



Original article

Plant diversity enhanced nematode-based soil quality indices and changed soil nematode community structure in intensively-managed agricultural grasslands

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ABSTRACT

Plant productivity, decomposition and nutrient cycling are controlled by plant-soil-biota interactions. However, it remains poorly understood how plant species diversity impacts belowground communities that modulate these processes in intensively-managed grassland systems. In managed grassland communities comprising species selected for agronomic performance, we investigated how plant species diversity affected the soil nematode community and associated ecological indices with a focus on assessing aboveground-belowground interactions. A total of 27 nematode taxa were identified from 61 experimental field plots in which plant species diversity was systematically manipulated from a pool of six plant species within three functional groups (FGs; grasses, legumes, herbs). In general, there were strong effects of plant species identity on the nematode community; interspecific interaction effects did not consistently occur, but where they did were best related to plant community evenness. The equi-proportional six-species plant community had a significantly higher nematode diversity, maturity index (MI), structure index (SI) and proportion of sensitive taxa (omnivore and predators) but a lower enrichment index (EI) than the individual monocultures. The two legumes (*Trifolium pratense* and *Trifolium repens*) had the highest EI but lowest abundance of fungivores and channel index, indicating a bacterial-dominated decomposition pathway. Moreover, the community structure of nematodes in the equi-proportional six-species community was significantly different from that in the monocultures. This change in community structure was associated with factors highly correlated with plant diversity, including higher aboveground biomass yield and total nitrogen in harvested biomass as well as lower biomass of weed species. Overall, our results show that multi-species forage sward mixtures that include grasses, legumes, and herbs can have a positive effect on the soil nematode community and nematode-based soil quality indices. This is of practical relevance for farmers and for EU agricultural policy targeted at sustainability, soil health and farming for biodiversity benefits.

1. Introduction

The biodiversity crisis is attributed to human impacts and is regarded as one of the most pressing global environmental challenges today [1], with approximately one million species at risk of extinction [2]. One of the main drivers of biodiversity loss is habitat destruction associated with activities such as deforestation, urbanization and land use change [3]. Biodiversity loss poses a great threat to human well-being, as it affects the delivery of critical ecosystem services such as climate regulation and nutrient cycling as well as food, water, and medicine provision [3–5]. It has therefore become imperative to take measures that will

preserve and protect biodiversity. As a vital component of ecosystems, soils play a critical role in maintaining biodiversity. They are regarded as a globally important reservoir of biodiversity, harbouring a greater variety of organisms (including many species that are yet to be discovered) than any other habitat on earth, with the biomass of soil microorganisms estimated to be more than 70 times greater than that of all terrestrial plants combined [8–10]. They serve as a habitat for a wide range of organisms, including bacteria, fungi, protozoa, nematodes, arthropods, and earthworms, that are essential for nutrient cycling and ecosystem functioning [6,7]. However, intensive agriculture, land use change, urbanization, and pollution have led to a decline in the abundance and

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diversity of soil biota, which has significant impacts on ecosystem functioning and services [4,11–14].

Plant-soil interactions are critically important for the maintenance of belowground soil biodiversity, and play a key role in shaping the structure and function of soil ecosystems. Plants can influence the diversity and composition of soil biological communities through root exudates and organic matter inputs, the creation of physical niches belowground and the modification of microenvironments, and a range of positive and negative selection processes [15]. However, in most temperate regions, food production and agricultural activity are primarily supported by intensively-managed grasslands which are sown as monocultures. Monoculture systems can reduce the diversity and abundance of other organisms within the ecosystems, with correspondingly negative impacts on ecosystem multifunctionality and crop resilience [17,18]. This includes a reduction in the diversity and abundance of soil microorganisms, and soil invertebrates, such as earthworms and springtails, leading to a decline in soil health and fertility [17–19]. Increasing plant species diversity can offer a sustainable alternative to monoculture systems, as they can improve biodiversity and ecosystem functioning [7,20]. While there is a growing evidence in literature suggesting that multi-species swards can improve aboveground biodiversity, maintain high yields with reduced fertiliser inputs, and even sometimes outperform the best performing monocultures [21–24], the effects of multi-species swards on belowground biodiversity (including soil microorganisms and invertebrates) are still not fully understood. Increasing plant species diversity can increase the abundance of soil microorganisms and soil invertebrates in semi-natural and natural grasslands [25–28] but there has been limited investigation of this in intensively managed grasslands. There is therefore a need for further research to study the effects of multi-species swards (particularly in intensively-managed grassland systems) on belowground biodiversity of other members of the soil biota, such as nematodes.

Nematodes are often used as indicators of soil health and ecosystem functioning because they are widely distributed, highly abundant, and sensitive to changes in environmental factors such as land use, management practices, soil chemistry and climate change [32–34]. Nematodes can be reliably studied in a quantitative manner and because they include a wide range of trophic groups and feeding types, they can give an indication of multiple aspects of the soil food web [29–31]. Several nematode-based soil quality indices are available that relate to soil function [35–37], making them especially attractive as a soil biodiversity indicator. For instance, the Maturity Index (MI) is an indicator of environmental disturbance resulting from perturbations. The Enrichment Index (EI) indicates food availability and nutrient enrichment, the Channel Index (CI) shows the predominant pathway of organic matter decomposition (lower values indicate decomposition pathway dominated by bacteria whereas higher values indicate decomposition pathway dominated by fungi) while the Structure Index (SI) indicates soil food web structure and complexity (higher values indicate a more structured soil food web) [38,45]. Previous studies found that increasing plant species diversity in grasslands can lead to an increase in the diversity and abundance of nematode taxa, changes in the nematode community structure, and improved functioning of the soil nematode community. This can lead to improved nutrient cycling, decomposition, and plant growth, which can have important implications for the overall health and function of the soil ecosystem [39–42]. However, none of these studies to date have analysed the effect of increased plant diversity on nematode community and their associated indices in an intensively-managed grassland.

This study aimed to quantify the effect of plant species diversity on the abundance and structure of the nematode community, and nematode-based soil quality indices. Plant diversity was systematically manipulated by sowing forage sward communities (from monocultures to six-species multi-species mixtures) from the following pool of six species: two grasses, perennial ryegrass (*Lolium perenne*) and timothy (*Phleum pratense*); two legumes, red clover (*Trifolium pratense*) and white

clover (*Trifolium repens*); and two herbs, chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*). We used Diversity-Interactions modelling [43] to investigate the effect of plant diversity (composition, richness and relative abundance) on selected responses. This is a multiple regression approach with predictors based on the plant species' proportions [44]. The simplex design creates the systematic variation in plant species' proportions (and therefore species composition, richness and relative abundance) that facilitates this type of analysis. Specifically, we aimed to 1) investigate the effect of plant diversity on nematode community composition and structure, and; 2) quantify the effect of plant diversity (through analysis of plant species' identity effects and interspecific interaction effects) on the abundance of nematode feeding types and nematode-based soil quality indices.

2. Materials and methods

2.1. Site description and field sampling

A plot-scale experiment was established on a previously barley crop site in September 2018 at Johnstown Castle, Wexford, in the south-east of Ireland (52°18'04.1"N 6°30'24.6"W, 75 m a.s.l.) The experiment was conducted on a sandy-loam soil with a pH of 5.7, and total carbon and nitrogen content of 5.4 and 2.2 g kg⁻¹ dry weight in the top 15 cm of soil, respectively. The field was ploughed and reseeded with a total of 61 plots, each 25 m² in size (10 m × 2.5 m). Treatments comprising systematically varying diversity of forage plant communities (≥3 replicates each of the six monocultures and multi-species mixtures) were randomly assigned to plots (Table S1). The plant species were chosen from high-yielding perennial forage species adapted to most Irish soils, climate and intensive management, and to represent three contrasting functional groups (FGs): grasses (FG1; *Lolium perenne*, *Phleum pratense*), legumes (FG2; *Trifolium pratense*, *Trifolium repens*) and herbs (FG3; *Cichorium intybus*, *Plantago lanceolata*). The sown communities comprised six monocultures and 13 mixtures, ranging from two to six forage species per plot. Using a simplex design [44], species proportions were as follows: 100% of the advised seeding weight for monocultures; 50% of each species from the same FG for the two-species mixtures; 25% of each species from two FGs for the four-species mixtures and 16.7% of each species for the six-species mixtures centroid (Fig. S1). The five-species mixtures were dominated by each species in turn such that there was 60% of one species and 10% of the four species from the other two FGs. Details of the experimental design of sown plant communities can be found in Table S1, and further information on the simplex design and DI modelling approach is provided in Fig. S1.

The plots received 150 kg ha⁻¹ yr⁻¹ of nitrogen fertiliser (150 N), split evenly into five applications. Background fertilisation of 60 kg ha⁻¹ yr⁻¹ of phosphorus and 300 kg ha⁻¹ yr⁻¹ of potassium was split in three applications, to ensure these nutrients were not limiting. Beginning from the year after establishment, annual dry matter yield, nitrogen content in harvested biomass, weed proportion and soil carbon and nitrogen contents in the top 15 cm layer, were recorded on each plot for two full growing seasons (i.e. from March 2019 to October 2020). An overview of the experimental and analytical approach adopted in this experimental trial is provided in Fig. S2.

2.2. Soil sampling, nematode extraction and identification

Soil samples were collected from the top 15 cm layer in March 2021 using a W-shaped sampling method. In each plot, 10 soil core samples were collected, mixed thoroughly to form a composite sample, and a representative subsample was taken for laboratory analyses. The samples were stored in the cold room at 4 °C until nematode extraction [45]. Nematodes were extracted from 100 g of fresh soil per sample with the Oostenbrink elutriator method [46] within three weeks after sampling. The extracted nematodes were counted, and identified to the genus or family level based on morphological features and identification keys,

using a light microscope [47–49].

2.3. Other data measured from the study site

Other measured data from the site were as follows. Aboveground biomass production was measured across six harvests per annum. Samples of about 100 g (fresh weight) of aboveground biomass were collected from each plot for each harvest, and used to assess total annual aboveground dry matter yield ($\text{t ha}^{-1} \text{yr}^{-1}$) and nitrogen content in harvested aboveground biomass ($\text{kg ha}^{-1} \text{yr}^{-1}$). Soil samples (0–15 cm) were taken at the same time as samples for nematode assessment, sieved at 2 mm and KCl extractions were performed to assess mineral nitrogen content (all measured in g kg^{-1} dry weight of soil). Samples were ball milled for 2 min at 23 Hz ($180\text{--}1500 \text{ min}^{-1}$) using a MM 200 Model Mixer Mill (Retsch, UK) before measuring total nitrogen (TN) and total carbon (TC) using the high-temperature combustion method by a LECO TruSpec CN analyser (Elementec, Ireland). Additionally, annual dry matter yield (proportion of weeds) of unsown species ($\text{t ha}^{-1} \text{yr}^{-1}$) was assessed through the sorting of additional aboveground biomass samples.

2.4. Calculation of nematode indices and statistical analyses

The nematode community dataset (the identified taxa and abundances) was used to assess community composition and structure. For the nematode community composition, we compared the nematode feeding groups across the six plant monocultures and the sown equi-proportional six species plant community (Kruskal-Wallis test based on the relative abundance of the feeding groups within each of the plant communities). This nonparametric test is suitable where there are unequal number of samples within treatment groups because it does not assume equal variance. To assess changes in the nematode community structure, we conducted an NMDS using the metaMDS function in ‘vegan’ [50]. A permutational multivariate analysis of variance (PERMANOVA) was performed with the adonis2 function in ‘vegan’ package using Bray-Curtis distance [50], to understand the change in community structure across treatments. If the PERMANOVA was significant, the pairwise.adonis function in ‘vegan’ was used to delineate which pairs were significantly different. To determine the nematode taxa that are driving the observed change in the nematode community structure, a similarity percentage (SIMPER) analysis was done using the ‘simper’ function in the vegan package [50]. Furthermore, an ‘indicator species’ analysis was carried using the ‘indicspecies’ package in R [71] to determine which nematode taxa preferentially occurred in each plant group. Redundancy analysis (RDA) was carried out on Hellinger-transformed nematode abundance data to identify other measured variables that are related to the changes in the nematode community structures. All measured variables were used in the model and only the significant variables were included in the final model taking multicollinearity into consideration. The significance of the overall model was tested using the anova.cca function ‘vegan’.

To calculate nematode-based indices, each genus was assigned to the “colonizer-persister” cp-scale [31], and the nematode-based indices were calculated.

The Maturity Index was calculated for the free-living nematodes following Bongers [31] as

$$MI = \frac{1}{n} \sum_{i=1}^n v_i f_i \quad (1)$$

where MI is the maturity index, v_i is the c-p value assigned to family i , f_i is the frequency of family i , and n is the total number of individuals of the free living taxa in the sample

The basal (b), enrichment (e), and structure (s) components of the nematode assemblages were calculated following Ferris et al. [45] as:

$$b = (BA_2 + FU_2) \times W_2 \quad (2)$$

$$e = (BA_1 W_1) + (FU_2 W_2) \quad (3)$$

$$s = (BA_m W_m) + (CA_2 W_2) + (CA_m W_m) + (FU_m W_m) + (OA_m W_m) \quad (4)$$

where BA_1 , BA_2 , and BA_m represent the abundances of bacterial-feeding nematodes belonging to c-p 1, 2, and m, respectively, FU_2 and FU_m and CA_1 and CA_m represent the abundances of fungal-feeding and carnivorous nematodes belonging to c-p 2 and m, respectively, OA_m represents the abundance of omnivorous nematodes belonging to c-p m, and W_1 , W_2 , and W_m are the weights assigned to nematodes belonging to c-p 1, 2, and m, respectively. Subsequently, the Enrichment and Structure indices were calculated as:

$$EI = 100 \times \frac{e}{e + b} \quad (5)$$

$$SI = 100 \times \frac{s}{s + b} \quad (6)$$

where EI is the enrichment index and SI is the structure index.

Using the regression-based DI modelling approach [44], the responses were regressed on the sown proportional contributions of the six plant species. The general formula of a DI model is:

$$y = \sum_{i=1}^S \beta_i P_i + \sum_{\substack{i,j=1 \\ i < j}}^S \delta_{ij} P_i P_j + \epsilon \quad (7)$$

Where y is the response variable, S is the number of species, and P_i the sown proportion of a species i . The β_i term represents a species identity effect i.e., the predicted response for $P_i = 1$ (a monoculture of species i) is β_i , and when $P_i < 1$, the contribution of that species' identity to the mixture is $P_i \beta_i$. The coefficient for the interaction effect δ_{ij} , reflects the strength of the pairwise interaction effect between species i and j , and ϵ is a normally distributed error term. The δ_{ij} value can be negative, neutral or positive, representing an antagonistic, neutral or synergistic mixing effect between a pair of species on the measured response. Based on relative abundances of the plant species, the response of a plant community with differing proportions of species can thus be estimated from the proportional combination of the identity effects and interaction effects. The DI Models package in R [44] can test different biological assumptions about the interaction effects [43]. We modelled each of the response variables using the following four models: 1) the species identity model, in which effects of plant diversity are explained by linear combinations of the plant species' identity effects (their estimated monoculture performance), with no additional effect of interspecific interactions (i.e. no synergistic or antagonistic effects); 2) the average pairwise interactions model, which includes plant identity effects and a mixing effect that is best explained by the average of the pairwise interspecific interactions; 3) the functional groups model, which includes plant identity effects and a mixing effect that is best explained by interactions between functional groups (i.e. grasses, legumes and herbs in this case), and; 4) the full pairwise model (plant identity effects and a mixing effect that is best explained by inclusion of all pairwise interspecific interactions) [43,44]. For each response, the models were compared and the best model selected (based on the F statistic). Predictions from the final fitted model were used to assess the effects of plant diversity on the response variables, and displayed using a ternary diagram. In this way, the sampling points across the design space represented by three functional groups in Fig. S1 can inform the visualisation of a response surface.

All statistical analyses were performed using the R software (v. 4.2) [51].

3. Results

3.1. Effect of plant diversity on nematode community composition and structure

A total of 27 nematode taxa were identified (Table S2). The top five most abundant taxa were *Cephalobus*, *Rhabditidae*, *Tylenchidae*, *Aphelenchus* and *Heterodera* (Table S2). Generally, the relative abundance of each feeding group across the plant communities was only significantly different in the equi-proportional six-species plant community. It had a significantly higher relative abundance of omnivores (except for chicory, $P > 0.05$) and predators but a lower relative abundance of herbivores, compared to the monocultures (Fig. 1). There was no significant difference in the relative abundance of bacterivores among all plant communities. The relative abundance of fungivores in chicory was significantly higher than in both *Trifolium* species (Fig. 1, Table S3). In general, there was a higher abundance of herbivorous nematodes in the grasses compared to the herbs and the six species mixture (Fig. S3).

The nematode abundance data was used to compute the NMDS (Fig. 2). All individual plant species monocultures were significantly different from the equi-proportional six species mixture (Fig. 2A; pairwise Adonis $P < 0.05$). SIMPER analysis showed that these differences were related to higher abundances of *Tripyla*, *Dorylaimus*, *Clarkus*, *Pristionchus* and *Alaimus* in the six species mixture compared to the plant monocultures (Table S2). Indicator species analysis showed that *Tripyla*, *Dorylaimus*, *Clarkus* were exclusively associated with the six species mixture, *Rotylenchus* was exclusively associated with timothy, while other nematode taxa could be strongly associated with more than one plant species e.g. *Helicotylenchus* was associated with both timothy and perennial ryegrass (Table 1). In addition, there was a highly significant effect of plant species richness on the nematode community structure (PERMANOVA; $P < 0.001$; Fig. 2B), with the plots with one plant species significantly different from those with two plant species. SIMPER analyses showed that this difference was associated with significantly higher abundances of *Pratylenchus* and *Helicotylenchus* in plots with one plant species compared to plots with two plant species (Table S2). Moreover, apart from plots with four species, all other plots were significantly different from the plots with equi-proportional six species forage swards (Fig. 2B; pairwise Adonis $P < 0.05$). These differences were attributed to significantly higher abundances of *Tripyla*, *Clarkus*,

Pristionchus, *Mononchus*, *Chiloplacus*, *Aporcelaimus* and *Criconema* in the plots with all six plant species (Table S2). Indicator species analysis based on the plant species richness revealed that *Tripyla*, *Pristionchus* and *Clarkus* were associated with plots with all six plant species (Table 1). There was also a significant (PERMANOVA; $P < 0.05$; Fig. 2C) effect of functional group richness on nematode community structure. Although plots with two and three functional groups were not different from each other (Fig. 2C; pairwise Adonis $P > 0.05$), plots with one functional group were significantly different from plots with two and three functional groups (Fig. 2C; pairwise Adonis $P < 0.05$). SIMPER analysis revealed that these differences were attributed to a significantly higher abundance of *Panagrolaimus*, *Helicotylenchus* and *Pratylenchus* in plots with one plant functional group, and a higher abundance of *Dorylaimus*, *Tripyla*, *Clarkus*, *Pristionchus*, *Alaimus* and *Chiloplacus* in plots with all three plant functional groups (Table S2).

3.2. Relationship between nematode community structure and other measured plant and soil variables

Variables that were significantly correlated with nematode community structure were identified and visualised in an RDA (Fig. 3). The total annual aboveground dry matter yield (ranging from 4735 to 14,132 t ha⁻¹ yr⁻¹), total annual nitrogen harvested in aboveground plant tissue (ranging from 73.3 to 448.8 kg ha⁻¹ yr⁻¹), and annual dry matter yield of unsown species (proportion of weeds) (ranging from 200 to 3824 t ha⁻¹ yr⁻¹) were the variables that correlated with the change in nematode community structure. Within this field experiment, these variables were highly correlated with plant diversity [22]. The nematode community in the equi-proportional six species mixture was positively associated with the total annual aboveground dry matter yield but was negatively associated with the annual dry matter yield of unsown species (Fig. 3A). The annual dry matter yield of unsown species (proportion of weeds) were mostly associated with nematode communities in the monocultures (Fig. 3B) and plots with only one functional group of plants present (Fig. 3C).

3.3. Strong effects of plant species diversity on nematode feeding groups and soil quality indices

The DI modelling allowed us to identify whether there were syner-

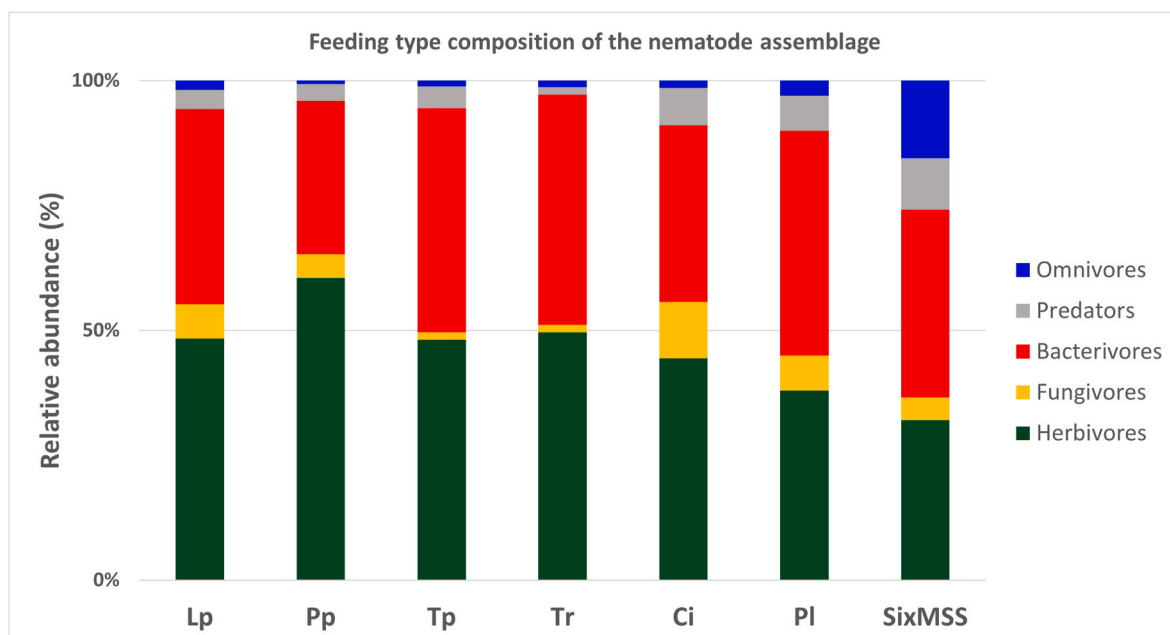


Fig. 1. Mean relative abundances (%) of the nematode feeding groups in the monocultures and the equi-proportional six-species multi-species swards.

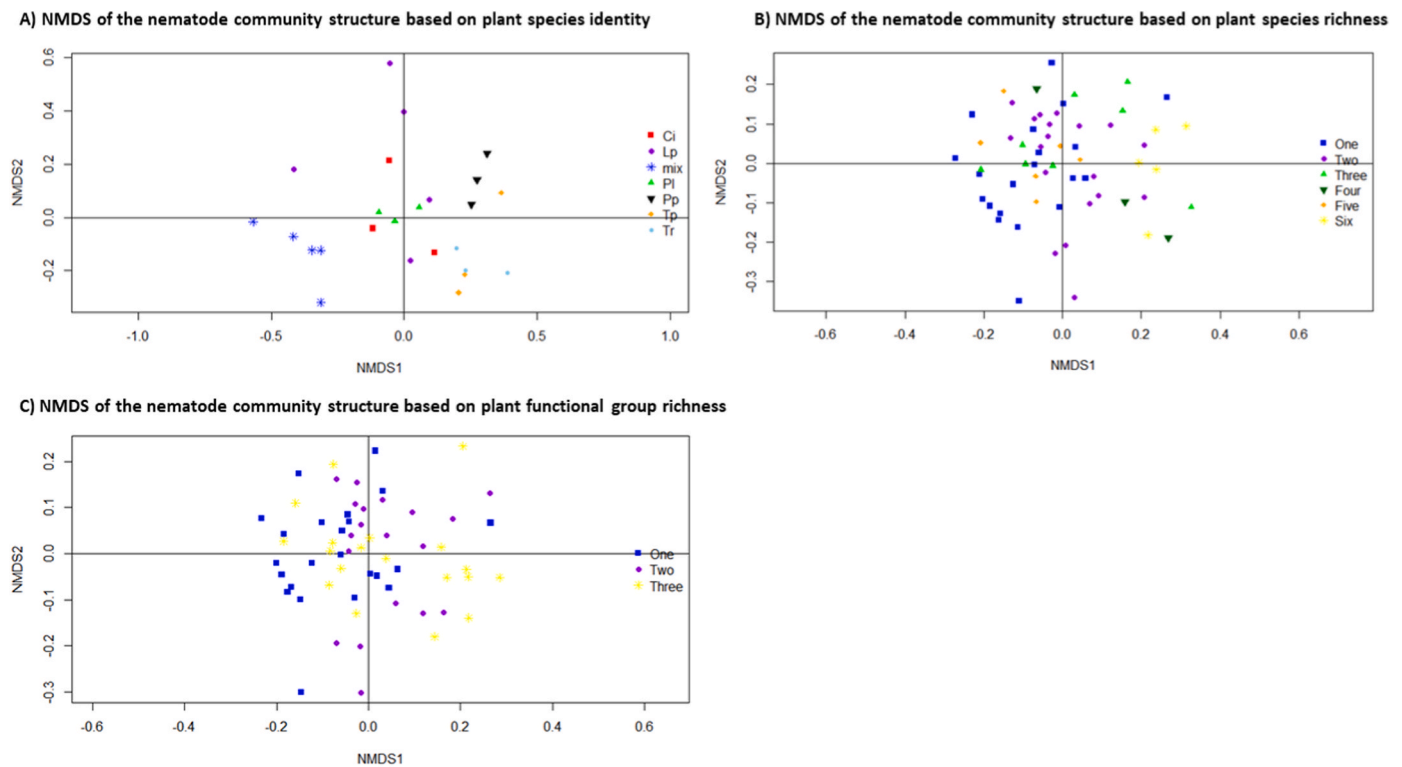


Fig. 2. NMDS of nematode community structure based on A: monocultures and the equi-proportional six-species multi-species swards; B: plant species richness; C: functional group richness.

Table 1

Indicator species (nematode taxa that preferentially significantly occurred within treatment groups) in aspects of plant diversity (monocultures and the six-species mixture, plant species richness and plant functional richness). This is based on ‘indicator species’ analyses. The ‘statistic’ quantifies the association between the presence/abundance of a taxa and a treatment group (helps determine whether a particular taxa is significantly associated with a certain group).

Treatment group	Indicator taxa	statistic	P value
Monocultures and six-species mixture			
Six-species mixture	<i>Tripyla</i>	0.931	0.0003
	<i>Dorylaimus</i>	0.808	0.0011
	<i>Clarkus</i>	0.740	0.0103
<i>Phleum pratense</i>	<i>Rotylenchus</i>	0.722	0.0181
<i>Lolium perenne, Phleum pratense</i>	<i>Helicotylenchus</i>	0.718	0.0126
<i>Plantago lanceolata, Phleum pratense</i>	<i>Trichodorus</i>	0.757	0.0088
<i>Cichorium intybus, Trifolium pratense, Trifolium repense</i>	<i>Pratylenchus</i>	0.68	0.0212
<i>Lolium perenne, Plantago lanceolata, six-species mixture</i>	<i>Cephalobus</i>	0.698	0.0162
Plant species richness			
Six-species mixture	<i>Tripyla</i>	0.920	0.0002
	<i>Pristionchus</i>	0.618	0.0076
	<i>Clarkus</i>	0.599	0.0126
Plant functional group richness			
One	<i>Helicotylenchus</i>	0.347	0.0053
Three	<i>Tripyla</i>	0.469	0.0009
	<i>Clarkus</i>	0.363	0.0100
	<i>Pristionchus</i>	0.314	0.0322

gistic or antagonistic effects of plant diversity on nematodes; it also enabled us to quantify and visualize the effect of plant diversity on nematodes as a continuous gradient of changes in relative abundance of plant functional groups that encompasses changes in composition (identity effects), richness and variation in relative abundance within richness levels (Fig. S1). Following model comparisons, the best DI model for the nematode-based soil quality indices, except for CI and

abundance of fungivores, was the average pairwise interaction model:

$$y = \beta_1 P L p + \beta_2 P P p + \beta_3 P T p + \beta_4 P T r + \beta_5 P C i + \beta_6 P P i + \delta_{AV} \sum_{i,j=1}^s P_i P_j + \epsilon \tag{8}$$

Where y is the response variable, S the number of species, and P_i the sown proportion of a species (with subscripts $Lp = L. perenne$, $Pp = P. pratense$, $Tp = T. pratense$, $Tr = T. repens$, $Ci = C. intybus$, $Pi = P. lanceolata$). Each β_i term stands for a species identity effect i.e., the predicted response for $P_i = 1$ (a monoculture of species i) is β_i , and when $P_i < 1$, the species identity contribution to the mixture is $P_i \beta_i$. The sum $\sum_{i < j} P_i P_j$ is a measure of the distribution of the relative abundances of the plant species in the community (a measure of evenness within a six-species plant community); the pairwise interaction effects did not differ significantly from each other in this model, and were best described by an average of the pairwise interaction coefficients, δ_{AV} [43]. Overall, there were strong effects of plant diversity on the nematode-based soil quality indices, which were derived from differences in plant species identity effects and, where they occurred (see values for ‘average interaction effect’ in Table 2 for MI, c-p 3–5, EI, SI and omnivores + predators), an interaction effect related to relative abundances of plant species in a six-species mixture (Table 2). The changes in relative proportions and composition across the plant communities had a strong effect on most of the measured responses for the nematode community (Fig. 4). The modelled performance of the equi-proportional six-species community was better than or equal to the best-performing monoculture for all of the nematode soil quality indices (Table 2, Fig. 4). For the responses with significant interaction effects, there was a relatively robust response of the nematode community metrics to variation in the plant community composition around the best-performing six-species community (Fig. 4).

Here, we look in more detail at the effect of plant community

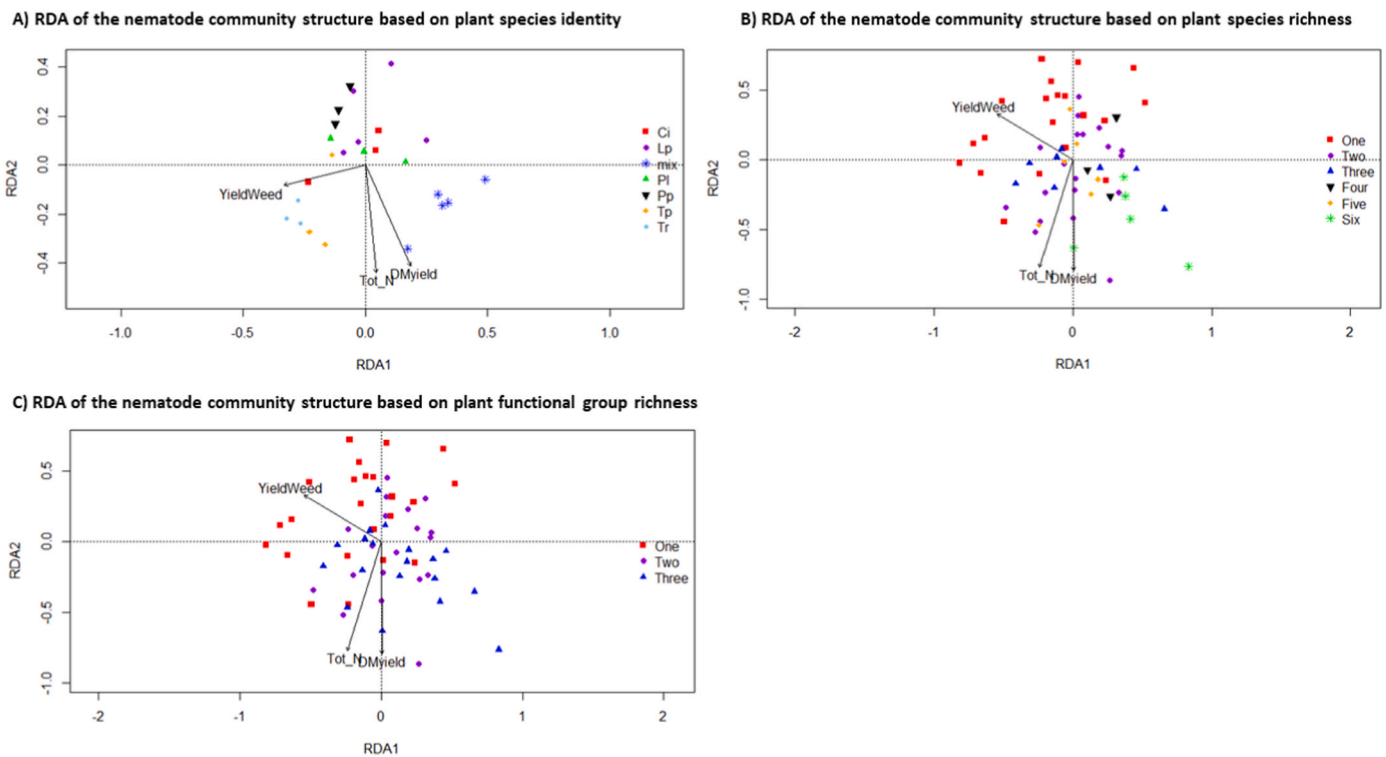


Fig. 3. Redundancy analysis (RDA) of the nematode abundance data across A: monocultures and the equi-proportional six-species multi-species swards; B: plant species richness; C: functional group richness. Direction and length of the fitted vectors (explanatory variables) are proportional to the correlation between the two RDAs and each measured variable ($P < 0.05$). The explanatory variables shown (DMyield = total annual aboveground dry matter yield, Tot_N = total annual nitrogen in harvested aboveground plant tissues; YieldWeed = annual dry matter yield of weeds) are those that are significantly impacting the nematode community structure.

Table 2

Coefficient estimates \pm standard errors for the identity effects and average interaction estimates from the best of the fitted DI models. These are presented for each of the responses, which were either a model with average interaction effects (values shown) or a model with identity effects only (without interaction effects and indicated by '-'). Within each response, we compared the values among the different species, and we also present and compare the estimated values for the equi-proportional six-species mixture.

Plant community	MI	c-p 3–5 (log 100 ⁻¹ g soil)	EI (%)	SI (%)	CI (log)	Bacterivores (log 100 ⁻¹ g soil)	Herbivores (log 100 ⁻¹ g soil)	Fungivores (log 100 ⁻¹ g soil)	Omnivores + Predators (log 100 ⁻¹ g soil)
<i>Lolium perenne</i>	1.86 \pm 0.10 ^{ab}	4.3 \pm 0.4 ^a	71.8 \pm 5.4 ^{ab}	43.0 \pm 6.8 ^a	2.16 \pm 0.4 ^b	6.36 \pm 0.2 ^a	6.53 \pm 0.1 ^a	3.89 \pm 0.6 ^b	4.3 \pm 0.4 ^a
<i>Phleum pratense</i>	1.84 \pm 0.13 ^{ab}	4.0 \pm 0.5 ^a	76.1 \pm 6.7 ^{ab}	50.7 \pm 0.5 ^b	2.00 \pm 0.5 ^b	5.93 \pm 0.2 ^a	6.37 \pm 0.2 ^a	3.39 \pm 0.7 ^b	4.0 \pm 0.5 ^a
<i>Trifolium pratense</i>	1.65 \pm 0.13 ^{ab}	3.3 \pm 0.5 ^a	81.2 \pm 6.7 ^b	41.1 \pm 8.5 ^a	0.68 \pm 0.5 ^a	5.98 \pm 0.2 ^a	6.06 \pm 0.2 ^a	1.20 \pm 0.7 ^a	3.3 \pm 0.5 ^a
<i>Trifolium repens</i>	1.58 \pm 0.12 ^a	3.7 \pm 0.5 ^a	81.2 \pm 6.4 ^b	36.4 \pm 8.1 ^a	0.87 \pm 0.5 ^a	6.33 \pm 0.2 ^a	6.08 \pm 0.2 ^a	2.00 \pm 0.7 ^a	3.7 \pm 0.5 ^a
<i>Cichorium intybus</i>	1.90 \pm 0.13 ^b	4.0 \pm 0.5 ^a	71.3 \pm 6.7 ^{ab}	44.3 \pm 8.5 ^a	3.05 \pm 0.5 ^b	6.00 \pm 0.2 ^a	5.87 \pm 0.2 ^a	5.14 \pm 0.2 ^b	4.0 \pm 0.5 ^a
<i>Plantago lanceolata</i>	2.01 \pm 0.13 ^b	4.6 \pm 0.5 ^{ab}	65.9 \pm 6.7 ^{ab}	50.3 \pm 8.5 ^a	3.04 \pm 0.5 ^b	6.13 \pm 0.2 ^a	6.25 \pm 0.2 ^a	4.90 \pm 0.7 ^b	4.6 \pm 0.5 ^{ab}
Average interaction effect	1.14 \pm 0.23	2.3 \pm 0.9	-40.6 \pm 11.9	30.8 \pm 15.1	-	-	-	-	2.3 \pm 0.9
Six-species mixture	2.50 \pm 0.04 ^c	4.9 \pm 0.5 ^b	59.6 \pm 4.4 ^a	75.1 \pm 1.6 ^b	1.97 \pm 0.5 ^b	6.21 \pm 0.2 ^a	6.19 \pm 0.2 ^a	3.42 \pm 0.7 ^b	4.9 \pm 0.5 ^b

Means \pm standard error; different letters within a column depict statistically significant differences ($P < 0.05$).

composition on individual responses. The modelled equi-proportional six-species mixtures had significantly higher MI and SI values than any of the plant monocultures. It had the lowest EI value (Table 2, Fig. 4) but was only significantly lower than the two *Trifolium* species. Compared to a selection of monocultures, the equi-proportional six-species mixture had significantly ($P < 0.05$) higher abundance of c-p 3–5 nematodes (sensitive taxa, k strategists) with the exception of *Plantago lanceolata*. *Trifolium pratense* and *Trifolium repens* had the lowest CI and

lowest abundance of fungivores (Table 2). For the abundances of bacterivores and herbivores, the effects of different plant species (the identity effects) were not significantly different from each other.

4. Discussion

Intensively managed grasslands cover a large area of temperate regions, and there is significant policy and societal pressure to improve

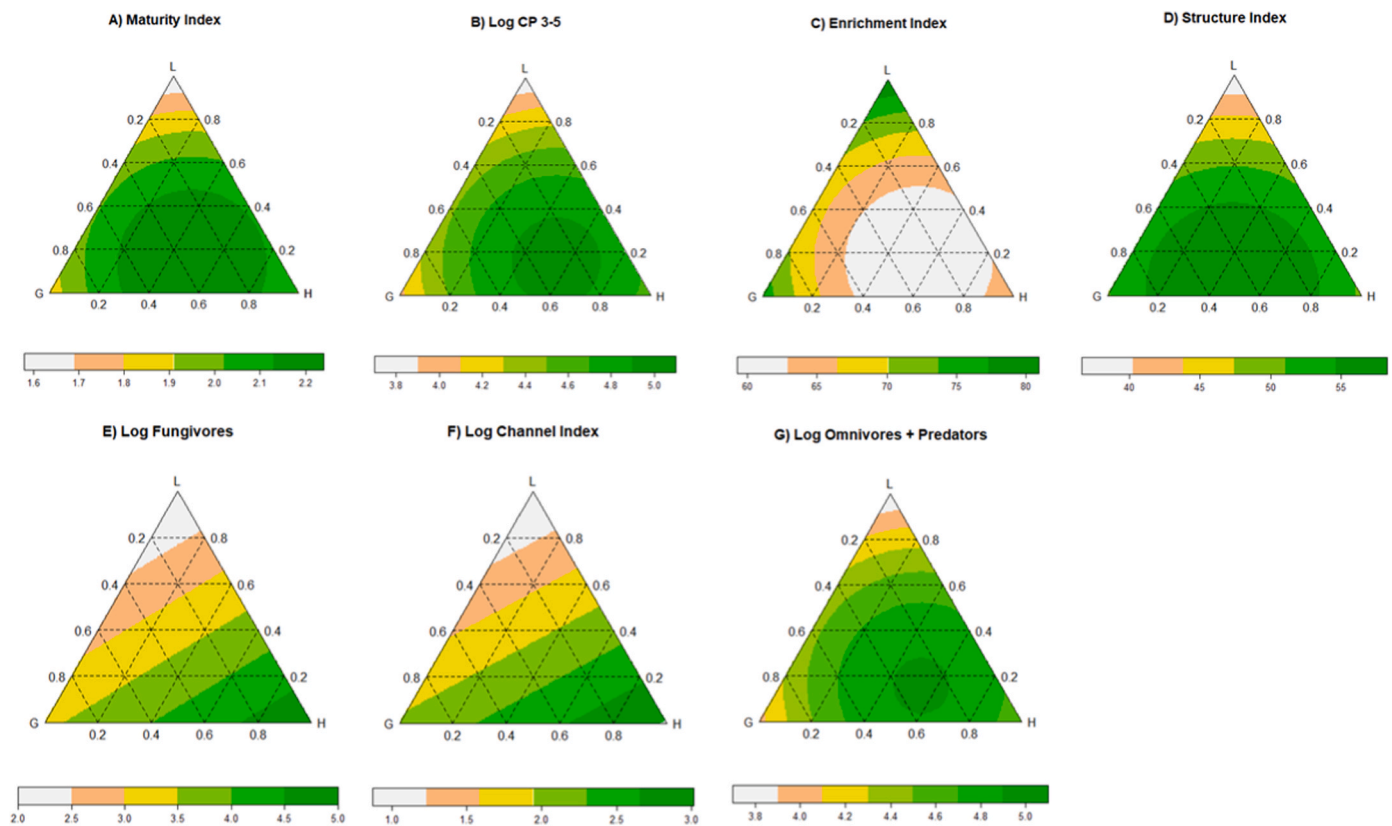


Fig. 4. Contour plots showing the model predictions of A) Maturity Index (MI), B) abundance of *c-p* 3–5 taxa 100 g^{-1} soil (log CP 3–5), C) Enrichment Index (EI, %), D) Structure Index (SI, %), E) abundance of fungivores (log Fungivores), F) Channel Index (log CI), and G) abundance of omnivores and predators (log Omnivores + Predators). Predictions are for two species (both from one functional group – the vertices in each ternary diagram), for four species (with two species from each of two functional groups – the sides of each ternary diagram) or for all six species (three functional groups – all interior points in the ternary diagrams). For example, prediction for 100% legume is for 50% *T. pratense* and 50% *T. repens*; prediction for 40% grass and 60% herb is for 20% *L. perenne*, 20% *P. pratense*, 30% *C. intybus* and 30% *P. lanceolata*; while prediction for 10% grass, 40% legume and 50% herb is for 5% *L. perenne*, 5% *P. pratense*, 20% *T. pratense*, 20% *T. repens*, 25% *C. intybus* and 25% *P. lanceolata*. The edges of the triangles labelled G, L, and H represent grass, legume, and herb, respectively.

their sustainability (agronomic, financial, social and environmental). To date, multi-species mixtures have demonstrated multiple benefits for agronomy and livestock production, and enhanced environmental outcomes; increased yield (including with lower nitrogen fertiliser application) [21–23], increased livestock production and animal health [65, 66], mitigation of drought, reduced N_2O emissions [22,63], and weed suppression [67]. However, there is a limited knowledge base on the effect of increased plant species diversity on belowground soil processes and biodiversity (positive effects on earthworms have been observed [64]); here, we conducted a novel manipulation experiment that quantified the effect of plant diversity on nematode communities in intensively managed temperate grassland communities.

In the present study, plant diversity had a significant impact on the nematode community composition and structure. These changes were mostly associated with higher abundances of sensitive nematode taxa (especially *Tripyla*, *Dorylaimus*, *Clarkus*, and *Pristionchus*) in the six-species mixtures and other plant communities with all three functional groups. These nematode taxa are mostly omnivorous and predatory nematodes and are linked to a higher complexity and structure in the soil food web [30]. Also, *Rotylenchus*, *Pratylenchus*, and *Helicotylenchus* were associated mostly with timothy, perennial ryegrass, plots with only one plant species, and plots with only one plant functional group. These are plant parasitic nematodes and they are highly prevalent in intensive agricultural systems especially in monocultures [68]. The changes in the nematode community structure were related to other measured soil and plant data using an RDA. In all, the changes in nematode community structure were significantly associated with total annual aboveground dry matter yield, total annual nitrogen harvested in aboveground tissue,

and annual dry matter yield of unsown species (proportion of weeds), factors that were also highly correlated with plant diversity [22]. Dry matter yield and N content in herbage have been associated previously with changes in nematode community structure [61]. Also, increased plant species diversity can enhance weed suppression [23]. Weeds can harbour specific nematode communities [62] which can lead to differences in nematode community structure.

An especially novel element of this study is the use of the DI modelling approach to quantify the effect of plant species on the nematode community, and quantification and visualisation of the effect of changes in the relative proportion of multiple plant species. To date, most experiments on plant diversity*nematode interactions have investigated the effects of plant species richness alone. In contrast, our design enabled us to simultaneously assess the effect of changes in plant species composition, richness and relative abundance (evenness). Our design and analysis allowed us to identify the separate effects of plant species identity and species interactions on nematode communities. Where it occurred, the strength of the interspecific interactions was sufficiently strong for the performance of the equi-proportional six-species community to be better than or equal to the best-performing monoculture for all of the indices. This means that no trade-offs among indices occurred and all impacts of diversifying the sward on nematode communities were positive. These results have strong implications for the potential role of multi-species grassland mixtures as a practical farm management option to maintain or improve soil health and belowground biodiversity in intensively managed grasslands.

Using a field trial, we found that increasing plant species diversity (from one to six species of three plant functional groups) resulted in an

increased abundance of omnivores and predators, and sensitive taxa (c-p 3–5 nematodes). Dietrich et al. [52] reported an indirect positive influence of plant species richness (in semi-natural grassland communities) on omnivorous and predatory nematodes. Higher shoot mass, and consequently a denser vegetation associated with increased plant species diversity can lead to a more stable microenvironment, enabling a higher abundance of large nematodes (K-strategists) comprising mainly of omnivores and predators [53]. In our study, there was no effect of increasing plant species diversity on the bacterivores, herbivores and fungivores. In contrast, De Deyn et al. [42] reported that increased abundance of bacterivores and herbivores was associated with specific plant species, while the abundances of omnivores and predators remained unaffected. However, their study had a higher plant species richness (up to 16 species) and was managed less intensively compared to our study. Also, Cortois et al. [54] reported that increased plant species diversity resulted in increased abundance of all nematode feeding groups, except for predatory nematodes; their species richness went as high as 60 plant species. Although a higher plant species richness is associated with extensively managed grasslands, maintenance of high plant species richness is difficult in intensively-managed grasslands.

Our study systematically manipulated the diversity of functional groups, which have distinctive properties that can affect nematode communities. *Trifolium pratense* and *Trifolium repens* (legumes) had the lowest abundance of fungivores and CI, but the highest value of EI. This indicated a bacterial-dominated decomposition pathway in the soils of the legume monocultures. Legumes can have higher values of EI as a result of increased abundance of r-selected nematode taxa belonging to c-p 1 [42]. Legumes can fix atmospheric N₂ leading to higher N in the rhizosphere [55], resulting in rapid bacterial growth. This can in turn lead to a higher abundance of r-selected bacterivores (enrichment opportunist taxa) and hence the higher EI but lower MI in the legumes. Increasing plant functional group diversity has been shown to also enhance taxon richness of predatory nematodes [55]. Predatory nematodes can control plant pests especially via feeding on plant parasitic nematodes. They can also release nutrients for plants by feeding on other nematodes [69]. Furthermore, specific nematode feeding groups are associated with specific plant functional groups [40–42], linked to differences in quality and quantity of resources [56]. Also, even species belonging to the same plant functional group can harbour different nematode communities [42,57]. Indeed, we found significant differences in the abundances of *Rotylenchus* and *Tylenchus* between timothy and perennial ryegrass (both grasses); and also significant differences in the abundances of *Eucephalobus* and *Trichodorus* between chicory and plantain (both herbs).

Plant diversity had strong effects on the nematode community. The equi-proportional six species forage swards (which included all three plant functional groups) had a higher abundance of omnivores and predators, higher c-p 3–5 taxa (large K- strategists, sensitive taxa), MI and SI compared to the individual monocultures and plant functional groups. A possible explanation may be increased variety and spatial deposition of root exudates linked to the higher plant diversity, which in turn can lead to increased growth and activity of the soil biota [58–60]. Different plant species can produce different types and quantities of root exudates, and these can be deposited at different depths, reflective of plant rooting depth and architecture [70]. Disentangling the mechanisms that produce the pattern of strong plant diversity effects is a fruitful topic for further investigation.

5. Conclusions

Within more intensively managed grasslands, multi-species forage mixtures that include grasses, legumes, and herbs can have a positive effect on the soil nematode community and nematode-based soil quality indices. The performance of the equi-proportional six-species mixture was better than or equal to the best-performing monoculture for all of

the indices, meaning no trade-offs in the positive effects occurred among the measured responses. Higher plant diversity was associated with a lower abundance of herbivores, the presence of which can negatively affect plant performance. Also, a higher abundance of predatory nematodes, that can control plant pests, was associated with higher plant diversity. This is of practical relevance for farmers seeking to enhance soil biodiversity and for EU agricultural policy aimed at sustainability, soil health and farming for biodiversity benefits.

Author contributions

JF co-designed the original field experiment. GG conducted field work. II performed the laboratory and data analyses. II, GG, JAF & FPB wrote the paper. All authors reviewed the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data and code is available from the Teagasc TStr repository at <http://hdl.handle.net/11019/3201>.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejsobi.2023.103542>.

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