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Combined effects of aboveground herbivores and belowground microorganisms on dynamics of soil nematode communities in grassland mesocosms

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ABSTRACT

Nematodes are the most abundant animals in soil. They are active in all trophic levels and functionally important for plant growth and plant diversity. Nematode community structure not only can be directly influenced by other belowground organisms such as soil microbes via trophic interactions, but also indirectly by aboveground organisms like herbivores through plant-mediated aboveground-belowground linkages. In the current study, by introducing foliar-feeding aphids (*Rhopalosiphum padi*) and soil microbial suspensions to mesocosms planted with 12 grassland plant species and where an identical nematode community was introduced in all mesocosms, we aimed to investigate the individual and combined effects of soil microbes and foliar herbivores on the dynamics of plant and the soil nematode community. Introduction of aphids reduced shoot and root biomass of the plant community, and in particular decreased the proportional biomass of the dominant plant species *Anthoxanthum odoratum*, resulting in a higher diversity of the plant community but without affecting the nematode communities. In contrast, the inoculation of soil microbes did not significantly alter plant composition structure, but it reduced the total nematode abundance and enhanced nematode diversity by increasing the abundance of carnivorous nematodes and decreasing the abundance of plant-feeding nematodes. There were no significant aboveground-belowground interactions in the current study via effects of aphids on the soil nematode communities or via soil microbes and nematodes on the plant communities. Collectively, our study indicates that soil nematode communities in grasslands can be strongly steered by soil microbial inoculations but weakly influenced by aboveground herbivory despite its resulting changes in plant communities, notwithstanding that these effects appeared to be largely independent.

1. Introduction

Nematodes are the most abundant animals in soil and of vital importance for a variety of ecosystem functions (van den Hoogen et al., 2019). Bacterial- and fungal-feeding nematodes can steer the soil microbial community via their feeding behavior, and contribute to carbon turnover and nutrient cycling as well as biotic interactions in soil (Ranoarisoa et al., 2020). Plant-feeding nematodes can directly affect the performance of specific plant species and thus can contribute to the dynamics and diversity of plant communities and to related ecosystem functioning (Wilschut and Geisen, 2021). Other nematode groups include omnivores and carnivores that influence ecosystems via trophic interactions with microbial- and plant-feeding nematodes. According to the reproductive and life history strategies of these nematode groups, a

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number of ecological indices have been proposed to indicate ecosystem states given the high sensitivity of nematode communities to environmental changes (Wang et al., 2021; Kamath et al., 2022). These nematode-based ecological indices, such as the maturity index (MI) and the structure index (SI), are widely considered reliable inferences to a variety of disturbance sources and the corresponding recovery of disturbed ecosystems can be inferred (Bongers, 1990; Ferris et al., 2001). Even though many studies have examined responses of soil nematode communities to a wide range of disturbances, such as grazing by livestock (Zhang et al., 2022), agricultural practices (Puissant et al., 2021), and water and nitrogen additions (Song et al., 2016), thus far that how the simultaneous occurrences of multiple disturbances interact to steer soil nematode communities remains poorly understood (Wang et al., 2020; Chen et al., 2021; Renčo et al., 2022).

Soil microbes are key component in soil communities and recognized as the main food resource for microbial-feeding nematodes, and therefore can directly influence the structure of soil nematode communities (Su et al., 2021). This has been reported by studies that focus on nematode-based soil food webs, in which the results indicate strong positive relationships between the diversity of microbial taxa and nematode genera, as well as covariation of their abundance (Briar et al., 2011; Papatheodorou et al., 2012; Neilson et al., 2020; Milkereit et al., 2021). Moreover, soil microbes contain soil-borne pathogens and symbionts, as well as free-living species that participate in vital soil processes, such as nutrient cycling, which all directly or indirectly feeds back to shifts in plant communities that may alter food availability to plant-feeding nematodes and thus the composition of the entire nematode community (Yeates and Coleman, 2021). Yet, thus far nearly all these studies simply reveal correlations between soil microbial and nematode communities, and very few have investigated how experimental inoculation with soil microbes can influence the community dynamics of soil nematodes.

In addition to belowground biota like soil microbes, aboveground biota, such as herbivores can also affect soil nematode communities via above-belowground interactions (Wardle et al., 2004a, 2004b; Mulder et al., 2008; Veen et al., 2010; Hu et al., 2015; Andriuzzi and Wall, 2017). Such effects induced by aboveground herbivory may take place via various pathways depending on the feeding mode of soil nematodes (Bennett et al., 2018; Frew et al., 2018). For example, shifts in plant-feeding nematodes can be driven by herbivore-induced changes in the plant community as some plant species are preferred over others by the nematodes, while effects on bacterial- and fungal-feeding nematodes can be driven by soil nutrient availability and the soil physiochemical environment that can be altered e.g. as a result of vertebrate grazing (Chen et al., 2013). Aboveground mammal and invertebrate herbivory of subalpine grasslands altered plant quantity and quality, and this steered the communities of soil nematodes, e.g. reducing the abundance of all nematode feeding types except plant feeders (Vandegehuchte et al., 2017). Although responses of soil nematodes to aboveground herbivory have been extensively explored, the majority of them have focused on responses to grazing by large mammals (Andriuzzi and Wall, 2017; Wang et al., 2020; Zhang et al., 2022; Pan et al., 2022). The consequences of aboveground herbivory by phytophagous insects remain elusive (De Deyn et al., 2007). More importantly, despite the potential impacts of both soil microbes and aboveground herbivory through changes in soil food webs or above-belowground linkages, their combined effects as well as their relative contribution to the dynamics of soil nematode communities have been less well studied (Wasof et al., 2019).

To understand the independent and combined effects of aboveground insect herbivory and soil microorganisms on structuring soil nematode communities, in the current study we established a mesocosm experiment in which we introduced soil microorganisms and aboveground aphids sequentially to a diverse plant community. We hypothesized that (1) inoculation of soil microorganisms particularly enhances abundance of microbial-feeding nematodes, thus leading to a shift in nematode abundance and community composition towards microbial-feeding nematodes; (2) introduction of aboveground aphids that feed on grasses reduces the productivity of the plant community that is dominated by grasses, leading to a lower abundance of the soil nematode community; (3) Feeding on grasses by aphids promotes the relative abundance of the often-subordinate forbs in the plant community, thus resulting in a higher diversity of plant community, and consequently also a higher diversity of soil nematode community; (4) the effects of soil microorganisms on the soil nematode community will be counteracted when aphids are present.

2. Materials and methods

2.1. Experimental design

Soil used in the study was a sandy loam soil (particle size: < 2 μm 3 %; 2–63 μm 17 %; > 63 μm 80 % and 3.5 % organic matter) that was collected from a perennial grassland at "de Born" in Wageningen (51°59′N, 5°40′E). The soil was taken using a surface-sterilized shovel from the 0–10 cm soil layer of soil after removal of all plantation on the ground, and immediately hand-mixed in plastic bags. A small subset of the collected soil was randomly sealed in a plastic bag and stored at 4 °C for nematode and microbial inoculation (see below). The remaining soil was sieved through a 1-cm mesh to remove stones and plant tissues and fully mixed before being sterilized with gamma irradiation of >25 K Gray (Cobalt 60 at Isotron Ede, The Netherlands). We prepared 40 8-L containers (17 cm × 17 cm × 20 cm, referred to mesocosms hereafter) that were each filled with 8 kg sterilized soil and randomly assigned to four treatments. Each of the filled containers was flushed with 2 L tap water to saturate the soil in order to remove nutrients released from sterilization (Troelstra et al., 2001). Afterwards, soil in all mesocosms was kept at 20 % (*w*/w) soil moisture to resemble the original soil moisture at collection, determined by weighing fresh and oven-dried sterilized soil. In each mesocosm, a plant community composed of 12 species was planted with one individual of each species. The species included three grasses (*Agrostis capillaris*, *Anthoxanthum odoratum, Festuca ovina*), eight forbs (*Achillea millefolium, Campanula rotundifolia, Cerastium fontana, Plantago lanceolata, Prunella vulgaris, Rumex acetocella, Jacobaea vulgaris, Tripleurospermum matricariae*) and one legume (*Lotus corniculatus*). These species typically co-occur in the natural restoration grassland where the experimental soil was collected. The artificial plant community consisted of fewer grass species than forb species and only included one legume, as such composition resembles a composition of natural Dutch grasslands (De Deyn et al., 2003). Also, due to the overall fast growth of grasses this design was chosen to prevent the rapid dominance of grasses in the composition (Wang et al., 2019). Seeds were surface sterilized using 4 % hypochlorite and rinsed with demineralized water for 5 min. Sterilized seeds were germinated on sterilized glass beads that were saturated by demineralized water and cultured in a climate chamber (16/8 h light/dark, 18/22 °C day/night). One 2-week-old seedling of each species was transplanted to each mesocosm in which the position of each species was randomized. Seedlings that died in the first week were replaced.

2.2. Experimental set-up

Preparation of soil microorganism inocula - Soil used for preparation of inocula was also collected from the site "de Born" in Wageningen. This site was grazed and fertilized until 1973 and regulated mown to collect hay twice a year afterwards and nitrogen was the limiting element. Soil was collected into plastic bags using a soil corer (5.3 cm in diameter) from the upper 10 cm layer and immediately transported to the laboratory. The soil microorganism inoculum was created by mixing 5 kg live soil with 5 L tap water. The soil suspension was thoroughly stirred for 1 min and left for 4 h to allow large soil particles to settle and the supernatant was sequentially sieved through one 75-μm sieve and two 45-μm sieves. This series of sieves removed most of the nematodes and other larger animals in soil, as well as the majority of arbuscular mycorrhiza (AM) but it allowed non-AM microorganisms and some eggs of nematodes to pass through. Therefore, the obtained inoculum of soil microorganisms via this approach may not include a vast abundance of AM and some small-sized nematode eggs (Wang et al., 2019).

Preparation of nematode inocula - Nematode inoculum was extracted from a total amount of 36 kg live soil suspended in 36 L tap water using the Cobbs' decantation and sieving method (Cobb, 1918). The extraction was performed on 6 kg of soil in 6 L water each time and repeated for 6 times. The soil suspensions were combined and went through one sieve of 180 μm, followed by one sieve of 75 μm and three sieves of 45 μm. Soil nematodes were collected from the 75- and 45-μm sieves and incubated for 48 h on two-layer filters (Hygia favorit, 220 mm in diameter; NIPA Instruments, Leeuwarden, The Netherlands) in tap water at room temperature. The obtained nematode suspension may not be free of soil microbes, but should include a relatively low abundance and diversity of soil microbes due to the high dilution used during the sieving procedure (Hol et al., 2010). Four samples of 1-ml suspension were collected and the nematode community was identified at genus or family level using an inverted light microscope (Olympus CK40, Germany) according to Bongers (1988) and further categorized to different feeding groups according to Yeates et al. (1993). The inoculated nematode community composition is presented in Table S1 (Wang et al., 2019).

Nematode and microorganism inoculation - Three weeks after transplantation, all mesocosms were inoculated with nematode communities. For the control treatment where only nematodes were introduced but no microorganisms (C), 20 mesocosms were each injected using a pipette with 1 ml of nematode suspension and 5 ml of tap water into soil adjacent to each of the 12 seedling positions. To create the soil microorganisms (MO) treatment, 20 other mesocosms were inoculated with 1 ml of nematode suspension, followed by 5 ml of microorganism inocula. All mesocosms were randomly placed in a climate-controlled greenhouse compartment with 60 % relative humidity, 16 h light: 8 h dark, and 20 ± 1 °C day: 14 ± 1 °C night. Natural daylight was supplemented by 400 W metal halide bulbs (1 per 1.5 m^2). Light intensity during the day was kept at ≥300 PAR (photosynthetic active radiation) throughout the experiment. The mesocosms were rotated once a week to avoid potential position effects within the greenhouse, and regularly watered by reweighing mesocosms to restore the soil to its initial humidity (20 %).

At 2, 4, and 6 months after the establishment of the soil treatments, aboveground biomass of the plant communities was clipped at 4 cm above soil surface, sorted to species, dried and weighed to estimate dynamics of the plant community following additions of soil microbial and nematode communities, and the results were reported in another study (Wang et al., 2019).

Aphid introduction - Ten weeks after the third clipping, all mesocosms were placed individually in a cylindrical mesh-cage with a zip that allows entrance into the cage (height 1.2 m, diameter 35 cm). In ten mesocosms per treatment (C and MO) we introduced 25 late-instar bird cherry-oat aphids (*Rhopalosiphum padi*) to create aphid treatments (AG and MO + AG, respectively). *R. padi* came from a culture initiated at the Laboratory of Entomology in Wageningen University & Research (Wageningen, The Netherlands). This aphid species exclusively feed on grasses and had been reared on barley (*Hordeum vulgare*).

Plant, soil and nematode measurements - Fourteen weeks after the introduction of aphids (12 months after the nematode and microorganism inoculation), the experiment was ended. All aboveground plant tissues were harvested using a scissor, sorted to species, and oven-dried at 70 °C to determine shoot biomass of each species. Four soil cores were taken using a soil sampler (2 cm in diameter) from each mesocosm. The subsamples were homogenized and analyzed to determine the concentration of available nitrogen (NO_3^- and NH_4^+), phosphorus (PO_4^-) after $CaCl_2$ extraction (Houba et al., 1996). Due to the dense root structure in each pot it was impossible to separate roots per species, and thus the whole root system in each mesocosm was rinsed, washed clean with tap water and oven-dried at 70 °C to determine root biomass of the whole plant community. Four additional soil cores (2 cm diameter) were collected from each mesocosm at harvest and gently mixed in a plastic bag. Nematodes were extracted from 100 g fresh soil using an Oostenbrink elutriator (Oostenbrink, 1960). Nematodes were counted and identified (ca. 150 individuals per sample) to genus or family and allocated to feeding groups. During the harvest, several mesocosms were missed due to the unexpected mistakes. Eventually, there were 9 replicates for treatment MO + AG, 9 replicates for treatment C and AG, and 8 replicates for treatment MO for plant measurement. Thus there were 9, 9, 9 and 8 replicated mesocosms per treatment for soil and nematode measurements.

2.3. *Plant and nematode community analyses*

Plant and nematode community diversity were calculated using the Shannon-wiener diversity index (H′): $H' = -\sum P_i \ln P_i$ (Shannon and Weaver, 1949), and Evenness index (J'): $J' = H'/\ln(S)$ (Pielou, 1974), where P_i represents the proportion of nematode abundance or aboveground biomass of the i^{th} individual nematode genus or plant species to the total nematode number or total aboveground biomass in a mesocosm, respectively, and S represents number of genus or family in a sample. The species richness index (SR) was also computed using $SR = (S-1)/\ln(N)$ (Heltshe and Forrester, 1983).

The identified nematodes were divided into five feeding groups: plant-feeding (PF), bacterial-feeding (BF), fungal-feeding (FF), omnivorous (OM) and carnivorous (CA) nematodes, and the abundance of each feeding group was calculated as the sum of abundance of nematode genera or families within the respective group. Nematodes can be classified along a colonizer-persister (c-p) scale that represents life-history strategy associated with r- or K-selection. Colonizers with high reproduction rates receive a low c-*p* value and persisters that reproduce slowly are given high c-*p* values (Bongers and Bongers, 1998). The maturity index (MI) was estimated as $MI = \sum v(i) \cdot f(i)$, in which $v(i)$ denotes the c-p value of the free living nematode taxon i, and $f(i)$ denotes the frequency of taxon *i* in the nematode community. The plant parasitic index (PPI) was estimated using a similar equation that was instead applied to plant parasitic genera or families (Bongers, 1990). The MI and PPI reflect recent disturbance history of a soil and the level of herbivore pressure on plant community grown in the soil, respectively. In theory, higher MI values suggest the more mature and stable and the less disturbed an ecosystem is, and higher PPI values indicate a higher chance of plants being fed by plant parasites. Finally, the Wasilewska index (WI) was estimated as $WI = (BF + FF)/PF$, where BF, FF and PF are the abundance of bacterial-feeding, fungal-feeding and plant-feeding nematodes, respectively. Lower WI values indicate the stronger impact of plant-feeding nematodes on nutrient mineralization, and thereby poor soil health (Wasilewska, 1994).

3. Data analysis

Data on nematode abundance, nematode-based ecological indices and plant biomass were analyzed using Two-way ANOVA, in which soil microorganism inoculation (MO), aboveground herbivory (AG) and their interaction were included as fixed factors. Tukey post hoc tests were performed when the fixed factors were significant. To meet assumptions of homogeneity and normality of data residuals, some of the plant and nematode data were log (X) or log(X + 1) transformed and soil data were inverse arcsine transformed. Non-parametric tests were used to analyze the data on abundance of fungal-feeding nematodes and the nematode plant parasite index (PPI) when the residuals of data did not meet either assumption. Effects of soil microorganisms and aboveground herbivory on plant and nematode community composition were analyzed using permutational multivariate analysis of variance

Table 1
Two-way ANOVA results for effects of addition of soil microorganisms (MO) and aboveground aphids (AG) on soil nutrients as well as characteristics of plant and nematode communities at the end of experiment. $N = 8$–9. P-H', Shannon index (H') of plant community; PF, plant feeding nematode abundance; BF, bacterial-feeding nematode abundance; FF, fungal-feeding nematode abundance; OM, omnivorous nematode abundance; CA, carnivorous nematodes; Total, total nematode abundance; SR, species richness; N-H', Shannon index (H') of soil nematode community; J', Pielou's evenness; MI, maturity index; PPI, plant parasite index; WI, Wasilewska index. Non-parametric Two-way ANOVA were used on FF and PPI given that the residuals did not meet the assumption of parametric methods. Bold numbers indicate significant differences at $p < 0.05$ level based on Tukey's post hoc test.

Properties			MO		AG		MO × AG	
		df	*F*	p	*F*	P	*F*	p
Plant	Shoot biomass	1, 32	1.07	0.309	**30.9**	**<0.001**	2.79	0.105
	Root biomass	1, 32	1.05	0.314	**9.05**	**0.005**	0.37	0.550
	Root:shoot ratio	1, 32	1.43	0.241	1.78	0.191	0.85	0.364
	P-H'	1, 32	0.00	0.978	**5.18**	**0.030**	1.17	0.287
Nematode	PF	1, 31	**5.91**	**0.021**	0.49	0.488	3.20	0.083
	BF	1, 31	0.24	0.628	0.39	0.538	0.43	0.518
	FF	1, 31	0.41	0.528	0.05	0.824	0.77	0.387
	OM	1, 31	0.07	0.790	1.49	0.232	0.00	0.937
	CA	1, 31	**16.1**	**<0.001**	1.10	0.303	3.18	0.084
	Total	1, 31	**6.36**	**0.017**	1.30	0.263	2.65	0.114
	SR	1, 31	**6.68**	**0,015**	0.29	0.591	1.49	0.231
	N-H'	1, 31	**6.61**	**0.015**	1.22	0.277	0.62	0.435
	J'	1, 31	**5.20**	**0.030**	1.55	0.222	0.47	0.500
	MI	1, 31	1.69	0.203	0.38	0.541	0.66	0.424
	PPI	1, 31	**11.7**	**0.002**	2.46	0.127	**4.32**	**0.046**
	WI	1, 31	**5.10**	**0.031**	0.13	0.719	2.07	0.160

(PERMANOVA) based on Bray–Curtis dissimilarity following 999 permutations (Anderson, 2001). A priori, a test of differences in dispersion among soil microorganism and aphid groups using PERMADISP procedure was performed by executing the 'betadisper' function in the "vegan" package. The non-significant result from PERMDISP in our study indicated that soil microorganism and aphid groups did not differ in dispersion, which rendered the rationale of using PERMANOVA to analyze our data. However, PERMANOVA and the employed Bray-Curtis dissimilarity is notoriously sensitive to unequal sample sizes (Anderson and Walsh, 2013), as we have in the current study due to the missing samples. Therefore, we used a random matching approach. Since there were 9 replicates in C, AG, MO + AG but 8 replicates in MO treatment, we randomly sampled 8 out of the 9 mesocosms in C, AG, and MO + AG and combined them with the MO to create a matrix of even sample sizes ($n = 8$). We then analyzed the data matrix using PERMANOVA based on Bray-Curtis dissimilarity. We repeated this procedure 1000 times and this yielded 1000 permutation results. The number of significant occurrences out of the 1000 repeats were summed and the proportion of non-significant occurrences was calculated (Pr). The result with Pr < 0.05 were considered significant. In these analyses, the proportion of aboveground biomass of each species to total shoot biomass of the corresponding plant community, and the proportion of abundance of each nematode taxonomic group to the total abundance of the corresponding nematode community were used. Patterns of nematode community composition were visualized using non-metric multidimensional scaling (NMDS, two axes) of Bray–Curtis dissimilarities (Bray and Curtis, 1957) calculated from nematode abundances among groups. Both the centroids and the individual samples within-groups were shown using the GGPLOT 2 package (Wickham, 2016). Redundancy analyses (RDAs) were conducted to reveal the relationship between nematode taxonomic groups and plant community or soil parameters. The ANOVA analyses were conducted using the "car" package, and PERMANOVAs, NMDS visualization and RDAs were performed using the "vegan" package (Oksanen et al., 2007). All the analyses were performed using R version 4.2.1. (R Core Development Team, 2022).

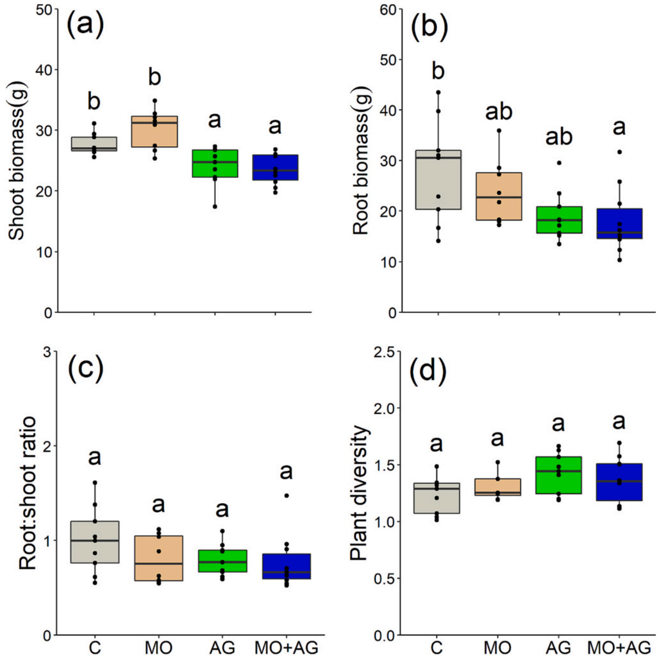

Fig. 1. Shoot biomass (a), root biomass (b), root:shoot ratio (c) and plant diversity (d) of plant communities in mesocosms with soil microorganisms (MO), with aboveground herbivores (AG), with both (MO + AG) or neither (C) at the final harvest 12 months after transplantation. Boxplots show the maximum, minimum and median values of the samples and the points in the plot area represent different replicates of the corresponding treatment. N = 8–10. Boxplot with identical letters are not significantly different based on Tukey's post hoc test. Statistics are shown in Table 1.

4. Results

4.1. Plant community

Introduction of aphids significantly reduced the total shoot biomass of the plant community compared to the community without aphids (Table 1, Fig. 1a), and in particular it reduced aboveground biomass of the dominant grass species *A. oderatum* by ca. 30 % (Fig. S1). Aphids also reduced the total root biomass of the plant community but the root:shoot ratio was not significantly influenced by aphid infestation (Table 1, Fig. 1b, c). Plant diversity (H') was enhanced by the introduction of aboveground aphids (Table 1, Fig. 1d). On the other hand, the inoculation of soil microorganisms did not significantly alter the above-

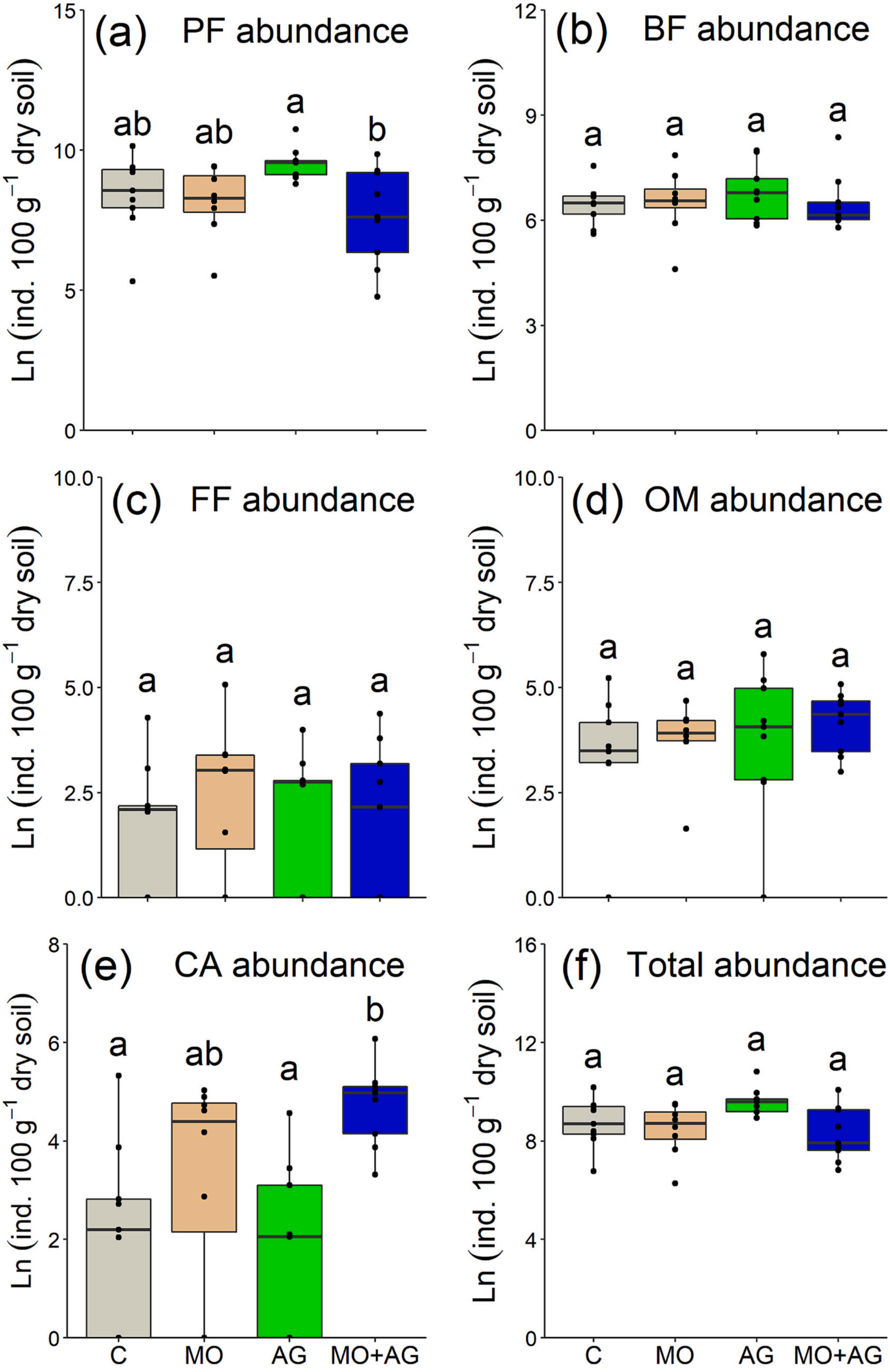

Fig. 2. The abundance of plant-feeding (PF, a), bacterial-feeding (BF, b), fungal-feeding (FF, c), omnivorous (OM, d), carnivorous (CA, e) nematodes, and the total abundance of soil nematode community (Total, f) in mesocosms with soil microorganisms (MO), with aboveground herbivores (AG), with both (MO + AG) or neither (C) at the final harvest 12 months after transplantation. Boxplot shows the maximum, minimum and median values of the samples and the points in the plot area represent different replicates of the corresponding treatment. Boxplot with identical letters are not significantly different based on Tukey's post hoc test. N = 8–9. Statistics are shown in Table 1.

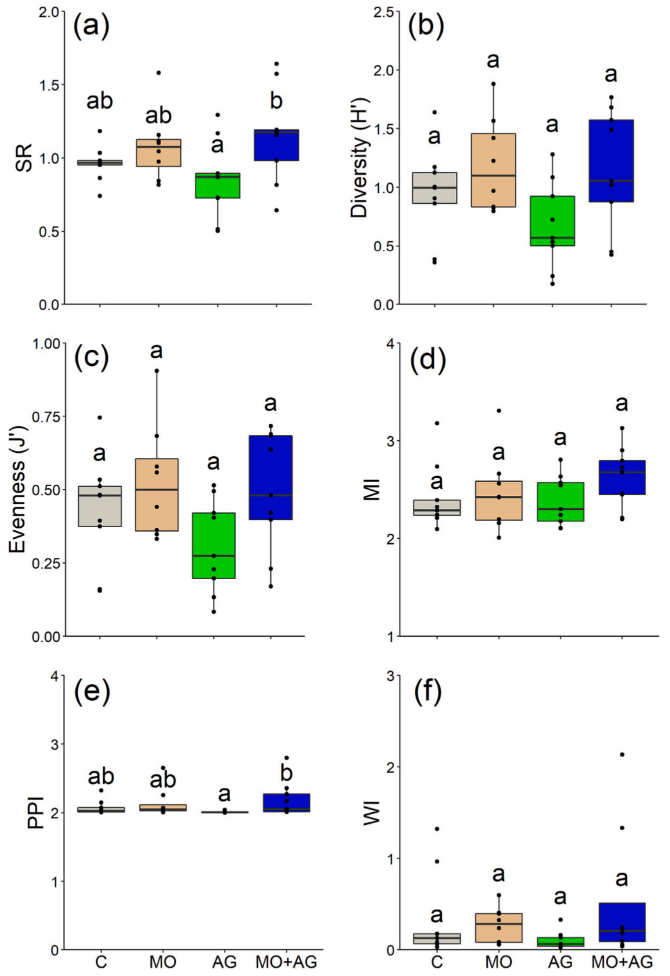

Fig. 3. Ecological indices of soil nematode communities, including species richness (SR, a), Shannon-Wiener index (H', b), Pielou's evenness (J', c), nematode maturity index (MI, d), plant parasite maturity index (PPI, e), and Wasilewska index (WI, f) in the experimental mesocosms with soil microorganisms (MO), with aboveground herbivores (AG), with both (MO + AG) or neither (C) at the final harvest 12 months after transplantation. Boxplot shows the maximum, minimum and median values of the samples and the points in the plot area represent different replicates of the corresponding treatment. Boxplot with identical letters are not significantly different based on Tukey's post hoc test. N = 8–9. Statistics are shown in Table 1.

mentioned attributes of the plant community (Table 1, Fig. 1a-d). The NMDS plot showed that plant community composition (quantified as Bray-Curtis dissimilarities in relative abundance of species) was not influenced by the inoculation of soil microorganisms, nor by the introduction of aphids ($Pr > 0.05$, Fig. S2).

4.2. *Soil nematode abundance*

The total number of soil nematodes in pots with soil microorganisms were lower than in pots without soil microorganisms (Fig. 2f). In particular, the abundance of plant feeding nematodes that accounted for 82–92 % of the total abundance of nematode community across treatments, was significantly lower in the presence of soil microorganisms (Table 1, Fig. 2a). The plant-feeding genus *Paratylenchus* spp. was dominant within the soil nematode community occupying 59–81 % of the total nematode abundance across the treatments, and this was reduced by 56 % in mesocosms with soil microorganisms (Table S2). Neither the abundance of microbial-feeding nematodes nor that of omnivorous nematodes was influenced (Table 1, Fig. 2b-d), but the abundance of carnivorous nematodes was higher when soil microorganisms were present (Fig. 2e). We did not observe significant effects of aphid introduction on the abundance of soil nematodes (Fig. 2a-f).

4.3. *Ecological indices of soil nematodes*

The species richness (SR), Shannon-Wiener diversity (H'), Pielou's evenness (J') and Wasilewska index (WI) of the soil nematode communities were higher in pots with soil microorganisms (Table 1, Fig. 3a, b, c, f). The plant parasite index (PPI) of the nematode community was also higher when soil microorganisms were present, but this effect was only marginally significant when the aphids were simultaneously introduced (Table 1, Fig. 3e). On the contrary, the maturity index (MI) was not influenced by either the presence of soil microorganisms or aphids (Table 1, Fig. 3d).

4.4. *Nematode community composition*

The NMDS plot showed that introduction of aphids tended to alter the composition of the nematode community (quantified as Bray-Curtis dissimilarities in relative abundance of each taxonomic group) in mesocosms that were previously inoculated with soil microorganisms, although this effect was not statistically significant (PERMANOVA test: $Pr > 0.10$, Fig. 4a). Results of redundancy analyses (RDA) showed that the first and second canonical axes accounted for only 8.1 % and 5.0 % of the total variance of the nematode community, respectively, and neither RD axes could significantly explain the variance (Fig. 4b).

4.5. *Soil nutrients*

By the end of the experiment the treatments with inoculation of soil microorganisms had significantly lower soil NH_4^+ levels than the other treatments, but soil NO_3^- or PO_4^- levels did not differ. There was no overall effect of inoculation of soil microorganisms or introduction of aphids on soil N:P ratio, but there was a significant MO × AG interaction (Table S3).

5. Discussion

Soil microorganisms can regulate the dynamics of plant and soil nematode communities via their key roles in belowground multitrophic interactions and nutrient cycling (van der Heijden et al., 2008; Delgado-Baquerizo et al., 2020). Aboveground herbivory can also contribute to these dynamics by influencing outcomes of plant-soil linkages (Liu et al., 2020; Pan et al., 2022). Our results partly support these views since inoculation with soil microorganisms mainly altered nematode communities while aboveground herbivory tended to affect plant communities only.

5.1. *Plant community responses to aboveground aphids and soil microorganisms*

Our results show that both shoot and root biomass of plant communities were significantly reduced by aboveground aphids. This is not surprising given that aphid species *Rhopalosiphum padi* is a specialist insect exclusively feeding on grass species that accounted for ca. 70 % of the shoot biomass of plant community across the treatments. For example, the aboveground biomass of the dominant species *Anthoxanthum odoratum* was strongly reduced (30 %) by aphid infestations (Fig. S1). The decrease in total root biomass may originate from the corresponding reduced photosynthetic efficiency of the plant community when exposed to aphids (Bilgin et al., 2010; Fyllas et al., 2022). Plant diversity was significantly increased by aphids, probably resulting from the reduced dominance of the grass species *Anthoxanthum odoratum* that releases the competitive pressure of subordinate species in the community (Engelkes et al., 2016). However, we did not observe a

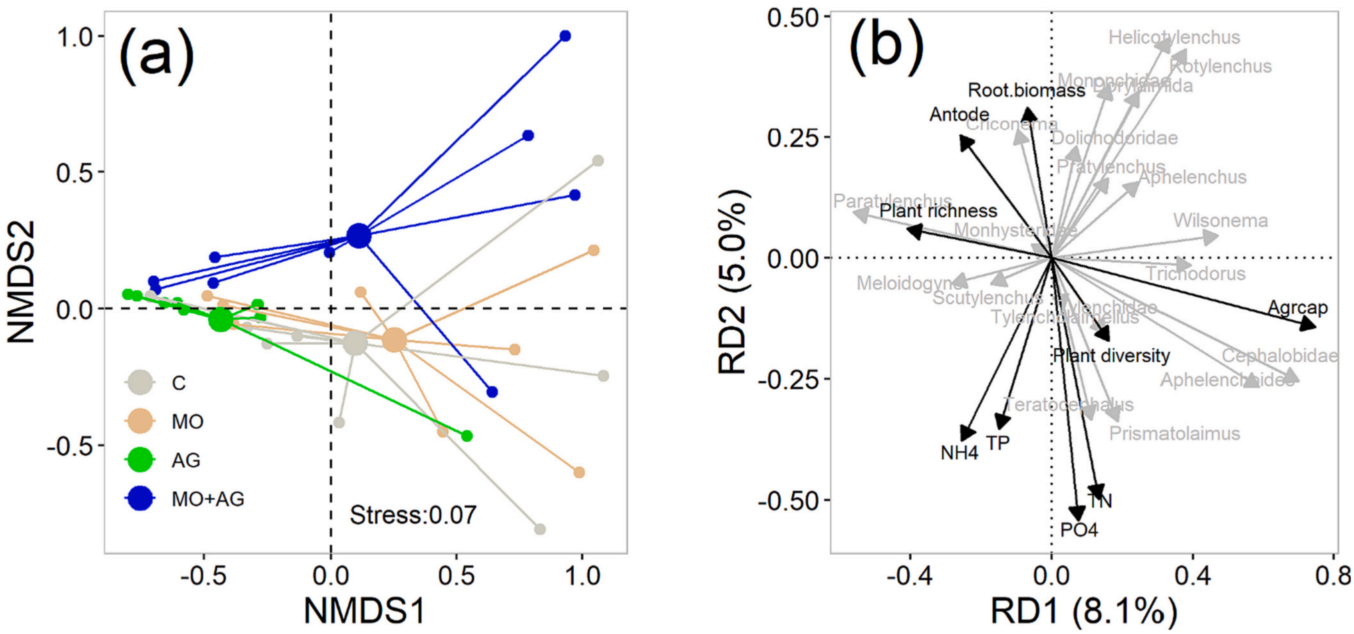

Fig. 4. Non-metric multidimensional scales (NMDS) showing the patterns for soil nematode community compositions (a) and biplot diagram of the redundancy analysis (RDA) in the nematode communities constrained by soil and plant traits (b), in the experimental mesocosms with soil microorganisms (MO), with aboveground herbivores (AG), with both (MO + AG) or neither (C) at the final harvest 12 months after transplantation. The soil physiochemical traits of the experimental treatments (C, MO, AG, MO + AG) used in the RDA were presented in Table S3. Small circles indicate replicates and large circles represent the centroid of each treatment in the panel (a). The black and gray arrows in panel (b) indicate plant/soil traits and relative abundance of taxonomic nematode groups in a mesocosm, respectively. N = 8–9.

significant influence of soil microorganisms on plant communities. This may be partly explained by the limitation of our sieving approach that may have not fully separated soil nematodes and microorganisms, which may have weakened the microbial inoculation effects on plant communities (de Vries and Wallenstein, 2017).

5.2. *Nematode community responses to aboveground herbivory and soil microorganisms*

In contrast to plant communities, soil nematode community were only weakly affected by introduction of aphids in our study. This result differs from a previous study that reported aphid infestation did not influence total plant biomass or productivity, but that it caused important effects on bacterial- and fungal-feeding nematodes, whereas not on plant-feeding and predatory nematodes (Wardle et al., 2004a, 2004b). This contradiction may be explained by the difference of aphid species or nematode species used in the two studies (Rasmann, 2022; Lima da Silva et al., 2020). The result that significant effects of aphid introduction on plant community were not extrapolated to soil nematodes via above-belowground linkages also contrasts with other studies (Borgström et al., 2018). This inconsistence may be due to the high complexity of plant community used in our study, in which each plant species may be specific in mediating the outcome of three-way interactions among aboveground aphids, plants, and soil nematodes, and this likely diminishes the overall above-belowground biotic linkages (Zhang et al., 2020). The small and uneven sample size in our study may increase the likelihood of type II errors that might skew the results, requiring caution in interpreting the results of the study, which should be replicated in balanced studies of larger samples.

We observed overall negative impacts of soil microorganisms on total soil nematode abundance and this effect appeared to result from a lower abundance of plant-feeding nematodes at soil microorganism inoculation, in particular due to a 56 % reduction in abundance of the dominant nematode genus *Paratylenchus* spp. that represented 59–81 % of the total abundance in soil nematode communities across the treatments. Since inoculation of soil microorganisms did not significantly alter the root biomass of plant community, its reduction effects on the abundance of plant-feeding nematodes may not have operated through effects on the plant community (Khanna et al., 2021). Instead, the diverse microbial community present in the inoculum may have directly contributed to the reduction in plant-feeding nematodes via biological control (Poveda et al., 2020). Interestingly, despite the reduction in herbivore abundance and the reduction of the total number of nematodes after inoculation with soil microorganisms, we noticed an increase in abundance of carnivorous nematodes. Considering this result violates the established trophic relations between the two nematode groups, such increase in abundance of carnivores may simply relate to the proportional decrease of other trophic groups in the community under this treatment (Topalović et al., 2020).

Our results show that a variety of ecological indices of the soil nematode community were altered after the addition of soil microorganisms. The higher species richness (SR) and Shannon diversity (H') of the nematode community following inoculation of the microbial community suggests that soil microorganisms may provide more diverse food sources to nematodes, in particular to the micro-feeding nematodes like bacterial feeders and fungal feeders (Zhang et al., 2022). Besides, this higher nematode diversity may also be caused by the induced reduction in ammonium-nitrogen in the soil with microbes. Indeed, many studies report decreased diversity of nematode communities by addition of ammonium fertilization, often due to increases in herbivores and decreases in the tertiary consumers like omnivores or predators (Wang et al., 2006; Wei et al., 2012). The enhancement in the plant-parasite index (PPI) of the nematode communities after inoculation of soil microorganisms in pots with aphids represents a higher belowground herbivore pressure for the plant community, indicating that the addition of soil microorganisms and aboveground aphids may interactively benefit the development of plant-feeding nematodes and the related ecosystem functions, such as plant diversity as observed in this study (Du Preez et al., 2022). Similarly, the higher Wasilewska index (WI) indicates a better health of plant communities grown in the soil after inoculation with soil microorganisms, is consistent with the results of a lower abundance of plant-feeding nematodes and a higher abundance of carnivorous nematodes in this treatment. All these results suggest that compared to aboveground herbivory by aphids, soil microorganisms are more important for conservation of soil biodiversity and related ecosystem functioning such as plant diversity and productivity (Bardgett and van der Putten, 2014).

6. Conclusion

Soil microorganisms and aboveground aphids only locally exerted effects on soil nematode and plant communities respectively, and there were only weak systemic above-belowground interactions between aphids and microorganisms. Since soil microorganisms can significantly reduce the abundance of primary consumers (e.g. plant-feeding nematodes) and enhance the abundance of tertiary consumers (predatory nematodes), they might strongly contribute to stability of soil food webs. Our study highlights that both aboveground herbivory and soil microorganisms may play important roles in ecosystem functioning, although their effects may be primarily independent.

CRediT authorship contribution statement

TMB conceived the idea of the study. GBDD and TMB collected the data. MW, NZ, HX and YJ analyzed the data and wrote the first draft of the manuscript. All authors contributed to the revision of the manuscript and approved its submission.

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 will be made available on request.

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Statement

The authors declare that the experiments comply with the current laws of the Netherlands where the experiments were performed.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.105097.

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