Title

Tidal flooding diminishes the effects of livestock grazing on soil micro-food webs in a coastal saltmarsh

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Abstract

Livestock grazing not only has a direct impact on plant productivity but also exerts an indirect influence on soil biota via various pathways. However, little is known about the effects of livestock grazing on soil food webs in saltmarsh ecosystems that are subject to regular tidal inundation stress. By exclosure experiments established at a frequently inundated middle marsh and a less inundated high marsh of Chongming Island (China), the responses of soil micro-food web components (microorganisms, protozoa, and nematodes) to cattle grazing in intertidal marshes were investigated. In the high marsh, cattle grazing significantly increased the biomass of soil microorganisms, protozoa, and the abundance of total nematodes by 30.0%, 97.3% and 76.2%, respectively, but did not significantly affect their biomass or abundance in the middle marsh. For low-trophic-level nematodes, the abundance of bacterial-feeding and algal-feeding nematodes increased more in the high marsh than in the middle marsh, and that of plant-feeding nematodes decreased more in the high marsh than in the middle marsh under grazing. In contrast, carnivorous and omnivorous nematodes at high trophic levels did not respond to cattle grazing along an elevational gradient. The nematode maturity index and structure index based on nematode functional guilds significantly decreased under grazing along the elevational gradient, suggesting that cattle grazing caused a more simplified and unstable soil micro-food web structure. Overall, low trophic levels in soil micro-food webs were most vulnerable under grazing and the response was strongest in the less inundated high marsh. Thus, cattle grazing leads to different changes in soil ecosystem processes at different elevations. These results indicate that the strength of the biotic grazing effect on soil micro-food webs and ecological functions might also depend on local abiotic disturbance such as tidal inundations in the saltmarsh.

Keywords: Large herbivore; Soil microbial biomass; Protozoa; Nematode; Tidal inundation; Phospholipid fatty acid.

1. Introduction

In terrestrial grasslands worldwide, livestock grazing has been a traditional land use for agricultural purposes (Doody, 2008). In general, grazing activities by livestock not only have a direct impact on plant shoot tissues but also exert an indirect influence on soil biota via various pathways, involving processes such as removal of plant biomass, dung and urine return, and trampling (Bardgett and Wardle, 2003; Chen et al., 2013). The removal of plant biomass and a reduced plant litter layer directly decreases plant material inputs into the soil (Ford and Grace, 1998; Lkhagva et al., 2013) while also possibly promoting root biomass and exudate production (Guitian and Bardgett, 2000). In turn, altered carbon resources from plants can positive or negative influence soil decomposer biomass and activity (Christensen et al., 2007; Kramer et al., 2012). Because grazing reduces the vegetation canopy, it affects soil temperature (Odriozola et al., 2014) and soil organisms (De Long et al., 2016). Inputs of dung and urine increase nutrient availability in the soil, which stimulates soil microbial activities (Bardgett et al., 1998). Trampling enhances soil compaction, i.e., it reduces soil pore size and increases soil waterlogging (limiting the availability of oxygen), thus it might negatively affect soil decomposers (Bardgett and Wardle, 2010). Overall, the effects of livestock grazing on soil biota are context dependent and vary depending on topographic conditions (Asner et al., 2009), ecosystem type (Bardgett et al., 1997), soil texture (Schrama et al., 2013) and soil fertility (Sankaran and Augustine, 2004).

Soil micro-food webs are important trophic networks in belowground decomposer systems, largely including microorganisms (bacteria and fungi), microbivores (protozoa and low-trophic-level nematodes etc.) and micropredators (high-trophic-level nematodes etc.) (Wardle, 1995). The food sources of these trophic groups are mainly subjected to the bottom-up control of carbon resources that enter the soil (Scharroba et al., 2012), while protozoa and nematodes feed on microorganisms and eventually affect nutrient liberation for plant uptake (Bonkowski et al., 2000; Griffiths, 1994; Hunt et al., 1987). Soil nematode communities are often used as bioindicators in soil assessment because they involve taxa at diverse trophic levels in decomposer food webs and are susceptible to habitat changes (Wu et al., 2002; Yeates and Bongers, 1999).

Saltmarshes differ from other terrestrial ecosystems because of periodic tidal flooding that leads to high soil water contents and consequently limits oxygen penetration into the soil (Ford et al., 2013). In contrast to arid soils, where soil microbial activity is found to increase with higher water availability (Iovieno and Baath, 2008), microbial activity is lower in waterlogged soils since poorly drained soil diminishes the oxygen supply (Schinner, 1982). Surprisingly, microbial activity is found to be greater in waterlogged soils of grazed saltmarsh, containing increased available carbon, in the UK (Ford et al., 2013; Olsen et al., 2011). The abundance of soil macrofauna such as arthropods is, however, strongly reduced in grazed temperate saltmarshes (Schrama et al., 2013; van Klink et al., 2015), probably because of a decrease in soil pore space for arthropod inhabitation. In saltmarshes, tidal inundation frequency is a significant factor affecting the distribution and development of vegetation (Bertness, 1991) and aboveground fauna such as spiders and insects (Andresen et al., 1990; Meyer et al., 1995). Despite some literature documenting the influence of livestock grazing on soil microbial activity in salt marshes (Ford et al., 2013; Olsen et al., 2011), it is unclear about livestock grazing interacts with tidal inundation in affecting the soil biota.

In this study, the impact of livestock grazing and tidal inundation on selective soil biota groups (microorganisms, protozoa and nematodes) that are essential components in the soil food web was investigated. By conducting an exclusion experiment in a coastal saltmarsh at Dongtan, Chongming Island, China, we aimed to assess the influence of dual-disturbance on the structure of soil micro-food webs in an experiment by comparing grazed and ungrazed treatments at two marsh elevations. Grazing often changes vegetation biomass or structure more intensively in the high marsh than in the middle marsh because it is subjected to less environmental stress, such as tidal inundation (Di Bella et al., 2014; Fariña et al., 2016), and the vegetation change exerts considerable influence on the organisms at lower trophic levels more directly than at higher trophic levels (Bardgett and Wardle, 2003; Scharroba et al., 2012). Therefore, we hypothesized that (1) under grazing, low trophic levels in soil micro-food webs are most vulnerable, as their main carbon resources are directly affected by grazing; and (2) grazing effects on soil organisms (microbial and protozoan biomass, nematode abundance) are stronger in the high marsh than in the middle marsh because of less frequent tidal inundation.

2. Materials and Methods

2.1 Site description

The study site was in the Dongtan saltmarsh (31°28′N, 121°56′E) of Chongming Island, which is located in the estuary of the Yangtze River, China. The climate is subtropical monsoon with mean annual temperature of 15.3 °C and precipitation of 1022 mm. Since the 1950s, the saltmarsh has been regularly and pervasively grazed by cattle. In the last decade after the reserve was established, cattle grazing was restricted to a 600 ha southeastern area of the Dongtan saltmarsh, which led to increased grazing intensity and an increased risk of ecosystem degradation (Yang et al., 2008). This area is grazed by approximately 1 cattle ha^{-1} from early April to late October each year. In the Dongtan marsh, the tides are irregularly semidiurnal with the range of two successive tides being unequal. The average tidal range is 2.5 m and around 3.5 m during spring tides; the highest astronomical tide is up to 5.2 m above the lowest astronomical tide (Yang et al., 2008). For the terms of the marsh, we follow the definition of Redfield (1972): the high marsh lies at approximately the mean high water level between spring tide and neap tide and the middle marsh lies below the mean high water and low water level of neap tide. In our study, the dominant plant species in the high marsh are *Phragmites australis* and *Carex scabrifolia*, while the middle marsh is dominated by sedges *Scirpus mariqueter* and *C. scabrifolia*.

2.2. Experimental design

A grazing-exclusion experiment was established in the grazed area of the Dongtan saltmarsh in April 2014. The experimental plots were set up in 12 blocks, with half in the high marsh and the other half in the middle marsh, respectively (Fig. 1). In our study area, the width of the grazed salt marsh (from marsh edge to seawall) is approximately 1.5 km and has a gentle slope. Based on this situation, the distance between the high and middle marsh blocks was chosen as long as possible to achieve a distinct discrimination between tidal regimes (inundation frequency and duration). The mean elevation is 380 cm above sea level for high-marsh blocks and 330 cm for middle-marsh blocks. Tidal inundations are relatively infrequent at the high marsh, with a frequency of 17 times on average and an accumulative duration of 43 h per month. At the middle marsh, tidal inundations are more frequent with around 39 inundations and an accumulative duration of 127 h per month.

Based on the observation on cattle activity and the counting of fresh cattle dungs, the stock cattle densities between the high and middle marsh were similar. Within both the high and middle marsh sites, we aimed to have all replicates on a similar elevation within a ±10 cm to ensure consistency of tidal inundation at a site and homogeneous soil environment. Therefore, in the high or middle marsh site, there is 50-100 m distance apart between every two blocks. Each block contained three experimental plots (15 × 15 m) that were assigned to one of three treatments: grazed without fence, grazed with short fence (the fence height is 50 cm) or ungrazed with tall fence (the fence height is 150 cm and the entire plot was surrounded with barbed wire). The distance was about 5 m between each two plots of all the three treatments. The grazed with short fence plots were used to eliminate the effects of the fence *per se* on soil biota.

2.3 Soil and plant characteristics

In September 2015, two growing seasons after the fences were established, soil pH, temperature and oxidation reduction potential (ORP) were measured *in situ* using a multiple meter (IQ Scientific Instruments, CA, USA). Soil conductivity was determined *in situ* as a proxy for salinity using a soil electrical conductivity (EC) meter (2265FS, Spectrum Technologies, Inc., IL, USA). Soil samples were collected to determine soil porosity and organic matter content using a splittable soil corer to take intact soil cores of 3.2 cm diameter and 15 cm depth. The entire core was dried at 70 °C for 72 h to determine soil bulk density. The specific gravity of soil was estimated using the density bottle method (Prakash et al., 2012). Soil porosity was calculated using the following formula (1 − ratio of bulk density and specific gravity). Loss-on-ignition (550 °C for 5 h) method was used for determining soil organic matter content (SOM) (Heiri et al., 2001). Total soil

C and N were measured by a NC Analyzer (Thermo Fisher Scientific, MA, USA).

Aboveground living plant materials were collected within three randomly positioned 25 × 25-cm quadrats in each plot, meanwhile, roots were collected in the same quadrats using a PVC corer of 15 cm diameter and 20 cm depth. Roots were washed to remove all soil and then both aboveground living plant materials and roots were dried at 70 °C for 72 h and weighed to determine above- and belowground biomass, respectively.

2.4 Soil organism analysis

For soil organism analysis, four soil cores (3.2 cm diameter and 15 cm depth) were taken from each plot and then mixed to form a composite sample. The composite sample was then divided into two subsamples for microorganism phospholipid fatty acids (PLFAs) and nematode analysis, respectively. The composition of PLFAs was analyzed to assess the soil microbial community structure and protozoa from 8 g freeze-dried soil subsample following the methods of Frostegård et al. (1993) with slight modifications (Li et al., 2012). Methyl nonadecanoate fatty acid 19:0 was used as the internal standard. The fatty acid methyl esters were divided and quantified with an Agilent 6890 Gas Chromatograph and identified by the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA) based on retention time. The PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω7, 16:1ω9, i17:0, a17:0, 17:0, cy17:0, 18:1ω7c and cy19:0 were summed to represent the biomass of bacterial biomass; and 18:2ω6 was used to indicate of the biomass of fungi (Frostegård et al., 1993; Frostegård and Bååth, 1996). Among these, i15:0, a15:0, i16:0, i17:0 and a17:0 were used for Gram-positive bacteria and cy17:0, 16:1ω7, 18:1ω7c and cy19:0 for Gram-negative bacteria indicators respectively (Ford et al., 2013). Other PLFAs such as 16:0 10-Me were used to identify sulphate-reducing bacteria (Dowing et al., 1986), 18:1ω7c for methanotrophs (Bull et al., 2000) and 20:4ω6c and 20:5 for protozoa (Fierer et al., 2003).

We extracted soil nematodes from about 200 g mixed soil subsample through Ludox® TM flotation method (Griffiths et al., 1990). The total numbers of nematodes individuals was counted for each sample. At least 100 nematode specimens were identified to genus level for each sample. The feeding types of nematodes were classified according to Yeates et al. (1993). To evaluate nematode diversity in each treatment, nematode genus richness (*S*, the number of nematode genera), Shannon's diversity index (*H'*, here calculation by using the numerical proportion of the taxon abundance) and Pielou's evenness index were calculated. The maturity index (MI) and structure index (SI) were used to assess the functional responses of soil nematodes to environmental changes and the complexities of the soil food web (Bongers, 1990; Ferris et al., 2001). High MI values represent a more stable soil environment. High SI values represent a more complex soil food web and a less disturbed environment. The calculation of MI index of nematodes follows Bongers (1990), which is based on a colonization–persistence (*c–p* values ranging from 1 to 5) classification of nematodes. Nematodes with higher *c–p* values represent longer generation times, larger body size, and low reproductive capacity, and are more sensitive to disturbance than those with lower *c–p* values (Bongers and Bongers, 1998). The SI index of nematodes was determined based on their feeding types, *c–p* values and the guild weighting values (Ferris et al., 2001).

2.5 Data analysis

An independent sample *t*-test method was conducted to test the difference between 'grazed without fence' and 'grazed with short fence' treatments. The results showed that there was no significant effect from the presence of fence, suggesting no fence artifacts. Therefore, the effects of the livestock grazing treatments (ungrazed with tall fence and grazed without fence), inundation (high marsh and middle marsh) and their interaction on soil characteristics, plant above- and belowground biomass, and soil organism communities (microorganism biomass, protozoan biomass and nematode abundance), were analyzed by two-way ANOVA. A post-hoc Tukey's HSD tests was performed if significant differences among treatments were found. The data were log(x+1) transformed to match the assumptions of ANOVA if necessary. Significance levels were set at $P <$ 0.05. The analyses were executed using the STATISTICA 8.0 (StatSoft Inc, Tulsa, OK, USA).

Two-way ANOSIM was applied to examine the effects of grazing treatments and inundation on nematode community structure. To examine the similarity in nematode community structure, ordination plots of non-metric multidimensional scaling (NMDS) analyses based on Bray-Curtis similarity measures were produced. The analyses were done using the PRIMER (Plymouth routines in multivariate ecological research) version 5.2 software package (Primer-E Ltd., Plymouth, UK). A redundancy analysis (RDA) was applied to interpret the relationship between soil organisms and environmental parameters using CANOCO 5.0 (ter Braak and Smilauer, 2012). To normalize data prior to the analyses, soil organism biomass or abundances were log(x+1) transformed when needed.

3. Results

3.1 Soil and vegetation characteristics

There was no significant difference observed for all parameters including soil characteristics, plant biomass and the biomass or abundance and indices of soil biota between the short-fence treatment and no-fence treatment. Therefore, our results were illustrated by using data of no-fence plot (grazed treatment) and tall-fence plot (ungrazed treatment). Soil temperature, SOM and soil C/N ratio were significantly affected by grazing and elevation but not by the interaction of the two factors (Table 1). Soil moisture and ORP were significantly affected only by elevation. Total soil porosity was significantly affected only by grazing. Post-hoc test showed that grazing significantly increased soil temperature and lowered the soil C/N ratio only in the high marsh, whereas SOM and total porosity were significantly reduced at both elevations (Table 1).

Aboveground plant biomass was significantly affected both by grazing and elevation, but not by their interaction (Table 1). Cattle grazing and frequent inundation decreased aboveground plant biomass by 75.6% and 28.5%, respectively, and when both were present they significantly decreased aboveground plant biomass by 91.2%. Belowground plant biomass was significantly affected only by grazing (Table 1). Cattle grazing decreased belowground plant biomass by 43.1%.

3.2 Soil microbial and protozoan PLFAs

Total microbial biomass and protozoan biomass (estimated as the amount of PLFAs) were affected by grazing and elevation but not by their interaction (Fig. 2). Cattle grazing increased total microbial biomass and protozoan biomass by 30.0% and 97.3%, respectively. Frequent inundation decreased total microbial biomass and protozoan biomass by 10.2% and 16.2%, respectively, and together they did not affect total microbial biomass and protozoan biomass. Grazing resulted in a significantly increased microbial biomass in the high marsh, but not in the middle marsh. For specific microbial group responses, in the high marsh, cattle grazing significantly stimulated biomass of total bacteria, Gram-negative bacteria, sulphate-reducing bacteria, methane-oxidizing bacteria, while it did not affect Gram-positive bacteria and fungi.

3.3 Composition and structure of nematode communities

Thirty-one nematode genera were identified in the marsh (Table 2). Elevation, grazing and their interaction significantly explained the variation in total abundance of nematode communities (Table 3). Grazing led to an increased nematode abundance by 76.2% in the high marsh, but not in the middle marsh (Table 3). Nematode genus richness, Shannon's diversity index and evenness index were not different between grazing treatments at both elevations. Nematode MI and SI were significantly reduced by grazing at both elevations (Table 3).

The abundance of different nematode feeding guilds responded to grazing across the elevations in different ways (Fig. 3). Elevation, grazing and their interaction significantly explained the variation in the abundance of bacterial-feeding nematodes. Their abundance was about 3.7 times higher in the grazed than in the ungrazed high marsh, while it was 2.1 times higher in the grazed than in the ungrazed middle marsh. Only grazing significantly explained the variation of algal-feeding and plant-feeding nematode abundance. Cattle grazing enhanced the abundance of algal-feeding nematodes by 16 times in the high marsh and by 5.7 times in the middle marsh. Cattle grazing suppressed the abundance of plant-feeding nematodes by 7.1 times in the high marsh and by 4.3 times in the middle marsh. The abundances of carnivorous and omnivorous nematodes were not significantly affected by grazing treatments along the elevational gradient.

Significant grazing (Global test: R = 0.68, P = 0.001) and elevation effects (Global test: R = 0.557, P = 0.001) were detected on nematode communities. The NMDS ordination of nematode communities clearly discriminated four plot groups of high and middle marsh, grazed and ungrazed treatments (Fig. 4).

3.4 The relationship between soil biota and environmental variables

The explanatory variables in the RDA analysis accounted for 81.3% of the total variance in the soil organisms (Fig. 5). The first axis had a positive correlation to total porosity, plant above- and belowground biomass, soil C/N ratio, SOM and pH, but negatively correlated with moisture. The second axis had a positive correlation to ORP, soil C/N ratio and had a negative correlation to temperature, conductivity, SOM, pH and moisture. For soil organisms at low trophic levels, the biomass of total bacteria, sulphate-reducing bacteria, methanotrophs, Gram-negative bacteria, Gram-positive bacteria and protozoa, as well as bacterial-feeding nematode abundance, was correlated positively with temperature, but negatively with soil C/N ratio. The plant-feeding nematode abundance was positively correlated with plant above- and belowground biomass, while the abundance of algal-feeding nematodes was negatively correlated with plant above- and belowground biomass. Carnivorous and omnivorous nematodes were positively correlated with ORP but negatively with SOM.

4. Discussion

4.1 Responses of soil biota at different trophic levels to grazing in high and middle marshes

The impact of grazing by cattle on the structure of soil micro-food webs was investigated at different saltmarsh elevations. In agreement with our hypotheses, cattle grazing markedly influenced low-trophic-level organisms (microbial communities, protozoa, bacterial-feeding nematodes, plant-feeding nematodes and algal-feeding nematodes) in the soil micro-food webs, and the grazing effects were stronger in the high marsh, which is inundated infrequently, than in the middle marsh.

Soil microbial biomass, based on PLFA analysis, was increased by grazing in the high marsh. This increase in biomass is probably because of the increased availability of labile root exudates caused by grazing (Bardgett and Wardle, 2003), and labile resource inputs through animal urine and feces (Bardgett et al., 1997). Microbial growth might also be stimulated by an increase in soil temperature because of more light penetration through the lower canopy to the soil surface. Correspondingly, the RDA results indicated that microbial biomass was positively related to soil temperature. In contrast to the high marsh, soil microbial communities were not affected by grazing in the middle marsh. This is probably caused by the higher tidal inundation frequency and duration in the middle marsh, which might inhibit microbial activity through diverse mechanisms, such as change of surrounding water chemistry and reduction of nutrient and gas exchange between the surrounding water and the sediment layer (Vargo et al., 1998). In our study area, the inundation frequency and duration of the middle marsh were almost three-fold greater than for the high marsh. Therefore, soil microorganisms were less sensitive to cattle grazing in the lower parts of the saltmarsh. In other ecosystems such as grasslands, it is also documented that abiotic factors along altitude gradient are more important in affecting soil microbial activity than human management (Paz-Ferreiro et al., 2010). Accordingly, the impact of management on some soil microbial processes should be considered in the context of other ambient factors such as water chemistry, gas and nutrient in salt marshes.

Cattle grazing significantly promoted the biomass of Gram-negative bacteria in the high marsh, which agrees with results obtained in temperate saltmarshes in northern Europe (Ford et al., 2013). They concluded that the growth rates of Gram-negative bacteria were restricted by the high reliance of this group on plant root exudates as their primary carbon resource. Cattle grazing did not significantly affect the biomass of Gram-positive bacteria presumably because Gram-positive bacteria could simultaneously use both multi-year cumulative SOM and fresh root exudates (Bird et al., 2011). Although root exudation could be promoted by grazing (Bardgett and Wardle, 2003), SOM decreased with grazing in this study. The biomass of sulphate-reducing bacteria were also increased by grazing in the high marsh, which could be explained by the change in soil characteristics and resources caused by cattle grazing activity that might lead to a decline in soil redox potential in waterlogged ground (Schrama et al., 2013). The RDA result also showed that sulphate-reducing bacteria were negatively related to ORP. Similarly, the abundance of methanotrophs increased in the grazed high marsh, which might be attributed to an increase in methane production through the input of feces and urine by livestock (Ford et al., 2012).

The response of soil protozoa to grazing by cattle was similar to that of bacteria across elevations, possibly because bacteria are the main food resource of protozoa. This is in agreement with some statements that protozoa are positively related to changes in the available food resources including rhizosphere bacteria that induce a bottom-up trophic control of the soil food web (Griffiths, 1994; Rønn et al., 2012).

The total nematode abundance increased with cattle grazing in the high marsh but not in the middle marsh. However, the various trophic groups of nematodes were affected differently by grazing. Bacterial-feeding nematodes were more abundant in grazed plots, because they are mostly colonizers or r-strategists, which typically have a high fecundity and are therefore tolerant to environmental changes (Bongers and Bongers, 1998). The RDA result indicated that bacterial-feeding nematodes were positively related to their main food resources of bacteria and protozoa.

Because bacterial-feeding nematodes are functionally active filter-feeders, bacteria as well as relatively small protozoan cells are ingested efficiently (Rønn et al., 2012). Plant-feeding nematodes were fewer in grazed than in ungrazed plots. The RDA result showed that plant-feeding nematode abundance had a positively relation to plant biomass, which was lower in grazed plots. Additionally, plant-feeding nematodes might be influenced by plant species identity, as *Dolichodorus* was the predominant taxon in the ungrazed high marsh dominated by the plant *Phragmites australis*, while *Tetylenchus* was the predominant taxon in the ungrazed middle marsh dominated by the plants *Scirpus mariqueter* and *Phragmites australis*. The association of a plant species with specific plant-feeding nematodes has previously been described (De Deyn et al., 2004). There was a large increase in algal-feeding nematode abundance under cattle grazing at both elevations. This might be because of the reduction in biomass and the creation of gaps in the vegetation canopy by grazing, which leads to more light reaching the soil surface that in turn promotes algae growth (Irving and Connell, 2002), thus providing abundant food sources for algal-feeding nematodes. In contrast, the higher trophic levels such as carnivores and omnivores, which are persisters or k-strategists and therefore have a low colonizing ability and are sensitive to environmental changes (Bongers and Bongers, 1998), tended not to be influenced by cattle grazing and tidal disturbance. The weak responses of carnivores and omnivores might be because the diverse range of their prey assures a relatively stable supply of food sources.

When the whole nematode community was considered, grazed and ungrazed plots were clearly separated for both the high and middle-marsh sites. This additionally demonstrated that both aboveground grazing activity and elevation differences could significantly change the structure of the soil organism community. Changes in structure of soil micro-food webs influence decomposition and nutrient cycling in saltmarsh ecosystems (Bardgett and Wardle, 2010; Ford et al., 2013).

4.2 The responses of soil biota to grazing in the saltmarsh compared with other ecosystems

Previous studies of grassland ecosystems revealed that large herbivores decreased soil microbial biomass through reducing C input of plant litter and SOM (Bardgett et al., 1997; Sankaran and Augustine, 2004), or promoted soil microbial biomass by stimulating plant root exudation (Guitian and Bardgett, 2000; Hamilton et al., 2008) or increased deposition of animal feces, which are often incorporated into SOM (Lovett and Ruesink, 1995). In this study, deposited animal feces were dispersed by tidal currents in the saltmarshes unlike mosaic feces deposition in the grasslands. This might lead to a decrease in SOM under cattle grazing disturbance rather than an increase. Therefore, the stimulated soil microbial biomass under cattle herbivory in the studied saltmarsh is likely because of the modification of root exudates rather than SOM. Our results indicated that the enhanced soil microbial biomass by cattle grazing can in turn promote bacterial-feeding nematodes and protozoa. This confirmed the findings from grassland ecosystems that grazing-induced changes in resource quantity and quality can influence various trophic groups in the soil food webs by bottom-up propagation (Bardgett and Wardle, 2003, 2010).

Functional index values of nematode communities indicate a response to resource and environmental changes under grazing disturbance. The nematode MI and SI values significantly decreased under grazing, which was similar to a previous study in a river-floodplain grassland (Veen et al., 2010). The decrease in MI and SI values suggested that the structure of the nematode community was deteriorating and the complexity of the soil food web declined with grazing. In terms of nematode diversity indices (genera richness, Shannon's index and evenness index), our study in saltmarshes demonstrated few effects of herbivore grazing, which is consistent with many studies in semi-natural steppe grasslands (Zolda, 2006), semiarid grasslands (Chen et al., 2013), alpine meadow ecosystems (Hu et al., 2015) and forest ecosystems (Wardle et al., 2001). This suggests that in all these ecosystems, large herbivores had greater impacts on the functional composition than on the overall diversity of soil nematodes.

It has been widely documented that aboveground herbivores influence soil micro-food webs and ecosystem processes by influencing plants and soil characteristics in grasslands (Chen et al., 2013; Wardle et al., 1999). The impact of cattle activity on the soil micro-food web structure in the saltmarsh was mediated by the hydrological conditions at different elevations. Since alterations in the soil micro-food web might play vital roles in regulating soil ecosystem functions including nutrient cycling and mineralization processes (Griffiths, 1994; Wardle, 1995), we suggest that cattle grazing will further induce different changes in soil ecosystem processes at different elevations.

5. Conclusions

Grazing effects on the community structure of soil organisms (microbial and protozoan biomass, nematode abundance) were generally greater in the high marsh than in the middle marsh. This might be attributed to tidal flooding that partially mediates the effect of grazing on the habitat of soil organism communities. Soil microorganisms were significantly influenced by cattle grazing, and soil protozoa and bacterial-feeding nematodes, consequently, were influenced through the bottom-up effect. In contrast, carnivorous and omnivorous nematodes at high trophic levels in the soil micro-food webs were not affected by grazing in this saltmarsh. Different characteristic nematode communities were found for the grazing treatments and the different elevations, which reflect the ecological differences imposed by both biotic disturbance and abiotic inundation stress. Therefore, the effects of livestock grazing on ecosystem functions need to be considered in the context of local abiotic disturbance in future wetland evaluation and conservation management.

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TABLE LEGENDS

Table 1

Soil characteristics and plant biomass in treatments with grazed (G) and ungrazed (UG) at two elevations. Significant effects (G=grazing, E=elevation, G×E=the interaction of grazing and elevation) in the ANOVA are indicated with *, ** and *** at $P < 0.05$, 0.01 and 0.001; ns=nonsignificant. Values with different letters represent significant differences among grazing treatments and between high and middle-marsh sites, according to a Tukey test at $P < 0.05$.

	High marsh		Middle marsh		
	G	UG	G	UG	ANOVA
Soil					
pH	4.22±0.04	4.24±0.03	4.23±0.07	4.15±0.06	G^{ns}, E^{ns}, G×E^{ns}
Temperature (°C)	25.08±0.19^{a}	24.35±0.17^{b}	23.21±0.32^{c}	23.08±0.12^{c}	G*, E***, G×E^{ns}
Moisture (%)	34.83±1.08	32.82±1.63	32.07±0.91	32.25±1.02	G^{ns}, E^{ns}, G×E^{ns}
Conductivity (mS cm^{-1})	0.81±0.05	0.89±0.06	0.79±0.06	0.76±0.05	G^{ns}, E^{ns}, G×E^{ns}
SOM (%)	3.94±0.16^{b}	4.64±0.17^{a}	3.17±0.18^{c}	3.82±0.26^{b}	G***, E***, G×E^{ns}
ORP (mV)	163.52±2.11	161.72±2.19	164.90±4.09	169.77±4.05	G^{ns}, E*, G×E^{ns}
Total porosity (%)	52.57±1.12^{b}	56.11±0.71^{a}	53.43±0.70^{b}	56.74±1.01^{a}	G***, E^{ns}, G×E^{ns}
C:N	14.15±0.52^{c}	19.06±1.43^{b}	21.63±1.08^{a}	23.25±1.00^{a}	G**, E***, G×E^{ns}
Vegetation					
Aboveground biomass (g m^{-2})	861.60±140.72^{b}	3533.97±230.39^{a}	311.97±20.27^{c}	2525.68±258.85^{a}	G***, E***, G×E***
Belowground biomass (g m^{-2})	249.76±38.68^{b}	439.28±38.66^{a}	188.59±29.06^{c}	376.61±24.47^{ab}	G***, E^{ns}, G×E^{ns}

Table 2

Genera composition and abundance (ind. g^{-1}) of nematode community with grazed (G) and ungrazed (UG) treatments at two elevations (mean±se). "-": not detected. Feeding guilds of soil nematodes characterized by feeding habits were assigned according to Yeates et al (1993). AF, algal-feeders; BF, bacterial-feeders; Ca, carnivores; Om, omnivores; PF, plant-feeders. *c–p* values (1–5) were presented following Bongers (1990).

Genera	Guild$_{c\text{-}p\ value}$	High marsh		Middle marsh	
		G	UG	G	UG
Polysigma	AF_3	0.55±0.17	0.03±0.02	0.54±0.14	0.10±0.02
Anaplectus	BF_2			0.01±0.01	
Anoplostoma	BF_2	0.01±0.01			
Camacolaimus	BF_3		0.02±0.02	0.01±0.01	0.01±0.01
Chronogaster	BF_3		0.05±0.05	0.01±0.01	0.03±0.02
Daptonema	BF_2	1.35±0.23	0.15±0.04	0.95±0.29	0.46±0.17

Dichromadora	BF_2	7.68±0.58	2.43±0.76	3.06±0.48	1.59±0.56
Diplolaimella	BF_2	0.07±0.04	0.08±0.08	0.02±0.02	0.13±0.06
Diplolaimelloides	BF_2	0.06±0.04	0.15±0.05	0.59±0.25	0.42±0.06
Disconema	BF_2	0.02±0.02		0.01±0.01	
Eucephalobus	BF_2		0.01±0.01	0.06±0.06	0.03±0.03
Halalaimus	BF_4	1.02±0.26	0.10±0.07	0.19±0.04	0.05±0.03
Metalinhomoeus	BF_2	0.17±0.15	0.06±0.06	0.07±0.04	0.02±0.01
Monhystera	BF_2	0.14±0.10	0.05±0.03	0.19±0.05	0.06±0.02
Panagrolaimus	BF_1	0.06±0.04	0.03±0.02	0.23±0.11	0.12±0.03
Parodontophora	BF_2	0.88±0.33	0.03±0.02	0.78±0.24	0.05±0.02
Terschellingia	BF_3	0.02±0.02			
Theristus	BF_2	0.06±0.03		0.01±0.01	
Adoncholaimus	Ca_3	0.08±0.05		0.23±0.07	0.02±0.01
Nygolaimus	Ca_5	0.24±0.11	0.30±0.10	0.04±0.02	0.13±0.02
Oncholaimus	Ca_4	0.01±0.01			
Sphaerolaimus	Ca_3	0.08±0.03	0.02±0.02	0.04±0.02	0.02±0.02
Tripyloides	Ca_3			0.04±0.04	0.02±0.01
Chrysonema	Om_5	0.12±0.05	0.19±0.07	0.75±0.17	1.22±0.35
Dorylaimus	Om_4	0.08±0.06	0.05±0.04	0.06±0.03	
Mesodorylaimus	Om_4	0.49±0.15	0.48±0.11	0.21±0.07	0.20±0.05
Criconemoides	PF_3	0.06±0.04	0.08±0.06		0.04±0.03
Dolichodorus	PF_3	0.08±0.02	2.06±0.68	0.05±0.03	0.40±0.18
Hirschmanniella	PF_3	0.16±0.10	0.26±0.07	0.16±0.05	0.43±0.06
Tetylenchus	PF_2	0.03±0.02	0.28±0.10	0.25±0.13	1.07±0.48
Tylenchus	PF_2	0.16±0.06	0.86±0.25	0.05±0.02	0.24±0.15

Table 3

Nematode community structure of with grazed (G) and ungrazed (UG) treatments at two elevations. Significant effects (G=grazing, E=elevation, G×E=the interaction of grazing and elevation) in the ANOVA are indicated with *, ** and *** at $P < 0.05$, 0.01 and 0.001; ns=nonsignificant. Values with different letters represent significant differences among grazing treatments and between high and middle-marsh sites according to a Tukey test at $P < 0.05$

Indices	High marsh		Middle marsh		ANOVA
	G	UG	G	UG	
Total abundance (ind. g^{-1})	13.68±1.06^{a}	7.76±0.77^{b}	8.61±0.51^{b}	6.88±0.53^{b}	G^{***}, E^{***}, $G×E^{*}$
Taxon richness (*S*)	14.33±1.17	12.67±1.15	16.17±1.01	16.67±0.88	G^{ns}, E^{*}, $G×E^{ns}$

Shannon's index (*H*′)	1.61±0.06	1.82±0.17	2.08±0.11	2.11±0.12	G ^{ns}, E **, G×E ^{ns}
Evenness index (*J*′)	0.61±0.02	0.72±0.05	0.75±0.03	0.75±0.03	G ^{ns}, E *, G×E ^{ns}
Maturity index (MI)	2.38±0.05 ^{c}	2.66±0.08 ^{ab}	2.49±0.05 ^{bc}	2.79±0.12 ^{a}	G *, E **, G×E ^{ns}
Structure index (SI)	45.20±4.14 ^{c}	67.37±5.21 ^{ab}	56.35±4.78 ^{b}	74.64±5.99 ^{a}	G *, E ***, G×E ^{ns}

FIGURE LEGENDS

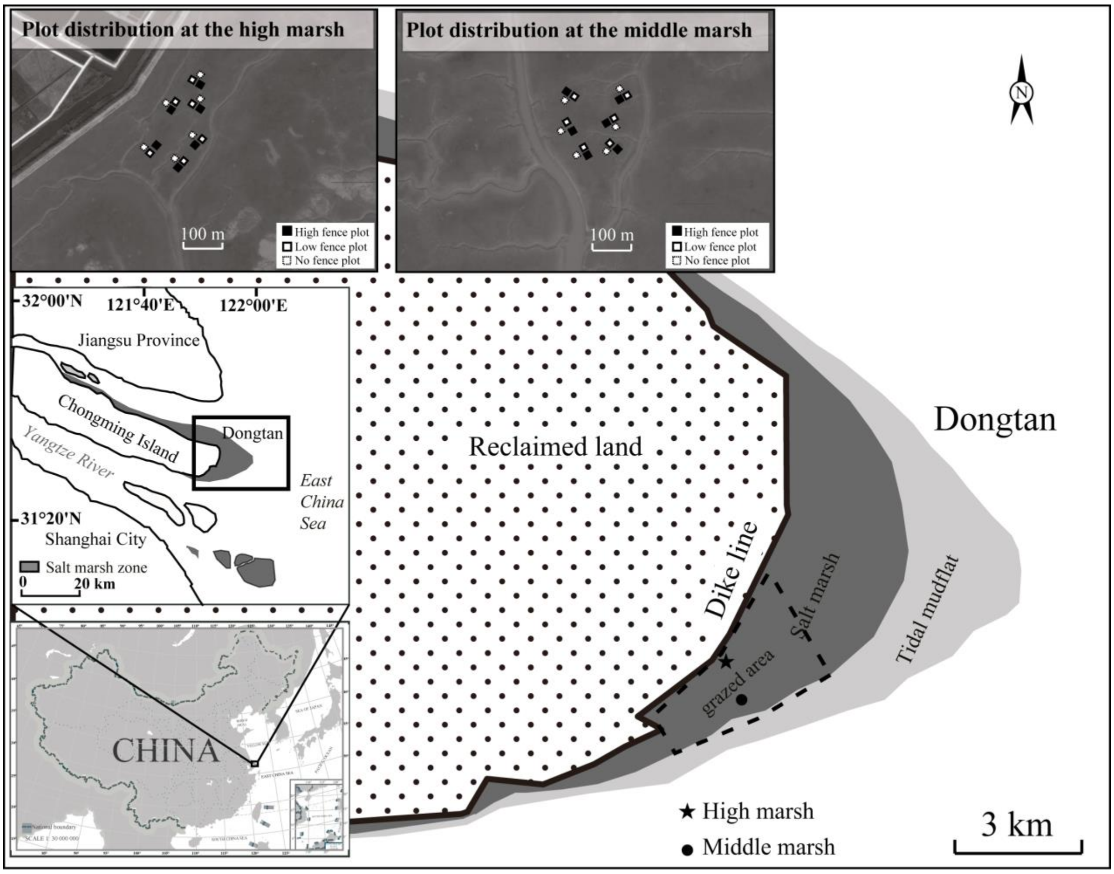

Fig. 1. Location of the experimental blocks and plots in the middle and high marsh of the Dongtan saltmarsh.

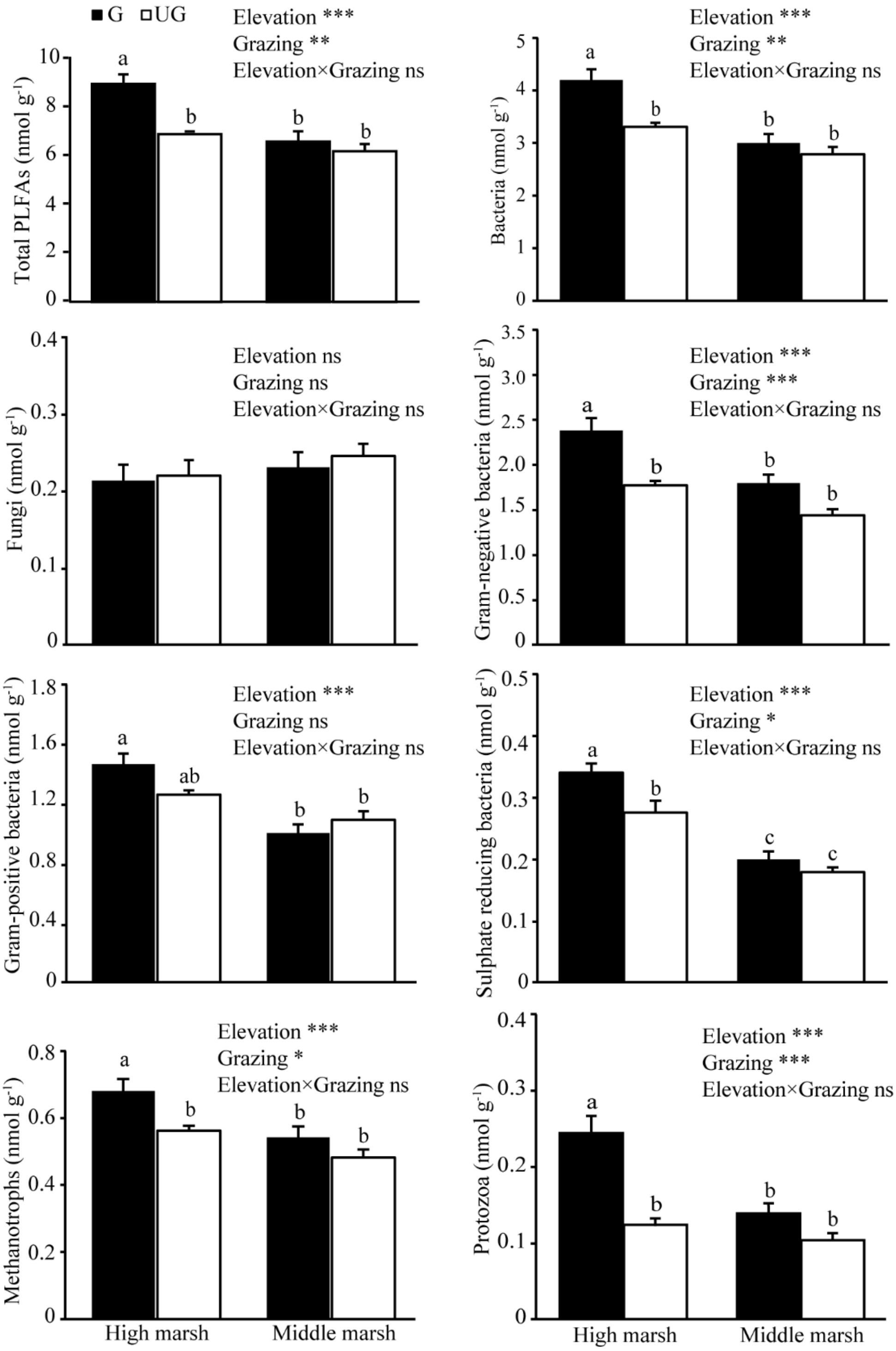

Fig. 2. Biomass of the soil microbial and protozoan community of grazed (G) and ungrazed (UG) treatment at two elevations. Significant effects (Elevation, Grazing, the interaction of Elevation and Grazing) in the ANOVA are indicated with *, ** and *** at $P < 0.05$, 0.01 and 0.001; ns=nonsignificant. The different letters above the bars are significantly different among grazing treatments and between

the high and middle-marsh sites according to a Tukey test at $P < 0.05$.

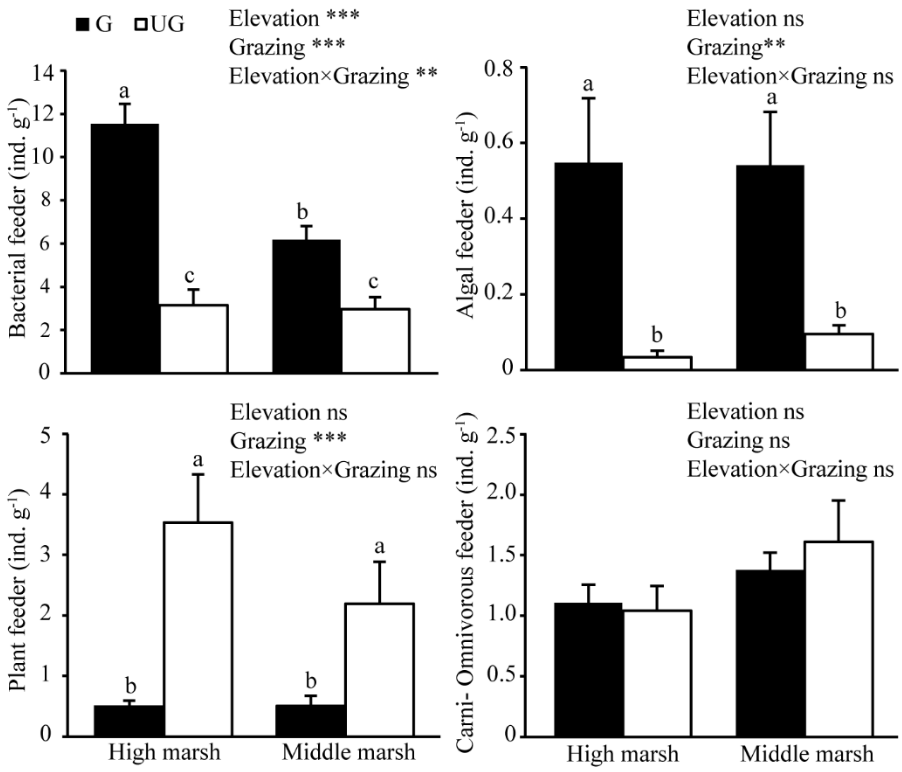

Fig. 3. The abundance of four nematode feeding types in grazed (G) and ungrazed (UG) treatments at two elevations. Significant effects (Elevation, Grazing, the interaction of Elevation and Grazing) in the ANOVA are indicated with *, ** and *** at $P < 0.05$, 0.01 and 0.001; ns=nonsignificant. The different letters above the bars are significantly different among grazing treatments and between the high and middle-marsh sites according to a Tukey test at $P < 0.05$.

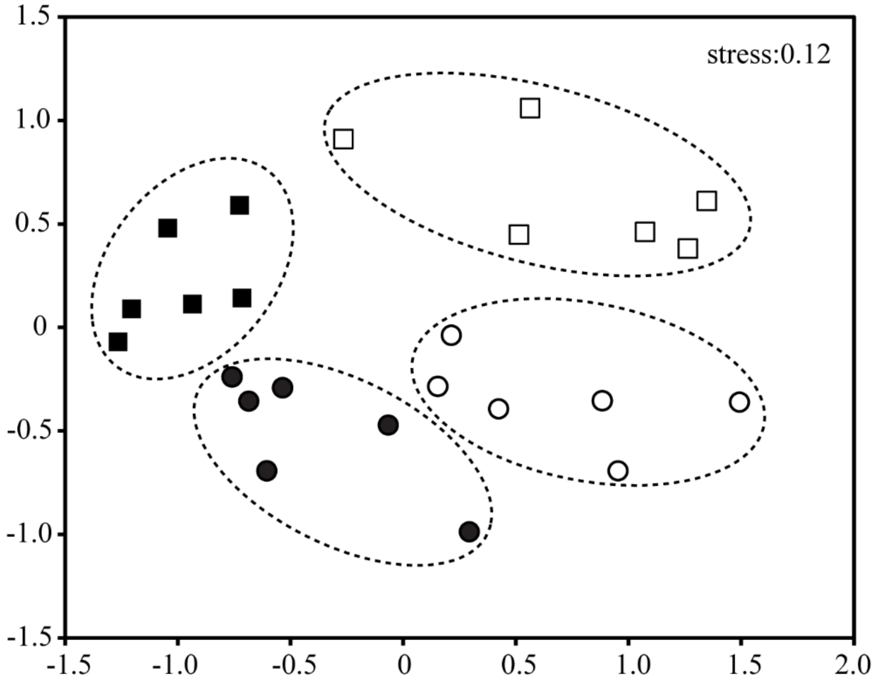

Fig. 4. Non-metric multidimensional scaling (NMDS) ordination of soil nematode communities of grazed and ungrazed treatments at two elevations. Squares represent plots located at the high marsh and circles represent plots located at the middle marsh; black symbols represent grazed plots and open symbols represent ungrazed plots.

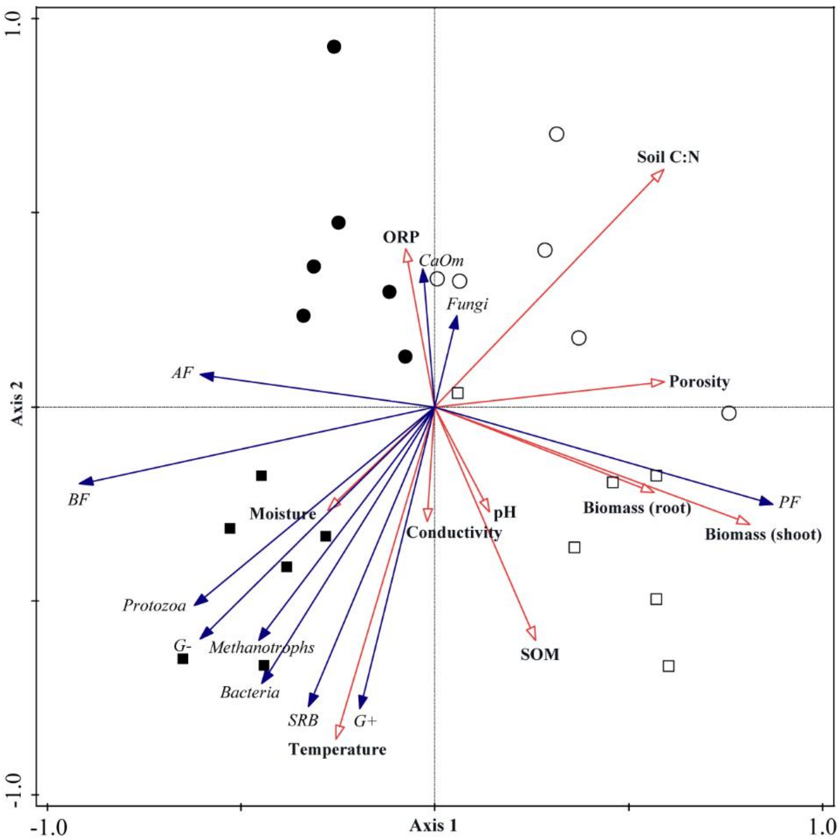

Fig. 5. Redundancy analysis (RDA) for different groups of soil organisms and environmental variables (Canonical eigenvalue is 0.813, Monte Carlo permutation test, *P* = 0.002). Closed arrows indicate the abundance of soil biota and open arrows indicate environmental variables. AF, algal-feeding nematodes; BF, bacterial-feeding nematodes; CaOm, carnivorous-omnivorous nematodes; PF, plant-feeding nematodes. Squares represent plots located at the high marsh and circles represent plots located at the middle marsh; black symbols represent grazed plots and open symbols represent ungrazed plots.