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Plant diversity and identity effects on predatory nematodes and their prey

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Abstract

There is considerable evidence that both plant diversity and plant identity can influence the level of predation and predator abundance aboveground. However, how the level of predation in the soil and the abundance of predatory soil fauna are related to plant diversity and identity remains largely unknown. In a biodiversity field experiment we examined the effects of plant diversity and identity on the infectivity of entomopathogenic nematodes (EPNs, *Heterorhabditis* and *Steinernema* spp.), which prey on soil arthropods, and abundance of carnivorous non-EPNs, which are predators of other nematode groups. To obtain a comprehensive view of the potential prey/food availability we also quantified the abundance of soil insects and non-predatory nematodes, and the root biomass in the experimental plots. We used structural equation modelling (SEM) to investigate possible pathways by which plant diversity and identity may affect EPN infectivity and the abundance of carnivorous non-EPNs. *Heterorhabditis* spp. infectivity and the abundance of carnivorous non-EPNs were not directly related to plant diversity or the proportion of legumes, grasses and forbs in the plant community. However, *Steinernema* spp. infectivity was higher in monocultures of *Festuca rubra* and *Trifolium pratense* than in monocultures of the other six plant species. SEM revealed that legumes positively affected *Steinernema* infectivity whereas plant diversity indirectly affected the infectivity of *Heterorhabditis* EPNs via effects on the abundance of soil insects. The abundance of prey (soil insects and root-feeding, bacterivorous, fungivorous nematodes) increased with higher plant diversity. The abundance of prey nematodes was also positively affected by legumes. These plant community effects could not be explained by changes in root biomass. Our results show that plant diversity and identity effects on belowground biota (particularly soil nematode community) can differ between organisms that belong to the same feeding guild and that generalizations about plant diversity effects on soil organisms should be made with great caution.

47

48 **Key words**: Belowground, Biodiversity, Natural enemies, Plant diversity, Predation, Soil biota

49

Introduction

Biodiversity is rapidly declining worldwide, and many studies have shown that this can result in significant negative effects on ecosystem processes, including economically important ecosystem services such as control of pest insects (Cardinale *et al*. 2003; Brussaard 2012). Most studies investigating the effects of species loss on ecosystem services and processes have focused on the aboveground effects of plant species richness hereafter named 'plant diversity', and show that a decline in plant diversity negatively affects the abundance or diversity of predators and parasitoids of foliar feeding herbivores (Thies & Tscharntke 1999; Haddad *et al*. 2009; Scherber *et al*. 2010). However, how the level of predation in the soil and the abundance of predatory soil organisms are related to the diversity and identity of the plant community is less well understood and the few studies addressing this question have focussed on carabid assemblages, predatory nematodes and predatory macrofauna (Wardle *et al*. 2003; Harvey *et al*. 2008; Scherber *et al*. 2010).

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* (Rhabditida: Steinernematidae and Heterorhabditidae) are natural enemies of insects or other arthropods that live in the soil or close to the soil surface (Kaya & Gaugler 1993). EPNs are present in the soil of most terrestrial ecosystems and used in pest management programs worldwide. They spend part of their life cycle in soil as free-living non-feeding infective juveniles and the other part in insect bodies which they infect and kill. EPNs are sensitive to abiotic factors, such as temperature and moisture, and biotic factors such as competition and natural enemies (e.g. nematophagous fungi, Collembolans and mites) (Lewis *et al*. 2006). Studies that have estimated the effects of intercropping on the presence and infectivity of EPNs show that heterogeneous vegetation in agricultural systems can serve as a refuge for EPNs (Lawrence, Hoy & Grewal 2006; Jabbour & Barberchek 2008). How the infectivity and

75 natural occurrence of EPNs is related to the diversity or composition of natural plant
76 communities is less well known.
77
78 Carnivorous non-EP nematodes feed predominantly on other nematodes and have evolved
79 special features for ingesting nematode prey, such as root-feeding, bacterivorous, fungivorous
80 and omnivorous nematodes (Yeates *et al*. 1993). Previous studies on effects of plant diversity
81 on non-EPNs mainly focused on functional shifts in nematode composition and have reported
82 weak or non-existing effects of plant diversity on carnivorous non-EPNs (e.g. De Deyn *et al*.
83 2004a; Viketoft *et al*. 2009; Eisenhauer *et al*. 2011). However, the mechanisms of these weak
84 responses have remained largely unclear.
85
86 Root-feeding insects and nematodes use plant roots as a food source and can be directly
87 affected by changes in root diversity or biomass production (De Deyn, Raaijmakers & van der
88 Putten 2004b). Increases in root biomass can also indirectly enhance the abundance of
89 organisms that are part of the decomposer subsystem of the soil food web, such as
90 bacterivorous and fungivorous nematodes, via increased amounts of litter or root exudates
91 that serve as the basal resource for decomposition (Wardle *et al*. 2003). According to the
92 diversity-trophic structure hypothesis (Hutchinson 1959), such increases in the abundance of
93 soil organisms that inhabit lower trophic levels may then positively affect predatory soil
94 organisms, as their prey density increases. Alternatively, increases in plant diversity and
95 biomass production may affect the abundance of soil predatory organisms directly, for
96 example, by providing habitat or refuge in the case of abiotic extremes or competition
97 (Lawrence *et al*. 2006). Therefore, the relationship between plant diversity, biomass and
98 higher trophic levels comprises a complex network of direct and indirect links and it is not
99 known how the interactions in these multitrophic networks operate. Here we use structural

100 equation modelling (SEM) to examine plant diversity effects on belowground multitrophic
101 networks with a particular focus on EPNs and other carnivorous nematodes. SEM is a
102 multivariate method that can be used to examine how alternative pathways in networks with
103 direct and indirect relationships may contribute to the observed species responses to
104 experimental treatments (Grace 2006).
105
106 Several studies have argued that the effects of plant diversity on other organisms are not
107 directly due to the number of plant species per se, but rather due to the abundance of certain
108 plant species or functional groups in the plant community (e.g. Spehn *et al*. 2000; Gastine,
109 Scherer-Lorenzen & Leadly 2003; Wardle *et al*. 2003; De Deyn *et al*. 2004a; Viketoft *et al*.
110 2009). For example, densities of aboveground invertebrates, including predatory arthropods,
111 are often higher in plant communities that contain leguminous species, most likely because
112 the nutritional quality of plant tissues is often higher in communities that contain nitrogen-
113 fixing plant species (e.g. Koricheva *et al*. 2000; Haddad *et al*. 2009). Many studies that have
114 examined effects of plant identity on the abundance of carnivorous nematodes in grasslands
115 did not find significant effects (Wardle *et al*. 2003; De Deyn *et al*. 2004a; Viketoft *et al*.
116 2009). However, plant diversity effects can be mediated by the changes in abundance of the
117 lower trophic level nematodes and hence the abundances of non-EP carnivorous nematodes
118 and infectivity of EPNs in relation to the diversity and composition of the plant community
119 warrant the inclusion of prey abundance.
120
121 In this study, we use a grassland biodiversity experiment, in which the diversity of the plant
122 communities was manipulated and maintained, to examine the effects of plant diversity and
123 identity on the infectivity of EPNs and abundance of carnivorous non-EPNs. To estimate the
124 potential prey or food availability for EPNs and carnivorous non-EPNs we also determined

125 root biomass, the number of root-feeding, fungivorous, bacterivorous and omnivorous
126 nematodes and root-feeding insects in soil samples. We hypothesized that (i) increased plant
127 diversity will enhance EPN infectivity, the abundance of carnivorous non-EPN and prey
128 nematodes, abundance of soil insects, and root biomass and that (ii) plant functional groups
129 and (iii) plant species in monocultures will strongly differ in their effect on the densities of
130 belowground organisms. In particular, we predict that the abundances of soil organisms will
131 be positively related to the cover of legumes in the plant community. Finally, we examined
132 whether the relationship between plant diversity, identity and predation in the soil could be
133 explained by changes in root biomass and/or prey abundances.
134
135 **Materials and methods**
136
137 *Field site*
138 A detailed description of the design of the field experiment has been presented elsewhere
139 (Kostenko *et al*. 2012). In brief, in 2008, 70 experimental plots of 3 × 3 m separated by 1-m-
140 wide lanes were set-up in a nature restoration grassland area that had been agricultural land
141 until 1996 (de Mossel, Ede, The Netherlands). The experimental area was fenced to exclude
142 large vertebrate herbivores. The plots were sown with 1, 2, 4, or 9 plant species drawn from a
143 pool of 12 grassland species including three grasses (*Anthoxanthum odoratum* L., *Agrostis*
144 *capillaris* L., and *Festuca rubra* L.), three legumes (*Lotus corniculatus* L., *Trifolium arvense*
145 L., and *Trifolium repens* L.), and six forbs (*Achillea millefolium* L., *Hypochaeris radicata* L.,
146 *Leucanthemum vulgare* Lamk., *Tanacetum vulgare* L., *Tripleurospermum maritimum* L.
147 W.D.J. Koch and *Plantago lanceolata* L.). Each diversity level was replicated with several
148 different mixtures in order to avoid confounding effects of species identity and diversity. Each
149 of the sown plant species mixtures and monocultures was replicated twice using a complete

150 randomized design. At the moment of sampling there were 16 monocultures, 18 plots with
151 two species, 22 plots with four, 6 plots with nine species, and 4 plots were kept free of all
152 vegetation and served as 'bare soil' treatment. Four remaining plots were excluded from the
153 experiment due to poor establishment. There were no monocultures of *A. odoratum*, *A.*
154 *capillaris*, *T. arvense* and *T. maritimum*, but these species were present in the mixtures.
155 Experimental plots were not mown, but hand-weeded during the growing season in 2009 and
156 2010 (from the end April until end August) to maintain the sown species composition. All soil
157 samples were collected in September 2010.
158
159 *Infection bioassay*
160 To assess the EPN infectivity in the experimental plots we used a standard laboratory
161 *Galleria*-bait method (Bedding & Akhurst 1975). Soil for the essay was collected from each
162 experimental plot by taking twenty five soil cores of 15 cm depth and 5 cm diameter from the
163 inner 2.5 × 2.5 m square in a regular 0.5 × 0.5 m grid. The samples were pooled per plot.
164 Plastic containers (10 × 10 × 5 cm) were filled with 250 g soil from each plot. The soil was
165 adjusted to field capacity (15%) by adding de-mineralized water. There were four containers
166 per plot. Into each container, four final instar *G. mellonella* larvae were placed on the soil
167 surface, the containers were closed and flipped over so that the larvae were covered by soil.
168 The insect larvae were obtained from Kreca V. O. F. (Ermelo, The Netherlands). The
169 containers were kept in a dark climate chamber under controlled conditions at 22 ^{o}C, 50-60%
170 humidity. After one week, all the larvae were retrieved from the soil and incubated
171 individually in the labelled plastic vials (3 cm diameter, 5 cm height) in the climate chamber.
172 Seven days later, all larvae were dissected and examined using a stereo microscope in order to
173 assess infection by *Heterorhabditis* or *Steinernema* EPNs. Assessments were based on the
174 colour of the cadaver and the morphology of adult nematodes found in the dissected larvae

(Stock & Hunt 2005). Because EPNs typically kill their hosts within 48 h (Kaya & Gaugler 1993), the two weeks scoring period virtually assured that we observed all nematode-imposed mortality. All EPN-infected larvae were dead before the dissection. We also recorded whether larvae died from fungal or bacterial infection. We grouped these larvae together as larvae that died from other causes.

Soil nematode extraction and identification

The soil for assessing the nematode community size and composition was a 100 ml subsample from the pooled soil collected for EPN infectivity bioassay. Soil moisture content was determined on another soil subsample of each plot by drying 50 g of fresh soil for three days at 120 ^{o}C. Nematodes were extracted from 100 ml fresh soil using Oostenbrink elutriators (Oostenbrink 1960, see Appendix A for details). Nematode densities were calculated per 100 g dry weight soil. Nematodes were categorized into feeding guilds according to Yeates *et al*. (1993), Andrassy (2005) and personal communication with a specialist in nematode taxonomy and biology (Prof Tom Bongers; Table S1, Appendix A). We considered nematodes as being carnivorous if there is evidence in literature that they consume other nematodes, although some of the listed carnivores might also feed on other organisms, e.g. bacteria (see Table S1 for details).

Root biomass

To determine community standing root biomass, three soil cores of 10 cm depth and 2.5 cm diameter were taken 1 m apart along a diagonal transect within each plot that started 50 cm from the edge of the plot. In the laboratory, the weight of the soil in each core was determined, and all root material was washed, oven-dried at 70 ^{o}C and weighed. Total root biomass was calculated as root dry weight per 100 g dry soil.

200

201 *Soil insects*

202 To estimate the abundance of soil-dwelling insects, four soil cores of 12.5 cm diameter and 15
203 cm deep were collected from four randomly selected locations within the inner 2.5 m × 2.5 m
204 square of each plot. In the laboratory, each soil sample was weighed and then hand-sorted. All
205 visible arthropods were collected and stored in 70% ethanol in labelled Eppendorf tubes. The
206 arthropods were categorized as white grub larvae (scarab beetle larvae), wireworms
207 (*Elateridae* beetle larvae), other insect larvae (*Lepidoptera*, *Diptera*, and other *Coleoptera*)
208 and adult beetles (*Coleoptera*). The abundance of soil insects was expressed per 100 g dry
209 weight soil.

210

211 *Statistical analyses*

212 All univariate analyses were performed using R statistical language, version 2.15.1 (R
213 Development Core Team 2012). Percentage data were arcsine square root-transformed;
214 biomass and prey nematode data were log-transformed; insect and carnivorous nematodes
215 data were square-root transformed to meet the requirements of normality and
216 homoscedasticity of errors. If the assumptions were still violated, non-parametric tests were
217 used to analyse the data (for these analyses χ^2 are reported). Because there were four
218 containers per plot, the effects of plant diversity, monoculture identity and proportion of
219 legumes, grasses or forbs in the vegetation on %EPN infectivity were analysed using linear
220 mixed models with plot identity as random factor. General linear models were used to test the
221 effects of plant diversity, monoculture identity and proportion of legumes, grasses or other
222 forbs in the vegetation on nematode and insect abundances, root biomass and soil moisture
223 content. Plant diversity was included as continuous variable to test for linear effects. We also
224 repeated the analyses by excluding the bare plots. Individual comparisons between

monocultures were based on a Tukey HSD test. Due to the low number of insects recovered from monocultures, the effects of monoculture identity on the soil insect abundance were not tested. To determine whether there was a relationship between prey nematode community composition and plant diversity we used multivariate principal component (PCA) and redundancy analysis (RDA) in CANOCO version 5.03 (Šmilauer & Lepš 2014).

Structural equation modelling

We tested three alternative pathways linking plant diversity and identity to EPN infectivity or predatory nematode abundance via changes in prey abundance (A, Fig. 1); via changes in root biomass (B, Fig. 1); and via changes in root biomass that subsequently controls prey abundance (C, Fig. 1). Separate models were developed for *Heterorhabditis* infectivity, *Steinernema* infectivity, and carnivorous non-EPN abundance. For EPN models, we included soil insects as prey; and for non-EPNs model, we included the total of root-feeding, bacterivorous and fungivorous nematodes as prey. Omnivorous nematodes were not included in the model as they also can feed on other food sources, such as bacteria or fungi. All plots were used in the analysis and data were transformed in the same way as for univariate analysis. The likelihood ratios and chi-squared tests were used to determine if the model-implied variance-covariance matrix differed from the observed variance-covariance matrix and to perform model simplification. The non-significant terms were removed from the initial model and the model that best fitted our data was selected. This model was used to determine which of the proposed hypothesis best explained the relationship between plant diversity and identity and EPN infectivity or carnivorous non-EPN abundance (see Appendix B for more details). SEM was performed using ‘sem’ package for R.

Results

Predator responses

Average total mortality of *Galleria* larvae in the bioassay was 78%, of which 21% were infected by *Heterorhabditis* and 12% by *Steinernema* while the other 43% died of other causes. Neither plant diversity nor the proportion of plant functional groups in the mixtures significantly affected infectivity by *Heterorhabditis* spp. (Table 1). However, the *Heterorhabditis* infectivity was on average three times lower in the bare compare to vegetated plots (0.11 ± 0.03% and 0.27 ± 0.03% respectively, Fig. 2). Infectivity of *Heterorhabditis* spp. did not differ among monocultures ($F_{7,8} = 0.31$, $P = 0.93$). There was no significant effect of plant diversity on the infectivity of *Steinernema* spp. (Table 1). However, the *Steinernema* infectivity was lower in plots where forbs were abundant; this effect was significant only when bare plots were excluded from the analysis (Table 1). The infectivity by *Steinernema* spp. varied significantly among monocultures ($F_{7,8} = 3.67$, $P = 0.044$; Fig. 2) and was highest in the monocultures of *F. rubra* and *T. repens*. The percentage of the larvae that died due to other causes was not affected by plant diversity or by the plant functional groups (Table 1) and did not differ among monocultures ($F_{7,8} = 1.27$, $P = 0.37$).

The abundance of carnivorous non-EPNs was not significantly affected by plant diversity or by the plant functional groups (Table 1), and did not differ among monocultures ($\chi^2_7 = 3.09$, $P = 0.88$). Nematodes of the family *Mononchidae*, and of the genera *Aporcelaimus* and *Dorylaimoides* were the most dominant carnivorous non-EPNs in our study (Table S1). The abundance of *Mononchidae* was highest in bare plots (236 ± 57 nematodes per 100 g soil) and lowest in nine species plots (89 ± 23 nematodes per 100 g soil), however, there was no significant effect of plant diversity on the *Mononchidae* abundance ($F_{1,64} = 0.39$, $P = 0.53$, Fig. 3). The abundance of *Aporcelaimus* was not affected by increase in plant diversity ($F_{1,64} = 1.02$, $P = 0.32$, Fig. 3), whereas *Dorylaimoides* nematode abundance increased with

increasing plant diversity ($F_{1,64} = 4.04$, $P = 0.048$, Fig. 3). Carnivorous nematodes of the genera *Nygolaimus*, *Paraxonchium* and *Sectonema* were not found in the bare plots (data not shown).

Prey responses

The abundance of all non-carnivorous non-EP nematodes increased significantly with plant diversity but the effect became non-significant when the bare plots were excluded from the analysis (Table 1, Fig. 4). The community composition of prey nematodes was also significantly related to plant diversity (RDA: $F = 2.5$, $P = 0.002$, Fig. 5). There was a positive relationship between the proportion of legumes in a plant community and abundance of root-feeding, bacterivorous and fungivorous nematodes. This was also true when bare plots were not included in the analysis (Table 1). The proportion of grasses negatively affected fungivorous nematode abundance but stimulated the abundance of root-feeding nematodes (Table 1). The abundance of root-feeding nematodes, however, decreased with increasing proportion of forbs (Table 1). Abundances of root-feeding ($\chi^2_7 = 10.68$, $P = 0.15$), bacterivorous ($\chi^2_7 = 10.50$, $P = 0.16$), fungivorous ($\chi^2_7 = 10.50$, $P = 0.16$) and omnivorous ($\chi^2_7 = 5.91$, $P = 0.55$) nematodes did not differ between the monocultures. The majority of root-feeding insects that were recovered from the soil were white grubs. No insects were recovered from the soil collected from bare plots (Fig. 4). There was a positive relationship between soil insect abundance and plant diversity when bare plots were included in the analysis (Table 1, Fig. 4). This relationship was marginally significant when bare plots were excluded from the model ($P = 0.06$). The density of soil insects was not affected by any of the three plant functional groups in the plant community (Table 1).

Plant community characteristics

There was no significant relationship between plant diversity and root biomass (Table 1, Fig. S1A). However, root biomass positively correlated with the proportion of grasses in the community (Table 1). Root biomass differed significantly between monocultures ($F_{7,8} = 5.48$, $P = 0.014$; Fig. S1B) and was highest in monocultures of *H. radicata* and *P. lanceolata*. Soil moisture content was not related to the diversity or identity of the plant community (all $P > 0.05$, Fig. S1, Appendix C).

Structural equation modelling

In the final SEM for *Heterorhabditis* spp. ($\chi^2_9 = 2.69$, $P = 0.98$), 11.5% of the variation in percentage EPN infectivity could be explained by plant diversity and soil insect abundances (Fig. 6A), which corresponds to hypothetical pathway A in Fig.1. For *Steinernema* spp. ($\chi^2_9 = 8.09$, $P = 0.53$), there was a significant pathway between the percentage of EPN infectivity and the proportion of legumes in the community (Fig. 6B). The pathway between plant diversity and soil insect abundance was also significant in this model ($P = 0.014$) and explained 8.6% of the variation in the soil insect abundance. The final SEM for carnivorous non-EPNs ($\chi^2_3 = 1.60$, $P = 0.66$) did not reveal a significant pathway associated with their abundance (Fig. 6C). There was a direct significant link between the abundance of non-carnivorous nematodes and plant diversity ($P = 0.0014$) and the proportion of legumes in the community ($P > 0.001$; Fig. 6C) that explained 26.7% of the variation in their abundance. In all models, there was no significant pathway between predators and root biomass thereby rejecting the hypothetical pathways B and C (Fig. 1). Root biomass was significantly associated with the proportion of plant functional groups in the community (Fig. 6A, B, C).

Discussion

In our study, plant diversity positively affected the abundance of soil insects and nematode prey. However, the functioning (infectivity) of EPN spp. and the abundance of carnivorous non-EPNs were not directly affected by plant diversity. Interestingly, although there was no direct effect of plant diversity on the infectivity of EPN spp. in our study, the structural equation modelling revealed a significant indirect effect of plant diversity on *Heterorhabditis* infectivity via changes in the abundance of soil insects. These effects of plant diversity on *Heterorhabditis* EPNs are in line with pathway A (Fig. 1) and the diversity-trophic structure hypothesis, which states that a greater number of resources support a greater number of consumers (Hutchinson 1959). Plant diversity, neither directly nor indirectly, affected the abundance of carnivorous non-EPNs and infectivity by *Steinernema*, suggesting that plant diversity effects might be genus-, or even species-specific and that generalizations about diversity effects on soil organisms should be made with great caution.

The effect of plant identity was not consistent among and between the two genera of EPNs and the carnivorous nematodes. The abundance of carnivorous nematodes was not affected by the presence of particular functional groups although the abundance of their prey (root-feeding, bacterivorous and fungivorous nematodes) was positively influenced by the proportion of legumes in the community. SEM also revealed a positive effect of legumes on the abundance of prey of the carnivorous nematodes. The positive effect of legumes might be explained by higher tissue nitrogen contents of plant roots or litter in presence of legumes that can lead to increased performance of root feeders and decomposers. Surprisingly, we did not observe an overall positive effect of legumes on the abundance of soil insects. This may be explained by the fact that root exudates of a large number of legumes contain isoflavonoids, which deter belowground insect larvae (Dakora 2003). It is important to note that the number of soil insects retrieved from the field plots in our study was low. *Steinernema* spp. infectivity

was relatively high in the monocultures of the leguminous species *T. repens*, and according to SEM *Steinernema* infectivity was positively affected by the presence of legumes. Increases in the abundance of predators in the soil can potentially lead to increased predation rates and as a result lower prey abundance (Siemann 1998; Preisser 2003). This suggests that potentially EPNs (in particular *Steinernema* species) could have reduced population densities of soil insects in legume plots. The infectivity of *Steinernema* spp. was also relatively high in the two monocultures of the grass species *F. rubra.* This might be explained by large amounts of fine roots produced by grass species altering soil structure and microclimate (but not soil moisture content) that potentially serves as beneficial habitat for EPNs (Lawrence *et al*. 2006). In our study, we could not discriminate between functional group and species identity effects for grasses as only the monoculture of *F. rubra* was included. Interestingly, no infection of wax moth larvae by *Steinernema* occurred in the monocultures of *A. millefolium*, whereas other study have shown that *A. millefolium* has a positive effect on free-living nematodes (Viketoft *et al*. 2005). For *Heterorhabditis* spp. infectivity we did not observe any significant effects of plant identity. As our results differ from those obtained in other studies (e.g. De Deyn *et al*. 2004a; Viketoft *et al.* 2005; Viketoft *et al.* 2009), it appears that site-specific differences such as pool of plant species, nematode species present and the history of the site are important for soil predatory invertebrates.

The infection rates of wax moth larvae by *Heterorhabditis* spp. were higher than by *Steinernema* spp. but in general the infection rates for both genera were low. Although EPNs are widely distributed in soils of all sorts of ecosystems, there is considerable variability in EPN distribution across seasons and habitats (Stuart & Gaugler 1994; Spiridonov, Moens & Wilson 2007). the low infectivity and inconsistent results for the two EPN genera in our study may be the result of differences in local densities and patchy distributions of EPN populations

374 (e.g. Lawrence *et al*. 2006; Spiridonov *et al*. 2007). Alternatively, the different responses of
375 EPNs could be due to local differences in abiotic conditions or prey availability in the field.
376 Soil moisture is one of the most important abiotic parameters for EPN survival (Lawrence *et*
377 *al*. 2006). In our study, there was no difference in the soil moisture content between different
378 plots and we cannot attribute the variation in the EPN abundances to variation in soil moisture
379 unless that operated at finer spatial and temporal scales than we could measure. The majority
380 of insect prey found in our study was scarab beetle larvae that are feeding on plant roots and
381 typical hosts of EPNs that are dispersed in deeper soil layers, such as *Heterorhabditis.*
382 Therefore, the difference in host availability and life histories between the two EPN genera
383 might explain differences in EPN responses in our study with *Heterorhabditis* responding
384 more strongly to general insect host abundance than *Steinernema*.
385
386 In contrast to our hypothesis and in line with several other studies (e.g. Spehn *et al*. 2000;
387 Gastine *et al*. 2003), root biomass was not affected by plant diversity at the time scale of our
388 experiment, while aboveground biomass increased with increasing plant diversity (Kostenko
389 *et al*. unpublished data). Correspondingly, the SEM also did not reveal a significant
390 relationship between abundance of nematodes and soil insects and root biomass. In contrast,
391 in aboveground communities the effects of plant diversity on consumer diversity and
392 abundance occur primarily via changes in plant biomass (Koricheva *et al*. 2001; Borer,
393 Seabloom & Tilman 2012). One possible explanation for this discrepancy with the
394 aboveground system is that soil organisms are generally not restricted by the quantity of
395 primary resources and that belowground plant diversity effects are generally not mediated
396 through root biomass (e.g. Bezemer *et al*. 2010). It is important to note that to maintain the
397 initial plant species composition the experimental communities were regularly hand-weeded.
398 It is almost inevitable that part of the roots of the removed plants remained in the soil, even

399 though the aboveground parts of these plants were removed entirely. This can also explain
400 why there was some root biomass present in the bare plots in our experiment. Therefore,
401 hand-weeding could cause perturbations in belowground systems that obscure the 'pure
402 effect' of plant biomass in synthetic biodiversity experiments (Bezemer & van der Putten
403 2007; Roscher *et al*. 2013). This will be the case in both seed addition and plant removal
404 experiments.
405
406 EPNs and predatory nematodes are broadly used in biological control programmes to suppress
407 pests of agricultural crops in soil and enhance crop yields (Peters 1996; Denno, Gruner &
408 Kaplan 2008). In our study, where plant communities were manually manipulated we could
409 not estimate the effect of predation on plant survival and productivity but our findings suggest
410 that increasing plant diversity will have an indirect positive effect on EPN infectivity (in
411 particular *Heterorhabditis* spp.). Studies in which the abundance of EPNs or other nematodes
412 was manipulated experimentally have demonstrated that increased levels of predation can
413 have a strong positive impact on plant survival, productivity and diversity (van der Putten &
414 van der Stoel 1998; Preisser 2003; Khan & Kim 2007). It should also be emphasized that
415 carnivorous non-EPN and EPNs are only a part of the predaceous soil fauna. Other important
416 groups of soil predators not estimated in our study (e.g. microarthropods, protists), can also be
417 affected directly or indirectly by plant diversity and identity. Ultimately, understanding the
418 relationships between plant diversity, plant community composition and natural populations
419 of predatory organisms in the soil may provide new insights in the functioning of soil
420 communities and their use as biological control agents in managed and natural systems.
421
422 In conclusion, our study shows that abundance of (non-EP) carnivorous nematodes is not
423 influenced by the diversity or identity of the community, although their prey is affected by

both characteristics of the plant community. However, increasing plant species diversity enhances the level of predation by *Heterorhabditis* EPNs in the soil but only indirectly by affecting the abundance of their prey. In contrast, the level of predation by *Steinernema* EPNs is not affected by an increase in prey abundance but is directly influenced by the composition/identity of the plant community. Thus, the responses of belowground organisms to manipulation in plant diversity and identity can be specific and may differ even between organisms that belong to different species but the same feeding guild, such as EPNs of the genera *Steinernema* and *Heterorhabditis*.

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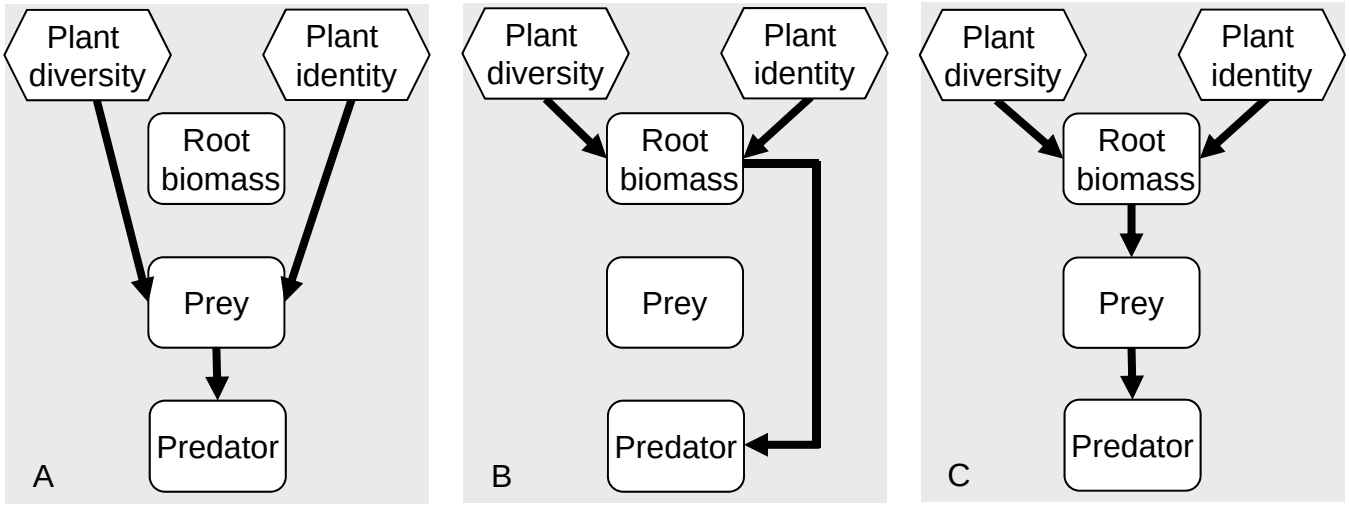
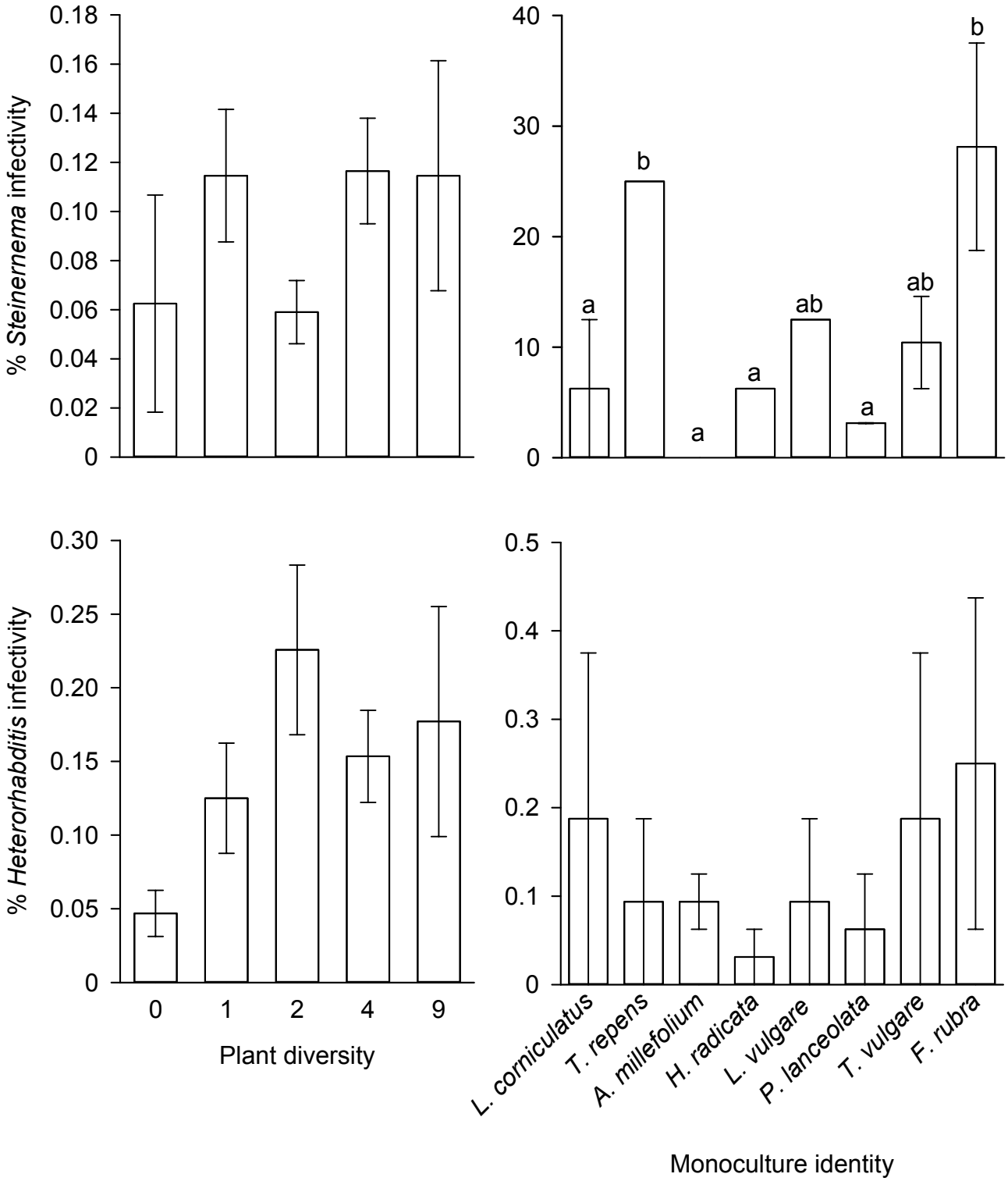
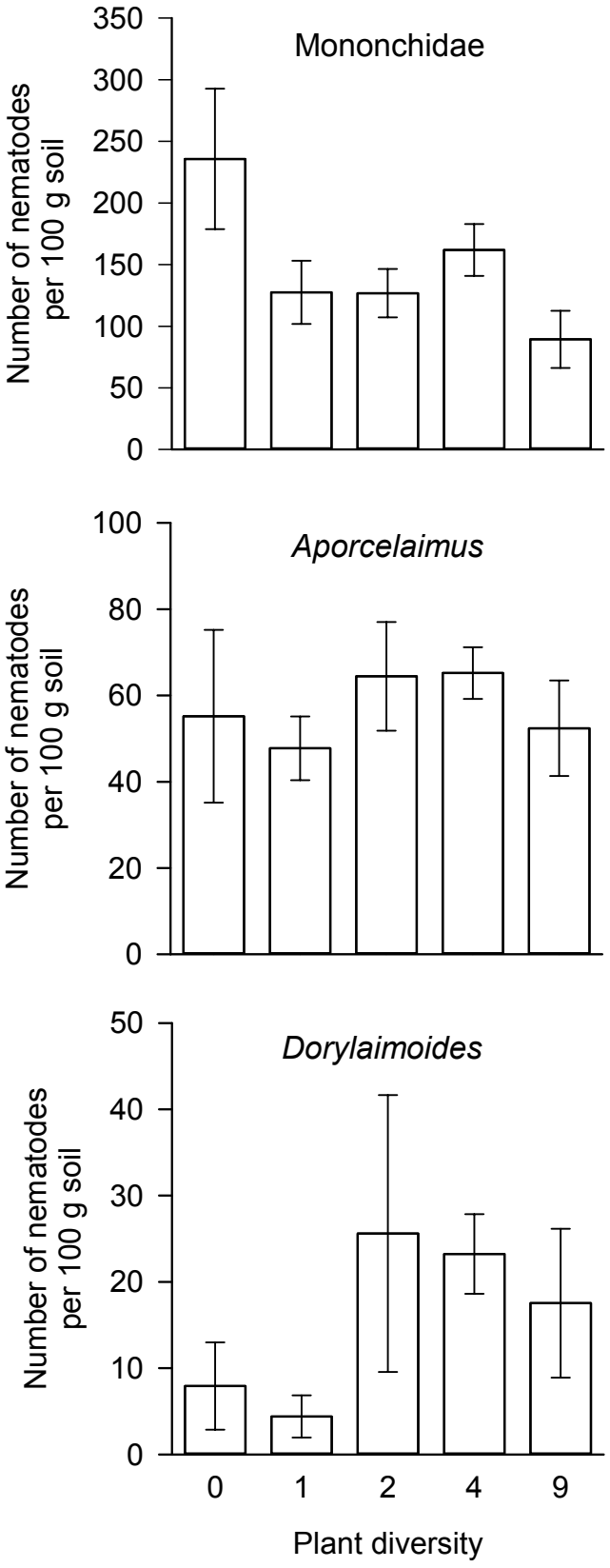
559 **Table 1.** Effects of plant diversity, proportion of legumes, grasses and forbs on the infectivity
560 of entomopathogens, abundance of other nematodes, soil insect abundance and community
561 root biomass. F-values are shown of linear mixed models for infectivity of EPNs and other
562 mortality causes and general linear models for other response variables. The respective
563 explanatory variable in those models was fitted first. Asterisks indicate significant effect at
564 *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; the brackets indicate marginally significant effect at P
565 < 0.06; the absence of asterisks indicates no significant effect. ↑ indicates positive effect and ↓
566 indicates negative effect.

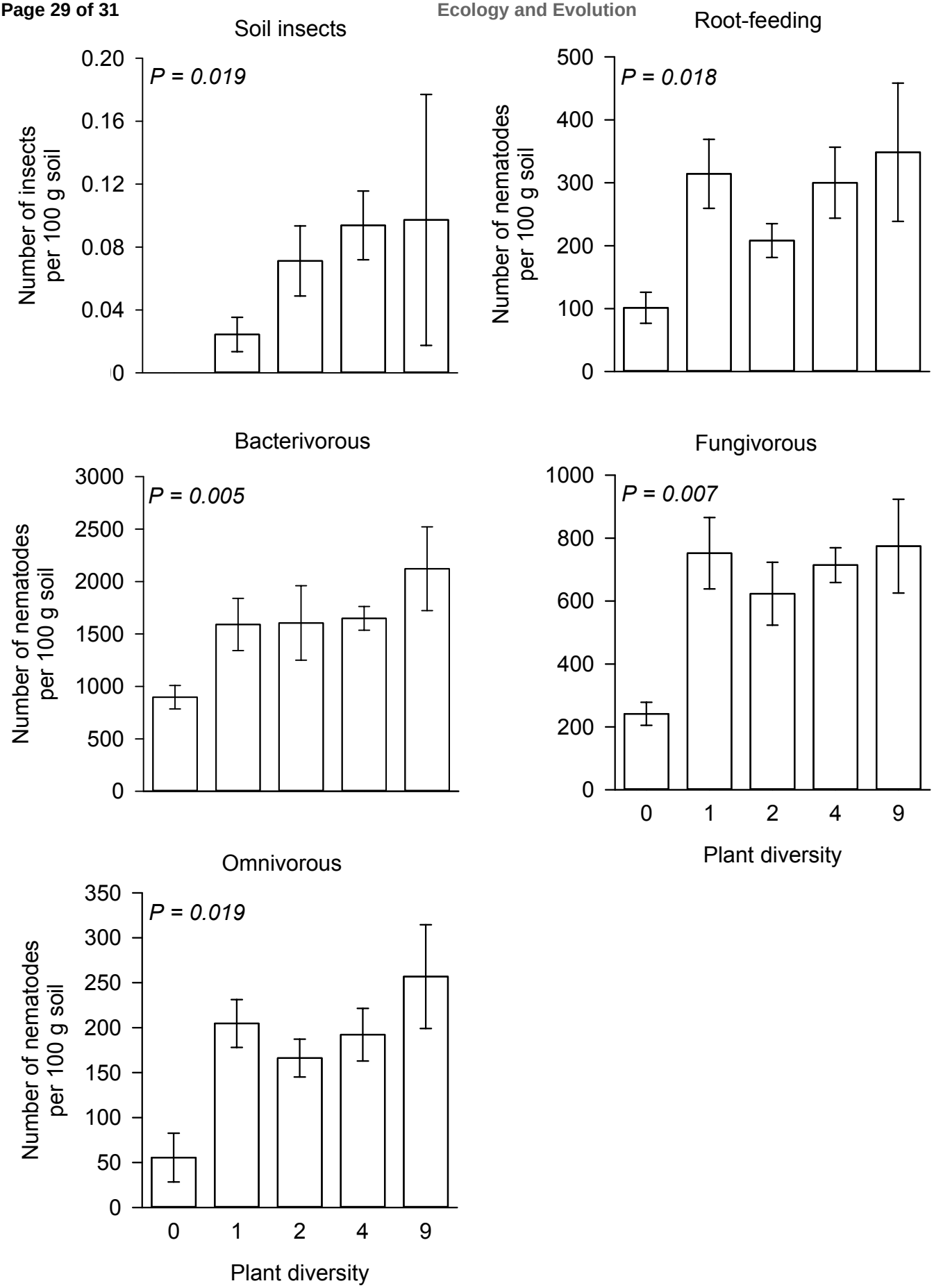
	Plant diversity	Legumes	Grasses	Forbs
Bare plots included				
Predator responses				
Heterorhabditis infectivity	1.15	0.003	1.85	0.09
Steinernema infectivity	1.25	3.26	1.32	3.27
Other mortality	0.46	0.22	0.19	0.013
Carnivorous nematodes	0.08	0.44	0.24	2.56
Prey responses				
Root-feeding nematodes	**↑5.95***	**↑16.18*****	**↑5.81***	**9.56**↓**
Bacterivorous nematodes	**↑8.68****	**↑9.30****	1.90	0.001
Fungivorous nematodes	**↑7.81****	**↑9.34****	**4.59*↓**	2.38
Omnivorous nematodes	**↑5.81***	1.88	0.95	2.32
Insect abundance	**↑5.83***	0.67	1.73	0.14
Community root biomass	1.74	0.0004	**↑4.51***	0.0003
Bare plots not included				
Predator responses				
Heterorhabditis infectivity	0.16	0.051	1.32	0.58
Steinernema infectivity	0.60	2.90	1.08	**4.88*↓**
Other mortality	0.01	0.10	0.08	0.23
Carnivorous nematodes	1.32	0.71	0.46	1.59
Prey responses				
Root-feeding nematodes	0.69	**↑13.34*****	**↑4.00***	**20.18***↓**
Bacterivorous nematodes	**↑(3.84)***	**↑7.58****	2.97	0.64
Fungivorous nematodes	0.52	**↑6.16***	**↑7.06***	0.06
Omnivorous nematodes	0.46	0.88	2.19	0.19
Insect abundance	**↑(3.62)***	0.98	1.21	0.01
Community root biomass	0.22	0.19	2.81	1.13

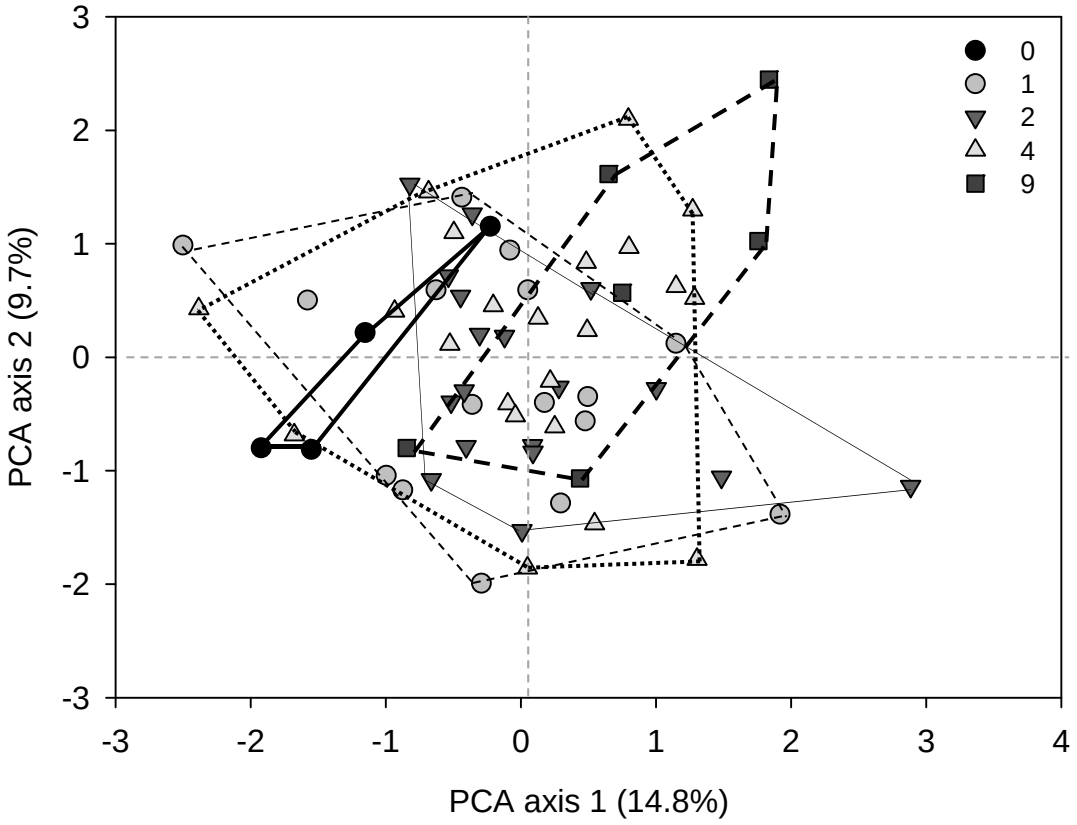
567

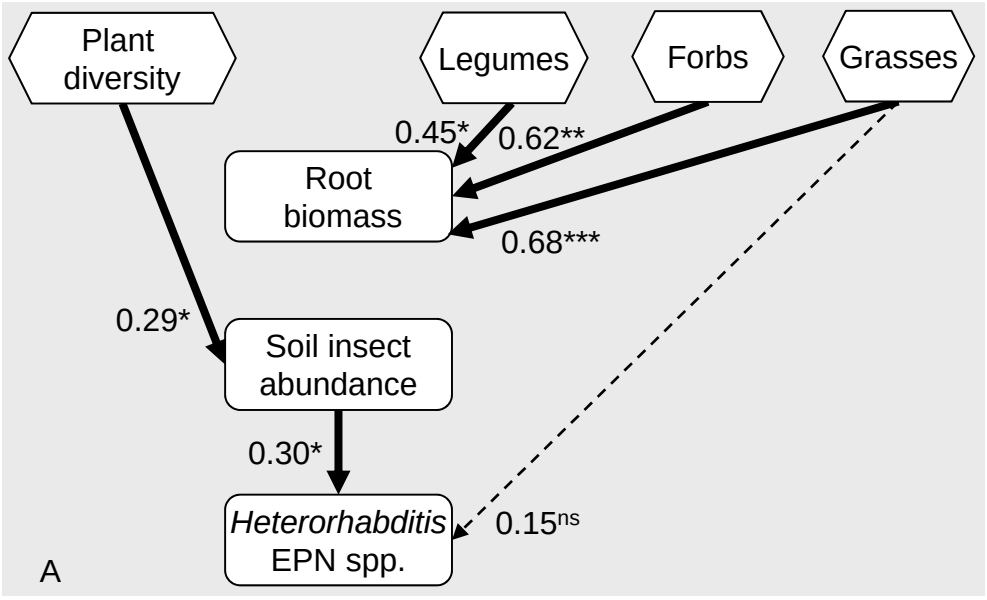

Plant diversity
Plant identity
Root biomass
Prey
Predator
A
Plant diversity
Plant identity
Root biomass
Prey
Predator
B
Plant diversity
Plant identity
Root biomass
Prey
Predator
C

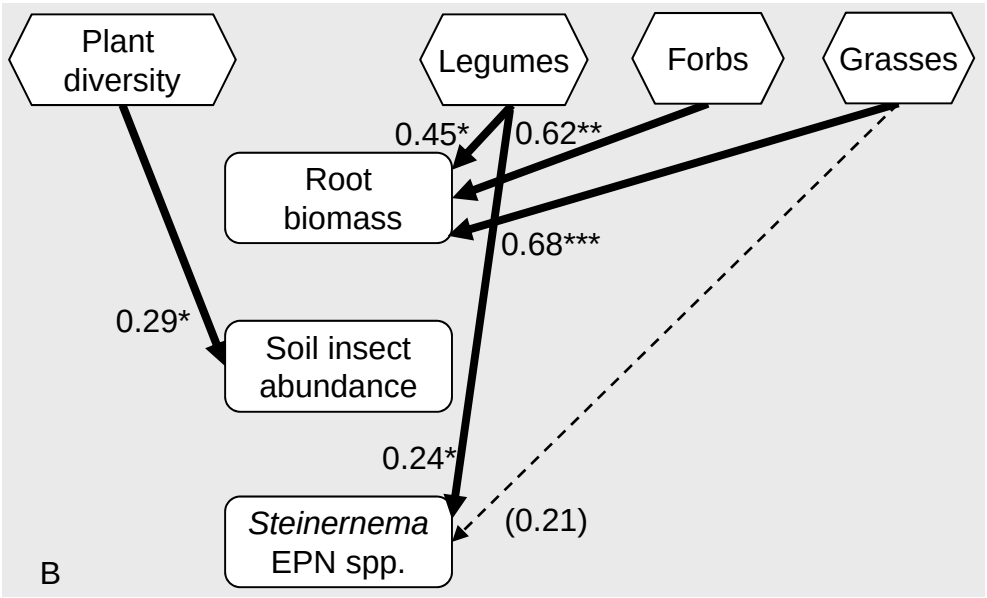

% Steinernema infectivity
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
40
30
20
10
0
a
b
a
a
ab
a
ab
b
% Heterorhabditis infectivity
0.30
0.25
0.20
0.15
0.10
0.05
0
0.5
0.4
0.3
0.2
0.1
0
0 1 2 4 9
Plant diversity
L. corniculatus
T. repens
A. millefolium
H. radicata
L. vulgare
P. lanceolata
T. vulgare
F. rubra
Monoculture identity

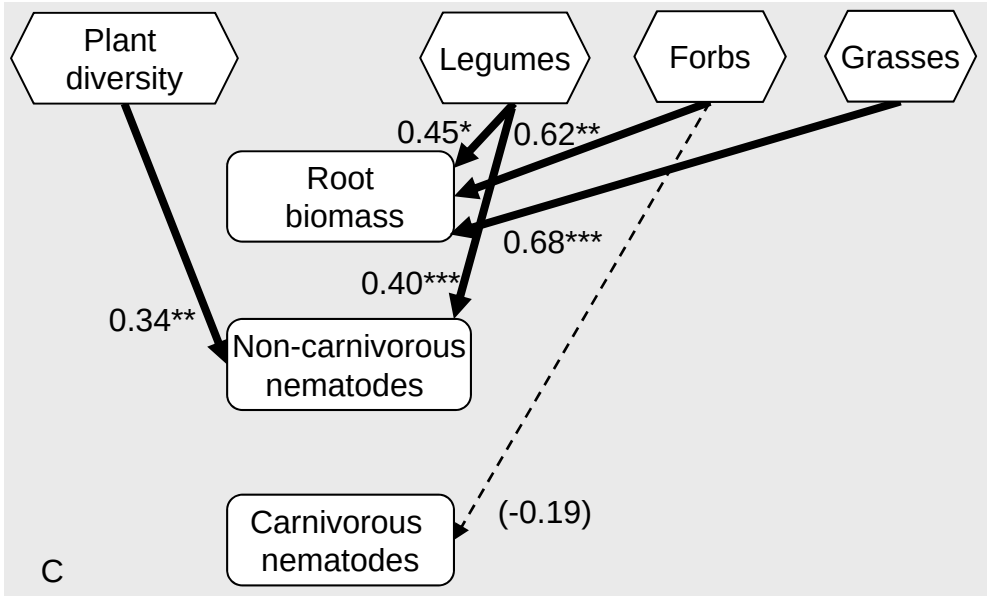

Mononchidae
Number of nematodes per 100 g soil
350
300
250
200
150
100
50
0
Aporcelaimus
Number of nematodes per 100 g soil
100
80
60
40
20
0
Dorylaimoides
Number of nematodes per 100 g soil
50
40
30
20
10
0
0
1
2
4
9
Plant diversity


Soil insects
P = 0.019
Number of insects per 100 g soil
0.20
0.16
0.12
0.08
0.04
0
Root-feeding
P = 0.018
Number of nematodes per 100 g soil
500
400
300
200
100
0
Bacterivorous
P = 0.005
Number of nematodes per 100 g soil
3000
2500
2000
1500
1000
500
0
Fungivorous
P = 0.007
1000
800
600
400
200
0
0
1
2
4
9
Plant diversity
Omnivorous
P = 0.019
Number of nematodes per 100 g soil
350
300
250
200
150
100
50
0
0
1
2
4
9
Plant diversity


0
1
2
4
9
PCA axis 2 (9.7%)
PCA axis 1 (14.8%)
3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2
3
4


Plant diversity
Legumes
Forbs
Grasses
0.45*
0.62**
Root biomass
0.68***
0.29*
Soil insect abundance
0.30*
Heterorhabditis EPN spp.
0.15ns
A


Plant diversity
Legumes
Forbs
Grasses
0.45*
0.62**
Root biomass
0.68***
0.29*
Soil insect abundance
0.24*
Steinernema EPN spp.
(0.21)
B


Plant diversity
Legumes
Forbs
Grasses
0.45*
0.62**
Root biomass
0.68***
0.40***
0.34**
Non-carnivorous nematodes
Carnivorous nematodes
(-0.19)
C