

1 Title

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

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24 **Abstract**

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

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

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## 49 **1. Introduction**

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

67 Soil micro-food webs are important trophic networks in belowground decomposer systems,

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

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

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

104

## 105 **2. Materials and Methods**

### 106 2.1 Site description

107 The study site was in the Dongtan saltmarsh (31°28'N, 121°56'E) of Chongming Island, which  
108 is located in the estuary of the Yangtze River, China. The climate is subtropical monsoon with mean  
109 annual temperature of 15.3 °C and precipitation of 1022 mm. Since the 1950s, the saltmarsh has  
110 been regularly and pervasively grazed by cattle. In the last decade after the reserve was established,  
111 cattle grazing was restricted to a 600 ha southeastern area of the Dongtan saltmarsh, which led to

112 increased grazing intensity and an increased risk of ecosystem degradation (Yang et al., 2008). This  
113 area is grazed by approximately 1 cattle ha<sup>-1</sup> from early April to late October each year. In the  
114 Dongtan marsh, the tides are irregularly semidiurnal with the range of two successive tides being  
115 unequal. The average tidal range is 2.5 m and around 3.5 m during spring tides; the highest  
116 astronomical tide is up to 5.2 m above the lowest astronomical tide (Yang et al., 2008). For the terms  
117 of the marsh, we follow the definition of Redfield (1972): the high marsh lies at approximately the  
118 mean high water level between spring tide and neap tide and the middle marsh lies below the mean  
119 high water and low water level of neap tide. In our study, the dominant plant species in the high  
120 marsh are *Phragmites australis* and *Carex scabrifolia*, while the middle marsh is dominated by  
121 sedges *Scirpus mariqueter* and *C. scabrifolia*.

122

## 123 2.2. Experimental design

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

134 Based on the observation on cattle activity and the counting of fresh cattle dung, the stock cattle  
135 densities between the high and middle marsh were similar. Within both the high and middle marsh  
136 sites, we aimed to have all replicates on a similar elevation within a  $\pm 10$  cm to ensure consistency  
137 of tidal inundation at a site and homogeneous soil environment. Therefore, in the high or middle  
138 marsh site, there is 50-100 m distance apart between every two blocks. Each block contained three  
139 experimental plots ( $15 \times 15$  m) that were assigned to one of three treatments: grazed without fence,  
140 grazed with short fence (the fence height is 50 cm) or ungrazed with tall fence (the fence height is  
141 150 cm and the entire plot was surrounded with barbed wire). The distance was about 5 m between  
142 each two plots of all the three treatments. The grazed with short fence plots were used to eliminate  
143 the effects of the fence *per se* on soil biota.

144

### 145 2.3 Soil and plant characteristics

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

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

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

162

#### 163 2.4 Soil organism analysis

164 For soil organism analysis, four soil cores (3.2 cm diameter and 15 cm depth) were taken from  
165 each plot and then mixed to form a composite sample. The composite sample was then divided into  
166 two subsamples for microorganism phospholipid fatty acids (PLFAs) and nematode analysis,  
167 respectively. The composition of PLFAs was analyzed to assess the soil microbial community  
168 structure and protozoa from 8 g freeze-dried soil subsample following the methods of Frostegård et  
169 al. (1993) with slight modifications (Li et al., 2012). Methyl nonadecanoate fatty acid 19:0 was used  
170 as the internal standard. The fatty acid methyl esters were divided and quantified with an Agilent  
171 6890 Gas Chromatograph and identified by the MIDI Sherlock Microbial Identification System  
172 (MIDI Inc., Newark, DE, USA) based on retention time. The PLFAs i15:0, a15:0, 15:0, i16:0,  
173 16:1 $\omega$ 7, 16:1 $\omega$ 9, i17:0, a17:0, 17:0, cy17:0, 18:1 $\omega$ 7c and cy19:0 were summed to represent the  
174 biomass of bacterial biomass; and 18:2 $\omega$ 6 was used to indicate of the biomass of fungi (Frostegård  
175 et al., 1993; Frostegård and Bååth, 1996). Among these, i15:0, a15:0, i16:0, i17:0 and a17:0 were  
176 used for Gram-positive bacteria and cy17:0, 16:1 $\omega$ 7, 18:1 $\omega$ 7c and cy19:0 for Gram-negative  
177 bacteria indicators respectively (Ford et al., 2013). Other PLFAs such as 16:0 10-Me were used to



178 identify sulphate-reducing bacteria (Dowing et al., 1986), 18:1ω7c for methanotrophs (Bull et al.,  
179 2000) and 20:4ω6c and 20:5 for protozoa (Fierer et al., 2003).

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

196

## 197 2.5 Data analysis

198 An independent sample  $t$ -test method was conducted to test the difference between 'grazed  
199 without fence' and 'grazed with short fence' treatments. The results showed that there was no

200 significant effect from the presence of fence, suggesting no fence artifacts. Therefore, the effects of  
201 the livestock grazing treatments (ungrazed with tall fence and grazed without fence), inundation  
202 (high marsh and middle marsh) and their interaction on soil characteristics, plant above- and  
203 belowground biomass, and soil organism communities (microorganism biomass, protozoan biomass  
204 and nematode abundance), were analyzed by two-way ANOVA. A post-hoc Tukey's HSD tests was  
205 performed if significant differences among treatments were found. The data were  $\log(x+1)$   
206 transformed to match the assumptions of ANOVA if necessary. Significance levels were set at  $P <$   
207  $0.05$ . The analyses were executed using the STATISTICA 8.0 (StatSoft Inc, Tulsa, OK, USA).

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

216

### 217 **3. Results**

#### 218 3.1 Soil and vegetation characteristics

219 There was no significant difference observed for all parameters including soil characteristics,  
220 plant biomass and the biomass or abundance and indices of soil biota between the short-fence  
221 treatment and no-fence treatment. Therefore, our results were illustrated by using data of no-fence

222 plot (grazed treatment) and tall-fence plot (ungrazed treatment). Soil temperature, SOM and soil  
223 C/N ratio were significantly affected by grazing and elevation but not by the interaction of the two  
224 factors (Table 1). Soil moisture and ORP were significantly affected only by elevation. Total soil  
225 porosity was significantly affected only by grazing. Post-hoc test showed that grazing significantly  
226 increased soil temperature and lowered the soil C/N ratio only in the high marsh, whereas SOM and  
227 total porosity were significantly reduced at both elevations (Table 1).

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

233

### 234 3.2 Soil microbial and protozoan PLFAs

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

244

### 245 3.3 Composition and structure of nematode communities

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

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

262 Significant grazing (Global test:  $R = 0.68$ ,  $P = 0.001$ ) and elevation effects (Global test:  $R =$   
263  $0.557$ ,  $P = 0.001$ ) were detected on nematode communities. The NMDS ordination of nematode  
264 communities clearly discriminated four plot groups of high and middle marsh, grazed and ungrazed  
265 treatments (Fig. 4).

266

### 267 3.4 The relationship between soil biota and environmental variables

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

280

## 281 4. Discussion

### 282 4.1 Responses of soil biota at different trophic levels to grazing in high and middle marshes

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

288 middle marsh.

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

307       Cattle grazing significantly promoted the biomass of Gram-negative bacteria in the high marsh,  
308 which agrees with results obtained in temperate saltmarshes in northern Europe (Ford et al., 2013).  
309 They concluded that the growth rates of Gram-negative bacteria were restricted by the high reliance

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

321 The response of soil protozoa to grazing by cattle was similar to that of bacteria across  
322 elevations, possibly because bacteria are the main food resource of protozoa. This is in agreement  
323 with some statements that protozoa are positively related to changes in the available food resources  
324 including rhizosphere bacteria that induce a bottom-up trophic control of the soil food web (Griffiths,  
325 1994; Rønn et al., 2012).

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

332 Because bacterial-feeding nematodes are functionally active filter-feeders, bacteria as well as  
333 relatively small protozoan cells are ingested efficiently (Rønn et al., 2012). Plant-feeding nematodes  
334 were fewer in grazed than in ungrazed plots. The RDA result showed that plant-feeding nematode  
335 abundance had a positively relation to plant biomass, which was lower in grazed plots. Additionally,  
336 plant-feeding nematodes might be influenced by plant species identity, as *Dolichodorus* was the  
337 predominant taxon in the ungrazed high marsh dominated by the plant *Phragmites australis*, while  
338 *Tetylenchus* was the predominant taxon in the ungrazed middle marsh dominated by the plants  
339 *Scirpus mariqueter* and *Phragmites australis*. The association of a plant species with specific plant-  
340 feeding nematodes has previously been described (De Deyn et al., 2004). There was a large increase  
341 in algal-feeding nematode abundance under cattle grazing at both elevations. This might be because  
342 of the reduction in biomass and the creation of gaps in the vegetation canopy by grazing, which  
343 leads to more light reaching the soil surface that in turn promotes algae growth (Irving and Connell,  
344 2002), thus providing abundant food sources for algal-feeding nematodes. In contrast, the higher  
345 trophic levels such as carnivores and omnivores, which are persisters or k-strategists and therefore  
346 have a low colonizing ability and are sensitive to environmental changes (Bongers and Bongers,  
347 1998), tended not to be influenced by cattle grazing and tidal disturbance. The weak responses of  
348 carnivores and omnivores might be because the diverse range of their prey assures a relatively stable  
349 supply of food sources.

350 When the whole nematode community was considered, grazed and ungrazed plots were clearly  
351 separated for both the high and middle-marsh sites. This additionally demonstrated that both  
352 aboveground grazing activity and elevation differences could significantly change the structure of  
353 the soil organism community. Changes in structure of soil micro-food webs influence decomposition



354 and nutrient cycling in saltmarsh ecosystems (Bardgett and Wardle, 2010; Ford et al., 2013).

355

356 4.2 The responses of soil biota to grazing in the saltmarsh compared with other ecosystems

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

370 Functional index values of nematode communities indicate a response to resource and  
371 environmental changes under grazing disturbance. The nematode MI and SI values significantly  
372 decreased under grazing, which was similar to a previous study in a river-floodplain grassland (Veen  
373 et al., 2010). The decrease in MI and SI values suggested that the structure of the nematode  
374 community was deteriorating and the complexity of the soil food web declined with grazing. In  
375 terms of nematode diversity indices (genera richness, Shannon's index and evenness index), our

376 study in saltmarshes demonstrated few effects of herbivore grazing, which is consistent with many  
377 studies in semi-natural steppe grasslands (Zolda, 2006), semiarid grasslands (Chen et al., 2013),  
378 alpine meadow ecosystems (Hu et al., 2015) and forest ecosystems (Wardle et al., 2001). This  
379 suggests that in all these ecosystems, large herbivores had greater impacts on the functional  
380 composition than on the overall diversity of soil nematodes.

381 It has been widely documented that aboveground herbivores influence soil micro-food webs  
382 and ecosystem processes by influencing plants and soil characteristics in grasslands (Chen et al.,  
383 2013; Wardle et al., 1999). The impact of cattle activity on the soil micro-food web structure in the  
384 saltmarsh was mediated by the hydrological conditions at different elevations. Since alterations in  
385 the soil micro-food web might play vital roles in regulating soil ecosystem functions including  
386 nutrient cycling and mineralization processes (Griffiths, 1994; Wardle, 1995), we suggest that cattle  
387 grazing will further induce different changes in soil ecosystem processes at different elevations.

388

## 389 **5. Conclusions**

390 Grazing effects on the community structure of soil organisms (microbial and protozoan biomass,  
391 nematode abundance) were generally greater in the high marsh than in the middle marsh. This might  
392 be attributed to tidal flooding that partially mediates the effect of grazing on the habitat of soil  
393 organism communities. Soil microorganisms were significantly influenced by cattle grazing, and  
394 soil protozoa and bacterial-feeding nematodes, consequently, were influenced through the bottom-  
395 up effect. In contrast, carnivorous and omnivorous nematodes at high trophic levels in the soil  
396 micro-food webs were not affected by grazing in this saltmarsh. Different characteristic nematode  
397 communities were found for the grazing treatments and the different elevations, which reflect the

398 ecological differences imposed by both biotic disturbance and abiotic inundation stress. Therefore,  
399 the effects of livestock grazing on ecosystem functions need to be considered in the context of local  
400 abiotic disturbance in future wetland evaluation and conservation management.

401

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408

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570

571

572 TABLE LEGENDS

573 **Table 1**

574 Soil characteristics and plant biomass in treatments with grazed (G) and ungrazed (UG) at two

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

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

579

580 **Table 2**

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

Genera	Guild <sub><i>c-p</i></sub> value	High marsh		Middle marsh	
		G	UG	G	UG
<i>Polysigma</i>	AF <sub>3</sub>	0.55±0.17	0.03±0.02	0.54±0.14	0.10±0.02
<i>Anaplectus</i>	BF <sub>2</sub>			0.01±0.01	
<i>Anoplostoma</i>	BF <sub>2</sub>	0.01±0.01			
<i>Camacolaimus</i>	BF <sub>3</sub>		0.02±0.02	0.01±0.01	0.01±0.01
<i>Chronogaster</i>	BF <sub>3</sub>		0.05±0.05	0.01±0.01	0.03±0.02
<i>Daptonema</i>	BF <sub>2</sub>	1.35±0.23	0.15±0.04	0.95±0.29	0.46±0.17

<i>Dichromadora</i>	BF <sub>2</sub>	7.68±0.58	2.43±0.76	3.06±0.48	1.59±0.56
<i>Diplolaimella</i>	BF <sub>2</sub>	0.07±0.04	0.08±0.08	0.02±0.02	0.13±0.06
<i>Diplolaimelloides</i>	BF <sub>2</sub>	0.06±0.04	0.15±0.05	0.59±0.25	0.42±0.06
<i>Disconema</i>	BF <sub>2</sub>	0.02±0.02		0.01±0.01	
<i>Eucephalobus</i>	BF <sub>2</sub>		0.01±0.01	0.06±0.06	0.03±0.03
<i>Halalaimus</i>	BF <sub>4</sub>	1.02±0.26	0.10±0.07	0.19±0.04	0.05±0.03
<i>Metalinhomoeus</i>	BF <sub>2</sub>	0.17±0.15	0.06±0.06	0.07±0.04	0.02±0.01
<i>Monhystera</i>	BF <sub>2</sub>	0.14±0.10	0.05±0.03	0.19±0.05	0.06±0.02
<i>Panagrolaimus</i>	BF <sub>1</sub>	0.06±0.04	0.03±0.02	0.23±0.11	0.12±0.03
<i>Parodontophora</i>	BF <sub>2</sub>	0.88±0.33	0.03±0.02	0.78±0.24	0.05±0.02
<i>Terschellingia</i>	BF <sub>3</sub>	0.02±0.02			
<i>Theristus</i>	BF <sub>2</sub>	0.06±0.03		0.01±0.01	
<i>Adoncholaimus</i>	Ca <sub>3</sub>	0.08±0.05		0.23±0.07	0.02±0.01
<i>Nygolaimus</i>	Ca <sub>5</sub>	0.24±0.11	0.30±0.10	0.04±0.02	0.13±0.02
<i>Oncholaimus</i>	Ca <sub>4</sub>	0.01±0.01			
<i>Sphaerolaimus</i>	Ca <sub>3</sub>	0.08±0.03	0.02±0.02	0.04±0.02	0.02±0.02
<i>Tripyloides</i>	Ca <sub>3</sub>			0.04±0.04	0.02±0.01
<i>Chrysonema</i>	Om <sub>5</sub>	0.12±0.05	0.19±0.07	0.75±0.17	1.22±0.35
<i>Dorylaimus</i>	Om <sub>4</sub>	0.08±0.06	0.05±0.04	0.06±0.03	
<i>Mesodorylaimus</i>	Om <sub>4</sub>	0.49±0.15	0.48±0.11	0.21±0.07	0.20±0.05
<i>Criconemoides</i>	PF <sub>3</sub>	0.06±0.04	0.08±0.06		0.04±0.03
<i>Dolichodorus</i>	PF <sub>3</sub>	0.08±0.02	2.06±0.68	0.05±0.03	0.40±0.18
<i>Hirschmanniella</i>	PF <sub>3</sub>	0.16±0.10	0.26±0.07	0.16±0.05	0.43±0.06
<i>Tetylenchus</i>	PF <sub>2</sub>	0.03±0.02	0.28±0.10	0.25±0.13	1.07±0.48
<i>Tylenchus</i>	PF <sub>2</sub>	0.16±0.06	0.86±0.25	0.05±0.02	0.24±0.15

586

587 **Table 3**

588 Nematode community structure of with grazed (G) and ungrazed (UG) treatments at two elevations.

589 Significant effects (G=grazing, E=elevation, G×E=the interaction of grazing and elevation) in the

590 ANOVA are indicated with \*, \*\* and \*\*\* at  $P < 0.05$ , 0.01 and 0.001; ns=nonsignificant. Values with

591 different letters represent significant differences among grazing treatments and between high and

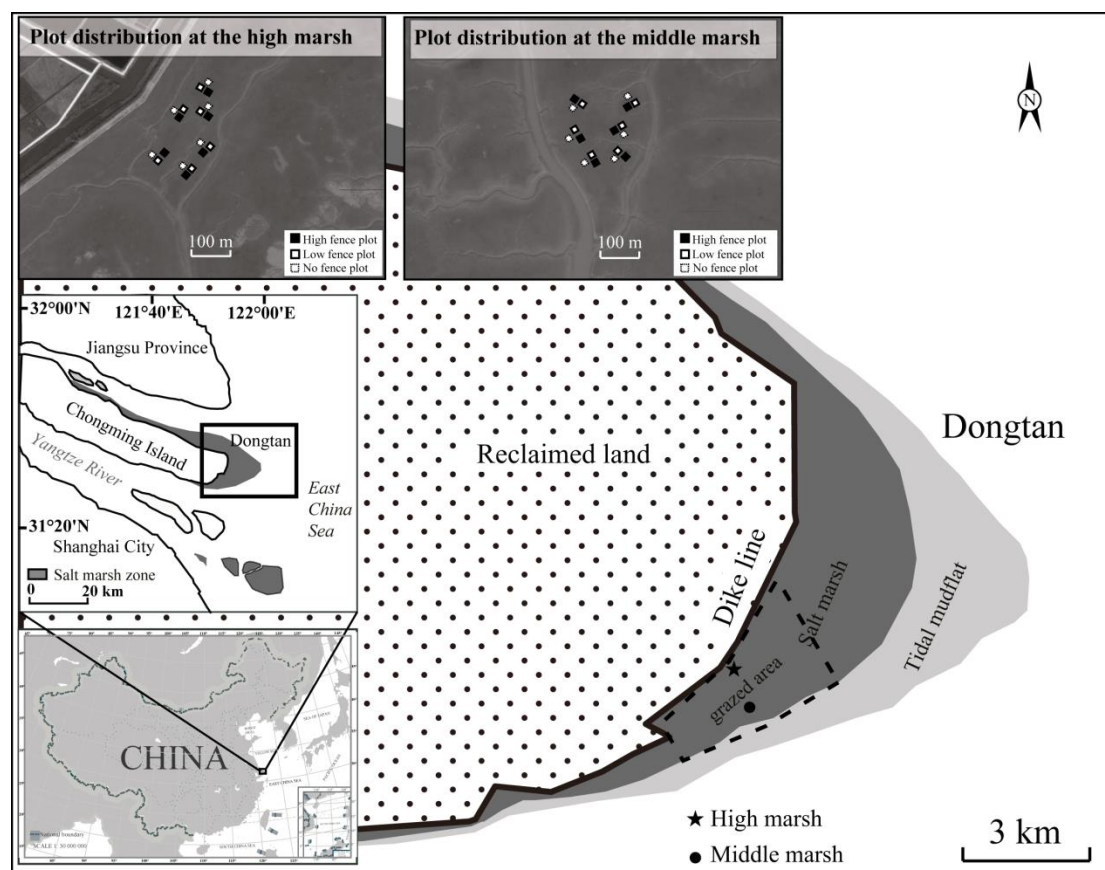
592 middle-marsh sites according to a Tukey test at  $P < 0.05$

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

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

593

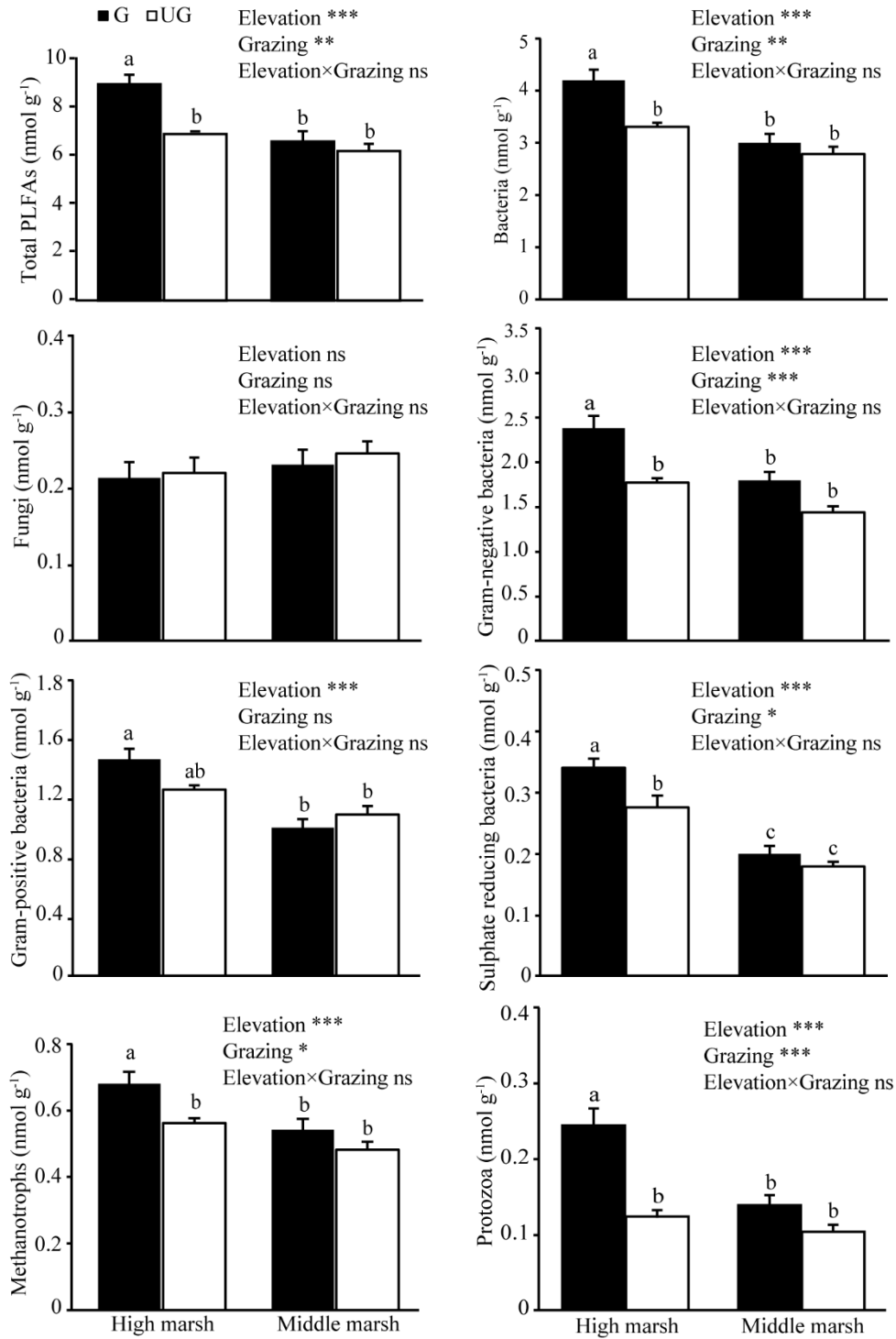
594 FIGURE LEGENDS



595

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

597 saltmarsh.



598

599 **Fig. 2.** Biomass of the soil microbial and protozoan community of grazed (G) and ungrazed (UG)

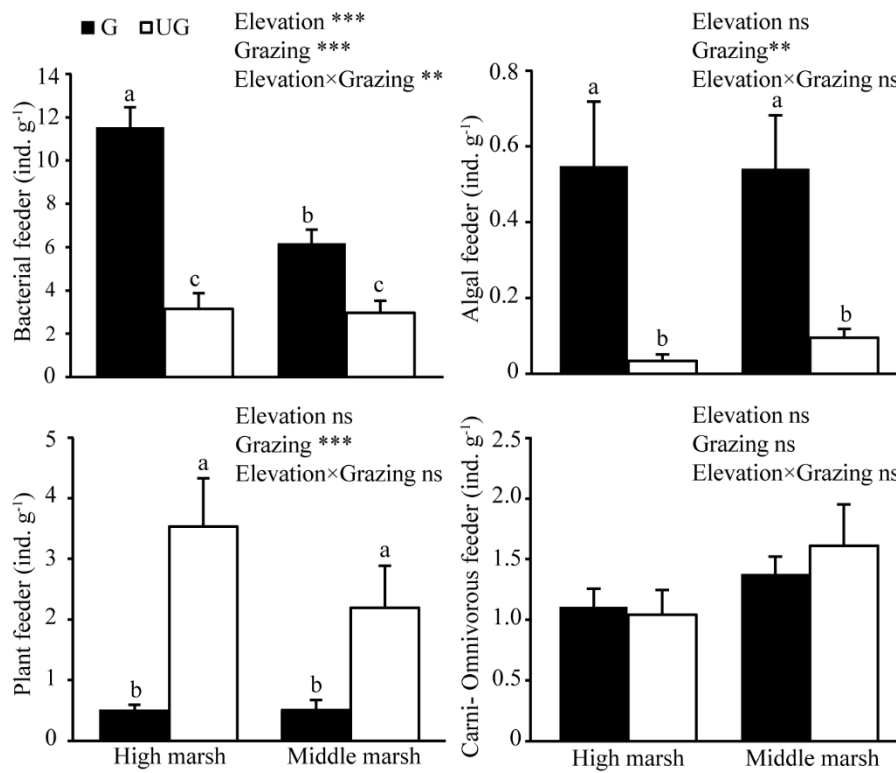
600 treatment at two elevations. Significant effects (Elevation, Grazing, the interaction of Elevation and

601 Grazing) in the ANOVA are indicated with \*, \*\* and \*\*\* at  $P < 0.05$ , 0.01 and 0.001; ns=nonsignificant.

602 The different letters above the bars are significantly different among grazing treatments and between

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

604



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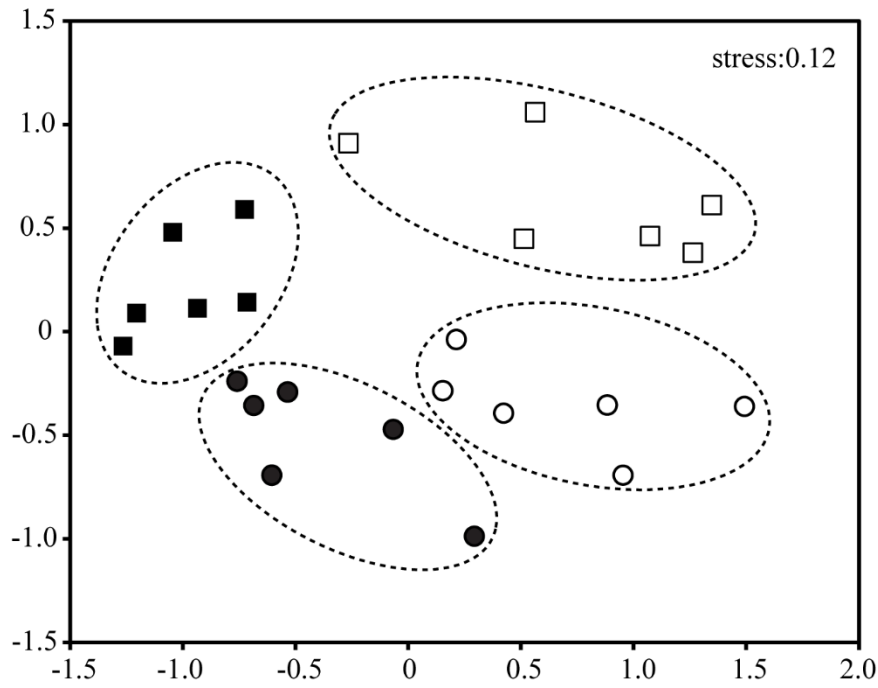
606 **Fig. 3.** The abundance of four nematode feeding types in grazed (G) and ungrazed (UG) treatments

607 at two elevations. Significant effects (Elevation, Grazing, the interaction of Elevation and Grazing)

608 in the ANOVA are indicated with \*, \*\* and \*\*\* at  $P < 0.05$ , 0.01 and 0.001; ns=nonsignificant. The

609 different letters above the bars are significantly different among grazing treatments and between the

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



611

612 **Fig. 4.** Non-metric multidimensional scaling (NMDS) ordination of soil nematode communities of

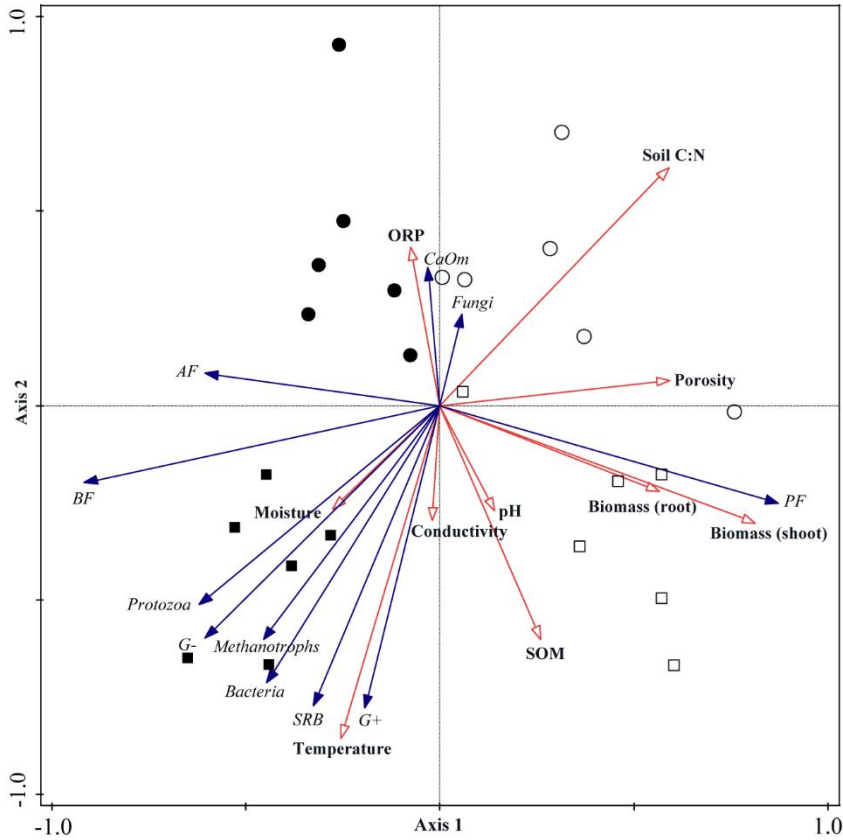
613 grazed and ungrazed treatments at two elevations. Squares represent plots located at the high marsh

614 and circles represent plots located at the middle marsh; black symbols represent grazed plots and

615 open symbols represent ungrazed plots.

616





617

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

625