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

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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 predatory nematodes, in turn, was positively related to numbers of plant-feeding nematodes, but not to the abundance of microbial feeders. 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.
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 quality.

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 m² 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 from the bulk soil sample per plot, using Oostenbrink elutriators (Oostenbrink 1960) to separate nematodes from the heavier soil particles. The nematodes from a subsample of 100 g of fresh soil, taken from the bulk soil sample per plot and weighed (Ravenek et al. 2014). 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 m² 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, and 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 CO₂ production. Briefly, O₂ 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 O₂-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 O₂ [g dry soil]⁻¹·h⁻¹) was calculated as the mean of the lowest three O₂ 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 (Smilauer 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
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: $0.14 \pm 0.05$, $P = 0.0022$; $2.06 \pm 0.48$, $P < 0.0001$; $0.23 \pm 0.07$, $P = 0.0028$; $0.26 \pm 0.08$, $P = 0.0009$, respectively; Fig. 1). The abundance of predatory nematodes was not significantly affected by plant species richness ($0.99 \pm 1.50$; $P = 0.51$; Fig. 1).

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

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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 ($^\ast P < 0.01$, $^{**} P < 0.001$, **ns, not significant; Bonferroni correction: $K = 5$; critical $P = 0.01$) based on general linear models. The open circles indicate data points that are not included between the whiskers of the box plots (i.e. outliers) at the respective plant species richness level.
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 abundance. There was no significant relationship between plant species richness and the abundance of predatory nematodes.

Fig. 2. The effect of plant functional group richness (1, 2, 3, or 4 functional groups/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 ($^*P < 0.01$, $^{**}P < 0.001$, ns, not significant; Bonferroni correction: $K = 5$; critical $P = 0.01$) based on general linear models. The open circles indicate data points that are not included between the whiskers of the box plots (i.e. outliers) at the respective plant species richness level.

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. $\chi^2$ 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
(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.

**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 below-
ground 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 nema-
tode 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 (Scher-
ber et al. 2010).

There have been a number of studies examin-
ing plant diversity effects on soil nematodes. Other plant diversity experiments showed either
no effects of plant community diversity on nema-
tode abundance, or effect sizes declined with
increasing trophic position of nematodes (De
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 com-

munity composition between plant communities
increased with sampling year, indicating below-
ground time lags of nematode responses to varia-
tions in plant community composition (Scherber
et al. 2010, Eisenhauer et al. 2012). This time lag
may also explain the lack of or weaker plant spe-
cies or functional group diversity effects on nema-
tode communities in relatively early stages of the
Jena Experiment and other outdoor plant biodiver-
sity 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 communi-
ties 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 bacteri-
vores 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 monocul-
tures in a well-replicated way, plant species iden-
tity 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 fur-
ther detail (Veen et al. 2015).

We did not observe a reduction in total root-
feeding nematodes in high-diversity plant com-
munities; however, there were fewer root-feeding
nematodes per gram root biomass in those commu-
nities. 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, Hen-
driks 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 com-
parison 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.

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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


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.


Supporting Information

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