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Grazing mediates soil microbial activity and litter decomposition in salt marshes

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Abstract

Salt marshes contribute to climate change mitigation because of their great capacity to store organic matter (OM) in soils. Most of the research regarding OM turnover in salt marshes in times of global change focuses on effects of rising temperature and accelerated sea-level rise, while effects of land-use change have gained little attention. The present work investigates the mechanisms by which livestock grazing can affect OM decomposition in salt marsh soils. In a grazing exclusion experiment at the mouth of the Yangtze estuary, China, we assessed soil microbial exo-enzyme activity (EEA), to gain insight into the microbial carbon (C) and nitrogen (N) demand. Additionally, we studied the decomposition of plant litter in soil using the Tea Bag Index (TBI), a widely used standardized litter bag assay to fingerprint soil

decomposition dynamics. Based on EEAs, grazing markedly reduced microbial C acquisition, whereas microbial N acquisition was strongly increased. These opposing grazing effects were also evident in the decomposition of standardized plant litter: The decomposition rate constant (k) and the stabilization (S) of litter were not inversely related, as would be expected, but instead both were reduced by livestock grazing. Our data suggest that grazing effects on EEAs and litter decomposition can just partly be explained by grazing-driven soil compaction and resulting lower oxygen availability, which has previously been hypothesized as a main pathway by which grazing can reduce microbial activity in wetland soils. Instead, grazing effects on microbial nutrient demand occurs to be an at least equally important control on soil decomposition processes.

Keywords: livestock, carbon sequestration, land-use change, blue carbon, enzyme stoichiometry, Tea Bag Index

1 Introduction

As “blue carbon” ecosystems, salt marshes store large amounts of carbon (C) in form of organic matter (OM) in their soils (Chmura, 2013) and are considered one of the most important long-term C sinks of the biosphere (Duarte et al., 2013; Mcleod et al., 2011). Salt marshes not only play an important role in coastal C sequestration, and thus contribute to climate change mitigation, they also provide other highly valued ecosystem services, like coastline protection and biodiversity support (Barbier et al., 2011; Möller et al., 2014). However, these ecosystem services of salt marshes are affected by global change (i.e. climate and land-use change), and therefore, there is a growing interest to understand how global change factors alter the potential for ecosystem-service delivery (Kirwan and Megonigal, 2013).

Several studies evaluated how C sequestration in salt marshes is affected by climate change factors, such rising temperatures and accelerated sea-level rise (Kirwan et al., 2013; Mueller et al., 2016;

Mueller et al., 2018; Rogers et al., 2019), whereas land-use change, such as the introduction or abandonment of livestock grazing, received much less attention. The use of salt marshes for livestock grazing has a long history especially in Europe, where it dates back to pre-historic times (Barr and Bell, 2016; Nolte et al., 2015; Tessier et al., 2003), but livestock grazing in salt marshes can also be found in South America (Di Bella et al., 2015; Sica et al., 2016) and East-Asia (He et al., 2015; Ning et al., 2019; Suzuki and Suzuki, 2010). Yet, the effect of this common land-use practice on C sequestration is unclear. Recent studies, mostly from Europe, provide equivocal results concerning grazing effects on soil C stocks and sequestration rates in salt marshes (Elschot et al., 2015; Ford et al., 2019; Harvey et al., 2019; Morris and Jensen, 1998). C sequestration in salt marshes is controlled by two primary processes, namely OM input via plant primary production and OM output via decomposition (Kirwan et al., 2013). Both of these processes are strongly affected by livestock grazing (Mueller et al., 2017), with a large number of studies outlining that livestock grazing reduces biomass (reviewed by Davidson et al. 2017). Yet, most of these studies quantified only effects on aboveground biomass, while those also quantifying belowground biomass are scarce (Davidson et al., 2017). Among these, Elschot et al. (2015) demonstrated that livestock grazing could in fact promote higher belowground biomass and thereby increase OM input to the soil.

In comparison to grazing effects on biomass production in salt marshes, its effects on OM decomposition is far less understood (Elschot et al., 2015; Mueller et al., 2017). To understand how livestock grazing regulates OM decomposition in salt marshes, particularly two mechanisms need consideration, namely grazing effects on the microbial substrate supply (i.e. changes in OM quality and quantity) and grazing effects on soil oxygen availability (Elschot et al., 2015). Grazing can affect the microbial substrate supply via several mechanisms including changes in plant primary production and species composition, which regulate the quality and amount of plant litter, root exudates, and allochthonous OM entering the soil OM pool of salt marshes (Ford et al., 2013; Mueller et al., 2019a;

Olsen et al., 2011). These changes in microbial substrate supply are potentially important controls of the microbial C and nutrient demand and thus affect OM decomposition (Sinsabaugh et al., 2008).

The probably most frequently hypothesized mechanism by which grazing affects decomposition in wetland soils is trampling-driven soil compaction (i.e. reduction of pore space) and the resulting reduction of soil oxygen availability (Elschot et al., 2015; Kauffman et al., 2004; Mueller et al., 2017; Schrama et al., 2013). This, in turn, can inhibit microbial exo-enzyme activity (EEA), metabolism, and ultimately OM decomposition (Davidson and Janssens, 2006; Freeman et al., 2001; Megonigal et al., 2004). Indeed, recent insights from salt marsh ecosystems could provide some evidence of trampling-driven soil compaction and resulting reductions in oxygen availability (Elschot et al., 2015). Yet, the link between trampling-driven soil compaction and decomposition has never been demonstrated.

The aim of this study is to improve the mechanistic understanding of how livestock grazing affects decomposition processes in salt marsh soils. The study was conducted in a grazing-exclusion experiment in a Chinese salt marsh ecosystem of the Yangtze estuary. Estimates of the total Chinese salt marsh area highly variable and range between 1.2×10^6 and 3.5×10^6 ha (Meng et al., 2019; Meng et al., 2017). Livestock grazing is a relatively common land-use in Chinese salt marshes (Davidson et al., 2017); however, data on grazing frequency and intensity is not available. We assessed the mechanisms by which grazing affects OM decomposition by quantifying the activity of microbial exo-enzymes and litter decomposition parameters. EEAs are regarded as the rate-limiting step of the decomposition processes and reveal insight into microbial C and nutrient demands (Sinsabaugh et al., 2008). However, EEA dynamics have yet poorly been studied in salt marsh ecosystems. We hypothesize (1) that grazing reduces EEAs by increasing soil bulk density and thereby lowering soil oxygen availability. We hypothesize (2) that grazing induced reductions in EEAs will translate into a higher degree of litter stabilization in soil and, inversely, lower rates of litter decomposition.

2 Methods

2.1 Site description and experimental design

This study was carried out in the Dongtan salt marsh on Chongming island, China (31°28'N, 121°56'E), close to the city of Shanghai (Fig. 1a, b). The Dongtan salt marsh covers an area of 4000 ha and is one of the largest tidal wetlands of the Yangtze estuary. It is a minerogenic salt marsh with high rates of sediment deposition and a typical feature of the Chinese salt-marsh landscape (Yang et al., 2001; Yang et al., 2008). The study area is exposed to subtropical humid monsoon climate, and the annual temperature and precipitation are 15.3°C and 1022 mm, respectively. The average spring tide range is approximately 2.5 - 3.5 m. The mean elevation of high marsh is 380 cm above sea level, resulting in an average monthly inundation frequency of 17. The mean elevation of the low marsh is 330 cm above sea level, resulting in an average monthly inundation frequency of 39. The salt marsh is grazed by cattle from early April to late October at a stocking density of approximately one cattle per ha (Yang et al., 2017). Dominant plant species in the high marsh are *Phragmites australis* and *Carex scabrifolia*, while *Scirpus mariqueter* and *C. scabrifolia* are dominant in the low marsh. Both marsh zones have similar grazing levels (Yang et al., 2017).

The design of the experiment has previously been described by Yang et al. (2017). Briefly, it includes two marsh zones (low vs. high) and land-use (grazed vs. ungrazed) as factors. In 2014, six replicate plots were established in each of the marsh zones. Each plot contains two sub-plots of 15 x 15 m, a grazed (control) and an ungrazed (exclusion) sub-plot. The total number of sub-plots is N = 24 (6 replicates x 2 marsh zones x 2 grazing treatments), and distance between plots within a marsh zone was 50-100 m.

2.2 Biomass and soil bulk density

Belowground biomass was sampled to understand potential differences in belowground OM input, and thus microbial substrate supply, between grazed and ungrazed sub-plots. Samples were collected in May of 2016 within three randomly positioned 25 cm x 25 cm quadrats using a PVC corer of 15 cm diameter into 20 cm depth in each sub-plot. Samples were washed to remove all soil with 0.5 mm sieve, dried at 70°C for 72 h, and weighed. This work is focused on belowground C dynamics. For aboveground biomass data, we refer the reader to previous work of our group (Yang et al., 2017). Soil bulk density (BD (g/cm^3) = soil dry weight (g) / soil volume (cm^3)) was determined as a proxy for soil compaction and thus oxygen availability (Schrama et al., 2013). One soil sample was collected in each sub-plot with three different soil depths (0-5 cm, 5-10 cm and 10-15 cm) using a 3.2-cm diameter soil corer. Samples were dried at 70 °C for 72 h and weighed.

2.3 Exo-enzyme assays

Following Sinsabaugh et al. (2009), we measured soil β -glucosidase activity for the assessment of microbial C acquisition. Leucine-aminopeptidase and chitinase activity were measured for the assessment of microbial N acquisition. Soil samples were collected in May 2016 using a 3.2-cm diameter corer to sample the topsoil (0-5 cm). EEAs were determined in fluorometric assays following Mueller et al. (2017). In brief, a 20-g subsample of fresh topsoil in each sub-plot was mixed in 20 mL deionized water, and the homogeneous slurry was stored at -20 °C until further analysis. Well-plate assays were conducted to measure potential enzyme activity. Plates were incubated in the dark at 20 °C for 16 h and read on a Multi-Detection Microplate Reader (Bio-tek Synergy™ HT, Winooski, USA). Activities reported refer to normalized EEAs per unit soil OM (i.e. specific EEA), in order to obtain a measure for organic matter decomposition rate (Morrissey et al., 2014; Mueller et al., 2017). OM contents of subsamples were assessed using loss on ignition following the protocol of Wang et al. (2011) for marine sediments (550 °C for 5 h).

2.4 Decomposition of standardized plant litter

We studied the decomposition of standardized litter to control for potential differences in native plant-litter quality (i.e. microbial substrate quality) between grazed and ungrazed treatments of our field experiment. Specifically, decomposition rate constant (k) and stabilization factor (S) were assessed following the Tea Bag Index (TBI) protocol (Keuskamp et al., 2013). The decomposition rate constant (k) describes the decomposition rate constant – i.e. the rate at which mass is lost over time – a parameter typically presented in litter-bag studies. However, in the TBI approach, k only refers to the labile (i.e. hydrolyzable) fraction of the deployed material. S describes the part of the labile, hydrolyzable fraction that did not decompose due to soil environmental factors leading to its stabilization (Keuskamp et al., 2013). The TBI is a standardized litter-decomposition assay using commercially available tea materials as standardized plant litter, which has been widely applied to characterize and compare decomposition dynamics within and across ecosystems (Keuskamp et al., 2013; Mueller et al., 2018). In each sub-plot, two polypropylene tea bags (55 mm x 50 mm) were buried from early June to late August 2015, one containing green tea and one containing rooibos tea. Tea bags were deployed at 5 cm soil depth. The initial weight of the contents was determined by subtracting the weight of empty bags. Bags were retrieved after an incubation period of 90 days, carefully separated from roots and soil, dried for 48 h at 70 °C, and weighed. The TBI parameters k and S were calculated following the tidal-wetland-adapted TBI protocol by Mueller et al. (2018):

$$(1) W_r(t) = a_r e^{-kt} + (1 - a_r),$$

$$(2) S = 1 - a_g / H_g,$$

$$(3) a_r = H_r(1 - S).$$

$W_r(t)$ the weight of the rooibos substrate after the incubation time (t in days); a_r is the labile; $1-a_r$ is the recalcitrant part of the rooibos substrate; k is the decomposition rate constant; S is the stabilization factor; a_g is the decomposable part of green tea substrate, and H_g is the hydrolyzable fraction of the green tea substrate. The decomposable part of the rooibos substrate is calculated in Eq. (3) based on the hydrolyzable fraction (H_r) and the stabilization factor S . We used the H_g and H_r values published in Mueller et al. (2018), because the tea materials used for the present study are from the same batches.

2.5 Statistical analyses

Two-way ANOVAs were conducted to test for effects of land-use (grazed vs. ungrazed) and marsh zone (high marsh vs. low marsh) on belowground biomass, soil bulk density, EEAs (β -glucosidase, leucine-aminopeptidase, chitinase and the ratio of β -glucosidase activity / (leucine-aminopeptidase activity + chitinase), and TBI parameters (k and S). Tukey HSD tests were conducted for pairwise comparisons. Normal distribution of residuals was assessed visually and equal sample sizes across groups assured robustness for parametric testing (McGuinness, 2002). Linear regression was used to test for the hypothesized relationships between bulk density, EEAs, and TBI parameters. All analyses were conducted using the statistical software OriginPro 2018 (OriginLab Corp. NorthamptonCity, USA).

3 Results

3.1 Plant biomass and soil bulk density

Belowground plant biomass was significantly decreased by grazing, but neither affected by marsh zone nor the interaction of grazing and marsh zone (Tab. 1). Grazing decreased belowground plant biomass by 31% in the high marsh and by 54% in the low marsh (Tab. 2). Bulk density was significantly higher in low-marsh vs. high-marsh sub-plots (Tab. 2). Grazing increased bulk density, whereas this effect was

only marginally significant in the topsoil ($p = 0.051$) and more pronounced with soil depth (Tab. 1). Grazing increased the bulk density at 5-10 cm depth by 10% in the low marsh and by 14% in the high marsh. Similarly, grazing increased bulk density at 10-15 cm soil depth by 16% in the low marsh grazing and 7% in the high marsh (Tab. 2)

3.2 Exo-enzyme activities

The activity of β -glucosidase was decreased by grazing (Tab. 1). Rates ranged from 2851-3300 $\text{nmol}\cdot\text{g OM}^{-1}\cdot\text{h}^{-1}$ in grazed sub-plots and 3665-3820 $\text{nmol}\cdot\text{g OM}^{-1}\cdot\text{h}^{-1}$ in ungrazed sub-plots. β -glucosidase activity was negatively related to bulk density (Tab. 3). There was also a significant and positive relationship between β -glucosidase activity and belowground biomass ($r = 0.70$; $p \leq 0.001$). Grazing strongly increased leucine-aminopeptidase activity in both the low and high marsh (Fig. 2b). Rates ranged from 4768-4943 $\text{nmol}\cdot\text{g OM}^{-1}\cdot\text{h}^{-1}$ in grazed sub-plots and 3707-3719 $\text{nmol}\cdot\text{g OM}^{-1}\cdot\text{h}^{-1}$ in ungrazed sub-plots. Chitinase activity was eight-times lower than leucine-aminopeptidase activity. In addition, chitinase activity was unaffected by grazing, marsh zone, and their interaction (Fig. 2c). In contrast to β -glucosidase activity, leucine-aminopeptidase activity was positively related to bulk density and not related to belowground biomass (Tab. 3). The ratio of C- vs. N-acquiring enzymes (activity of β -glucosidase / activity of leucine-aminopeptidase plus chitinase) was strongly decreased by grazing (Tab. 1). Grazing strongly reduced the ratio of C- vs. N-acquiring enzymes by 38% in the low marsh and by 21% in the high marsh (Fig. 2d). The ratio of C- vs. N-acquiring enzymes was negatively related to soil bulk density (5-10 cm and 10-15 cm) (Tab. 3) and positively related to belowground biomass ($r = 0.66$; $p \leq 0.001$).

3.3 Standardized litter decay based on TBI and its relation to EEAs

Both S and k were significantly decreased by livestock grazing (Tab. 1). S ranged from 0.018-0.031 in grazed sub-plots and 0.071-0.073 in ungrazed sub-plots. k ranged from 0.0105-0.0106 in grazed sub-plots and 0.0107-0.0110 in ungrazed sub-plots. Grazing significantly decreased S by 75 % in the low marsh and by 57 % in the high marsh (Fig. 3). Grazing decreased k slightly, but significantly, by 3% in the low marsh and by 2% in the high marsh. S was negatively related to leucine-aminopeptidase activity, but was positively related to β -glucosidase activity and the ratio of C- vs. N-acquiring enzymes, whereas k was negatively related to leucine-aminopeptidase activity and positively to chitinase activity (Tab. 3).

4 Discussion

4.1 Grazing effects on microbial exo-enzyme activity

In line with our first hypothesis, the activity of β -glucosidase was lower in grazed than ungrazed sub-plots. Generally, β -glucosidase activity is considered the key enzyme of microbial C acquisition and therefore controls the soil C turnover of ecosystems (Sinsabaugh et al., 2008). Previous studies suggested that grazing in salt marshes affects the activity of EEAs involved in C cycling via two mechanisms, i.e. reducing soil oxygen availability as terminal electron acceptor for microbial respiration and changing the supply or quality of OM as microbial substrate (Mueller et al., 2017). Grazing-driven soil compaction and resulting lower oxygen availability has previously been hypothesized as a main pathway by which grazing can reduce microbial activity in wetland soils (Elschot et al., 2015; Mueller et al., 2017). Our data can support this mechanism as grazed sub-plots showed significantly higher bulk density, providing evidence of trampling-driven soil compaction (Tab. 3), and more importantly, bulk density was negatively related to β -glucosidase activity (Tab. 3). It needs to be noted here, however, that grazing also led to lower belowground biomass. This unexpected effect is not commonly observed

in salt marshes (Davidson et al., 2017), but it would lead to higher bulk density independent of trampling-driven compaction.

Our data also provide evidence for a second, alternative pathway by which grazing can reduce soil β -glucosidase activity. Livestock grazing can lead to large changes in plant productivity and diversity affecting both the quantity and quality of microbial substrates. For instance, grazing effects on belowground biomass have been argued to affect the input of labile organic C compounds in form of root exudates, thereby controlling the microbial C turnover (Olsen et al., 2011). This effect of grazing on decomposition via changes in belowground biomass production is supported by our results, which show a reduction of belowground biomass with grazing and a positive relation between β -glucosidase activity and belowground biomass. Consequently, grazing induced reduction in belowground biomass could be an alternative explanation for the observed reduction in microbial C acquisition.

Leucine-aminopeptidase and chitinase mediate the microbial N acquisition from OM and therefore reflect the microbial N demand (Moorhead and Sinsabaugh, 2006; Sinsabaugh et al., 2008). In contrast to β -glucosidase activity, the activity of leucine-aminopeptidase was increased by livestock grazing. This result thus controverts our hypothesis that lower oxygen availability in grazed soils decreases EEA generally. The activity of chitinase was negligibly low and therefore considered unimportant for understanding microbial N demand. Both microbial N acquisition based on leucine-aminopeptidase activity and microbial C vs. N demand based on the ratio of C- vs. N-acquiring enzymes clearly point to higher microbial N demand in grazed soils.

Microbial N demand based on EEAs is often explained by the elemental stoichiometry of C and N in soils (Sinsabaugh et al., 2009; Sinsabaugh et al., 2008). However, in our study system soil C:N-stoichiometry cannot explain a higher microbial N demand under grazing, because soil C:N ratios are in fact markedly lower in grazed vs. ungrazed sub-plots of our field site (Yang et al., 2017).

Increased microbial N demand under grazing could also be induced by the input of labile C compounds to the soil that would not necessarily be reflected in soil C:N ratios because of their fast turnover. Plants can increase the input of C substrates to the soil via root exudation to increase microbial nutrient acquisition and plant nutrient uptake; a mechanism previously discussed in the context of rhizosphere priming effects (Jones et al., 2004; Kuzyakov et al., 2000). Indeed, higher rates of root exudation in grazed vs. ungrazed salt marsh soils have been evoked as an important control of microbial activity (Olsen et al., 2011). However, our data cannot support this mechanism because belowground biomass was decreased by grazing.

Lastly, the mixing of autochthonous vs. allochthonous OM is another major control of microbial C- vs. N-acquisition activities in salt marshes, irrespective of the soil C:N stoichiometry (Mueller et al., 2020). More importantly it is also strongly affected by grazing (Mueller et al., 2017; Mueller et al., 2019b). Allochthonous OM inputs can represent a major fraction of the soil OM pool in minerogenic, sediment-rich salt marshes (Mueller et al., 2019a; Van de Broek et al., 2018). Considering the extreme rates of sediment-driven accretion of several centimeters per year in our study site (Yang et al., 2008), allochthonous OM input likely exerts important control over microbial C vs. nutrient acquisition. Furthermore, grazing has been shown to reduce annual sediment deposition and accretion drastically by >30 kg dry weight m^{-2} and 2 cm, respectively (Yang, 2017). It is therefore possible that lower inputs of allochthonous OM input under grazing – a mechanisms previously demonstrated by Mueller et al. (2017) for European salt marshes – is also driving the stimulated microbial N demand in the grazed plots of the present study. However, additional research is needed to assess quality and mixing of allochthonous OM in the marshes of Yangtze estuary before any conclusions can be drawn on its implications on soil microbial ecology and biogeochemistry.

4.2 Litter decomposition

In line with our second hypothesis, we found a grazing-induced reduction of k , the decomposition rate constant of the deployed plant litter. Lower k in grazed sub-plots could be ascribed to lower soil oxygen availability, as we also demonstrate a negative relationship between k and soil bulk density (Tab. 3), a proxy for trampling-driven soil compaction and oxygen availability in grazed wetland soils (Elschot et al., 2015; Schrama et al., 2013). Contrary to our hypothesis, however, S , describing the stabilization of plant litter in soil, was also decreased in grazed sub-plots and not inversely related to k . Even though the TBI parameters k and S do not necessarily show a strong inverse correlation (Keuskamp et al., 2013; Mueller et al., 2018), the unidirectional decrease of both parameters in response to our grazing treatment was unexpected. Yet, this finding clearly highlights the importance to distinguish litter decomposition rate from litter stabilization in the litter decomposition process. The factors controlling litter decomposition rate and stabilization, as well as the ecological implications of the two parameters, can be quite different (Althuizen et al., 2018; Petraglia et al., 2018). While there is a wealth of studies providing insight into the controls of litter decomposition rate, far less is known about the controls of litter stabilization (review provided by Prescott (2010)). From a C-sequestration perspective, litter stabilization is the more relevant parameter, as it describes the fraction of litter that gets ultimately transformed to stable soil OM (Córdova et al., 2018; Paul, 2016).

In the present study, a lower degree of plant-litter stabilization cannot be explained by lower soil oxygen availability under grazing. We therefore argue that grazing effects on other factors controlling microbial activity are responsible for the observed effect. Specifically, S was negatively related with leucine-aminopeptidase activity and positively related with microbial C vs. N acquisition (based on EEA stoichiometry), suggesting a negative effect of microbial N demand on litter stabilization. In support of this notion, low stabilization potential for organic material in soils with high microbial N demand is in line with several observations on soil OM cycling in terrestrial ecosystems (Doetterl et al., 2018; Wild et

al., 2017). Similarly, N additions have previously been hypothesized to increase plant litter stabilization to soil OM (Prescott, 2010).

It is possible that the TBI stabilization factor, S , is particularly sensitive to the N demand of the soil microbial community, given that the green-tea substrate used to assess S is relatively N-rich (C:N = 12) (Keuskamp et al., 2013). Yet, S has proven useful to explain variability in the soil C-sequestration capacity across ecosystems, suggesting that it can be used as a proxy for plant litter stabilization in soils (Keuskamp et al., 2013; Mueller et al., 2018). Our study provides further support for this, because grazing-induced reductions of S are in accordance with lower soil OC contents and densities in grazed vs. ungrazed plots (Yang et al., 2017).

4.3 Summary and implications

The present work demonstrates marked effects of livestock grazing on decomposition processes in salt marsh soils with potentially important implications for C sequestration. Negative effects of livestock grazing on soil C stocks and sequestration rates in salt marshes have previously been ascribed to reductions in plant biomass (Davidson et al., 2017). Here we suggest that livestock grazing can stimulate the early OM decomposition processes in salt marsh soils by reducing litter stabilization via increased microbial N demand, and therefore provide an alternative explanation for negative grazing effects on C stocks and sequestration. Our data show that grazing effects on EEAs and litter decomposition can just partly be explained by grazing-driven soil compaction and resulting lower oxygen availability, which has previously been hypothesized as a main pathway by which grazing can reduce microbial activity in wetland soils (Elschot et al., 2015; Mueller et al., 2017). Instead, grazing effects on microbial nutrient demand appears to be an equally important control on soil decomposition processes. In addition, the present study is the first to provide insight into the relations between soil enzymic processes and TBI, an increasingly recognized, standardized belowground litter assay used to understand soil OC formation

and compare decomposition dynamics across ecosystems at a global scale (Djukic et al., 2018; Keuskamp et al., 2013). The identified interactions between microbial EEAs and TBI parameters warrant further investigation to improve the mechanistic understanding that can be derived from TBI with respect to soil OC formation. Particularly, if the observed negative effect of microbial N demand on S applies more generally, it yields important implications for linking and modeling nutrient, litter, and soil OC dynamics based on TBI.

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PM, SN, and KJ conceived the study. ZJ and JW designed the field experiment. ZJ and SN conducted the field work. HT conducted the lab work, analyzed the data, and wrote the first draft of the manuscript. PM supervised lab work, data analysis, and writing. All authors read and approved the final manuscript.

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The authors declare no conflict of interest.

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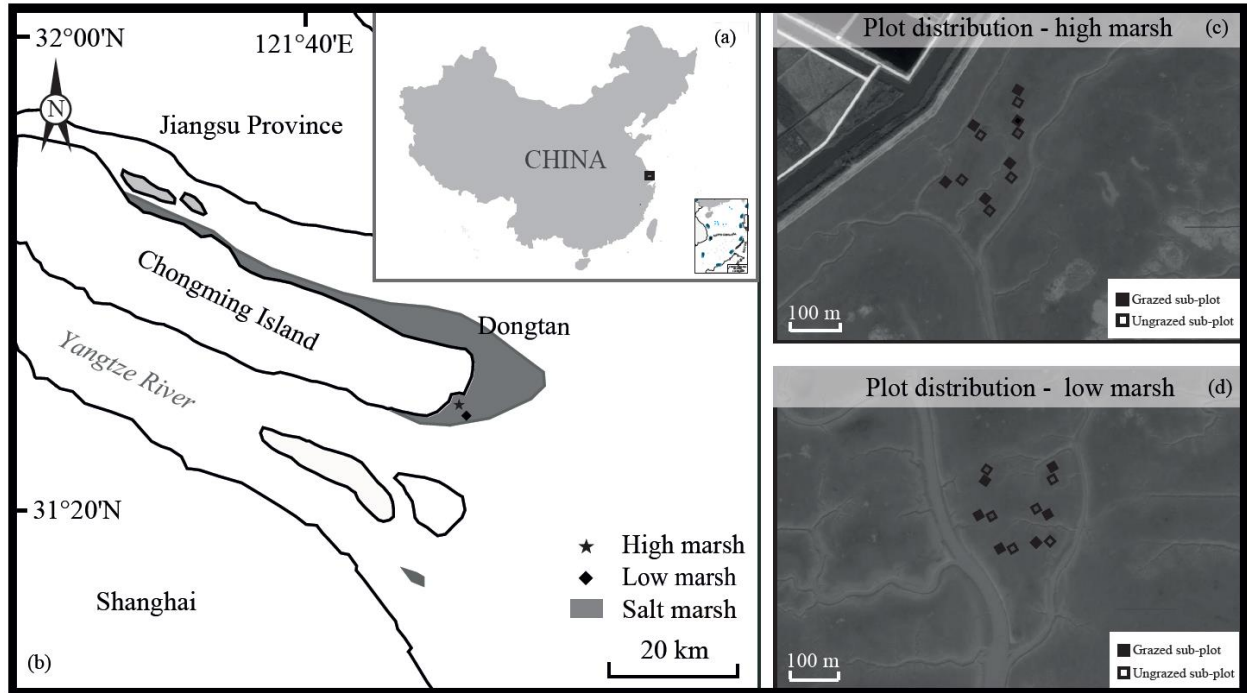


Fig. 1 Location of experimental site (a, b) and sub-plots distribution in low- and high-marsh zones (c, d).

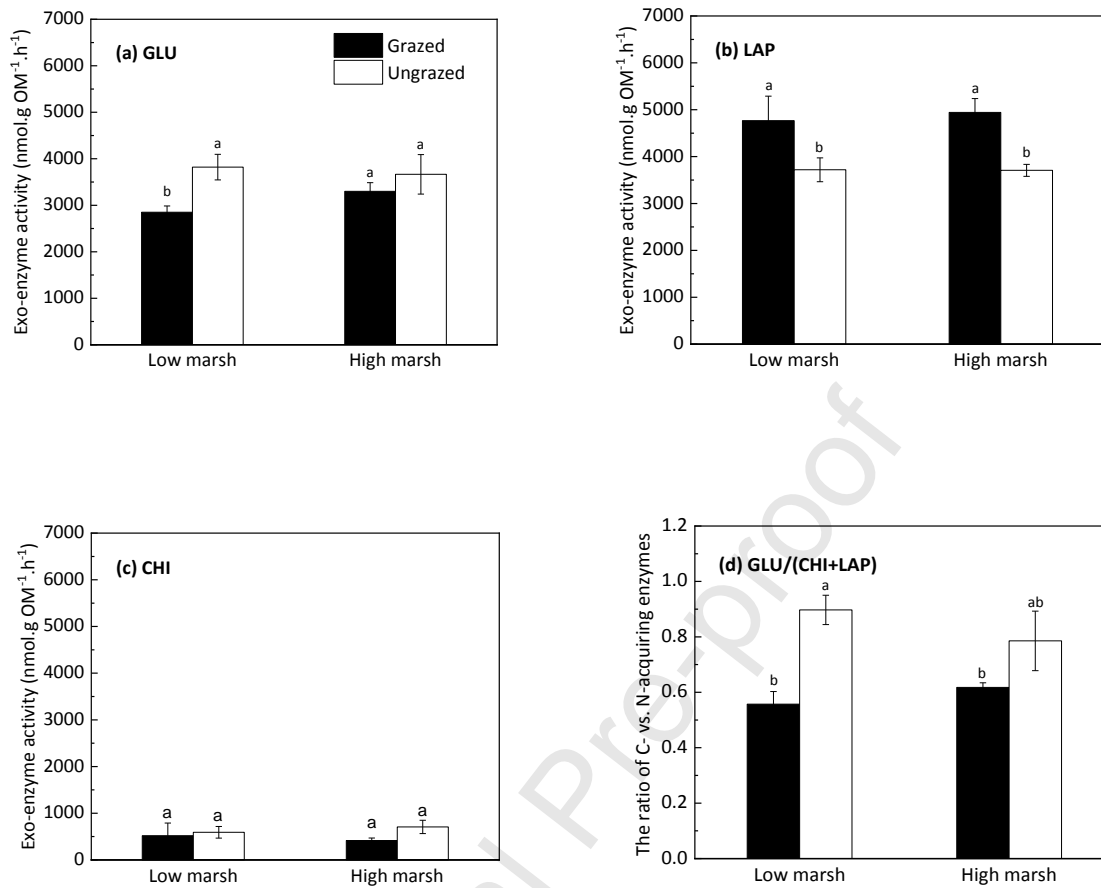


Fig. 2 The enzyme activity of β -glucosidase (GLU), leucine-aminopeptidase (LAP), chitinase (CHI) and the ratio of C- vs. N-acquiring enzymes (GLU/(CHI+LAP)) in grazed and ungrazed sub-plots of low and high marsh zone. Values are means and SE (n=6), bars not connected by the same letter are significantly different at $p \leq 0.05$ based on Tukey's HSD.

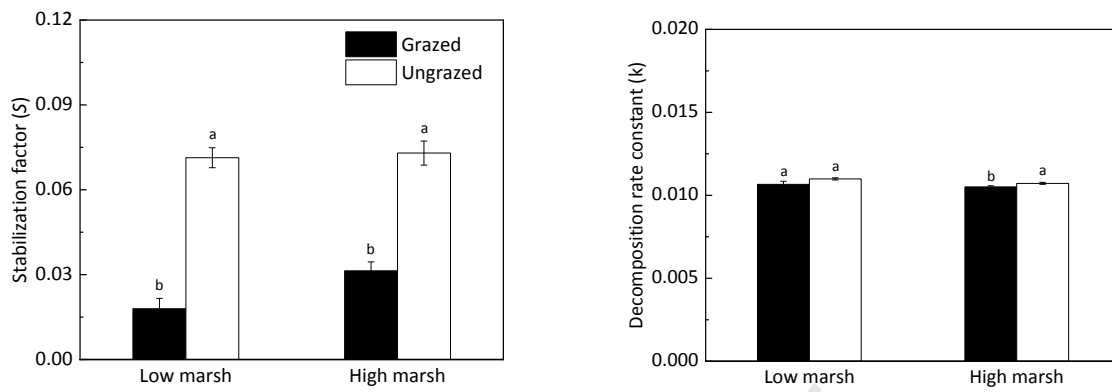


Fig. 3 Stabilization factor (S) and decomposition rate constant (k) in grazed and ungrazed sub-plots of the low and the high marsh zone. Values are means and SE ($n=6$), bars not connected by the same letter are significantly different at $p \leq 0.05$ based on Tukey's HSD.

Table 1 ANOVA table for two-way (marsh zone and grazing treatment) analysis. Response variables are belowground biomass (BB), bulk density (BD), β -glucosidase activity (GLU), leucine-aminopeptidase activity (LAP), Chitinase (CHI), the ratio of C- vs. N-acquiring enzymes (GLU/(CHI+LAP)), Stabilization factor (*S*), and decomposition rate constant (*k*). n = 6, degrees of freedom for main- and interaction-effect tests = 1

	Marsh zone		Grazing		Interaction	
	F	p-value	F	p-value	F	p-value
BB	1.45	ns	5.10	<0.05	0.22	ns
BD (0-5 cm)	7.94	<0.05	4.31	<0.1	2.02	ns
BD (5-10 cm)	6.56	<0.05	22.63	<0.001	0.02	ns
BD (10-15 cm)	3.21	<0.1	18.13	<0.001	2.35	ns
GLU	0.28	ns	5.79	<0.05	1.18	ns
LAP	0.06	ns	11.88	<0.05	0.08	ns
CHI	0.00	ns	1.18	ns	0.43	ns
GLU/(CHI+LAP)	0.16	ns	15.45	<0.001	1.78	ns
<i>S</i>	4.22	<0.1	167.89	<0.001	2.57	ns
<i>k</i>	3.84	ns	6.38	<0.05	0.32	ns

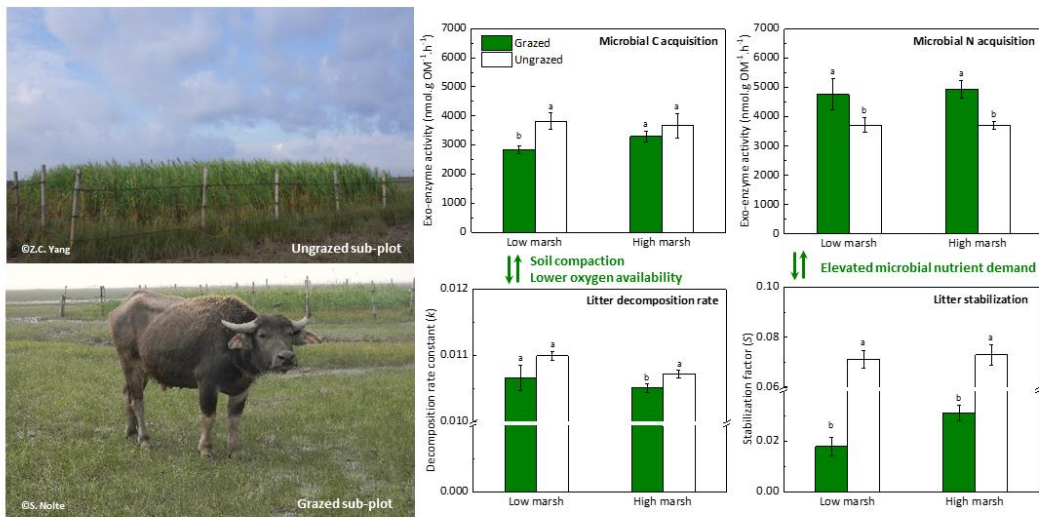
Table 2 Belowground biomass and bulk density of different soil depths in grazed and ungrazed sub-plots of low and high marsh zones. belowground biomass (BB); bulk density (BD). Values are means \pm SE (n=6). Letters indicate significant differences at $p \leq 0.05$ within rows based on Tukey's HSD.

Section	Low marsh		High marsh	
	Grazed	Ungrazed	Grazed	Ungrazed
BB (g)	5.74 \pm 0.99 b	12.50 \pm 1.35 a	9.89 \pm 2.72 a	14.32 \pm 3.79 a
BD (0-5 cm) (g·cm ⁻³)	1.29 \pm 0.01 a	1.27 \pm 0.02 a	1.25 \pm 0.05 a	1.13 \pm 0.05 a
BD (5-10 cm) (g·cm ⁻³)	1.36 \pm 0.03 a	1.22 \pm 0.02 b	1.29 \pm 0.04 a	1.13 \pm 0.02 b
BD (10-15 cm) (g·cm ⁻³)	1.56 \pm 0.03 a	1.35 \pm 0.05 b	1.44 \pm 0.04 a	1.34 \pm 0.02 a

Table 3 Linear regression results for relationships between **(A)** bulk density (BD) at different soil depths and enzyme activities; and **(B)** enzyme activities in the topsoil and the TBI parameters *S* and *k*. R^2 -values are bold-typed at $p \leq 0.05$.

	GLU			LAP			CHI			GLU/(LAP+CHI)		
	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept
(A) bulk density vs. enzyme activities												
BD (0-5 cm)	0.08	-2093	5994	0.10	3006	573	0.08	-1096	1912	0.12	-0.68	1.56
BD (5-10 cm)	0.23	-3170	7379	0.41	5530	-2642	0.17	-1480	2413	0.44	-1.20	2.23
BD (10-15 cm)	0.35	-3624	8559	0.27	4094	-1534	0.09	-996	1974	0.50	-1.17	2.39
(B) enzyme activities vs. <i>S</i> and <i>k</i>												
<i>S</i>	0.19	2×10^{-5}	-0.01	0.29	-1×10^{-5}	0.11	0.00	3×10^{-6}	0.05	0.47	0.09	-0.02
<i>k</i>	0.02	6×10^{-8}	0.01	0.44	-2×10^{-7}	0.01	0.26	4×10^{-7}	0.01	0.20	1×10^{-3}	0.01

Notes: β -glucosidase (GLU), leucine-aminopeptidase (LAP), chitinase (CHI), the ratio of C- vs. N-acquiring enzymes (GLU/(CHI+LAP))



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Highlights

1. Grazing exerted opposing effects on soil microbial C- and N-acquisition activity
2. Litter stabilization was strongly negatively correlated with soil microbial N demand
3. TBI parameters S and k were both reduced by grazing and not inversely related

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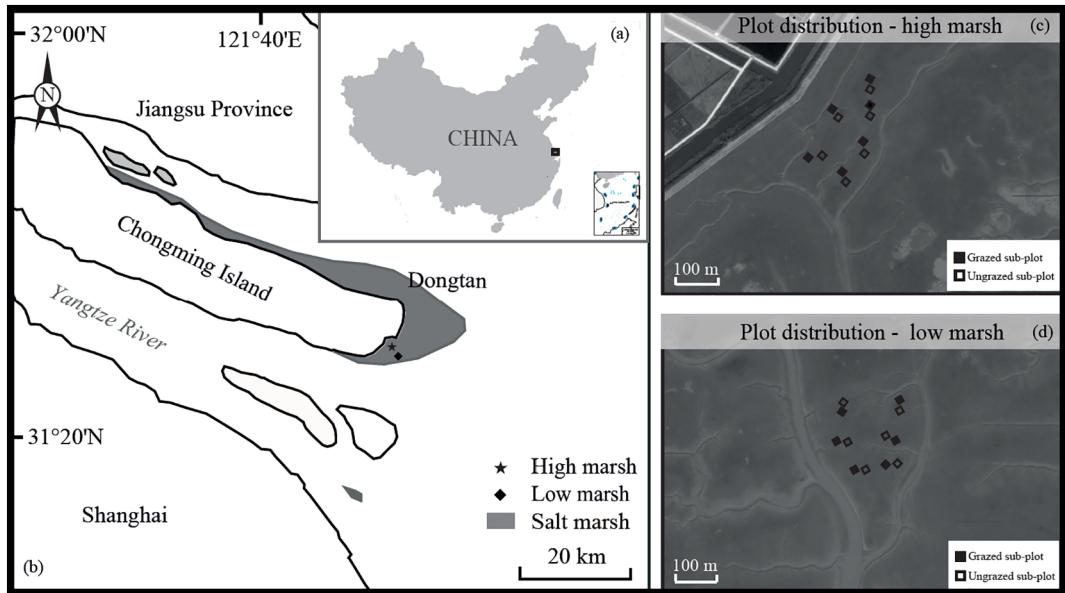


Figure 1

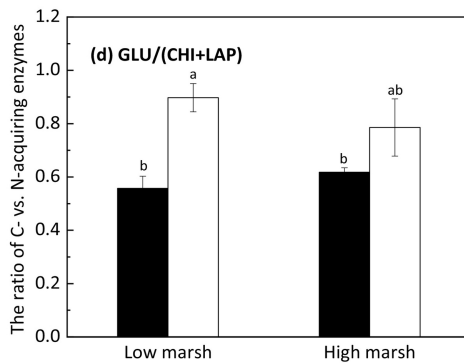
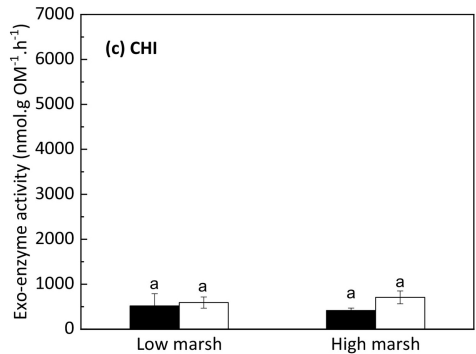
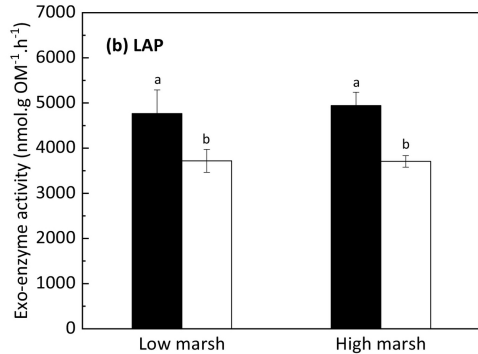
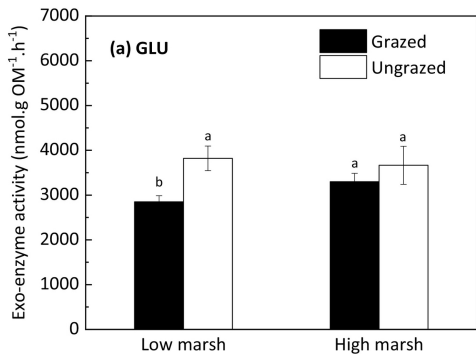


Figure 2

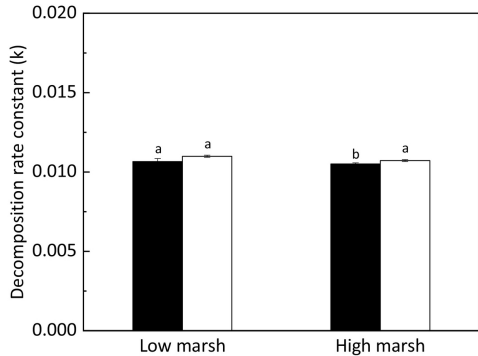
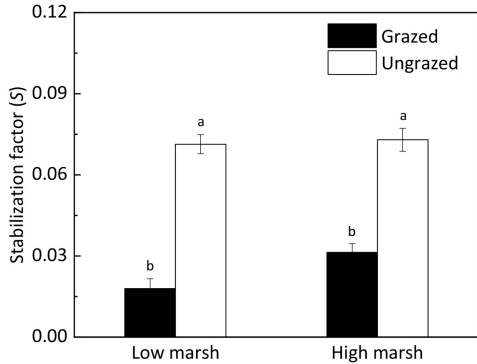


Figure 3