil microbial population, composition, and community structure (Zhou et al., 2020).

N input positively affects plant growth and soil C storage in nutrient-poor ecosystems (van Der Heijden et al., 2008; Bolinder et al., 2010). In this study, plant growth, soil C storage, and microbial biomass were stimulated in the NO-treated soil, supporting the significant positive correlations between plant biomass, SOC, and MBC (Table 2). Higher levels of NO and NH input resulted in greater plant biomass, height, and density. However, neutralizing effects on plant growth were observed in the NN-treated soil (Fig. 2), suggesting that the fertilization effect is N-form dependent in ecosystems where plant growth is limited by the soil nutrients (Tian et al., 2019).

4.2. The response of SOC decomposition to N-related changes

Previous studies reported contradictory effects of N input on SOC decomposition via fluxes in the CO_2 and CH_4 emission rates (Neff et al., 2002; Janssens et al., 2010; Tu et al., 2013; Wang et al., 2017; Fang et al., 2019). Our study is consistent with prior results; both NH_4^+-N and NO_3^--N stimulated SOC decomposition in the YRD coastal wetlands (Tao et al., 2013; Tao et al., 2018). We used the SEM analysis to test our hypothesis that the N input-induced stimulation of SOC decomposition is associated with N-related soil nutrients, vegetation, soil C storage, and the soil environment (Table 2 and Fig. 6). Higher total N and NO_3^--N were observed in the N-treated soils, both of which were positively correlated with SOC decomposition. Increased N availability and a decreased C:N ratio (Tables S2 and S3) stimulates the microbes to produce more C-degrading enzymes relative to the N-degrading enzymes, further accelerating SOC decomposition (Taylor et al., 1989; Allison and Vitousek, 2005). N-induced plant growth increased C input and stimulated SOC formation through soil-root interactions, indicating that root exudation leads to rhizosphere priming effects on SOC decomposition (Kuzyakov et al., 2000; Dijkstra and Cheng, 2007). Our results suggest that N input accelerates SOC decomposition through higher plant biomass and soil C storage. Higher SOC contents increase SOC decomposition according to the kinetic model of SOC decomposition (Fang et al., 2019). Meanwhile, N-related increases in MBC also played an important role in stimulating SOC decomposition. N inputs alter the microbial allocation to enzyme production, thereby affecting soil C cycling (Guan et al., 2019). We observed a significant positive correlation between MBC and SOC decomposition throughout the incubation period (Fig. 8), supporting the key role of the microbial population in SOC decomposition (Treseder, 2008; Min et al., 2011). Some studies have illustrated limited SOC decomposition due to high soil EC or salinity (Liu et al., 2017; Qu et al., 2019), stemming from lower plant productivity and microbial abundances (Setia et al., 2013;
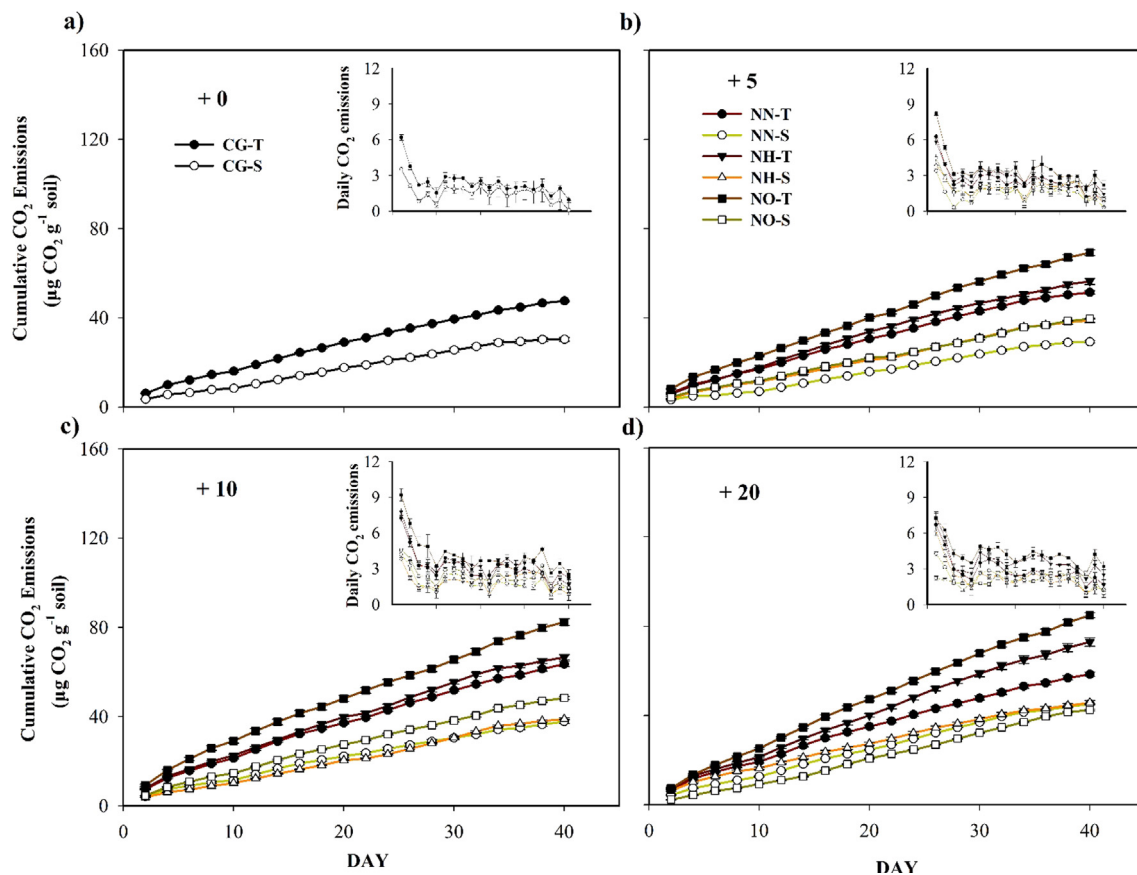
Fig. 3. Cumulative emissions of CO_2 during the 40-day incubation of treatments of (a) control group (no added N), (b) low N addition ($+5$ g N m^{-2} yr^{-1}), (c) medium N addition ($+10$ g N m^{-2} yr^{-1}) and (d) high N addition ($+20$ g N m^{-2} yr^{-1}:d). The error bars indicate the standard deviations of four measurements (n = 4). The subgraph represents daily CO_2 emissions for each treatment. CG, NO, NH and NN represent control group, adding KNO_3, NH_4Cl and NH_4NO_3, respectively. T and S represent topsoil (0–10 cm) and subsoil (10–20 cm), respectively.

Belleveau et al., 2015). We observed a negative correlation between SOC decomposition and EC (Table 2 and Fig. 6), suggesting that N input alleviates soil salinization via plant growth stimulation (Guan et al., 2019) and accelerated soil C losses in the coastal wetlands.

4.3. Different responses of SOC decomposition to the dominant-form N input

Prior work has shown that the form of N input affects soil C mineralization via biological (enzyme activities) and chemical (pH) changes (Evans et al., 2008; Min et al., 2011). Min et al. (2011) reported neutralizing effects of N input on soil pH in alkaline soil, consistent with our results from the topsoil. Therefore, we assume SOC decomposition responds to the dominant-form N through N-induced increases in the vegetation, soil C storage, and microbial function. Puri and Ashman (1999) found that microorganisms prefer NH_4^+-N over NO_3^--N because of the low energy costs, indicating higher SOC decomposition rates with NH_4^+-N input. We did not find a strong correlation between NH_4^+-N and SOC decomposition, but NO_3^--N had a significant positive correlation with SOC decomposition. NO input had the greatest effects on soil NO_3^--N and SOC decomposition (compared to the NN- and NH-treated soils) (Fig. 5). Perhaps, NO_3^--N had a stronger fertilization effect on plant growth than NH_4^+-N, and the effect increased with the N supply level (Guan et al., 2019). The highest SOC was also observed in the NO-treated soil (Tables S2 and S3). The kinetic model of SOC decomposition suggests that NO input more easily decomposes SOC relative to NN and NH, which contributes to the higher SOC contents. Increased MBC was observed under N input and was related to the microbial population and structure. High MBC in the

NO-treated soils provides evidence for the strongest stimulation of SOC decomposition among the dominant N chemical forms. NO input resulted in a microbial shift from fungal to bacterial (Allison et al., 2009), with an increase in SOC decomposition (Six et al., 2006). A prior short-term N input experiment also reported positive effects of NH_4^+ on the ammonia-oxidizing bacteria (AOB) and a role for AOB in nitrification (Ying et al., 2017). This may explain the lower allocation of microbial activities in SOC decomposition compared to the NO_3^--N soil.

4.4. Implications for soil C balance in the coastal wetlands

Nutrient enrichment, a global problem in coastal ecosystems (Canfield et al. 2010), can lead to salt marsh loss (Deegan et al. 2012) and C cycle regulation (Min et al. 2011). Along with sea-level rise, nutrient loading results in variable soil properties and C exchange between the soil and atmosphere. We found higher SOC decomposition rates in the N-treated soils, with increases of 41.6% and 37.7% in topsoil and subsoil (Fig. 5), respectively. This indicates an increased C pool function driven by N input. However, continuous N input increased the vegetation productivity and fixed more C from the atmosphere into the soil, thereby acting as a C sink. Consistent with prior work (Liu et al. 2017), N input played an important but complex role in regulating the soil C dynamics of the coastal wetlands, interacting with the vegetation, microbial activity, and soil environment. Therefore, a better understanding of the feedback processes of N deposition and C dynamics in the coastal wetlands requires more empirical studies (Morillas et al. 2015). Moreover, forced factors, such as the N input forms and levels, experiment duration, and the soil depth, may moderate the SOC decomposition response. Nevertheless, the effects of
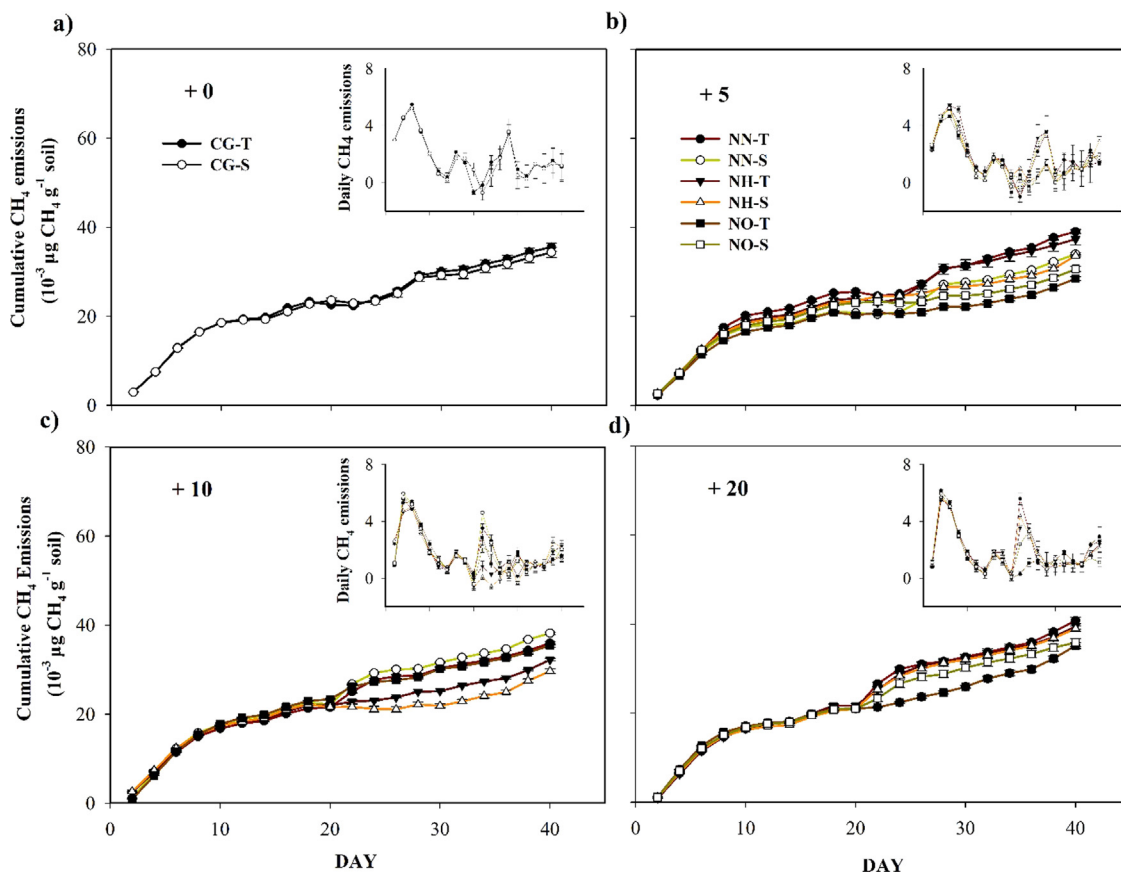
Fig. 4. Cumulative emissions of CH_4 during the 40-day incubation of treatments of (a) control group (no added N), ((b) low N addition ($+5$ g N m^{-2} yr^{-1}), (c) medium N addition ($+10$ g N m^{-2} yr^{-1}) and (d) high N addition ($+20$ g N m^{-2} yr^{-1}:d). The error bars indicate the standard deviations of four measurements (n = 4). The subgraph represents daily CH_4 emissions for each treatment. CG, NO, NH and NN represent control group, adding KNO_3, NH_4Cl and NH_4NO_3, respectively. T and S represent topsoil (0–10 cm) and subsoil (10–20 cm), respectively.

different N supply forms and levels on SOC decomposition was demonstrated in this study, with implications for C sequestration in coastal wetlands.

4.5. Limitations

Factors that may have interfered with the experiment are listed here. First, removing plants may create uncertainty in the experiment results. A previous study reported a rhizosphere priming effect, where
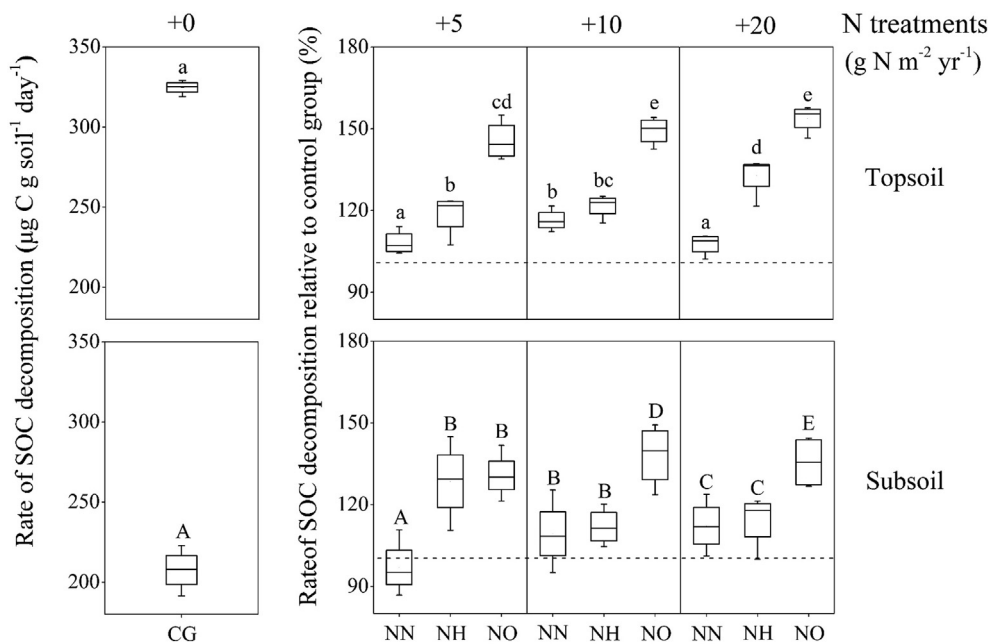

Fig. 5. Box plots (showing the median, surrounded by a 50% quantile box and 100% quantile whiskers) of the rate of soil organic carbon decomposition (SOCD) during 40-day incubation, relative to the control group, for each nitrogen (N) chemical form, level and soil type. CG, NO, NH and NN represent control group, adding KNO_3, NH_4Cl and NH_4NO_3, respectively. $+0$, $+5$, $+10$ and $+20$ represent no N input, adding 5, 10 and 20 g N m^{-2} yr^{-1}, respectively. Different lower case and upper case letters represent a highly significant difference for topsoil and subsoil ($p < 0.05$), respectively.

Table 2
Pearson correlation coefficients (r) between plant biomass, soil properties and soil organic carbon decomposition in incubation.

	SOCD	MBC	TC	SOC	DOC	TN	NH$_4^+$-N	NO$_3^-$-N	pH	EC	PB
SOCD	1	**0.46****	**0.340***	**0.670****	−0.184	**0.714****	0.119	**0.694****	**0.485****	**−0.401****	**0.732****
MBC		1	**0.347***	**0.483****	**−0.336***	**0.383****	0.030	**0.539****	**0.303***	**−0.462****	**0.286***
TC			1	**0.314***	**−0.279***	**0.616****	−0.023	**0.663****	−0.013	**−0.355***	0.039
SOC				1	−0.022	**0.416****	−0.249	**0.399****	**0.575****	0.158	**0.506****
DOC					1	−0.062	0.163	−0.256	**−0.392***	**0.509****	−0.024
TN						1	0.08	**0.829****	0.072	0.076	**0.499****
NH$_4^+$-N							1	**0.355***	−0.213	0.228	0.046
NO$_3^-$-N								1	0.190	−0.010	**0.400****
pH									1	−0.070	**0.334***
EC										1	**−0.414****
PB											1

SOCD: soil organic carbon decomposition, MBC: microbial biomass carbon, TC: total carbon, DOC: dissolved organic carbon, TN: total nitrogen, NH$_4^+$-N: ammonium, NO$_3^-$-N: nitrate, EC: electrical conductivity, PB: plant biomass. Significant correlations marked in bold, n = 5, where ** $p < 0.01$, * $p < 0.05$.

interactions between the soil and plant roots accelerate SOC decomposition (Dijkstra and Cheng, 2007). Increased N availability stimulated soil C loss with a higher rate of SOC decomposition and fixed more C into the plants and the soil, acting as a driver of C sequestration. Therefore, more field experiments, involving the soil, plants, and their interactions, are required to evaluate the role of N input on the soil C balance. Second, the soil salinity (Gao et al., 2019) and drying-rewetting frequency (Morillas et al. 2015) moderates the response of SOC decomposition to N input. This study examined only the forms and levels of N input and controlled the potential effects of soil salinity and water conditions. Long-term coupling experiments are required for more meaningful results. Furthermore, N input is a regulatory factor of CH$_4$ oxidation and may enhance CH$_4$ emission from the soils and sediments into the atmosphere (Bodelier & Laanbroek 2004). We reported variability in the CH$_4$ emissions of the different treatments but lack an empirical explanation of this phenomenon. Additional microbial experiments are required to investigate the responses of bacterial and methanotrophic communities to different chemical forms and levels of N input.

5. Conclusions

Our results suggest that six years of N input accelerated the SOC decomposition in the YRD coastal wetland. This was related to N-induced shifts in the soil properties and plant growth. Increased N availability significantly stimulated plant growth, increased soil C
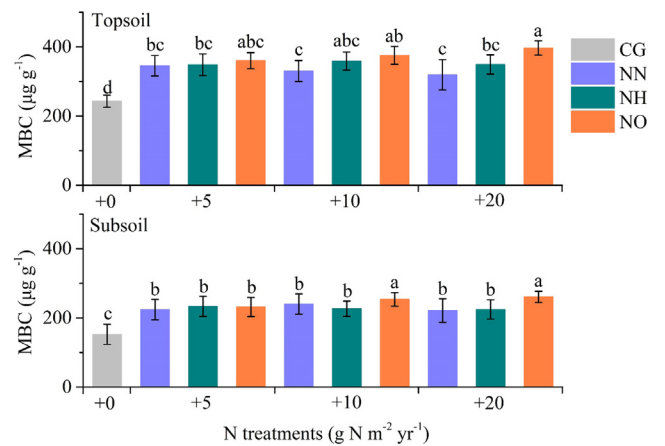
Fig. 7. Effects of nitrogen (N) input on the microbial biomass carbon (MBC) of all nitrogen-addition treatments in topsoil (0–10 cm) and subsoil (10–20 cm), respectively. The bar graphs represent average MBC for day 10, 20 30 and 40. Different lower case letters represent a highly significant difference for average MBC ($p < 0.05$). CG, NO, NH and NN represent control group, adding KNO$_3$, NH$_4$Cl and NH$_4$NO$_3$, respectively. +0, +5, +10 and +20 represent no N input, adding 5, 10 and 20 g N m^{-2} yr^{-1}, respectively.
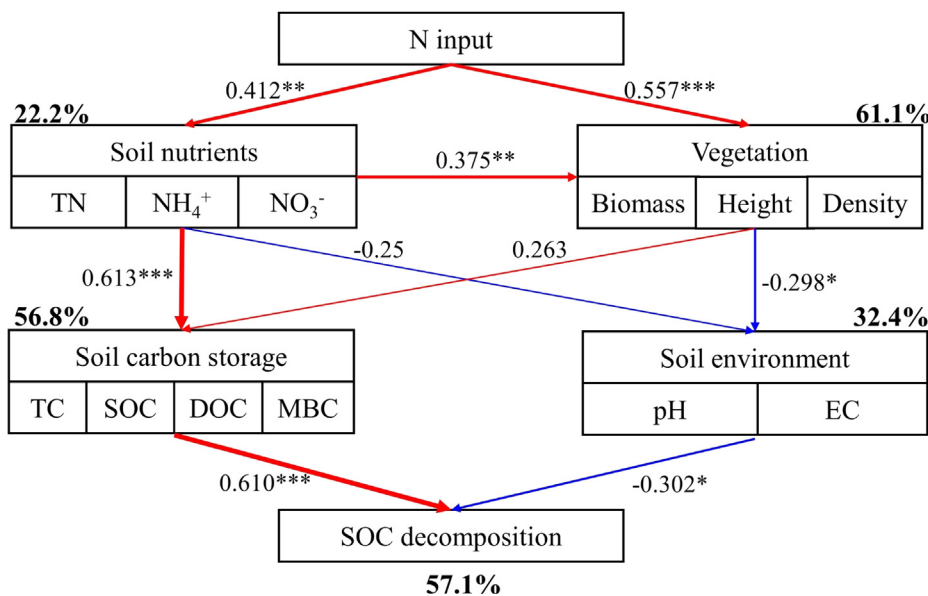
Fig. 6. Structural equation model (SEM) analysis describing the effects of soil nutrients, vegetation, soil carbon storage, and soil environment on soil organic carbon decomposition under nitrogen input. TN: total nitrogen, NH$_4^+$-N: ammonium, NO$_3^-$-N: nitrate, TC: total carbon, SOC: soil organic carbon, DOC: dissolved organic carbon, MBC: microbial biomass carbon, EC: electrical conductivity. Path coefficients (correlation coefficients) along arrows are standardized by the mean of each parameter. Numbers on the arrows are standardized direct path coefficients. Red arrow lines indicate positive effects, and blue arrow lines indicate negative effects. The width of the arrows is proportional to the strength of the relationship, and the numbers adjacent to arrows are standardized path coefficients. Percentages close to variables refer to the variance accounted for by the model (R^2). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
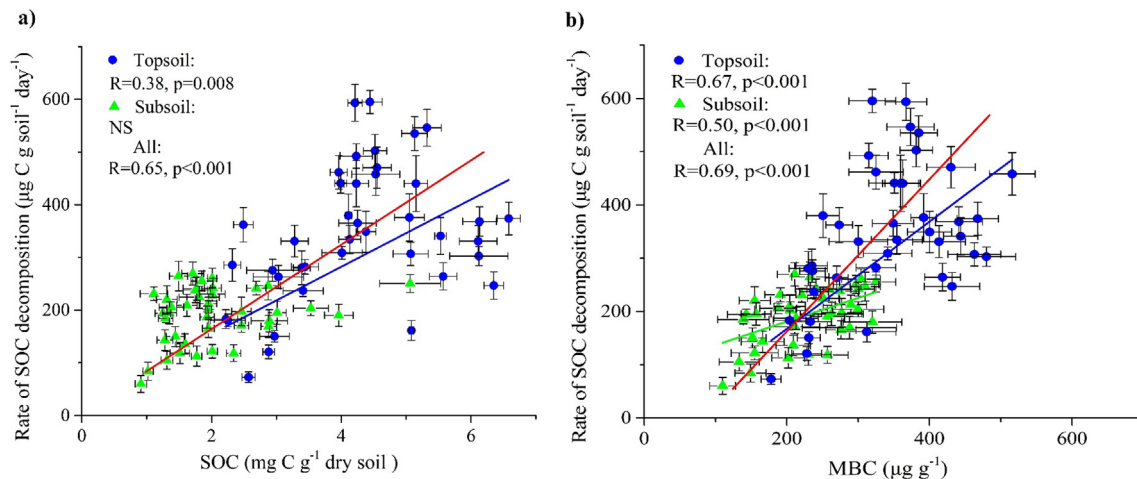
Fig. 8. Relationships between (a) soil organic carbon (SOC), (b) microbial biomass (MBC) and rate of SOC decomposition for all nitrogen-treated soil samples (topsoil: 0–10 cm; subsoil: 10–20 cm) in four stages of incubation (day 10, 20, 30 and 40). Significance was set at $p < 0.05$.

storage, and decreased the soil EC. SOC decomposition was positively associated with SOC, MBC, and NO_3^--N, and negatively correlated with soil EC. The NO-treated soil had the highest rate of SOC decomposition. Differences in the stimulatory effects of the dominant-form N inputs increased with increasing N levels (NO > NH > NN), and the optimal level of N input to maximize SOC decomposition was N-form dependent. MBC helped to explain differences in SOC decomposition among the different N-form soil samples during the incubation period. From the SEM analysis, we conclude that N input stimulates SOC decomposition via effects on the soil nutrients, vegetation, soil C storage, and soil environment.

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.

Acknowledgements

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catena.2020.104672.

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