esa

ECOSPHERE

Possible mechanisms underlying abundance and diversity responses of nematode communities to plant diversity

Roeland Cortois,[1] G. F. (Ciska) Veen,[1] Henk Duyts,[1] Maike Abbas,[2] Tanja Strecker,[3] Olga Kostenko,[1] Nico Eisenhauer,[4,5] Stefan Scheu,[3] Gerd Gleixner,[6] Gerlinde B. De Deyn,[7] and Wim H. van der Putten[1,8,†]

[1]*Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50, 6700 AB Wageningen, The Netherlands*
[2]*AG Planktologie, Institut für Chemie und Biologie des Meeres, Carl von Ossietzky Universität Oldenburg, Schleusenstraße 1, 26382 Wilhelmshaven, Germany*
[3]*JFB Institute of Zoology and Anthropology, University of Göttingen, Berliner Straße 28, Göttingen, Germany*
[4]*German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany*
[5]*Institute of Biology, Leipzig University, Johannisallee 21, 04103 Leipzig, Germany*
[6]*Max Planck Institute for Biogeochemistry, Jena, Germany*
[7]*Department of Soil Quality, Wageningen University, 6700 AA Wageningen, The Netherlands*
[8]*Laboratory of Nematology, Wageningen University, 6700 ES Wageningen, The Netherlands*

Citation: Cortois, R., G. F. Veen, H. Duyts, M. Abbas, T. Strecker, O. Kostenko, N. Eisenhauer, S. Scheu, G. Gleixner, G. B. De Deyn, and W. H. van der Putten. 2017. Possible mechanisms underlying abundance and diversity responses of nematode communities to plant diversity. Ecosphere 8(5):e01719. 10.1002/ecs2.1719

Abstract. Plant diversity is known to influence the abundance and diversity of belowground biota; however, patterns are not well predictable and there is still much unknown about the driving mechanisms. We analyzed changes in soil nematode community composition as affected by long-term manipulations of plant species and functional group diversity in a field experiment with plant species diversity controlled by sowing a range of 1–60 species mixtures and controlling non-sown species by hand weeding. Nematode communities contain a variety of species feeding on bacteria, fungi, plants, invertebrates, while some are omnivorous. We analyzed responses of nematode abundance and diversity to plant species and functional diversity, and used structural equation modeling (SEM) to explore the possible mechanisms underlying the observed patterns. The abundance of individuals of all nematode feeding types, except for predatory nematodes, increased with both plant species and plant functional group diversity. The abundance of microbial-feeding nematodes was related positively to aboveground plant community biomass, whereas abundance of plant-feeding nematodes was related positively to shoot C:N ratio. The abundance of predatory nematodes, in turn, was positively related to numbers of plant-feeding nematodes, but not to the abundance of microbial feeders. Interestingly, the numbers of plant-feeding nematodes per unit root mass were lowest in the high-diversity plant communities, pointing at reduced exposure to belowground herbivores when plants grow in species-diverse communities. Taxon richness of plant-feeding and microbial-feeding nematodes increased with plant species and plant functional group diversity. Increasing plant functional group diversity also enhanced taxon richness of predatory nematodes. The SEM suggests that bottom-up control effects of plant species and plant functional group diversity on abundance of nematodes in the various feeding types predominantly involve mechanistic linkages related to plant quality instead of plant quantity; especially, C:N ratios of the shoot tissues, and/or effects of plants on the soil habitat, rather than shoot quantity explained nematode abundance. Although aboveground plant properties may only partly serve as a proxy for belowground resource quality and quantity, our results encourage further studies on nematode responses to variations in plant species and plant functional diversity in relation to both quantity and quality of the belowground resources.

Key words: C:N ratio; functional diversity; mechanistic linkages; nematode diversity; plant diversity; plant–soil interaction; resource quality; resource quantity; structural equation modeling.

Received 6 December 2016; accepted 12 December 2016. Corresponding Editor: Debra P. C. Peters.

† **E-mail:** w.vanderputten@nioo.knaw.nl

Introduction

A number of studies have analyzed effects of plant diversity on belowground community composition (Zak et al. 2003, De Deyn et al. 2004, 2011, Viketoft et al. 2009, Eisenhauer et al. 2010, 2011). However, the results are variable and the underlying mechanisms by which plant diversity influences belowground communities have been poorly resolved. Understanding the variations in the patterns in relation to the mechanistic role of how plant community composition influences belowground communities is important in order to predict the consequences of biodiversity gains and losses in plant communities, through feedback interactions with the soil biota, for plant community dynamics and the resulting ecosystem processes (Wardle et al. 2011).

Plant community biomass as well as plant community composition and diversity all have been identified as potential drivers of belowground community composition (Bardgett and Wardle 2010). The relative importance of these different factors for belowground community composition may depend on the functions of the soil biota considered (e.g., Wardle et al. 1999, De Deyn et al. 2011). Effects of plant community composition on soil biota may depend on their trophic position (Scherber et al. 2010). Lower trophic levels of soil biota, such as plant feeders, have been shown to be more responsive to changes in plant species diversity and composition than organisms from higher trophic levels in the soil food web (Wardle et al. 2003, De Deyn et al. 2004, Viketoft et al. 2009, Scherber et al. 2010). However, less is known about the mechanisms that may drive these responses (Eisenhauer et al. 2012, Hines et al. 2015).

Plant community effects on soil biota may operate through a number of factors, including resource quantity and resource quality (Wardle 2002). The traditional view that soil biota are generalists in their responses to plant quality is changing due to the increasing evidence on resource specialization below ground (Veen et al. 2015, Ali and Agrawal 2017). It has been well established that many bacteria and some soil fungi predominantly consume the easily decomposable components of the soil organic matter, whereas others can utilize more recalcitrant compounds (Kramer and Gleixner 2006). Results from detailed sampling in the rhizosphere of plant individuals growing in mixed vegetation have pointed at substantial degrees of specialization in the detritus-based component of the soil food web (Bezemer et al. 2010). Moreover, there are reports showing that the root zone of plant species can contain soil biota that decompose their own, conspecific, litter better than heterospecific litter (Ayres et al. 2009, Veen et al. 2015).

Specialization may also occur among root herbivores, soil pathogens, and, perhaps to a lesser extent, symbiotic mutualists (Klironomos 2003). Specialized soil biota contribute to specificity in plant–soil feedback interactions, of which it is increasingly demonstrated that they are a major driver of spatio-temporal dynamics in plant communities (Bever et al. 1997, 2015). It has been shown that dilution of negative feedback effects in diverse plant communities could explain positive plant diversity–productivity relationships (Maron et al. 2011, Schnitzer et al. 2011, Kulmatiski et al. 2012, Hendriks et al. 2013).

The sheer diversity of soil biota complicates a full and comprehensive analysis of plant diversity effects on soil biodiversity (Bardgett and van der Putten 2014). However, there are some phyla of soil biota, such as nematodes, which include a wide variety of feeding types and trophic groups that can be studied in a quantitatively reliable way (Bongers 1990, Yeates et al. 1993, Kardol et al. 2010). It has been shown that plant functional groups can promote specific nematode feeding groups (Wardle et al. 1999, Viketoft et al. 2009, Sohlenius et al. 2011), presumably because of differences in resource quality (Orwin et al. 2010). However, also plant species within the same plant functional group can host quite different nematode communities (De Deyn et al. 2004, Viketoft et al. 2005, Sohlenius et al. 2011)

and plant diversities may affect nematodes in a species-specific way (Kostenko et al. 2015). None of the previous studies on plant diversity–nematode interactions have examined nematode exposure to manipulated plant diversities for a very long time, and no study has attempted to relate results to both resource quantity and quantity.

The aim of the present study was to determine how long-term variations in plant species and functional group diversity influence abundance and richness of belowground nematodes, and to relate the observed patterns to resource quantity and quality. We tested a number of hypotheses. First, that nematode abundance responses to plant (functional) diversity will be strongest among the nematode feeding types in the lower trophic levels of the soil food web (Scherber et al. 2010). As increasing plant (functional) diversity will result into more shoot and root biomass, as well as total microbial biomass (Marquard et al. 2009, Eisenhauer et al. 2010, Ravenek et al. 2014), we expected the abundance of plant feeders and microbial feeders to increase with plant diversity. As increasing plant (functional) diversity might increase the diversity of resources, for example, due to the variety of plant chemical diversity represented in the vegetation, we expected taxon richness of all nematode feeding types (plant, bacterial, and fungal feeders; omnivores; and predators) to increase with higher plant species and plant functional group diversity. Finally, we expected nematodes to respond more strongly to differences in plant species diversity than to plant functional group diversity.

We performed our study in the long-term Jena Experiment, which is a large-scale biodiversity experiment in Germany (Roscher et al. 2004), where plant monocultures and mixtures from 1, 2, 4, 8, 16, and 60 species were established in the field eight years before our sampling. A previous study of nematode communities in the Jena Experiment (Eisenhauer et al. 2011), three and five years after establishing the experiment, pointed at increases in nematode taxon richness, but no effects on nematode abundance with increasing species richness of the plant community. Absence of nematode abundance response might have been due to a longer response time needed for the belowground biota. Moreover, as indicated above, results from this and other studies examining how plant diversities influence nematode community composition have not attempted to relate nematode responses to plant quality. Therefore, we performed a new sampling campaign, now after eight years of plant community development, and elaborate on the approach of Eisenhauer et al. (2011) by explicitly testing for various potential pathways linking plant community diversity to nematode abundance and diversity of different trophic groups.

We used structural equation modeling (SEM) in order to test whether the mechanistic linkages between plant community diversity-related parameters and soil nematode abundance and taxon richness depend on the nematode feeding type and trophic level. Structural equation modeling enables testing the fit of data to a priori formulated hypotheses when assuming a particular organization among variables (Shipley 2000, Grace and Kelley 2006). Structural equation modeling allows for testing multivariate hypotheses in which some plant and nematode variables can act as both predictor and response variables at the same time (e.g., Veen et al. 2010). We assumed predominant bottom-up control of soil biota by plant resource input in both detritus-based and living plant-based components of the soil food web (Wardle 2002). In line with our hypotheses, we expected that bottom-up effects would work via plant biomass (we used shoot biomass as proxy) and plant quality (we used shoot C:N ratio as proxy). We expected that these would relate directly to the plant feeder abundance and diversity (as first trophic level) and, indirectly, via soil microbial biomass to the microbivore abundance and diversity. As we observed relations between shoot quality and the response of various nematode feeding types, we discuss implications for further studies that may study quality of belowground resources in a more direct way.

Materials and Methods

Study site and soil sampling

We performed our study in the long-term grassland biodiversity field experiment at Jena, Germany (50°55′ N, 11°35′ E). The experimental field site is located on the floodplain of the River Saale and has been established in 2002 on former fertilized arable land that had been used for the production of wheat and vegetables prior to installing the biodiversity experiment. Soil is Eutric Fluvisol

developed from loamy sediments. The experimental treatments include monocultures, mixtures of all 60 plant species in the species pool, and mixtures of 2, 4, 8, and 16 plant species. Functional group richness also varies near-orthogonally with species richness from 1 to 4, comprising grasses, tall herbs, small herbs, and legumes. All 60 plant species are typical for mesophilic meadows of Central-Western Europe. Further details on experimental design and field conditions are provided by Roscher et al. (2004).

In September 2010, we collected soil samples in the 82 main plots of the Jena Experiment (Roscher et al. 2004). Five soil cores of 2 cm diameter and 15 cm depth were taken from all plots: four cores at the corners of a 1 m^2 square and one core in the center of that square; the square itself was placed >50 cm away from the plot edges. The five soil samples were homogenized so that there was one bulk sample per plot, leaving the plot replicates as the true replicates. Soil samples were transported to The Netherlands Institute of Ecology at Wageningen and stored at 4°C for a maximum of two weeks until nematode extraction. Nematodes were extracted from a subsample of 100 g of fresh soil, taken from the bulk soil sample per plot, using Oostenbrink elutriators (Oostenbrink 1960) to separate the nematodes from the heavier soil particles. The floating nematodes were collected on a stack of sieves, consisting of one sieve of 75 μm and three sieves of 45 μm mesh size. The nematodes on the sieves were rinsed off onto a double cotton filter that was placed in 100 mL tap water for 24 h at room temperature to let the nematodes migrate through the cotton filter into the tap water. All nematodes in the 100 mL nematode suspension were concentrated in 2 mL water after which they were fixated by diluting the suspension with 4 mL hot and 4 mL cold formalin of 4% (v/v). Total numbers of nematodes in each sample were counted in 1 mL (i.e., 10% of the total sample), and 150 nematodes were identified to family or genus level using an inverted light microscope. Due to process errors, we had to discard three out of 82 samples, so that we lost one 16-species plot, one two-species plot, and one monoculture plot (*Trifolium repens*), leaving 79 samples for data analysis. Nematode taxa were assigned to feeding groups according to Yeates et al. (1993; Appendix S1: Table S1). A 100 g fresh soil subsample, taken from the bulk soil sample composed of the five cores per plot, was weighed before and after drying at 105°C to determine soil moisture levels and to be able to express nematode densities per 100 g dry soil.

Plant and soil parameters

We compiled a data set of plot-level plant and soil parameters from published and non-published data sets of the Jena Experiment. Root mass data were available of 2008 and 2011. We used root biomass of the following year (2011) published by Ravenek et al. (2014); that study points at good correlation of root mass data of subsequent years, as well as between root and shoot biomass within years. Briefly, standing root biomass was collected from 0 to 40 cm depth in all 1-, 2-, 4-, 8-, 16-, and 60-species plots. Three soil cores of 3.5 cm diameter and 10 cm depth were collected from every plot. Soil cores were stored cool at 4°C until further processing. The bulk material of the pooled soil cores was weighed and subsequently washed for root material. Remaining soil particles were removed by hand. Roots were dried at 60–70°C before weighing (Ravenek et al. 2014).

Aboveground plant community biomass of all plots was harvested in late May and August by clipping plants in two rectangles of 0.1 m^2 at 3 cm above the soil surface. The harvested biomass was separated into species sown in the plot, cleaned from weeds, dried at 70°C for 72 h, weighed as biomass per species per plot, and summed per plot (for details, see Weigelt et al. 2010).

The C and N concentrations of aboveground plant tissue were determined as described in Abbas et al. (2013). In short, aboveground biomass was harvested in 2010 in late May prior to mowing. Plants were clipped at 3 cm above ground level in four rectangles of 20 × 50 cm. Sample location was selected randomly, leaving out the outer 70 cm of the plot. Biomass was dried at 70°C for at least 48 h. The concentrations of C and N were measured by analyzing the mixture of pooled plot biomass using an elemental analyzer (EA, Vario EL III; Elementar Analysen systeme GmbH, Hanau, Germany).

Microbial biomass in soil was determined using a soil subsample of the bulk sample per plot and expressed as microgram soil microbial carbon per gram dry soil (μg microbial C/g dry soil) based on

rates of oxygen use and CO_2 production. Briefly, O_2 consumption of soil microorganisms in fresh soil equivalent to 3.5 g dry soil was measured over a period of 24 h at 22°C using an electrolytic O_2-microcompensation apparatus (Scheu 1992). Substrate-induced respiration (Anderson and Domsch 1978) was determined by adding D-glucose to saturate catabolic enzymes of the microorganisms according to preliminary studies (4 mg D-glucose/g dry soil solved in 400 μL deionized water; Eisenhauer et al. 2010). Maximum initial respiratory response (MIRR; μL O_2·[g dry soil]$^{-1}$·h^{-1}) was calculated as the mean of the lowest three O_2 consumption values within the first 10 h after glucose addition. Microbial biomass (μg microbial C/g dry soil) was calculated as 38 × MIRR (Beck et al. 1997).

Soil organic matter content was determined according to Steinbeiss et al. (2008). Briefly, in April 2008, three samples of 4.8 cm diameter and 30 cm depth were collected from the core area of each plot. Subsequently, the soil samples were dried at 40°C, passed through a sieve with a mesh size of 2 mm, and sieved further using 1-mm mesh size according to common root removal methods (Allard et al. 2005, Ostonen et al. 2005, Stevens and Jones 2006). Total carbon concentration was analyzed on ball-milled subsamples (time 4 min, frequency 30 s^{-1}) by an elemental analyzer at 1150°C (Elementar analysator vario Max CN; Elementar Analysen systeme GmbH). To determine the organic carbon concentration, either the carbonate or the organic compounds need to be removed (Bisutti et al. 2004). Inorganic carbon concentration was measured by elemental analysis at 1150°C after removal of organic carbon for 16 h at 450°C in a muffle furnace (Hirota and Szyper 1975, Keefe 1994). Organic carbon concentration was then calculated as the difference between both measurements.

Data analysis

Effects of species richness and functional group richness level of plant communities on abundance or taxon richness of plant-feeding, bacterial-feeding, fungal-feeding, omnivorous, and predator nematodes were tested using general linear models. Plant species (log-transformed) and functional group richness were included as continuous variables to test for linear effects. The critical P value was adjusted for multiple testing of significance by Bonferroni correction (critical P-value = 0.01). The abundance of fungal-feeding nematodes was square-root-transformed, the abundance of predators was cube-root-transformed, and abundance data of other nematode groups were log-transformed to meet the requirements of normality and homoscedasticity of errors. As in almost all plots the number of taxa of fungal-feeding nematodes was the same, the effects of plant species or functional group richness level on the taxon richness of fungal-feeding nematodes were not tested. To determine whether there was a relationship between nematode community composition and species richness or functional group richness level, we used multivariate principal component analysis and redundancy analysis (RDA) in CANOCO version 5.03 (Šmilauer and Lepš 2014).

Our use of the root mass data of 2011 (Ravenek et al. 2014) enabled testing whether numbers of plant-feeding nematodes in the subsequent year were linearly related to species and functional group richness of the plant community. These data were used to calculate the log ratio of the number of plant-feeding nematodes per g dry mass of roots in the top 20 cm of the soil, which was considered as a proxy of plant exposure to nematode feeding. This exposure was used as dependent variable in general linear models, testing for linear effects of (log-transformed) species richness and functional group richness of plant communities.

We constructed SEM to analyze possible mechanistic pathways of plant species and plant functional group richness influences on abundance and taxon richness of plant feeders, microbial feeders (sum of bacterial and fungal feeders), and predatory nematodes. We wanted to distinguish effects of resource quantity and quality, but had C:N data for shoots only. As shoot and root biomass were well correlated (Ravenek et al. 2014), we used shoot biomass data in the SEM. Three outlier plot samples were excluded as the nematode numbers were extreme outliers. Based on the general linear model analyses, in- or exclusion of these data did not influence that statistics. We started SEM by including all pathways from plant species or functional richness to nematode abundance or taxon richness. We compared the model-implied and observed variance–covariance matrix in order to test the model fit to the data using a maximum-likelihood estimation method. By stepwise removal of non-significant

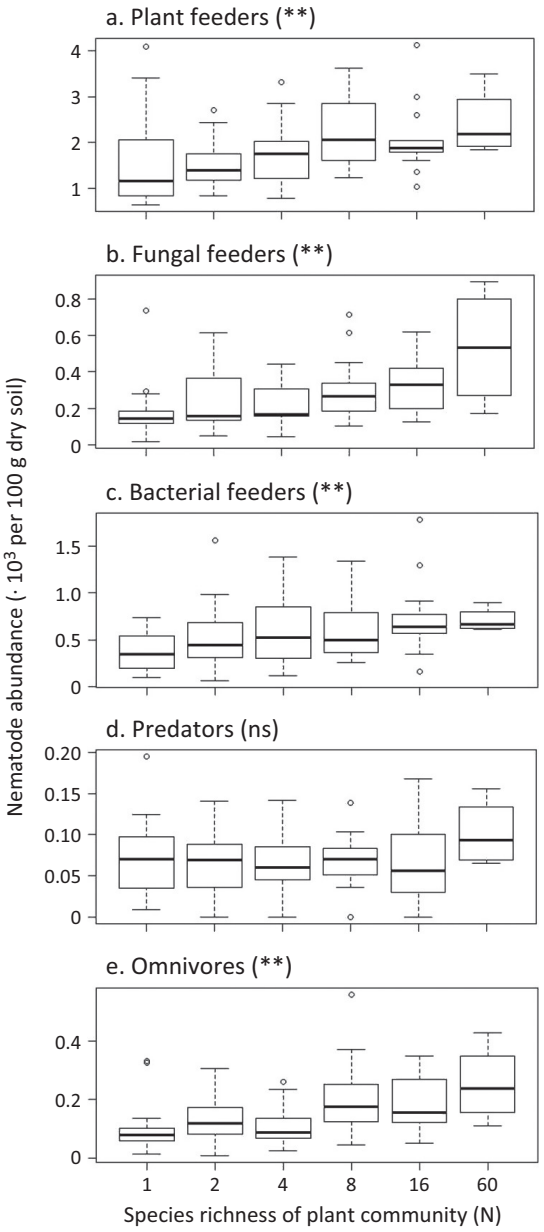

Fig. 1. The effect of sown plant species richness (1, 2, 4, 8, 16, 60 species/community) on the abundance (numbers $\times$ 10^3 per 100 g dry soil) of (a) plant-feeding nematodes, (b) fungal-feeding nematodes, (c) bacterial-feeding nematodes, (d) predatory nematodes, and (e) omnivorous nematodes. Number of asterisks above each subpanel denotes significance of relationship between number of plant species and number of nematodes ($^{*}P < 0.01$, $^{**}P < 0.001$, ns, not significant; Bonferroni correction: $K = 5$; critical $P = 0.01$) based on general linear models. The open circles indicate data

(*Fig. 1. Continued*)
points that are not included between the whiskers of the box plots (i.e. outliers) at the respective plant species richness level.

paths from the initial model, we selected the model that best fitted our data.

Results

Nematode abundance

The numbers of plant feeders, fungal feeders, bacterial feeders, and omnivores increased significantly with increasing plant species richness (regression slope: 0.14 ± 0.05, $P = 0.0022$; 2.06 ± 0.48, $P < 0.0001$; 0.23 ± 0.07, $P = 0.0028$; 0.26 ± 0.08, $P = 0.0009$, respectively; Fig. 1). The abundance of predatory nematodes was not significantly affected by plant species richness (0.99 ± 1.50; $P = 0.51$; Fig. 1).

With increasing plant functional group richness, regression slopes were significantly positive for fungal feeders and omnivores (regression slope: 1.68 ± 0.47, $P = 0.0007$; and 0.21 ± 0.07, $P = 0.005$, respectively; Fig. 2). The abundance of plant feeders and bacterial feeders increased marginally with plant functional group richness (regression slope: 0.11 ± 0.04, $P = 0.019$; and 0.17 ± 0.07, $P = 0.013$, respectively), whereas the abundance of predators was not significantly related to plant functional group richness (regression slope: 2.46 ± 1.41; $P = 0.09$; Fig. 2). The log ratio of the number of plant-feeding nematodes per gram dry root decreased significantly with plant species richness (regression slope: -0.37 ± 0.07; $P < 0.0001$, and plant functional group richness (-0.21 ± 0.07; $P = 0.0043$; Fig. 3).

Structural equation modeling indicated that the positive effect of plant species richness on plant feeder abundance may have operated via increased plant C:N ratios (Fig. 4a). The positive effect of plant species richness on the abundance of microbial feeders, however, was explained by increased shoot biomass (Fig. 4a). Increased microbial biomass was related positively to both plant species richness and the amount of soil organic matter; however, it did not explain enhanced abundance of microbial feeders in the species-rich plant communities (Fig. 4a). The abundance of predators was explained by the abundance of plant-feeding nematodes, but not by microbial-feeding nematode

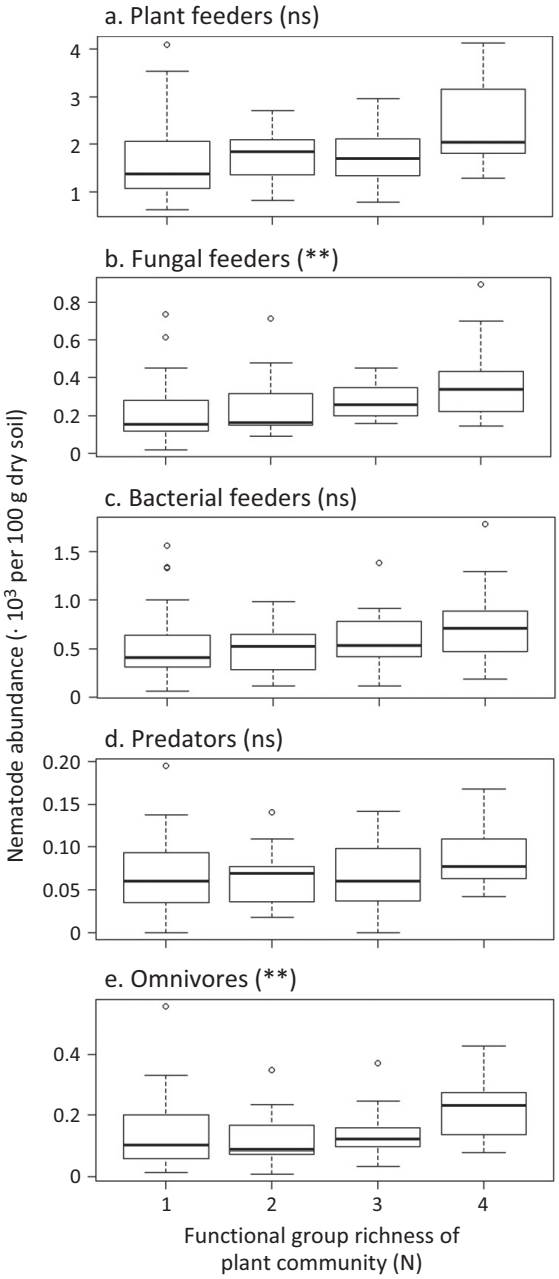

Fig. 2. The effect of plant functional group richness (1, 2, 3, or 4 functional groups/community) on the abundance (numbers × 10^3 per 100 g dry soil) of (a) plant-feeding nematodes, (b) fungal-feeding nematodes, (c) bacterial-feeding nematodes, (d) predatory nematodes, and (e) omnivorous nematodes. Number of asterisks above each subpanel denotes significance of relationship ($^*P < 0.01$, $^{**}P < 0.001$, ns, not significant; Bonferroni correction: $K = 5$; critical $P = 0.01$) based on general linear models. The open circles indicate data

(*Fig. 2. Continued*)
points that are not included between the whiskers of the box plots (i.e. outliers) at the respective plant species richness level.

abundance. There was no significant relationship between plant species richness and the abundance of predatory nematodes. Structural equation modeling also indicated that—similar to plant species richness—plant functional group richness enhanced plant-feeding nematode abundance by increasing plant C:N ratios and abundance of microbial feeders by increased plant biomass (Fig. 4a, b, respectively).

Nematode taxon richness

Nematode community composition was significantly related to plant species richness (RDA: pseudo-$F = 7.5$, $P = 0.001$, 8.9% explained variation) and plant functional group richness RDA: pseudo-$F = 6.7$, $P = 0.001$, 8.0% explained variation). Together these two variables explained 11.5% of variation in the nematode community

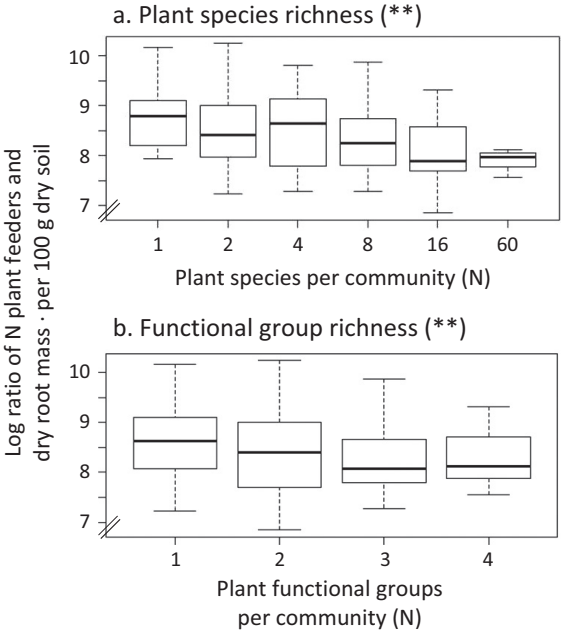

Fig. 3. Log ratio of the number of plant feeders and dry root mass per 100 g dry soil as affected by (a) species richness and (b) functional group richness of plant communities. Number of asterisks denotes significance of relationship ($^{**}P < 0.001$) based on general linear models.

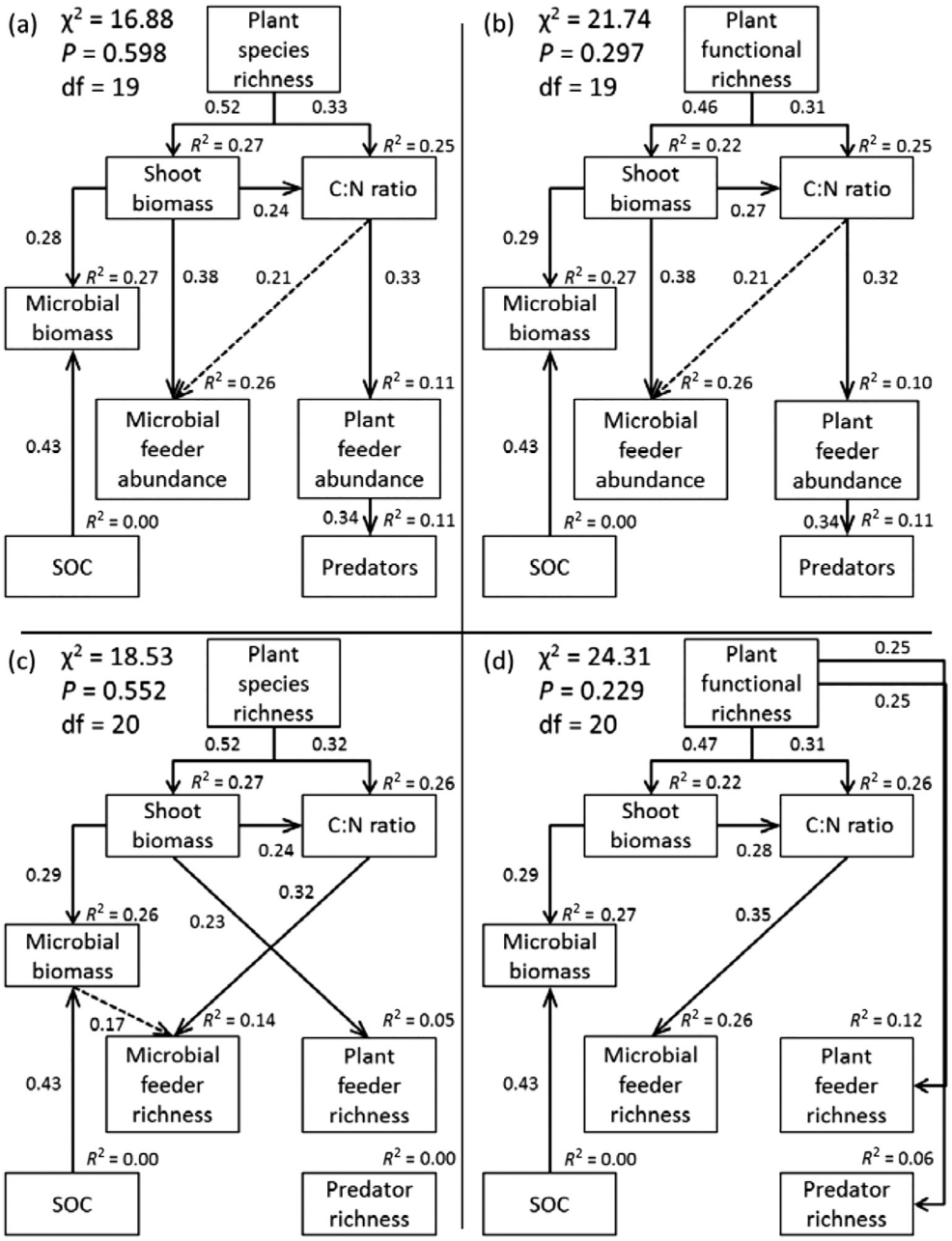

Fig. 4. Model results of the structural equation modeling analyses showing the influence of plant species richness on (a) nematode abundance and (c) nematode taxon richness, and of plant functional group richness on (b) nematode abundance and (d) nematode taxon richness. χ^2 and P are the test results from the comparison between the model-implied and observed variance–covariance matrices, with $P > 0.05$, indicating that there is no difference between model-implied and observed variance–covariance matrices. Square boxes display variables included in the model: species richness (number of plant species/community); functional richness (number of plant functional groups/community); shoot biomass as a proxy for root biomass (g dry weight/m^2 in 2010); organic matter (percentage of soil organic matter); microbial biomass (μg microbial C/g dry soil); C:N ratio in shoot tissue in 2010; microbial feeders (number of microbial-feeding nematodes per 100 g dry soil); plant feeders

(*Fig. 4. Continued*)
(number per 100 g dry soil); predators (number per 100 g dry soil); microbial feeder richness (number of taxa); plant feeder richness (number of taxa); predator richness (number of taxa). Solid arrows represent significant relationships at $P < 0.05$; dashed arrows represent relationships at $P < 0.10$. R^2-values associated with the response variables indicate the proportion of explained variation by the relationship with the other variables. Values associated with the arrows represent standardized path coefficients.

composition (RDA: pseudo-$F = 5.0$, $P = 0.002$; 8.9% explained by plant species richness and 2.6% by plant functional group richness). The total list of observed nematode taxa is presented in Appendix S1: Table S1.

There was a positive relation between plant species richness and taxon richness of microbivorous nematodes (regression slope: 0.50 ± 0.16; $P = 0.0018$). Taxon richness of all other nematode feeding groups (plant feeders, omnivores, carnivores) was not significantly explained by plant species richness ($P > 0.05$ in all cases, data not shown). Plant functional group richness enhanced taxonomic richness of bacterivorous nematodes (regression slope: 0.40 ± 0.15; $P = 0.0099$) and marginally significantly affected taxonomic richness of predators (0.18 ± 0.07; $P = 0.019$). Taxon richness of all other nematode feeding groups (plant feeders and omnivores) was not significantly explained by plant functional group richness ($P > 0.05$ in all cases, data not shown).

Structural equation modeling indicated that the effect of plant species richness on microbial feeder taxon richness operated via the C:N ratio (Fig. 4c). In SEM, we did not separate between effects on bacterivores and fungivores, because bacterial biomass could not be separated from fungal biomass in the microbial biomass assay that we applied. There was also a significant effect via plant shoot biomass and, albeit marginally significant ($P < 0.10$), via microbial biomass on microbial-feeding nematode richness (Fig. 4c). Structural equation modeling also could explain only a small percentage of variation in plant feeder taxon richness through plant species richness (Fig. 4c). Structural equation modeling did not explain which factors are driving taxon richness of all other nematode feeding groups (Fig. 4c). Taxon richness of both plant feeders and predators was explained by plant functional group richness, although the percentage explained variation was low for the predators (Fig. 4d).

Discussion

We investigated how plant species and plant functional group diversity influence the abundance, richness, and community composition of nematodes in a long-term grassland biodiversity experiment at Jena, Germany. In a number of previous studies, among others based on samples collected from the Jena Experiment, effects of plant biomass (resource quantity) and plant species identity or diversity (resource quality) have been considered separately as potential drivers of abundance and composition of plant-associated communities of soil biota (De Deyn et al. 2004, Lange et al. 2014). However, understanding the mechanisms of such effects of plant diversity on belowground community components is still in its infancy, just as for aboveground community components (Ebeling et al. 2014). In the present study, we explored which quantity- vs. quality-related mechanisms might underlie effects of plant community diversity on soil nematodes by testing a priori hypothesized mechanistic pathways between plant community diversity and the abundance and species richness of different nematode feeding types, which occupy different trophic positions in the soil food web.

Effects of plant community diversity on different nematode feeding groups

In support of our hypothesis, we found that the abundance of all nematode feeding types, except that of predatory nematodes, was positively related to plant species and, less strongly, to plant functional group richness. Although this points at abundance control of nematodes by resources, also named bottom-up control, further experiments are needed in order to establish whether not other factors, such as direct or apparent competition, could explain the observed patterns as well. In a previous study in the same experiment, it has been shown that plant diversity effects on

the abundance of both aboveground and belowground soil biota decrease with increasing trophic level (Scherber et al. 2010). In our study, however, we only observed a weaker response of predatory nematodes to plant community diversity. According to our SEM results, predatory nematode responses were mainly explained by plant-feeding nematodes. As this is one trophic level closer to the plant roots than when the predators are feeding on bacterivores or fungivores, it might explain why the predators still show a weak response to plant diversity. Nevertheless, the weaker response of the predators than of the plant feeders is in line with predictions based on a larger variety of belowground species groups (Scherber et al. 2010).

There have been a number of studies examining plant diversity effects on soil nematodes. Other plant diversity experiments showed either no effects of plant community diversity on nematode abundance, or effect sizes declined with increasing trophic position of nematodes (De Deyn et al. 2004, Viketoft et al. 2009). The results may be due to the length of the studies, or to other contextual aspects. For example, in soils with low amounts of organic matter, plant diversity has been shown to impact on microbial-feeding nematodes via microbial biomass (Eisenhauer et al. 2013). Sohlenius et al. (2011) demonstrated that differences in nematode community composition between plant communities increased with sampling year, indicating belowground time lags of nematode responses to variations in plant community composition (Scherber et al. 2010, Eisenhauer et al. 2012). This time lag may also explain the lack of or weaker plant species or functional group diversity effects on nematode communities in relatively early stages of the Jena Experiment and other outdoor plant biodiversity experiments (e.g., Korthals et al. 2001, Gastine et al. 2003, Eisenhauer et al. 2011) than in our study, which is based on the longest exposure of plant diversity treatments on nematode communities that have been examined thus far.

Interestingly, the numbers of plant-feeding nematodes per unit root dry mass declined with species richness of the plant communities. This supports the view that the nematode community shifts from an herbivory-based to a detrital-based food web when plant species richness increases (Eisenhauer et al. 2011). A possible mechanism that could underlie this finding is that there is a stronger degree of host plant species specificity of plant-feeding nematode species than of bacterivores and fungivores. The experimental design of the Jena Experiment does not allow us to detect such plant species specificity, but in a study where all plant species have been included in monocultures in a well-replicated way, plant species identity indeed has been reported to be an important predictor of nematode community composition (e.g., De Deyn et al. 2004). Growing plant species that accumulate root-feeding nematodes with plant species that reduce their abundance may lead to a reduction in plant-feeding nematodes. Such plant species-specific effects have also been reported for the decomposer subsystem of the soil food web (Bezemer et al. 2010), suggesting that the role of specificity for the functioning of soil food web interactions should be explored in further detail (Veen et al. 2015).

We did not observe a reduction in total root-feeding nematodes in high-diversity plant communities; however, there were fewer root-feeding nematodes per gram root biomass in those communities. This supports the findings of other plant diversity experiments that pointed at dilution effects of soil-borne enemies in high-diversity plant communities (Maron et al. 2011, Schnitzer et al. 2011, Kulmatiski et al. 2012, Hendriks et al. 2013). In long-term plant diversity experiments, it has been demonstrated that diversity–productivity relationships became stronger when the duration of the experiment increased (Reich et al. 2012). Our results in comparison with previous nematode inventories in the Jena Experiment suggest that such patterns can be due to some time lag in response of root-feeding nematode development.

In search for underlying mechanisms

We performed SEM to further explore whether the positive effect of plant species diversity and plant functional group diversity on nematode abundance and nematode taxon richness was determined via pathways related to plant quality (e.g., tissue C:N) or quantity (e.g., plant biomass). These are two key plant traits that are known to influence belowground processes (Wardle 2002). In contrast to our hypothesis, however, microbial feeder abundance was more strongly explained by shoot biomass than by microbial biomass.

There are several possible explanations. One explanation is that the standing biomass is high, but microbial production is low. Another possibility is that the standing biomass may be high, but that the microbial feeders select specific microbes, which are present only at low biomass. Testing those assumptions requires further labeling studies. As shoot biomass correlates with root biomass (Ravenek et al. 2014), it is well possible that microbial feeders are influenced by resource quantity, as shoot biomass also may correlate with the rate of carbon input into the soil with increased plant diversity (De Deyn et al. 2012).

In addition to resource quantity, a role of resource quality in the response of microbial-feeding nematodes cannot be excluded, as the only pathway in our SEM that could explain the increased abundance of plant-feeding nematodes with increasing plant species or plant functional group diversity was an increase in the C:N ratio of aboveground plant tissue. We used shoot C:N ratio as a proxy for resource quality as there were no data on root C:N ratios. Taking into account the limitations of our use of shoot C:N as a proxy for root C:N, our findings suggest that the abundance of plant-feeding nematodes was controlled more by resource quality than by resource quantity, albeit that this explanation pointed toward an opposite direction (higher C:N) than we expected (lower C:N). Besides the possible caveat that shoot C:N might not necessarily predict root C:N, high C:N ratio itself is not likely to have caused high plant-feeding nematode abundance, community-level herbivory rates are expected to increase with decreasing nutritional value of consumed biomass (e.g., Cebrian et al. 2009).

It should be borne in mind that plant-feeding nematodes are sap feeders, whereas the C:N ratio of the shoots is primarily related to the proportion of supportive tissue such as stems. These are relatively high in C and tend to increase with increased plant diversity (see also Abbas et al. 2013), whereas plant feeders are likely to respond more to rates of phytosynthate flow to the roots, which has been found to increase from monocultures to species mixtures (De Deyn et al. 2012). This aspect might also play a role in interpreting similar results reported in a SEM analysis of aboveground herbivorous invertebrates in the Jena Experiment. This showed that their abundance also increased with increasing C:N ratio of aboveground plant tissue, instead of via aboveground biomass (Ebeling et al. 2014). The authors proposed that an increase in habitat volume (stem material) with increasing stem height in diverse plant communities (see also Abbas et al. 2013) caused increased abundance of aboveground invertebrate herbivores.

Conclusions

In the present study, we demonstrated that eight years of experimental manipulation of both plant species and plant functional group diversity has resulted in strong bottom-up effects of plant diversity on nematode abundance in all nematode feeding types, except predatory nematodes. This suggests that the demonstrated weaker effects of plant diversity on higher trophic levels of soil biota (Scherber et al. 2010) could be due, at least to some extent, to a time lag in belowground responses to plant diversity. Using SEM, we showed that abundance of microbial feeders and plant feeders could not be explained by total resource quantity: Microbial feeder abundance increased with plant biomass, but not with microbial biomass. This may be explained by lower productivity of the entire microbial community, or by a subset that is being selectively fed upon. The abundance of plant feeders increased with C:N ratio of aboveground biomass. We could not establish in full detail to what extent shoot C:N is a proxy for root C:N, but our SEM analysis in any case suggests that it can be worthwhile to further examine the relationship between plant species diversity, root quality, and root-feeding nematode abundance. As results of SEM are largely explorative, our results do not reveal direct evidence on mechanisms, but they suggest that bottom-up control effects of plant species and functional group diversity on abundance of nematodes in the various feeding types predominantly involve mechanistic linkages related to plant quality and/or effects of plants on the soil habitat, rather than direct effects of plant quantity.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (FOR 1451) and by NWO-ALW VIDI through financial support to GBDD (Grant

No. 864.11.003), and NWO additional fund (Grant No. 832.13.009) to WvP. We thank anonymous referees for their helpful suggestions on a previous version of the manuscript. This is NIOO-KNAW publication no. 6225. RC, GBDD, and WvdP designed the study, analyzed the data, and wrote the manuscript. GFV performed SEM analyses. OK did all other statistical analyses. HD performed nematode analyses. MA analyzed C:N. TS, NE, and SS analyzed microbial biomass. GG analyzed soil organic matter; all have read and commented on the manuscript.

Literature Cited

Abbas, M., A. Ebeling, Y. Oelmann, R. Ptacnik, C. Roscher, A. Weigelt, W. W. Weisser, W. Wilcke, and H. Hillebrand. 2013. Biodiversity effects on plant stoichiometry. PLoS ONE 8:e58179.

Ali, J. G., and A. A. Agrawal. 2017. Trade-offs and tritrophic consequences of host shifts in specialized root herbivores. Functional Ecology 31:153–160.

Allard, V., P. C. D. Newton, M. Lieffering, J.-F. Soussana, R. A. Carran, and C. Matthew. 2005. Increased quantity and quality of coarse soil organic matter fraction at elevated CO_2 in a grazed grassland are a consequence of enhanced root growth rate and turnover. Plant and Soil 276: 49–60.

Anderson, J. P. E., and K. H. Domsch. 1978. Physiological method for quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10:215–221.

Ayres, E., H. Steltzer, B. L. Simmons, R. T. Simpson, J. M. Steinweg, M. D. Wallenstein, N. Mellor, W. J. Parton, J. C. Moore, and D. H. Wall. 2009. Home-field advantage accelerates leaf litter decomposition in forests. Soil Biology & Biochemistry 41: 606–610.

Bardgett, R. D., and W. H. van der Putten. 2014. Belowground biodiversity and ecosystem functioning. Nature 515:505–511.

Bardgett, R. D., and D. A. Wardle. 2010. Aboveground-belowground linkages: biotic interactions, ecosystem processes and global change. Oxford University Press, New York, New York, USA.

Beck, T., R. G. Joergensen, E. Kandeler, F. Makeschin, E. Nuss, H. R. Oberholzer, and S. Scheu. 1997. An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biology & Biochemistry 29:1023–1032.

Bever, J. D., S. A. Mangan, and H. M. Alexander. 2015. Maintenance of plant species diversity by pathogens. Pages 305–325 *in* D. J. Futuyma, editor. Annual review of ecology, evolution, and systematics. Volume 46. Annual Reviews, Palo Alto, California, USA.

Bever, J. D., K. M. Westover, and J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology 85:561–573.

Bezemer, T. M., et al. 2010. Divergent composition but similar function of soil food webs of individual plants: plant species and community effects. Ecology 91:3027–3036.

Bisutti, I., I. Hilke, and M. Raessler. 2004. Determination of total organic carbon – an overview of current methods. TrAc Trends in Analytical Chemistry 23: 716–726.

Bongers, T. 1990. The maturity index – an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14–19.

Cebrian, J., J. B. Shurin, E. T. Borer, B. J. Cardinale, J. T. Ngai, M. D. Smith, and W. F. Fagan. 2009. Producer nutritional quality controls ecosystem trophic structure. PLoS ONE 4:e4929.

De Deyn, G. B., H. Quirk, and R. D. Bardgett. 2011. Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. Biology Letters 7:75–78.

De Deyn, G. B., H. Quirk, S. Oakley, N. J. Ostle, and R. D. Bardgett. 2012. Increased plant carbon translocation linked to overyielding in grassland species mixtures. PLoS ONE 7:e45926.

De Deyn, G. B., C. E. Raaijmakers, J. Van Ruijven, F. Berendse, and W. H. Van der Putten. 2004. Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. Oikos 106:576–586.

Ebeling, A., S. T. Meyer, M. Abbas, N. Eisenhauer, H. Hillebrand, M. Lange, C. Scherber, A. Vogel, A. Weigelt, and W. W. Weisser. 2014. Plant diversity impacts decomposition and herbivory via changes in aboveground arthropods. PLoS ONE 9: e106529.

Eisenhauer, N., T. Dobies, S. Cesarz, S. E. Hobbie, R. J. Meyer, K. Worm, and P. B. Reich. 2013. Plant diversity effects on soil food webs are stronger than those of elevated CO_2 and N deposition in a long-term grassland experiment. Proceedings of the National Academy of Sciences USA 110: 6889–6894.

Eisenhauer, N., V. D. Migunova, M. Ackermann, L. Ruess, and S. Scheu. 2011. Changes in plant species richness induce functional shifts in soil nematode communities in experimental grassland. PLoS ONE 6:e24087.

Eisenhauer, N., P. B. Reich, and S. Scheu. 2012. Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. Basic and Applied Ecology 13:571–578.

Eisenhauer, N., et al. 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. Ecology 91:485–496.

Gastine, A., M. Scherer-Lorenzen, and P. W. Leadley. 2003. No consistent effects of plant diversity on root biomass, soil biota and soil abiotic conditions in temperate grassland communities. Applied Soil Ecology 24:101–111.

Grace, J. B., and J. E. Kelley. 2006. A structural equation model analysis of postfire plant diversity in California shrublands. Ecological Applications 16: 503–514.

Hendriks, M., L. Mommer, H. de Caluwe, A. E. Smit-Tiekstra, W. H. van der Putten, and H. de Kroon. 2013. Independent variations of plant and soil mixtures reveal soil feedback effects on plant community overyielding. Journal of Ecology 101: 287–297.

Hines, J., et al. 2015. Towards an integration of biodiversity-ecosystem functioning and food web theory to evaluate relationships between multiple ecosystem services. Pages 161–199 *in* G. Woodward and D. A. Bohan, editors. Ecosystem services: from biodiversity to society. Pt 1. Elsevier Academic Press Inc., San Diego, California, USA.

Hirota, J., and J. P. Szyper. 1975. Separation of total particulate carbon into inorganic and organic components. Limnology and Oceanography 20:896–900.

Kardol, P., M. A. Cregger, C. E. Campany, and A. T. Classen. 2010. Soil ecosystem functioning under climate change: plant species and community effects. Ecology 91:767–781.

Keefe, C. W. 1994. The contribution of inorganic compounds to the particulate carbon, nitrogen, and phosphorus in suspended matter and surface sediments of Chesapeake Bay. Estuaries 17:122–130.

Klironomos, J. N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301.

Korthals, G. W., P. Smilauer, C. Van Dijk, and W. H. Van der Putten. 2001. Linking above- and belowground biodiversity: abundance and trophic complexity in soil as a response to experimental plant communities on abandoned arable land. Functional Ecology 15:506–514.

Kostenko, O., H. Duyts, S. Grootemaat, G. B. De Deyn, and T. M. Bezemer. 2015. Plant diversity and identity effects on predatory nematodes and their prey. Ecology and Evolution 5:836–847.

Kramer, C., and G. Gleixner. 2006. Variable use of plant- and soil-derived carbon by microorganisms in agricultural soils. Soil Biology & Biochemistry 38:3267–3278.

Kulmatiski, A., K. H. Beard, and J. Heavilin. 2012. Plant-soil feedbacks provide an additional explanation for diversity-productivity relationships. Proceedings of the Royal Society of London B: Biological Sciences 279:3020–3026.

Lange, M., et al. 2014. Biotic and abiotic properties mediating plant diversity effects on soil microbial communities in an experimental grassland. PLoS ONE 9:e96182.

Maron, J. L., M. Marler, J. N. Klironomos, and C. C. Cleveland. 2011. Soil fungal pathogens and the relationship between plant diversity and productivity. Ecology Letters 14:36–41.

Marquard, E., A. Weigelt, V. M. Temperton, C. Roscher, J. Schumacher, N. Buchmann, M. Fischer, W. W. Weisser, and B. Schmid. 2009. Plant species richness and functional composition drive overyielding in a six-year grassland experiment. Ecology 90: 3290–3302.

Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. Pages 85–102 *in* J. N. Sasser and W. R. Jenkins, editors. Nematology. The University of North Carolina Press, Chapel Hill, North Carolina, USA.

Orwin, K. H., S. M. Buckland, D. Johnson, B. L. Turner, S. Smart, S. Oakley, and R. D. Bardgett. 2010. Linkages of plant traits to soil properties and the functioning of temperate grassland. Journal of Ecology 98:1074–1083.

Ostonen, I., K. Lõhmus, and K. Pajuste. 2005. Fine root biomass, production and its proportion of NPP in a fertile middle-aged Norway spruce forest: comparison of soil core and ingrowth core methods. Forest Ecology and Management 212:264–277.

Ravenek, J. M., et al. 2014. Long-term study of root biomass in a biodiversity experiment reveals shifts in diversity effects over time. Oikos 123: 1528–1536.

Reich, P. B., D. Tilman, F. Isbell, K. Mueller, S. E. Hobbie, D. F. B. Flynn, and N. Eisenhauer. 2012. Impacts of biodiversity loss escalate through time as redundancy fades. Science 336:589–592.

Roscher, C., J. Schumacher, J. Baade, W. Wilcke, G. Gleixner, W. W. Weisser, B. Schmid, and E. D. Schulze. 2004. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic and Applied Ecology 5:107–121.

Scherber, C., et al. 2010. Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. Nature 468:553–556.

Scheu, S. 1992. Automated measurement of the respiratory response of soil microcompartments – active

microbial biomass in earthworm feces. Soil Biology & Biochemistry 24:1113–1118.
Schnitzer, S. A., et al. 2011. Soil microbes drive the classic plant diversity-productivity pattern. Ecology 92:296–303.
Shipley, B. 2000. A new inferential test for path models based on directed acyclic graphs. Structural Equation Modeling – A Multidisciplinary Journal 7:206–218.
Šmilauer, P., and J. Lepš. 2014. Multivariate analysis of ecological data using CANOCO 5. Second edition. Cambridge University Press, Cambridge, UK.
Sohlenius, B., S. Boström, and M. Viketoft. 2011. Effects of plant species and plant diversity on soil nematodes – a field experiment on grassland run for seven years. Nematology 13:115–131.
Steinbeiss, S., H. Beßler, C. Engels, V. M. Temperton, N. Buchmann, C. Roscher, Y. Kreutziger, J. Baade, M. Habekost, and G. Gleixner. 2008. Plant diversity positively affects short-term soil carbon storage in experimental grasslands. Global Change Biology 14:2937–2949.
Stevens, G. N., and R. H. Jones. 2006. Patterns in soil fertility and root herbivory interact to influence fine-root dynamics. Ecology 87:616–624.
Veen, G. F., G. T. Freschet, A. Ordonez, and D. A. Wardle. 2015. Litter quality and environmental controls of home-field advantage effects on litter decomposition. Oikos 124:187–195.
Veen, G. F., H. Olff, H. Duyts, and W. H. van der Putten. 2010. Vertebrate herbivores influence soil nematodes by modifying plant communities. Ecology 91:828–835.
Viketoft, M., J. Bengtsson, B. Sohlenius, M. P. Berg, O. Petchey, C. Palmborg, and K. Huss-Danell. 2009. Long-term effects of plant diversity and composition on soil nematode communities in model grasslands. Ecology 90:90–99.
Viketoft, M., C. Palmborg, B. Sohlenius, K. Huss-Danell, and J. Bengtsson. 2005. Plant species effects on soil nematode communities in experimental grasslands. Applied Soil Ecology 30:90–103.
Wardle, D. A. 2002. Communities and ecosystems: linking the aboveground and belowground components. Princeton University Press, Princeton, New Jersey, USA.
Wardle, D. A., R. D. Bardgett, R. M. Callaway, and W. H. Van der Putten. 2011. Terrestrial ecosystem responses to species gains and losses. Science 332:1273–1277.
Wardle, D. A., K. I. Bonner, G. M. Barker, G. W. Yeates, K. S. Nicholson, R. D. Bardgett, R. N. Watson, and A. Ghani. 1999. Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. Ecological Monographs 69:535–568.
Wardle, D. A., G. W. Yeates, W. Williamson, and K. I. Bonner. 2003. The response of a three trophic level soil food web to the identity and diversity of plant species and functional groups. Oikos 102:45–56.
Weigelt, A., et al. 2010. The Jena experiment: six years of data from a grassland biodiversity experiment. Ecology 91:930–931.
Yeates, G. W., T. Bongers, R. G. M. De Goede, D. W. Freckman, and S. S. Georgieva. 1993. Feeding habits in soil nematode families and genera – an outline for soil ecologists. Journal of Nematology 25:315–331.
Zak, D. R., W. E. Holmes, D. C. White, A. D. Peacock, and D. Tilman. 2003. Plant diversity, soil microbial communities, and ecosystem function: Are there any links? Ecology 84:2042–2050.

Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1719/full