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Short communication

Soil nematode assemblage responds weakly to grazer exclusion on a nutrient-rich seabird island

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Abstract

The effects of aboveground herbivores on plant-soil interactions are highly context dependent and a key underlying factor controlling this is thought to be nutrient availability. Here, we tested whether the effects of vertebrate grazing on the soil food web varied with nutrient availability and hypothesised that soil food web structure would be driven more by the exclusion of vertebrate grazers than by nutrient enrichment. An 8-year long grazer exclusion experiment was performed in grasslands on a small Scottish island near soil nutrient-enriching seabird colonies at the coast and in less fertile conditions inland. We investigated the trophic structure of the soil nematode assemblage as a proxy for soil food web structure. Across all eight study sites the bacterial energy channel was predominant over the fungal

channel. Grazer exclusion strongly enhanced plant biomass accumulation and although this tended to be associated with a somewhat lower abundance of bacterial-feeders, this effect was non-significant and surprisingly weak given the observed changes aboveground. Indeed, plant species identity, diversity and dominance were, just as any other vegetation descriptor, weak predictors of nematode trophic structure. Instead, site specific conditions were important, despite the small island area and apparently homogenous sampling conditions.

Keywords

Soil food web; Nematode feeding groups; Grazing; Trophic structure; Aboveground-Belowground interaction; Exclosure

Introduction

Effects of aboveground primary consumers on the soil food web are context-dependent [1]. Herbivores may either promote plant species that produce recalcitrant litter and enhance the fungal energy channel [2], or benefit plants that produce easily decomposable detritus, thereby promoting the bacterial energy channel [3]. This may have important implications for ecosystem functioning, e.g. the response of soil processes to drought [4-5]. Comparison of studies across different ecosystems suggests that the direction of change in soil webs resulting from aboveground grazing may be depend on the fertility of the ecosystem [1]; this hypothesis, however, has rarely been tested.

Long-term exclosures have been used to investigate the effects of herbivores on plant-soil linkages in field conditions [6-7], and by sampling such systems repeatedly in time, both short and long term effects of herbivore removal can be studied. This could reveal trends and mechanisms that are otherwise difficult to detect, either early on in a short term experiment or later through comparing an advantage stage to a control. For instance, effects of a plant

species on soil can persist years after that species has been replaced, i.e. a plant legacy effect [8]. Herbivores can change the plant species composition of an ecosystem and therefore induce such legacies. Longer term experiments that focus on the interactions between herbivores, plants and soil in different environmental conditions are needed to progress from context-dependent generalisations to context-specific predictions.

Here we report on an herbivore exclusion study at sites with contrasting levels of nutrient enrichment to test whether nutrient availability regulates the effects of aboveground herbivory on the soil food web. In particular, we studied the effects of vertebrate herbivores and natural nutrient enrichment on the soil nematode trophic structure on the Isle of May, a small (1.5×0.5 km) seabird island in the Firth of Forth, eastern Scotland, UK ($56^{\circ}11'9''$ N, $2^{\circ}33'27''$ W). Because the coastal areas of the island receive greatest nutrient inputs from seabird colonies [9], inland and coastal sites were used to represent low and high levels of nutrient enrichment, respectively. Exclosures ($n=8$) were compared to neighbouring plots that were intensively grazed by rabbits (*Oryctolagus cuniculus*). The main hypothesis was that removal of grazers would override seabird nutrient enrichment in driving the soil food web structure given the profound effect of rabbits on the island's vegetation. We also expected the relative abundance of bacterial-feeders to be greater in grazed areas than in the exclosures, due to a combination of increased rhizodeposition and input of labile organic compounds through faeces and urine, and that such a difference would be weaker at coastal sites as a consequence of greater nutrient availability. While shorter term effects (i.e. over three years following grazing exclusion) of grazing exclusion on the nematode community have been previously reported [10], here we report longer term (eight years) effects. Furthermore, we investigated whether quantitative and qualitative changes in vegetation could explain patterns in nematode trophic structure.

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 enclosure 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 enclosure 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 $\text{pH}_{\text{H}_2\text{O}}$. 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 i th 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 i th 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

models. To explore differences in feeding group relative abundances across all sample locations, non-metric multidimensional scaling (NMDS) was performed on a matrix of Bray-Curtis dissimilarities based on feeding group proportions. PERMANOVA [14] was carried out on the Bray-Curtis matrix to determine the extent to which grazing, location and site explained variance in feeding group proportions. Co-Correspondence analysis [15] with plant species as predictors and nematode feeding groups as response variable was also performed. All statistical analyses were performed in R [16] (packages *vegan* [17], *cocorresp* [18] and *nlme* [19]). Model distributional assumptions and homogeneity were checked by plotting standardised residuals against fitted values and producing normal quantile-quantile plots. In the linear models Nematode Channel Ratio was transformed as $\log(x+1)$ to improve normality; residual variance was allowed to differ among levels of a factor when needed to improve homogeneity. Feeding group densities and plant species cover were log-transformed (as $\ln(x+1)$) to reduce skewedness for Co-Correspondence analysis. Data are expressed as mean \pm standard error.

Results and discussion

A summary of the biotic and abiotic data is provided in Table 1. We found that the fences had effectively excluded rabbits, as no droppings were found in exclosures. Vegetation height was dramatically affected by grazer exclusion ($F_{1,12} = 476.87$, $p < 0.0001$), being 14× taller inside the fences along with a 3× deeper litter layer than in the controls ($F_{1,12} = 116.45$, $p < 0.0001$). Grazing exclusion led to a less diverse and even grassland community at both coastal and inland sites: the Shannon-Wiener index was higher in the controls than in the exclosures (0.89 ± 0.08 vs. 0.43 ± 0.10 , $F_{1,12} = 11.07$, $p = 0.006$), while the reverse was found for the Berger-Parker index (0.43 ± 0.04 vs. 0.59 ± 0.04 , $F_{1,12} = 7.60$, $p = 0.02$).

Nematode density was not significantly affected by grazing or location (Table 1). Nematode Trophic Diversity was not affected by grazing, but was higher at inland ($2.88 \pm$

0.19) than coastal sites (2.27 ± 0.13 ; $F_{1,13} = 7.06$, $p = 0.02$). Nematode Channel Ratio appeared highest in grazed conditions (Exclosure: 0.76 ± 0.04 , Control: 0.82 ± 0.02), but this response was not significant ($F_{1,12} = 2.55$, $p = 0.13$), and there was no difference in this measure between coastal and inland sites ($F_{1,12} = 1.81$, $p = 0.20$). The hypothesis that differences in Nematode Channel Ratio between control and exclosures would be weaker at coastal than inland sites was not supported, as the interaction of grazing and location was also non-significant ($F_{1,12} = 0.04$, $p = 0.83$). The Nematode Channel Ratio was always greater than 0.5, pointing to a dominance of the bacterial energy channel across all grassland sites.

No clear differences in nematode trophic structure among treatments were detected by NMDS, although PERMANOVA indicated a different nematode trophic structure between coastal and inland samples ($F_{1,14} = 3.83$, $p = 0.03$), but not between grazed and exclosure samples ($F_{1,14} = 0.59$, $p = 0.61$). Co-Correspondence analysis confirmed that plant species did not explain patterns in the nematode assemblage (first axis 10.41% fit, $p = 0.58$, 9999 permutations), and neither Shannon-Wiener index nor Berger-Parker index had detectable effects on Trophic Diversity or Nematode Channel Ratio. Therefore, the trophic structure of the nematode community was little affected by differences in the vegetation aboveground, despite dramatic effects of grazer exclusion on vegetation height and litter layer depth (Table 1). Instead, between-site variation was important, with significant effects on nematode density ($F_{1,7} = 4.08$, $p = 0.03$), Trophic Diversity ($F_{1,7} = 5.70$, $p = 0.01$) and the Nematode Channel Ratio ($F_{1,7} = 32.22$, $p < 0.001$).

Overall, the hypothesis that the Nematode Channel Ratio would be higher in the grazed plots relative to those in exclosures was not supported by the data. This may reflect a weak below-ground effect of grazing, as also found by Wright et al. in the first three years of the experiment [10]. Although several studies have shown increases in bacterial-feeding nematodes under grazed conditions [3, 20], the opposite effect has also been reported [2, 21].

The lack of effect in our study, however, is surprising given the large effects of rabbits on plant growth (vegetation height) and biomass accumulation (litter layer depth). An explanation could be that our sample size ($n=8$ exclosures) was too small to overcome the spatial heterogeneity characterising nematode assemblages [22] and, in fact, site was the main factor that explained patterns in nematode trophic structure. The occurrence of such small-scale spatial variability was unexpected, because the island is relatively small, all study sites were ecological replicates in that they were all placed in one plant community, and all sample plots were grass-dominated. However, the absence of a grazer exclusion effect is more likely to be genuine than an artefact of our study design, as no such effect was detected even when controlling for between-site variability. It is also possible that grazing effects occurred in the litter layer (here not sampled) but failed to extend to the soil underneath.

Vegetation data did not significantly explain variance in nematode trophic structure. Plant identity has been shown to be an important determinant of the soil nematode community [23-24], but our sites were rather homogeneous in plant species composition. All samples were dominated by the same grass species (Table 1). Therefore, litter quality - a well-known driver of soil food web structure [1] - may not have differed much between treatments. Litter quantity, however, was considerably greater in non-grazed plots, but did not explain variance in nematode trophic structure in the soil below the litter layer. Shifts in species composition that could not be detected by the chosen level of identification might however have occurred.

Both univariate and multivariate analyses showed some differences in soil nematode trophic structure between coastal and inland sites. This might be due to differences in soil fertility induced by the seabirds, as the inland sites were not subject to the high levels of guano and ammonia-derived N deposition that characterised coastal sites [9]. Seabirds 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 \pm 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 \pm 1.61	65.25 \pm 8.85	54.70 \pm 6.48	40.37 \pm 8.72
pH	4.88 \pm 0.36	5.15 \pm 0.50	5.31 \pm 0.52	5.56 \pm 0.03
Salinity (dS/m)	3.35 \pm 0.15	3.72 \pm 0.18	3.65 \pm 0.62	3.25 \pm 0.26
Vegetation height (cm)	24.65 \pm 1.81	26.30 \pm 1.19	1.80 \pm 0.14	1.72 \pm 0.07
Litter depth (cm)	7.75 \pm 0.25	8.87 \pm 0.72	2.25 \pm 0.59	3.50 \pm 0.29
Rabbit droppings	–	–	21.82 \pm 3.99	12.72 \pm 1.84
Soil nematodes				
Density (ind./cm²)	519.38 \pm 151.04	748.46 \pm 150.53	670.68 \pm 196.80	752.26 \pm 207.14
Bacterial-feeders (%)	38.50 \pm 6.12	60.50 \pm 7.92	49.00 \pm 4.56	59.75 \pm 3.350
Fungal feeders (%)	15.25 \pm 4.33	15.00 \pm 5.69	13.00 \pm 2.86	10.50 \pm 2.99
Plant feeders (%)	38.25 \pm 9.63	18.75 \pm 7.23	26.00 \pm 0.71	22.50 \pm 3.59
Omnivores (%)	5.25 \pm 2.06	1.75 \pm 0.63	6.00 \pm 4.06	3.00 \pm 0.71
Predators (%)	2.75 \pm 1.11	4.25 \pm 2.36	6.25 \pm 2.87	4.00 \pm 1.08
Plants species cover (%) [†]				
<i>Festuca rubra</i>	56.75 \pm 4.50	50.50 \pm 13.26	46.75 \pm 7.73	39.75 \pm 5.42
<i>Silene uniflora</i>	1.5 \pm 0.64	0.5 \pm 0.50	0.3 \pm 1.78	5.0 \pm 1.4719601
<i>Agrostis stolonifera</i>	5.00 \pm 1.87	27.50 \pm 15.16	16.00 \pm 2.97	23.75 \pm 4.66
<i>Holcus lanatus</i>	–	0.75 \pm 0.75	0.25 \pm 0.25	2.50 \pm 1.89
<i>Rumex acetosa</i>	1.00 \pm 1.00	1.00 \pm 1.00	1.50 \pm 0.64	1.00 \pm 1.00
<i>Potentilla erecta</i>	0.25 \pm 0.25	–	–	–

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