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1 *Short communication*

2 **Soil nematode assemblage responds weakly to grazer exclusion on a**

3 **nutrient-rich seabird island**

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16

17 **Abstract**

18 The effects of aboveground herbivores on plant-soil interactions are highly context dependent

19 and a key underlying factor controlling this is thought to be nutrient availability. Here, we

20 tested whether the effects of vertebrate grazing on the soil food web varied with nutrient

21 availability and hypothesised that soil food web structure would be driven more by the

22 exclusion of vertebrate grazers than by nutrient enrichment. An 8-year long grazer exclusion

23 experiment was performed in grasslands on a small Scottish island near soil nutrient-

24 enriching seabird colonies at the coast and in less fertile conditions inland. We investigated

25 the trophic structure of the soil nematode assemblage as a proxy for soil food web structure.

26 Across all eight study sites the bacterial energy channel was predominant over the fungal

27 channel. Grazer exclusion strongly enhanced plant biomass accumulation and although this

28 tended to be associated with a somewhat lower abundance of bacterial-feeders, this effect was

29 non-significant and surprisingly weak given the observed changes aboveground. Indeed, plant

30 species identity, diversity and dominance were, just as any other vegetation descriptor, weak

31 predictors of nematode trophic structure. Instead, site specific conditions were important,

32 despite the small island area and apparently homogenous sampling conditions.

33

34 **Keywords**

35 Soil food web; Nematode feeding groups; Grazing; Trophic structure; Aboveground-

36 Belowground interaction; Exclosure

37

38 *Introduction*

39 Effects of aboveground primary consumers on the soil food web are context-dependent [1].

40 Herbivores may either promote plant species that produce recalcitrant litter and enhance the

41 fungal energy channel [2], or benefit plants that produce easily decomposable detritus,

42 thereby promoting the bacterial energy channel [3]. This may have important implications for

43 ecosystem functioning, e.g. the response of soil processes to drought [4-5]. Comparison of

44 studies across different ecosystems suggests that the direction of change in soil webs

45 resulting from aboveground grazing may be depend on the fertility of the ecosystem [1]; this

46 hypothesis, however, has rarely been tested.

47 　　　　Long-term exclosures have been used to investigate the effects of herbivores on plant-

48 soil linkages in field conditions [6-7], and by sampling such systems repeatedly in time, both

49 short and long term effects of herbivore removal can be studied. This could reveal trends and

50 mechanisms that are otherwise difficult to detect, either early on in a short term experiment or

51 later through comparing an advantage stage to a control. For instance, effects of a plant

52 species on soil can persist years after that species has been replaced, i.e. a plant legacy effect

53 [8]. Herbivores can change the plant species composition of an ecosystem and therefore

54 induce such legacies. Longer term experiments that focus on the interactions between

55 herbivores, plants and soil in different environmental conditions are needed to progress from

56 context-dependent generalisations to context-specific predictions.

57 Here we report on an herbivore exclusion study at sites with contrasting levels of

58 nutrient enrichment to test whether nutrient availability regulates the effects of aboveground

59 herbivory on the soil food web. In particular, we studied the effects of vertebrate herbivores

60 and natural nutrient enrichment on the soil nematode trophic structure on the Isle of May, a

61 small (1.5×0.5 km) seabird island in the Firth of Forth, eastern Scotland, UK (56°11′9″ N,

62 2°33′27″ W). Because the coastal areas of the island receive greatest nutrient inputs from

63 seabird colonies [9], inland and coastal sites were used to represent low and high levels of

64 nutrient enrichment, respectively. Exclosures (n=8) were compared to neighbouring plots that

65 were intensively grazed by rabbits (*Oryctolagus cuniculus*). The main hypothesis was that

66 removal of grazers would override seabird nutrient enrichment in driving the soil food web

67 structure given the profound effect of rabbits on the island's vegetation. We also expected the

68 relative abundance of bacterial-feeders to be greater in grazed areas than in the exclosures,

69 due to a combination of increased rhizodeposition and input of labile organic compounds

70 through faeces and urine, and that such a difference would be weaker at coastal sites as a

71 consequence of greater nutrient availability. While shorter term effects (i.e. over three years

72 following grazing exclusion) of grazing exclusion on the nematode community have been

73 previously reported [10], here we report longer term (eight years) effects. Furthermore, we

74 investigated whether quantitative and qualitative changes in vegetation could explain patterns

75 in nematode trophic structure.

76 *Materials and methods*

The study was carried out at four sites located near seabird colonies (coastal sites),

subject to high deposition of ammonia-derived N [9-10] and another four approximately 100

m from the colonies (inland sites) subject to lower levels of nutrient supply. Each site was

composed of a 5×5 m exclosure which effectively kept out rabbits since its erection in April

2003, and an adjacent designated control area of the same size grazed by rabbits. In June

2011, two grass-dominated plots (50×50 cm), one in the exclosure and the other in the

control, were randomly located at each site. In each plot, the litter layer was removed and

three soil turves (4×4 cm, 10 cm deep) were collected with a knife and pooled to obtain a

single composite sample from which to extract nematodes and measure soil moisture, salinity

and pH_{H2O}. For each plot we also measured the depth of the litter layer (average of three

measurements), plant species % cover and vegetation height, and counted the number of

rabbit droppings (as indication of grazing intensity). Nematodes were extracted as in [10] and

specimens were counted to estimate density. In each sample, 100 random specimens were

attributed to one of the following feeding groups: bacterial-feeders, fungal-feeders, plant-

feeders, omnivores, and predators [11]. The relative abundance of different feeding groups

were used to calculate Trophic Diversity ($1/\sum p_i^2$, where p_i is the proportion of the ith feeding

group [12]), and Nematode Channel Ratio (B/(B+F), where B and F are the proportions of

bacterial- and fungal-feeders [13]). Plant species data were used to calculate the Shannon-

Wiener index of diversity ($-\sum[p_i \ln(p_i)]$, where p_i is the frequency of the ith species) and the

Berger-Parker index of dominance (N_{max}/N, where N_{max} and N are the abundance of the

dominant species and of all species, respectively).

The effects of grazing (fence vs. no fence), location (coastal vs. inland) and their

interaction on vegetation height, litter layer depth, plant diversity, nematode density,

Nematode Channel Ratio, and Trophic Diversity were investigated with linear models. To

determine the extent of between-site variability, site was also used as predictor in separate

102   models. To explore differences in feeding group relative abundances across all sample

103   locations, non-metric multidimensional scaling (NMDS) was performed on a matrix of Bray-

104   Curtis dissimilarities based on feeding group proportions. PERMANOVA [14] was carried

105   out on the Bray-Curtis matrix to determine the extent to which grazing, location and site

106   explained variance in feeding group proportions. Co-Correspondence analysis [15] with plant

107   species as predictors and nematode feeding groups as response variable was also performed.

108   All statistical analyses were performed in R [16] (packages *vegan* [17], *cocorresp* [18] and

109   *nlme* [19]). Model distributional assumptions and homogeneity were checked by plotting

110   standardised residuals against fitted values and producing normal quantile-quantile plots. In

111   the linear models Nematode Channel Ratio was transformed as $\log(x+1)$ to improve

112   normality; residual variance was allowed to differ among levels of a factor when needed to

113   improve homogeneity. Feeding group densities and plant species cover were log-transformed

114   (as $\ln(x+1)$) to reduce skewedness for Co-Correspondence analysis. Data are expressed as

115   mean ± standard error.

116   ***Results and discussion***

117   A summary of the biotic and abiotic data is provided in Table 1. We found that the

118   fences had effectively excluded rabbits, as no droppings were found in exclosures. Vegetation

119   height was dramatically affected by grazer exclusion ($F_{1,12} = 476.87$, $p < 0.0001$), being 14×

120   taller inside the fences along with a 3× deeper litter layer than in the controls ($F_{1,12} = 116.45$,

121     $p < 0.0001$). Grazing exclusion led to a less diverse and even grassland community at both

122   coastal and inland sites: the Shannon-Wiener index was higher in the controls than in the

123   exclosures (0.89 ± 0.08 vs. 0.43 ± 0.10, $F_{1,12} = 11.07$, $p = 0.006$), while the reverse was found

124   for the Berger-Parker index (0.43 ± 0.04 vs. 0.59 ± 0.04, $F_{1,12} = 7.60$, $p = 0.02$).

125   Nematode density was not significantly affected by grazing or location (Table 1).

126   Nematode Trophic Diversity was not affected by grazing, but was higher at inland ($2.88 \pm$

127   0.19) than coastal sites (2.27 ± 0.13; $F_{1,13}$ = 7.06, p = 0.02). Nematode Channel Ratio

128   appeared highest in grazed conditions (Exclosure: 0.76 ± 0.04, Control: 0.82 ± 0.02), but this

129   response was not significant ($F_{1,12}$ = 2.55, p = 0.13), and there was no difference in this

130   measure between coastal and inland sites ($F_{1,12}$ = 1.81, p = 0.20). The hypothesis that

131   differences in Nematode Channel Ratio between control and exclosures would be weaker at

132   coastal than inland sites was not supported, as the interaction of grazing and location was also

133   non-significant ($F_{1,12}$ = 0.04, p = 0.83). The Nematode Channel Ratio was always greater than

134   0.5, pointing to a dominance of the bacterial energy channel across all grassland sites.

135   No clear differences in nematode trophic structure among treatments were detected by

136   NMDS, although PERMANOVA indicated a different nematode trophic structure between

137   coastal and inland samples ($F_{1,14}$ = 3.83, p = 0.03), but not between grazed and exclosure

138   samples ($F_{1,14}$ = 0.59, p = 0.61). Co-Correspondence analysis confirmed that plant species did

139   not explain patterns in the nematode assemblage (first axis 10.41% fit, p = 0.58, 9999

140   permutations), and neither Shannon-Wiener index nor Berger-Parker index had detectable

141   effects on Trophic Diversity or Nematode Channel Ratio. Therefore, the trophic structure of

142   the nematode community was little affected by differences in the vegetation aboveground,

143   despite dramatic effects of grazer exclusion on vegetation height and litter layer depth (Table

144   1). Instead, between-site variation was important, with significant effects on nematode

145   density ($F_{1,7}$ = 4.08, p = 0.03), Trophic Diversity ($F_{1,7}$ = 5.70, p = 0.01) and the Nematode

146   Channel Ratio ($F_{1,7}$ = 32.22, p < 0.001).

147   Overall, the hypothesis that the Nematode Channel Ratio would be higher in the

148   grazed plots relative to those in exclosures was not supported by the data. This may reflect a

149   weak below-ground effect of grazing, as also found by Wright et al. in the first three years of

150   the experiment [10]. Although several studies have shown increases in bacterial-feeding

151   nematodes under grazed conditions [3, 20], the opposite effect has also been reported [2, 21].

152 The lack of effect in our study, however, is surprising given the large effects of rabbits on

153 plant growth (vegetation height) and biomass accumulation (litter layer depth). An

154 explanation could be that our sample size (n=8 exclosures) was too small to overcome the

155 spatial heterogeneity characterising nematode assemblages [22] and, in fact, site was the main

156 factor that explained patterns in nematode trophic structure. The occurrence of such small-

157 scale spatial variability was unexpected, because the island is relatively small, all study sites

158 were ecological replicates in that they were all placed in one plant community, and all sample

159 plots were grass-dominated. However, the absence of a grazer exclusion effect is more likely

160 to be genuine than an artefact of our study design, as no such effect was detected even when

161 controlling for between-site variability. It is also possible that grazing effects occurred in the

162 litter layer (here not sampled) but failed to extend to the soil underneath.

163   Vegetation data did not significantly explain variance in nematode trophic structure.

164 Plant identity has been shown to be an important determinant of the soil nematode

165 community [23-24], but our sites were rather homogeneous in plant species composition. All

166 samples were dominated by the same grass species (Table 1). Therefore, litter quality - a

167 well-known driver of soil food web structure [1] - may not have differed much between

168 treatments. Litter quantity, however, was considerably greater in non-grazed plots, but did not

169 explain variance in nematode trophic structure in the soil below the litter layer. Shifts in

170 species composition that could not be detected by the chosen level of identification might

171 however have occurred.

172   Both univariate and multivariate analyses showed some differences in soil nematode

173 trophic structure between coastal and inland sites. This might be due to differences in soil

174 fertility induced by the seabirds, as the inland sites were not subject to the high levels of

175 guano and ammonia-derived N deposition that characterised coastal sites [9]. Seabirds

176 transfer nutrients from sea to land, often considerably enhancing soil fertility and primary

productivity of coastal areas and impacting on higher trophic levels where forming large

colonies [25-26]. However, the relative importance of differences in soil fertility in our study

is uncertain, as other factors might have been at play. For example, soil moisture was higher

at inland than coastal sites ($F_{1,6} = 6.85$, $p = 0.04$), but did not significantly explain variance of

any nematode response variable (not shown), and neither did other predictor variables.

Therefore, unmeasured factors, perhaps related to greater environmental stress on the plant-

soil system near the coast, might have partly driven the differences in the soil food web

associated with close proximity to coastal seabird colonies.

In conclusion, weak linkages between grazing and the trophic structure of soil

nematode assemblages were found on the Isle of May after eight years of herbivore

exclusion. In contrast with what was found in the first three years after erection of the

enclosures [10], proximity to seabird colonies was more important than grazer exclusion for

the overall trophic structure of the nematode community, despite strong effects of grazer

exclusion on plant height and litter build-up. No clear relationships between nematode

trophic structure and plant species were found. Notwithstanding apparent homogeneity in the

grass-dominated plots we sampled, important between-site differences in nematode density

and trophic structure were found, adding to the evidence of high spatial variability of

nematode assemblages.

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Table 1. Estimates (mean ± SE, n=4) of abiotic and biological variables on the Isle of May, Scotland, in relation to distance from the main seabird cliffs (coastal or inland location) and grazing (exclosure or unfenced control)

	Inland Exclosure	Coastal Exclosure	Inland Control	Coastal Control
Environmental properties				
Moisture (%)	67.27 ± 1.61	65.25 ± 8.85	54.70± 6.48	40.37 ± 8.72
pH	4.88 ± 0.36	5.15 ± 0.50	5.31 ± 0.52	5.56 ± 0.03
Salinity (dS/m)	3.35 ± 0.15	3.72 ± 0.18	3.65 ± 0.62	3.25 ± 0.26
Vegetation height (cm)	24.65 ± 1.81	26.30 ± 1.19	1.80 ± 0.14	1.72 ± 0.07
Litter depth (cm)	7.75 ± 0.25	8.87 ± 0.72	2.25 ± 0.59	3.50 ± 0.29
Rabbit droppings	–	–	21.82 ± 3.99	12.72 ± 1.84
Soil nematodes				
Density (ind./cm^2)	519.38 ± 151.04	748.46 ± 150.53	670.68 ± 196.80	752.26 ± 207.14
Bacterial-feeders (%)	38.50 ± 6.12	60.50 ± 7.92	49.00 ± 4.56	59.75 ± 3.350
Fungal feeders (%)	15.25 ± 4.33	15.00 ± 5.69	13.00 ± 2.86	10.50 ± 2.99
Plant feeders (%)	38.25 ± 9.63	18.75 ± 7.23	26.00 ± 0.71	22.50 ± 3.59
Omnivores (%)	5.25 ± 2.06	1.75 ± 0.63	6.00 ± 4.06	3.00 ± 0.71
Predators (%)	2.75 ± 1.11	4.25 ± 2.36	6.25 ± 2.87	4.00 ± 1.08
Plants species cover (%) [†]				
Festuca rubra	56.75 ± 4.50	50.50 ± 13.26	46.75 ± 7.73	39.75 ± 5.42
Silene uniflora	1.5 ± 0.64	0.5 ± 0.50	0.3 ± 1.78	5.0 ± 1.4719601
Agrostis stolonifera	5.00 ± 1.87	27.50 ± 15.16	16.00 ± 2.97	23.75 ± 4.66
Holcus lanatus	–	0.75 ± 0.75	0.25 ± 0.25	2.50 ± 1.89
Rumex acetosa	1.00 ± 1.00	1.00 ± 1.00	1.50 ± 0.64	1.00 ± 1.00
Potentilla erecta	0.25 ± 0.25	–	–	–

† Only species in more than 2 samples with 5% cover are shown