

Alfalfa and maize intercropping enhances soil nematode structure and food web complexity in low-nitrogen soils

Abile Teshita ^{a,b}, Yuanyuan Feng ^a, Rui Qian ^a, Xinyu Wang ^a, Waqif Khan ^a, Yingzhi Gao ^{a,c,*}

^a Institute of Grassland Science, Key Laboratory of Vegetation Ecology, Ministry of Education, State Environmental Protection Key Laboratory of Wetland Ecology and Vegetation Restoration, Jilin Songnen Grassland Ecosystem National Observation and Research Station, Northeast Normal University, Changchun 130024, China

^b Department of Biology, College of Natural and Computational Sciences, Bule Hora University, Bule Hora 144, Oromia National Regional State, Ethiopia

^c Key Laboratory of Grassland Resources and Ecology of Western Arid Desert Area of the Ministry of Education, College of Grassland Science, Xinjiang Agricultural University, Urumqi 830052, China



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ABSTRACT

Soil nematodes play key roles in soil ecosystem processes and functions. Although agricultural practices such as intercropping and nitrogen (N) fertilization are known to have a strong influence on soil nematodes, less is known about the responses of the soil nematode community to the combined effects of intercropping and N fertilization. This study was conducted to test whether the intercropping of maize and alfalfa can enhance the nematode community and quantified the effects of intercropping and N fertilization on nematode abundance, diversity, and metabolic footprints compared with soils under monoculture systems. An experiment involving maize and alfalfa was conducted in a 2×3 factorial completely randomized block design with four replications. The factors consisted of two N levels, namely, no N fertilization and N fertilization ($225 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), and three cropping modes: monoculture maize, monoculture alfalfa and maize intercropped with alfalfa. The results showed that, compared with the monoculture systems, intercropping increased total nematode and omnivore-predator abundances but reduced herbivore abundance. The total nematode biomass and metabolic footprint of omnivores and predators significantly increased in the intercropping treatments, regardless of N fertilization. The soil food web in the intercropping system without N fertilization was mature, structured, and enriched, as indicated by high maturity, structure and enrichment index values. However, all these effects decreased in the monoculture treatments, particularly after N fertilization. These results indicated that, compared with the monoculture systems, maize/alfalfa intercropping in low-N soil can improve the structural complexity of the soil food web. The observed complexity was attributed to the high soil moisture content, soil organic carbon and total N under the intercropping and low-N conditions. Therefore, this study provides a promising way to improve soil health and ecosystem stability, revealing that alfalfa intercropping in low-N soils can improve resource flow in the soil food web through the increase in total nematode biomass and the metabolic footprints of nematodes.

1. Introduction

Intensive agricultural systems have substantially contributed to rising global food requirements through the use of high-yielding crop varieties that require high levels of inorganic nitrogen (N) fertilizer inputs (Jackson et al., 2013; Matson et al., 1997). However, there have been significant environmental costs associated with agricultural intensification, which include the degradation of soils that ultimately leads to loss of soil biodiversity (De Graaff et al., 2019; Postma-Blaauw et al.,

2012; Tiemann et al., 2015). Considering the multiple ecosystem functions and services provided by soil biota, such as carbon (C) and N cycling, soil biodiversity loss has been identified as a major environmental issue of global concern (Bardgett and van der Putten, 2014; Wagg et al., 2014). This calls for a shift in agricultural practices to an approach that promotes soil biodiversity or beneficial organisms (e.g., soil nematodes) and enhances soil health while simultaneously sustaining high levels of production (Lavelle et al., 2014).

Soil nematodes represent vital components of soil organisms and

* Corresponding author at: Institute of Grassland Science, Key Laboratory of Vegetation Ecology, Ministry of Education, State Environmental Protection Key Laboratory of Wetland Ecology and Vegetation Restoration, Jilin Songnen Grassland Ecosystem National Observation and Research Station, Northeast Normal University, Changchun 130024, China.

E-mail address: gaoyz108@nenu.edu.cn (Y. Gao).

occupy key positions in the soil food web (van den Hoogen et al., 2019). The composition and abundance of soil nematodes reflect food web structure, ecosystem processes and functions (Ritz and Trudgill, 1999; van den Hoogen et al., 2019; Zhao and Neher, 2014). For example, the activity of bacterivorous and fungivorous nematodes regulates organic matter decomposition and nutrient mineralization (Ferris et al., 2001), whereas omnivores and predators are indicative of soil disturbances, and an increase in their density and diversity is associated with improved soil health (Ferris et al., 2001, 2004). Moreover, in addition to diversity in their life history traits, nematodes are susceptible to changes in food sources and thus differ in the type of food sources they utilize (Sánchez-Moreno et al., 2006; Sánchez-Moreno et al., 2009). Hence, the metabolic activity of different functional groups of nematodes provides an indication of the ecological structure and resource flow of the soil food web (Hodson et al., 2014). The metabolic footprint of nematodes is thus derived to represent the metric of nematode metabolic activity and ecosystem function (Ferris, 2010b).

Studies have shown that intercropping systems, particularly those involving legume species, contribute to the reduction in the use of inorganic N fertilizers (Jensen et al., 2020; Weih et al., 2022) while maintaining crop production potential. In a recent meta-analysis by Li et al. (2020), intercropping systems were shown to produce 16–29 % more yield per unit area than did intensive monoculture systems while necessitating 19–36 % less fertilizer. There is a substantial amount of data on grain/legume intercropping in China (e.g., maize/soybean, maize/faba bean, and maize/peanut) (Knörzer et al., 2009), but only recently has the intercropping of perennial forage legumes (e.g., alfalfa) with annual cereals (e.g., maize) received considerable attention because of increasing demands for meat and milk production (Sun et al., 2014).

Some intercropping studies have also focused on how various cropping practices affect soil nematode density and trophic group diversity. For instance, greater total nematode (TNem) density (Santiago et al., 2012) and diversity (Liu et al., 2016c) were found under maize intercropped with the perennial legume *Canavalia ensiformis* and wheat intercropped with jujube trees. Crop diversity increases the amount and diversity of litter returned to soil (Tiemann et al., 2015), and increased heterogeneity of the C substrate entering the soil has been shown to be associated with increased belowground faunal density and diversity (Albrecht et al., 2004; Hooper et al., 2000; Zhao et al., 2014). In addition to the differences in their response to diverse cropping practices, nematodes also differ in their response to the plant identity/species involved. For instance, the density of herbivorous, bacterivorous and fungivorous nematodes was shown to increase under monoculture alfalfa and alfalfa grown in a mixture with grass (*Paspalum wetsfeteini*) compared with grass grown alone (Ye et al., 2020). This suggested that the response of nematodes also varies with the quality of plant detritus entering the soil (Bonkowski et al., 2009). Legumes contain N-rich organic substrates (Ye et al., 2020; Zhao et al., 2014), and the N-rich substrates in legumes (e.g., root exudates) can be readily used by soil organisms at low trophic levels in the food web, altering their community composition. For example, legumes were shown to increase the abundance of bacterivorous nematodes, particularly that of members of opportunistic genera such as *Rhabditis* and *Panagrolaimus* (Viketoff et al., 2009; Viketoff et al., 2005). These changes in the abundance and composition of the soil community at low trophic levels could propagate and affect species at higher trophic levels (e.g., soil nematodes) (Ye et al., 2020). Although there exist several reports on the effects of intercropping on soil nematodes, little is known about how the intercropping of perennial leguminous species such as alfalfa with annual cereal species such as maize influences the nematode community structure, especially the nematode metabolic footprint and energy flow in the soil food web.

Many studies have shown that N fertilization also greatly alters the abundance, diversity and composition of soil nematodes (Liu et al., 2016a; Shaw et al., 2019; Song et al., 2016). Song et al. (2016) found that N fertilization enhanced the abundance of bacterivores but

suppressed that of fungivores and omnivores/predators. Negative effects on soil nematode generic richness (Shaw et al., 2019; Song et al., 2016) and diversity (Liu et al., 2016a; Shaw et al., 2019) have been reported after N fertilization, particularly at high N levels. The negative effects of fertilization were attributed to soil acidification (Velthof et al., 2011) and/or a reduction in soil macroaggregation (Cai et al., 2020), and the positive effects were associated with increased plant biomass and microbial biomass carbon (MBC) and N (Hu et al., 2017).

Previous studies have clearly shown that intercropping or N fertilization can affect the composition and abundance of nematode communities directly or indirectly via alterations to soil physicochemical and/or microhabitat conditions. However, the comprehensive understanding of the responses of the nematode community and nematode-regulated C and energy flow under the combined effects of intercropping and N fertilization remain unclear, especially in perennial legume-dominated cereal/legume intercropping systems. Therefore, it was hypothesized (1) that monoculture treatments would detrimentally affect soil nematode abundance and community composition, with the effect being more pronounced under high N fertilization, and (2) that intercropping would alleviate the damage to the soil food web complex caused by high N fertilization and may be more advantageous under conditions of low soil N, since intercropping can create a complex and stable food web by increasing the diversity of higher-trophic level fauna. The objective of this study was to evaluate the effects of cropping mode, N fertilization and their interaction on the community structure of soil nematodes in pastoral areas of the Northeast China Plain.

2. Materials and methods

2.1. Study site and experimental design

The study was conducted at the Grassland Ecological Research Station of Northeast Normal University (also known as the Songnen Grassland). This grassland, a typical farming and pastoral area of northeastern China, is located at 44° 40' N and 123° 44' E at an elevation of 137.8–144.8 m above sea level. The grassland is characterized by a semiarid, temperate monsoon climate. This climate is cold and dry in the winter but hot and humid in the summer, and the annual mean temperature ranges from 5.4 to 7.1 °C. The average annual precipitation ranges from 300 to 500 mm, with >80 % occurring between May and September (Supplementary Fig. S1). Precipitation differed between the two experimental years. The annual total rainfall in 2017 was 420 mm, and the peak amount (228 mm) occurred in August, while the annual precipitation in 2018 was 383 mm; there is a clear seasonal distribution, and most rain events occurred in June and July (Supplementary Fig. S1). The soil is a soda-saline type comparable to Solonchak soils in the FAO-UNESCO soil classification (FAO, 2001). The site comprised 8.81 g kg⁻¹ organic C and 0.84 g kg⁻¹ total N, and the pH value was 8.36 in the top 20 cm soil layer before the experiment was performed (Wang et al., 2019).

This study was carried out in an existing experimental setup. The platform was established in 2014 with maize (*Zea mays* L. cv. Zhengdan 958) and alfalfa (*Medicago sativa* L. cv. Dongmu No. 1). The field experiment was conducted in accordance with a completely randomized block design with four blocks, and each block consisted of two N levels and three cropping modes. The two N levels were no N fertilization (N0) and N fertilization (N1). For the N0 treatment, the maize plots received 120 kg P ha⁻¹ yr⁻¹ (as triple superphosphate) and 60 kg K ha⁻¹ yr⁻¹ (as potassium chloride) each year. The alfalfa plots were supplemented with 135 kg P ha⁻¹ (as triple superphosphate) and 90 kg K ha⁻¹ (as potassium chloride) as basal nutrients. For the N1 treatment, the maize plots were supplemented with 112.5 kg N ha⁻¹ yr⁻¹ (half of the N fertilizer) together with P and K fertilizers before sowing, and the other half of the N fertilizer was applied during the tasseling stage of maize each year. The alfalfa plots were supplemented with 53 kg N ha⁻¹ in 2014, and no additional fertilizer was applied to the alfalfa in the subsequent years of

the experiment. The three cropping modes were (i) monoculture alfalfa (MA), consisting of 13 alfalfa rows with a 30 cm row spacing having an area of $3.6 \times 6 \text{ m}^2$ per plot; (ii) monoculture maize (MM), consisting of 6 maize rows with a 65 cm row spacing and having an area of $3.9 \times 6 \text{ m}^2$ per plot; and (iii) maize intercropped with alfalfa (IMA), having an area of $11.25 \times 6 \text{ m}^2$ per plot with three strips, with each strip consisting of 4 rows of maize intercropped with 6 rows of alfalfa and 30 cm between the two strips of species. In the intercropping system, plants were selected from alfalfa plots in the alfalfa treatments adjacent to the intercropped maize, and vice versa for the intercropped maize treatments. Hence, the intercropping treatments involved intercropped alfalfa (IMAM) and intercropped maize (IMAY).

2.2. Soil sampling and analysis

Soil samples (0–30 cm depth) were collected from the plant rhizosphere during the maize flowering period in August of 2017 and 2018. The plants in the maize and alfalfa monoculture systems selected for rhizosphere soil sampling were randomly selected from within the inside rows according to a five-point sampling method. However, for each plot of maize and alfalfa in the intercropping systems, the plants were chosen from the central rows (Zhang et al., 2018). The selected plants were gently uprooted, and any loosely attached soil was removed by careful shaking of the roots. Soil tightly adhering to the roots was collected by brushing it off the roots into a plastic tray (Barillot et al., 2013). The rhizosphere soils from alfalfa and maize were collected, placed into polyethylene bags, and transported to the laboratory in a cooler to avoid desiccation. In the laboratory, the samples were stored at 4 °C for subsequent soil analysis and nematode extraction (which was performed within a week after sampling). Soil pH of a soil: water suspension (1:2 w/v) was measured with a pH meter. Soil moisture content (SMC) was determined gravimetrically by oven drying 50 g of moist soil overnight at 105 °C. Soil organic carbon (SOC) was measured via the potassium dichromate oxidation method. Total soil nitrogen (TN) and soil mineral nitrogen (soil NO₃⁻-N) were measured by the use of a fully automated discrete analyzer (SmartChem 450, AMS, Italy). Soil NO₃⁻-N was extracted by dissolving 10 g of fresh soil in 100 ml of 2.0 M KCl. The soil C:N ratio was calculated by dividing the concentrations of SOC by the TN. Soil MBC was analyzed according to chloroform fumigation extraction procedures (Vance et al., 1987).

2.3. Nematode extraction and identification

Nematodes were extracted from 100 g of fresh rhizosphere soil using the modified Baermann funnel method (Hooper et al., 2005), heated (causing death) in a water bath at 65 °C, and preserved in an equal ratio of 4 % hot formaldehyde. Following extraction, nematodes were counted via a stereomicroscope (E200, Nikon), and the first 100 individuals encountered (or all if the total number was lower than 100) were identified to the family and/or genus level using the identification keys of Bongers (1988) and Li et al. (2017). The nematodes were then assigned to five trophic groups (herbivores (He), bacterivores (Ba), fungivores (Fu), omnivores (Om) and predators (Pr)) on the basis of stomatal and esophageal morphology (Yeates et al., 1993). Furthermore, the nematode genera were categorized into colonizer-persister classes (cp classes), ranging from cp1 (r-strategist nematodes) to cp5 (K-strategist nematodes) (Bongers, 1990).

2.4. Calculations of ecological indices

TNem abundance was expressed as individuals 100 g⁻¹ dry soil. Nematode functional feeding groups were quantified as the proportion of each trophic group relative to the total number of nematodes identified in the sample. Nematode genera whose relative abundance was ≥5 % of the total population and present under all cropping modes were considered dominant (Zhong et al., 2015). Ecological and food web

indices such as the maturity index (MI), enrichment index (EI), structure index (SI), and channel index (CI) were used to estimate the dynamics of the soil food web and nematode community under stressed, enriched, stable, and structured conditions and the predominant decomposition pathway. The EI, SI, and CI were calculated based on the weighted abundances of nematode guilds representing enrichment (*e*), structure (*s*) and basal (*b*) characteristics according to the methods of Ferris et al. (2001): $EI = 100 \times (e/e + b)$; $e = 3.2 \times (Ba_1) + 0.8 \times (Fu_2)$, $b = \Sigma k_{bnb}$, $SI = 100 \times (s/s + b)$; $s = 1.8 \times (Ba_3 + Fu_3 + OP_3) + 3.2 \times (Ba_4 + Fu_4 + OP_4) + 5 \times (Ba_5 + Fu_5 + OP_5)$, and $CI = 100(0.8Fu_2) / (3.2Ba_1 + 0.8Fu_2)$, where *kb* are the weightings of the nematodes that exhibit characteristics of basal food web conditions and where *nb* is the abundance of nematodes in those guilds. The MI was calculated as $MI = \Sigma v_i \times f_i$, where *v_i* is the *c-p* value of the *i*th taxon and where *f_i* is the frequency of the *i*th taxon. Nematode metabolic footprints were used to evaluate nematode-regulated C and energy flows (Ferris, 2010b). All nematode indices and metabolic footprints were computed via the nematode indicator joint analysis (NINJA) tool available at <https://shiny.wur.nl/ninja/> (Sierienbriennikov et al., 2014).

2.5. Statistical data analysis

Before data analysis, nematode count data were $\ln(x + 1)$ transformed to meet normality and homogeneity criteria for statistical data analyses. All statistical analyses were carried out using SPSS 23 (IBM Corp., Armonk, NY, USA). Two-way analysis of variance (ANOVA) was conducted separately for each experimental year since a three-factor interaction was observed for the majority of the measured nematode and environmental parameters. For each specific parameter, if the interaction between N fertilization and cropping mode was significant, one-way ANOVA was then conducted to evaluate the effects of N fertilization and cropping mode. Significant differences between the means were assessed with Fisher's least significant difference (LSD) test. The effects of N fertilization were tested by independent-sample *t*-tests. The significance level was set at $p < 0.05$. Redundancy analysis (RDA) was performed to assess the contributions of soil properties to nematode community variation using Canoco 4.5 software (Šmilauer and Lepš, 2014). Ordination triplots were constructed via CanoDraw (Microcomputer Power, Ithaca NY, USA), and an unrestricted full-model Monte Carlo permutation test ($n = 999$) was performed to test the significance of the axes. Structural equation modeling (SEM) was conducted to test a hypothetical pathway that explains the direct and indirect effects of cropping mode and N fertilization on the energy flow channeled by soil nematode trophic groups. SEM was performed by comparing the model implied variance-covariance matrix with the observed variance-covariance matrix. Data-model fit was tested by the use of the two most popular methods: the root mean square error of approximation (with $p > 0.05$ and standardized root mean squared residual (SRMR) < 0.08 indicating a good fit of the model) and the χ^2 goodness-of-fit statistic (with $0 \leq \chi^2/\text{df} \leq 2$ and $0.05 < p \leq 1.00$ indicating a good fit of the model) (Hu and Bentler, 1999; Lei and Wu, 2007).

3. Results

3.1. Soil physicochemical properties

Across the two study years, cropping mode significantly affected all the measured soil properties ($p < 0.05$) except for pH (Table 1). Compared with the monoculture treatments, intercropping significantly increased the SMC, SOC, and TN in both 2017 and 2018. However, it significantly lowered the concentration of soil NO₃⁻-N (Table 1). On the other hand, compared with no N fertilization, N fertilization increased the SOC, TN, and soil NO₃⁻-N but significantly decreased pH in 2017. In 2018, only TN and NO₃⁻-N were increased after N fertilization.

Table 1

Results (mean \pm S.E) of two-way analyses of variance (ANOVA) on the effects of N addition and cropping modes (CM), and their interaction (N \times CM) on soil physicochemical properties: soil pH; soil moisture content (SMC — %); soil organic carbon (SOC — g kg $^{-1}$); soil total nitrogen (TN — g kg $^{-1}$); the soil carbon to nitrogen ratio (C:N); and concentration of soil nitrate (NO $_3^-$ -N — mg kg $^{-1}$).

Study years	Soil properties	Cropping modes (CM)				Fertilization (N)		ANOVA (<i>p</i> values)		
		MA	IMAM	MM	IMAY	N0	N1	N	CM	N \times CM
2017	pH	7.8 \pm 0.15 ^a	7.7 \pm 0.16 ^a	7.8 \pm 0.14 ^a	7.8 \pm 0.15 ^a	8.1 \pm 0.04 ^A	7.4 \pm 0.03 ^B	<0.001	ns	ns
	SMC	17.9 \pm 0.24 ^b	21.6 \pm 0.64 ^a	15.8 \pm 0.30 ^c	18.1 \pm 0.31 ^b	18.6 \pm 0.62 ^A	18.2 \pm 0.59 ^A	ns	<0.001	ns
	SOC	9.4 \pm 0.22 ^c	12.4 \pm 0.14 ^a	8.1 \pm 0.26 ^d	11.6 \pm 0.27 ^b	9.9 \pm 0.47 ^B	10.7 \pm 0.44 ^A	<0.001	<0.001	ns
	TN	0.9 \pm 0.04 ^b	1.1 \pm 0.03 ^a	0.6 \pm 0.05 ^c	0.9 \pm 0.04 ^b	0.8 \pm 0.05 ^B	0.9 \pm 0.05 ^A	<0.001	<0.001	ns
	C:N	10.8 \pm 0.44 ^c	11.2 \pm 0.25 ^c	14.9 \pm 0.65 ^a	13.3 \pm 0.57 ^b	13.4 \pm 0.57 ^A	11.7 \pm 0.43 ^B	<0.001	<0.001	ns
	NO $_3^-$ -N	2.9 \pm 0.03 ^a	2.2 \pm 0.07 ^c	2.9 \pm 0.05 ^a	2.5 \pm 0.09 ^b	2.5 \pm 0.09 ^B	2.8 \pm 0.07 ^A	<0.001	<0.001	<0.001
2018	pH	6.9 \pm 0.15 ^a	7.2 \pm 0.19 ^a	7.2 \pm 0.21 ^a	7.3 \pm 0.17 ^a	7.2 \pm 0.13 ^A	7.1 \pm 0.13 ^A	ns	ns	ns
	SMC	16.9 \pm 0.18 ^c	21.3 \pm 0.56 ^a	15.7 \pm 0.19 ^d	19.2 \pm 0.23 ^b	18.0 \pm 0.62 ^A	18.5 \pm 0.59 ^A	ns	<0.001	ns
	SOC	10.1 \pm 0.12 ^b	12.3 \pm 0.11 ^a	8.9 \pm 0.28 ^c	11.9 \pm 0.19 ^a	10.7 \pm 0.42 ^A	10.9 \pm 0.33 ^A	ns	<0.001	ns
	TN	0.9 \pm 0.01 ^b	1.1 \pm 0.03 ^a	0.6 \pm 0.03 ^c	0.9 \pm 0.01 ^b	0.8 \pm 0.04 ^B	0.9 \pm 0.04 ^A	0.010	<0.001	ns
	C:N	10.7 \pm 0.12 ^c	11.0 \pm 0.19 ^c	14.2 \pm 0.24 ^a	12.9 \pm 0.30 ^b	12.4 \pm 0.38 ^A	12.0 \pm 0.39 ^A	ns	<0.001	ns
	NO $_3^-$ -N	3.3 \pm 0.08 ^a	2.4 \pm 0.08 ^c	2.7 \pm 0.07 ^b	1.8 \pm 0.04 ^d	2.4 \pm 0.13 ^B	2.7 \pm 0.15 ^A	<0.001	<0.001	ns

MA: monoculture alfalfa; IMAM: intercropped alfalfa; MM: monoculture maize; IMAY: intercropped maize. N0: without N fertilization; N1: with N fertilization. Different small letters in each row show significant difference between cropping modes (CM), while different capital letters show significant difference between the two N levels. ns indicate nonsignificant values. Means were separated using Fisher's LSD test ($n = 4$).

3.2. Total nematode and trophic group abundances

In total, 46 nematode genera (39 in 2017 and 45 in 2018) were recorded in the present study (Tables S1 and S2). Bacterivorous nematodes constituted the most diverse trophic group (17 taxa), followed by omnivores/predators (13 taxa), fungivores (9 taxa), and herbivores (7 taxa). The dominant genera included *Panagrolaimus* (*c-p1* bacterivore), *Plectus* and *Prismatolaimus* (*c-p2* and *c-p3* bacterivores), and *Dorylaimellus* and Dolichodoridae the family (*c-p5* and *c-p3* herbivores) (Tables S1 and S2). TNem abundance was significantly affected by cropping mode and N addition (Table 2), with a significant increase in the intercropping treatments (i.e., in the IMAY treatment in 2017 and in the IMAM and IMAY treatments in 2018) (Fig. 1a). Similarly, N addition increased TNem abundance, with the highest score recorded in the IMAM treatment under N addition and the lowest score recorded in the MM treatment under no N addition.

With respect to nematode trophic groups, bacterivores were more abundant in the MA treatment under no N addition than in the IMAM treatment in both 2017 and 2018 (Fig. 1b). However, the abundance of bacterivores in the MA treatment varied between years following N fertilization, with a significant decrease (11.91 %) occurring in 2017 but an increase of 12 % occurring in 2018 (Fig. 1b). The relative abundance of fungivorous nematodes in the MM treatment was significantly higher than that in the IMAY treatment under no N addition, and their abundance increased after N addition (Fig. 1c). Moreover, under no N addition, the abundance of herbivores in the MM treatment in 2017 was significantly (105 %) higher than that in the IMAY treatment and was 18.99 % higher in 2018 (Fig. 1d); N fertilization had little effect on herbivore abundance under each planting mode during the two years. In

contrast to herbivores, omnivores and predators were significantly more abundant in the intercropping treatments than in the monoculture treatments, except for predators under N0 in 2018. N addition had a pronounced negative effect on omnivore-predator abundance under each planting mode during the two years (Fig. 1e-f).

3.3. Nematode diversity and ecological and food web indices

The effects of cropping mode on nematode diversity and species richness (SR) were slightly weaker than those of N addition (Table 3). Compared with the monoculture systems, intercropping significantly increased nematode SR in the IMAY treatment in 2018 under no N addition (Fig. 2a). On the other hand, N fertilization significantly reduced SR in all the treatments (Fig. 2a). Moreover, both cropping mode and N fertilization significantly affected the food web indices (Table 3), with intercropping without N addition resulting in increased SI and MI values compared with those in the monoculture systems in both 2017 and 2018 (Fig. 2b-c). Unlike the MI and SI, the basal index (BI) was lower in the IMAM and IMAY treatments, regardless of N fertilization (Fig. 2d). Compared with that in the MM treatment, the EI value in the IMAY treatment increased in 2017 regardless of N fertilization (Fig. 2e). The results of the nematode faunal analysis indicated that both the EI and the SI decreased under all cropping modes after N fertilization, particularly in the MM treatment, which was mapped to quadrant D in the faunal analysis graph (Fig. 3). Similarly, the CI was significantly reduced in the IMAY treatment, but the IMAM treatment induced a significant decrease in the CI only under the N0 condition in 2018 (Fig. 2f).

Both cropping mode and N addition significantly altered the total

Table 2

Results (mean \pm S.E) of two-way analyses of variance (ANOVA) on the effects of N fertilization (N), cropping modes (CM), and their interaction (N \times CM) on TNem abundance (individuals 100 g $^{-1}$ dry soil) and the relative abundance of bacterivores (Ba%), fungivores (Fu%), herbivores (He%), omnivores (Om%), and predators (Pr %).

Nematode variables	2017			2018		
	N	CM	N \times CM	N	CM	N \times CM
TNem	57.48**	131.32**	6.21**	29.32**	28.29**	1.31ns
Ba	8.53**	26.15**	4.68*	5.56*	30.14**	2.75ns
Fu	233.45**	12.8**	3.2*	60.75**	133.46**	6.89**
He	17.34**	30.45**	0.85ns	5.56*	4.14*	1.19ns
Om	258.62**	68.81**	2.75ns	61.34**	20.11**	10.66**
Pr	10.48**	8.46**	4.13*	96.99**	23.97**	0.73ns

Values indicate *F*-values and the level of significance. ns, not significant.

* $p < 0.05$.

** $p < 0.01$.

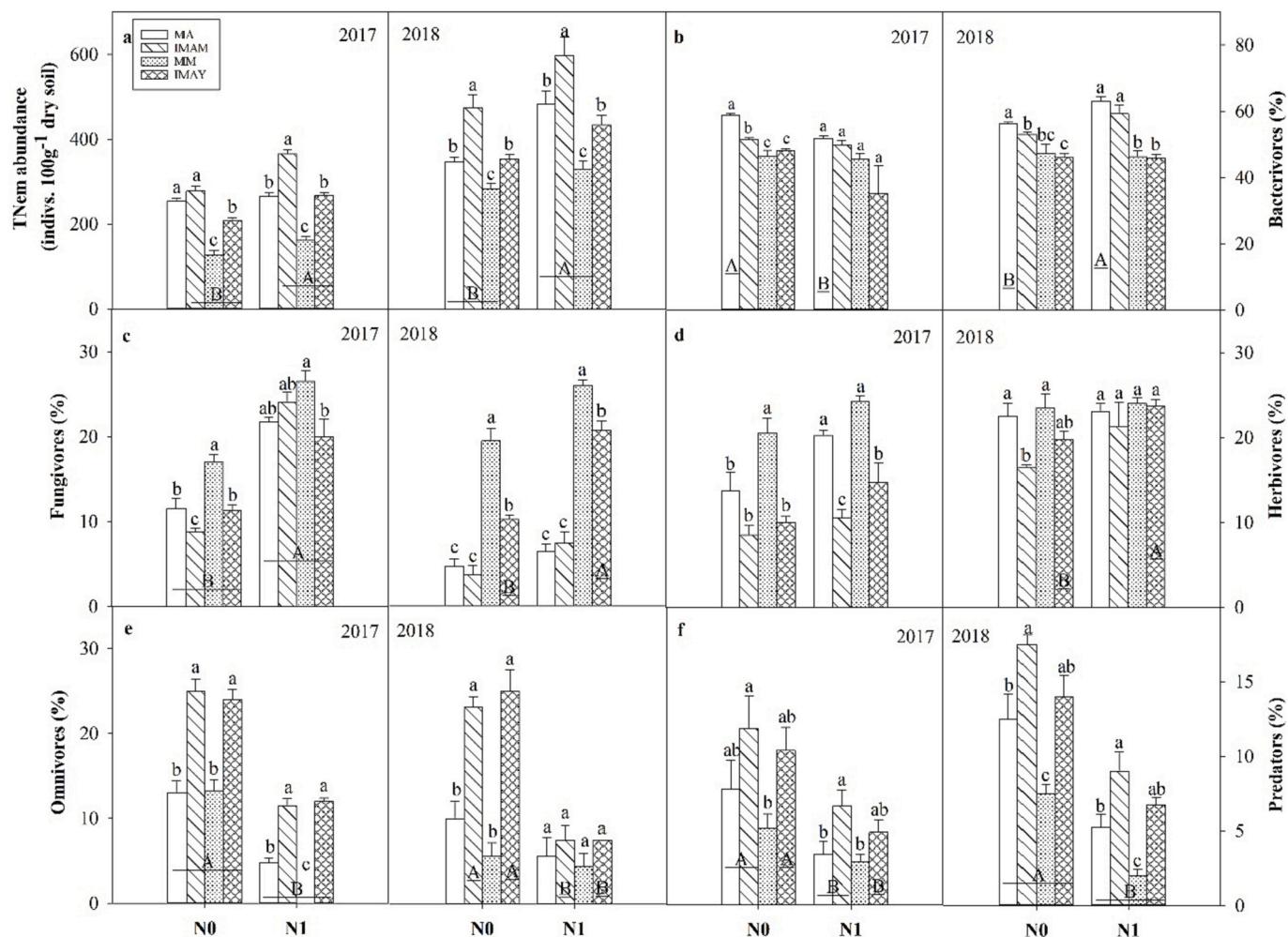


Fig. 1. Total nematode (TNem) abundance (a) (individuals 100 g⁻¹ dry soil) and relative abundance (%) of the nematode trophic groups: bacterivores (b), fungivores (c), herbivores (d), omnivores (e) and predators (f) under different cropping modes and N fertilization rates in 2017 and 2018. MA: monoculture alfalfa, MM: monoculture maize, IMAM: intercropped alfalfa, IMAY: intercropped maize. N0: without N fertilization; N1: with N fertilization. Different letters above bars represent a statistically significant difference ($p < 0.05$) between cropping modes (small letters) and N fertilization rates (capital letters).

Table 3

Results (mean \pm S.E) of two-way analyses of variance (ANOVA) on the effects of N fertilization (N), cropping modes (CM), and their interaction (N \times CM) on species richness (SR), and ecological indices including maturity index (MI), basal index (BI), channel index (CI), enrichment index (EI), and structure index (SI).

Nematode variables	2017			2018		
	N	CM	N \times CM	N	CM	N \times CM
SR	117.57**	9.78ns	2.96ns	200.34**	31.90**	3.37ns
MI	37.42**	59.05**	13.16**	37.07**	18.95**	1.41ns
BI	390.26**	59.69**	19.2**	52.85**	16.9**	2.78ns
CI	531.74**	87.91**	14.45**	28**	31.31**	4.98**
EI	250.98**	16.79**	0.42ns	28.03**	10.42**	1.55ns
SI	192.39**	47.94**	16.46**	54.26**	19.79**	2.03ns

Values indicate F -values and the level of significance. ns, not significant.

* $p < 0.05$.

** $p < 0.01$.

biomass and metabolic footprints of nematodes. Regardless of N fertilization, intercropping significantly increased the TNem biomass, nematode composite footprint and metabolic footprint of omnivores and predators compared with those under the monoculture systems, except for the IMAM treatment under N1 in 2017. In contrast, N addition significantly decreased the TNem biomass, composite footprint and metabolic footprints of omnivores and predators under all cropping modes, except for the IMAM treatment in 2018 (Table 4). Similarly, N

addition had a significant negative effect on the metabolic footprints of bacterivores in all the treatments in 2017 and in the MA and IMAY treatments in 2018 (Table 4); however, it positively affected the metabolic footprints of fungivores in all the treatments except the IMAM and IMAY treatments in 2018 and those of herbivores in the MA and IMAY treatments in 2017 and 2018 (Table 4). The flow of energy and matter through the soil food web is mainly driven by the feeding interrelationships of soil biota (Lenoir et al., 2007). SEM showed that N

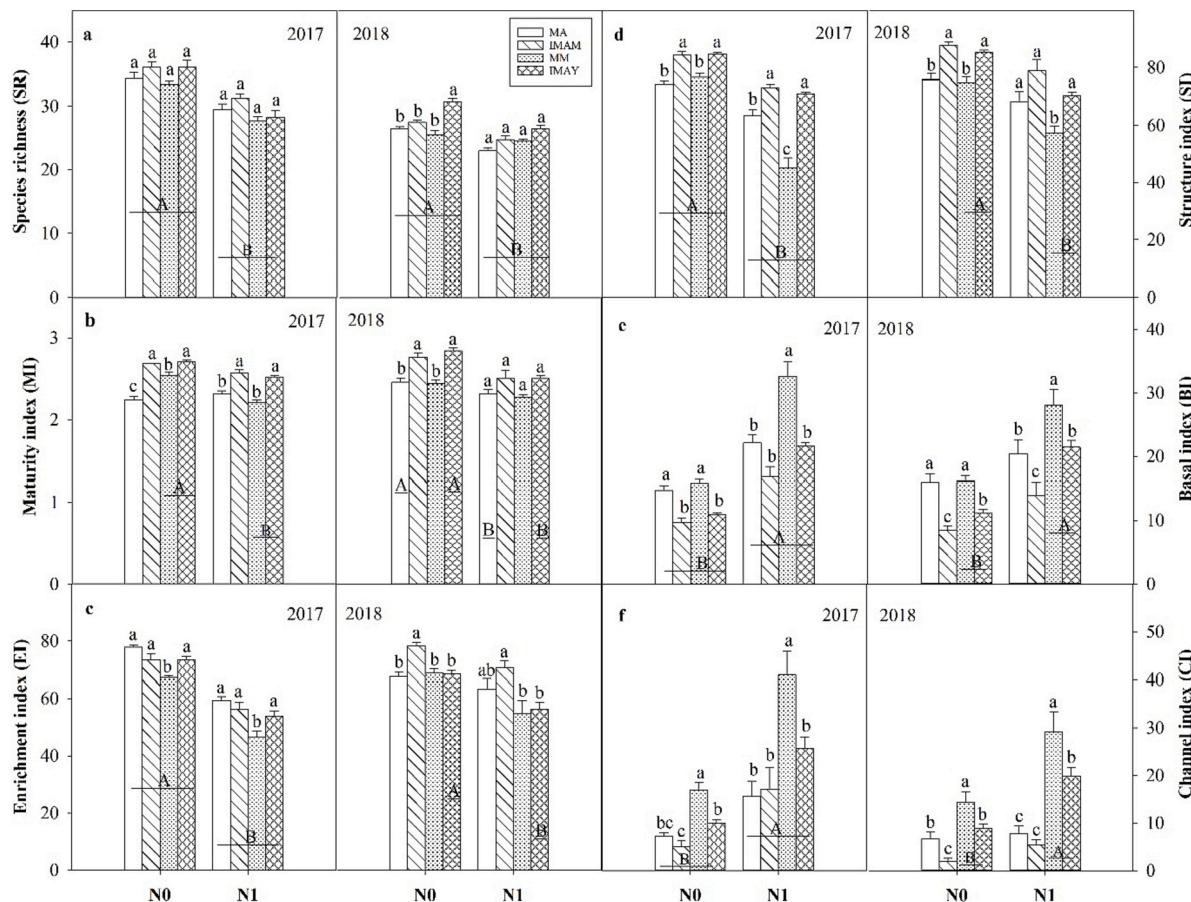


Fig. 2. Effect of cropping modes and N fertilization on nematode (a) species richness (SR), (b) structure index (SI), (c) maturity index (MI), (d) basal index (BI), (e) enrichment index (EI), and (f) channel index (CI) in 2017 and 2018. MA: monoculture alfalfa, MM: monoculture maize, IMAM: intercropped alfalfa, IMAY: intercropped maize. N0: without N fertilization; N1: with N fertilization. Different letters above bars represent a statistically significant difference ($p < 0.05$) between cropping modes (small letters) and N fertilization rates (capital letters).

addition explained 51.3 % and 28.6 % of the variation in C flow via bacterivores and fungivores, respectively (Fig. 4). The relationship between predators and herbivores in terms of C flow was stronger than that between other trophic groups, but this flow of energy and matter between the metabolic footprints of omnivores was negative. In addition, there was a significant negative correlation between the metabolic footprints of bacterivores and fungivores (Fig. 4).

3.4. Relationships between soil properties and nematode abundance

The responses of the total and trophic group abundances of soil nematodes differed among cropping modes under the two N levels, as depicted by the results of an RDA (Fig. 5a–b). Overall, the first two axes of the RDA explained over 90 % of the total variation observed in the soil nematode communities in the treatments without N addition and over 95 % in those with N addition. In the treatments without N addition, Axis 1 of the RDA plot separated the MM treatment from the IMAY treatment and explained 88.7 % of the variation in the nematode communities. In the N addition treatments, Axis 1 of the RDA plot explained 95.3 % of the variation and separated the MM and MA treatments from the IMAM and IMAY treatments. Under the N0 condition, Axis 2 separated the MA and MM treatments from their matching intercropping treatments; this was true for the N1 condition but only in the MA and IMAY treatments. Axis 2 explained only approximately 1.1 % and 0.2 % of the total variation in soil nematode community composition under the N0 and N1 conditions, respectively. Moreover, under the two N levels, the IMAM and IMAY treatments were associated with omnivorous and predatory nematodes and were positively correlated with TN, SMC, and

SOC. However, MM was associated with herbivorous nematodes and positively correlated with $\text{NO}_3\text{-N}$ (Fig. 5a–b, Table S5) and soil pH, whereas fungivores were positively correlated with the C:N ratio (Fig. 5a–b, Table S5). MA was associated with bacterivorous nematodes.

4. Discussion

4.1. Effects of intercropping and N fertilization on soil nematode community composition

Crop diversification (such as intercropping) promotes diversity in agricultural systems, and this has been shown to be associated with increases in soil C return and an improved soil environment, which in turn is associated with increased belowground faunal density and diversity (Albrecht et al., 2004; Hooper et al., 2000). Ferris et al. (2001) stated that a food web with abundant and diverse resource inputs is more speciose and has more trophic links, resulting in a complex and stable food web characterized by the development of higher-trophic level fauna (i.e., omnivores and predators). In line with the above findings, we found that there were significant increases in TNem abundance and omnivore and predator abundance in the intercropping systems, particularly in the absence of N fertilization (Fig. 1a, e–f). The increases were related to the increases in SMC, TN, and SOC (Fig. 4). In contrast to that of omnivorous and predatory nematodes, the abundance of herbivorous nematodes decreased significantly in the intercropping treatments. There are two possible explanations for this: 1) intercropping systems may regulate root-feeding nematodes, as also indicated by previous studies (Atandi et al., 2017; Hooks et al., 2010; Santiago et al.,

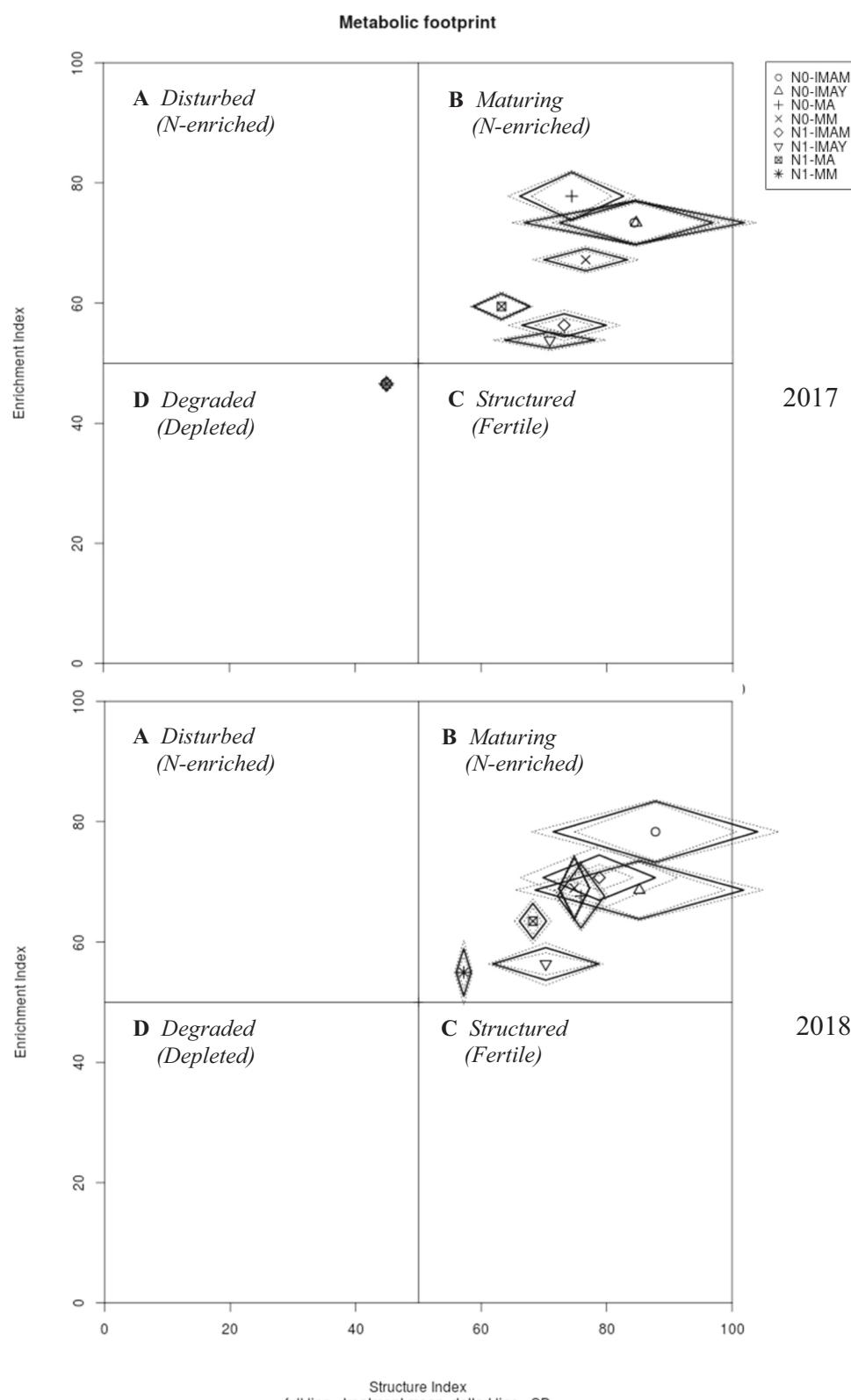


Fig. 3. Effects of cropping modes and N fertilization on metabolic footprints of soil nematodes in the maize/alfalfa intercropping systems in 2017 and 2018. A rhombus represents the metabolic footprints of soil nematodes in the maize/alfalfa agro-ecosystem. The length of the vertical and horizontal axes of the rhombus corresponds to the footprints of enrichment and structure components depicted as enrichment index and structure index, respectively (Ferris, 2010b). The full line and the dotted lines around the full line of the rhombus represents mean values and standard deviation of the cropping modes. The point in the middle of a rhombus represents the intersection of enrichment and structure footprints (Ferris et al., 2001; Sieriebrennikov et al., 2014). NO-MA, NO-IMAM, NO-MM, NO-IMAY and N1-MA, N1-IMAM, N1-MM, N1-IMAY represents monoculture alfalfa, intercropped alfalfa, monoculture maize, and intercropped maize without N fertilization (NO) and with N fertilization (N1), respectively.

2012) showing that intercropping increased nematode density and diversity but suppressed the incidence of plant-parasitic nematodes, and 2) omnivores and predators may impose top-down control on herbivorous nematodes. For instance, Djigal et al. (2012) reported top-down control of plant-feeding nematodes when lower trophic groups enhanced higher trophic groups. Similarly, in a grassland soil in

Scotland, an increase in the density of predatory and omnivorous nematodes was found to be positively correlated with soil microbes but negatively correlated with bacterivores (Neilson et al., 2020), suggesting that suppression of herbivores and bacterivores by predatory and omnivorous nematodes occurred (Djigal et al., 2012; Neilson et al., 2020).

Table 4

Results (mean \pm S.E) of two-way analyses of variance (ANOVA) on the effect of N fertilization (N), cropping modes (CM), and their interactions (N \times CM) on TNemB, nematode composite footprint (CFP), metabolic footprint of bacterivores (BFP), fungivores (FFP), herbivores (HFP), and omnivore-predators (OPFP).

	Fertilization	Cropping modes	TNemB	CFP	BFP	FFP	HFP	OPFP	
2017	N0	MA	0.4 \pm 0.05 ^{bA}	74.5 \pm 5.05 ^{cA}	26.6 \pm 0.74 ^{aA}	1.5 \pm 0.19 ^{bB}	1.7 \pm 0.27 ^{bB}	44.7 \pm 5.04 ^{cA}	
		IMAM	0.9 \pm 0.05 ^{aA}	122.8 \pm 5.54 ^{aA}	24.3 \pm 0.48 ^{aA}	1.1 \pm 0.16 ^{bB}	1.0 \pm 0.15 ^{bA}	96.4 \pm 5.37 ^{aA}	
		MM	0.3 \pm 0.04 ^{cA}	53.7 \pm 5.84 ^{dA}	13.8 \pm 1.24 ^{bA}	2.2 \pm 0.08 ^{aB}	2.7 \pm 0.23 ^{aA}	34.9 \pm 4.85 ^{cA}	
		IMAY	0.6 \pm 0.03 ^{bA}	93.1 \pm 3.08 ^{bA}	23.7 \pm 0.79 ^{aA}	1.5 \pm 0.20 ^{bB}	1.4 \pm 0.13 ^{bA}	66.4 \pm 3.12 ^{bA}	
	N1	MA	0.2 \pm 0.02 ^{aB}	44.9 \pm 1.58 ^{aB}	16.4 \pm 0.98 ^{aB}	3.2 \pm 0.18 ^{aA}	2.8 \pm 0.12 ^{aA}	22.6 \pm 1.18 ^{bB}	
		IMAM	0.3 \pm 0.05 ^{aB}	54.3 \pm 4.61 ^{aB}	15.1 \pm 1.30 ^{bB}	3.2 \pm 0.23 ^{aA}	1.3 \pm 0.14 ^{cA}	34.7 \pm 5.71 ^{aB}	
		MM	0.1 \pm 0.01 ^{bB}	19.2 \pm 1.40 ^{bB}	9.7 \pm 0.55 ^{cB}	2.9 \pm 0.21 ^{aA}	3.1 \pm 0.13 ^{aA}	3.4 \pm 0.70 ^{cB}	
		IMAY	0.3 \pm 0.04 ^{aB}	54.3 \pm 4.50 ^{bB}	12.3 \pm 0.95 ^{bcB}	2.8 \pm 0.23 ^{aA}	1.9 \pm 0.31 ^{bA}	37.3 \pm 4.77 ^{aB}	
ANOVA (p-values)									
Fertilization (N)									
<0.001									
Cropping modes (CM)									
<0.001									
N \times CM									
<0.001									

	Fertilization	Cropping modes	TNemB	CFP	BFP	FFP	HFP	OPFP	
2018	N0	MA	0.1 \pm 0.01 ^{bA}	39.2 \pm 1.40 ^{bA}	24.1 \pm 0.86 ^{aA}	0.3 \pm 0.05 ^{cB}	2.8 \pm 0.27 ^{aA}	11.9 \pm 1.03 ^{bA}	
		IMAM	0.5 \pm 0.06 ^{aA}	82.3 \pm 5.80 ^{aA}	22.1 \pm 0.65 ^{abA}	0.7 \pm 0.19 ^{cA}	3.2 \pm 0.26 ^{aA}	56.2 \pm 6.13 ^{aA}	
		MM	0.1 \pm 0.00 ^{bA}	34.7 \pm 0.87 ^{bA}	22.2 \pm 0.58 ^{abA}	2.8 \pm 0.29 ^{aB}	3.6 \pm 0.32 ^{aA}	6.1 \pm 0.45 ^{aA}	
		IMAY	0.5 \pm 0.05 ^{aA}	83.1 \pm 5.60 ^{aA}	21.0 \pm 0.49 ^{bA}	1.8 \pm 0.14 ^{bA}	3.6 \pm 0.37 ^{aB}	56.7 \pm 5.53 ^{aA}	
	N1	MA	0.1 \pm 0.00 ^{bB}	26.6 \pm 0.93 ^{bB}	17.3 \pm 1.19 ^{aB}	0.7 \pm 0.12 ^{cA}	3.2 \pm 0.06 ^{bA}	5.4 \pm 1.41 ^{bB}	
		IMAM	0.3 \pm 0.06 ^{aA}	53.5 \pm 6.03 ^{aB}	18.8 \pm 2.97 ^{aA}	1.4 \pm 0.37 ^{cA}	4.4 \pm 0.84 ^{abA}	28.9 \pm 6.05 ^{aB}	
		MM	0.1 \pm 0.01 ^{bB}	27.1 \pm 2.15 ^{bB}	18.1 \pm 2.61 ^{aA}	3.8 \pm 0.05 ^{aA}	3.3 \pm 0.15 ^{bA}	1.9 \pm 0.51 ^{bB}	
		IMAY	0.3 \pm 0.02 ^{aB}	49.8 \pm 2.21 ^{aB}	13.5 \pm 1.64 ^{aB}	2.9 \pm 0.32 ^{bA}	5.6 \pm 0.31 ^{aA}	27.7 \pm 1.27 ^{aB}	
ANOVA (p-values)									
Fertilization (N)									
<0.001									
Cropping modes (CM)									
<0.001									
N \times CM									
0.026									

Different small letters in table columns indicate significant difference between cropping modes (CM), while the capital letters show significant difference between the two N levels; ns, not significant. Means were separated using Fisher's LSD test ($n = 4$). See Table 1 for other abbreviations.

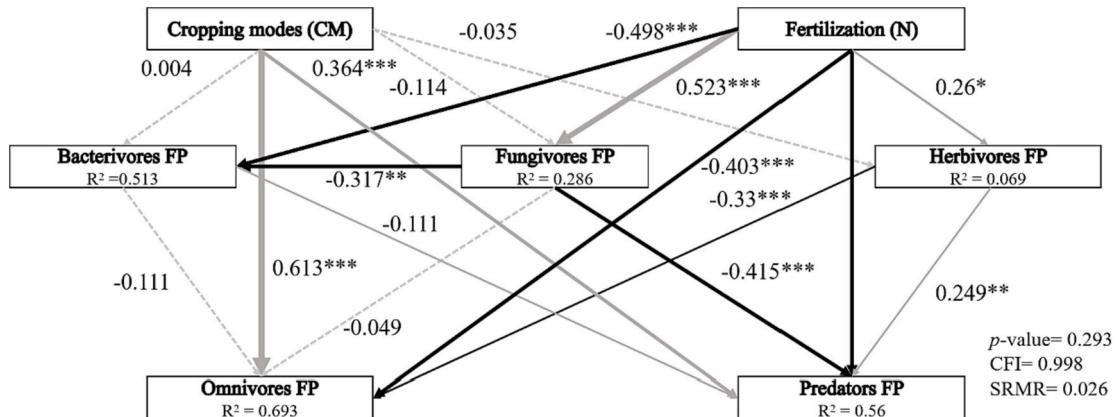


Fig. 4. Structural equation modeling (SEM) of the effect of cropping modes (CM) and N fertilization on metabolic footprints (MFP) of nematode trophic groups (bacterivore FP, fungivore FP, herbivore FP, omnivore FP, and predator FP) in the rhizosphere of maize/alfalfa intercropping systems. The model adequately fitted the data on the metabolic footprints of nematode trophic groups ($\chi^2 = 2.454$, $p = 0.293$, CFI = 0.998, SRMR = 0.026). The numbers at the arrows are standardized path coefficients, and the arrow thickness represents the magnitude of the path coefficient. The black and grey solid arrows indicate significant ($p < 0.05$) negative and positive paths, respectively. Dashed arrows indicate non-significant ($p > 0.05$) paths. R^2 values associated with response variables indicate the proportion of variation explained by the model.

Bacterivorous and fungivorous nematodes are associated with decomposition and nutrient cycling processes, and their abundances reflect the quality and quantity of the food resources (i.e., plant residue and root exudates) available (Freckman, 1988; Griffiths, 1994). Previous studies have shown that organic substrates with lower C:N ratios decompose rapidly and produce better-quality organic matter and that bacteria utilize such organic matter quickly (Kou et al., 2018; Lynch et al., 2016), leading to large populations of bacterivorous nematodes (Ferris and Matute, 2003). Substrates with a high C:N ratio (i.e., those containing low nutrient and high fiber contents) provide poor-quality

foods to soil-dwelling organisms (Scheunemann et al., 2010). In accordance with the above findings, the soil in the MA treatment had a relatively low C:N ratio, resulting in a relatively high bacterivore abundance (Table 1). However, the soil in the MM treatment had a significantly higher C:N ratio than did that in the IMAY treatment. This condition could result in less decomposition by soil microorganisms, requiring an abundance of fungal soil organisms that favor slow decomposition (Rousk and Baath, 2007), subsequently leading to more fungivorous nematodes in the MM treatment than in the IMAY treatment (Table 1). Furthermore, consistent with the finding of Ferris and Bongers (2003), the soil in the IMAY treatment had a significantly higher C:N ratio than did that in the MA treatment. This condition could result in less decomposition by soil microorganisms, requiring an abundance of bacterial soil organisms that favor fast decomposition (Rousk and Baath, 2007), subsequently leading to more bacterivorous nematodes in the IMAY treatment than in the MA treatment (Table 1).

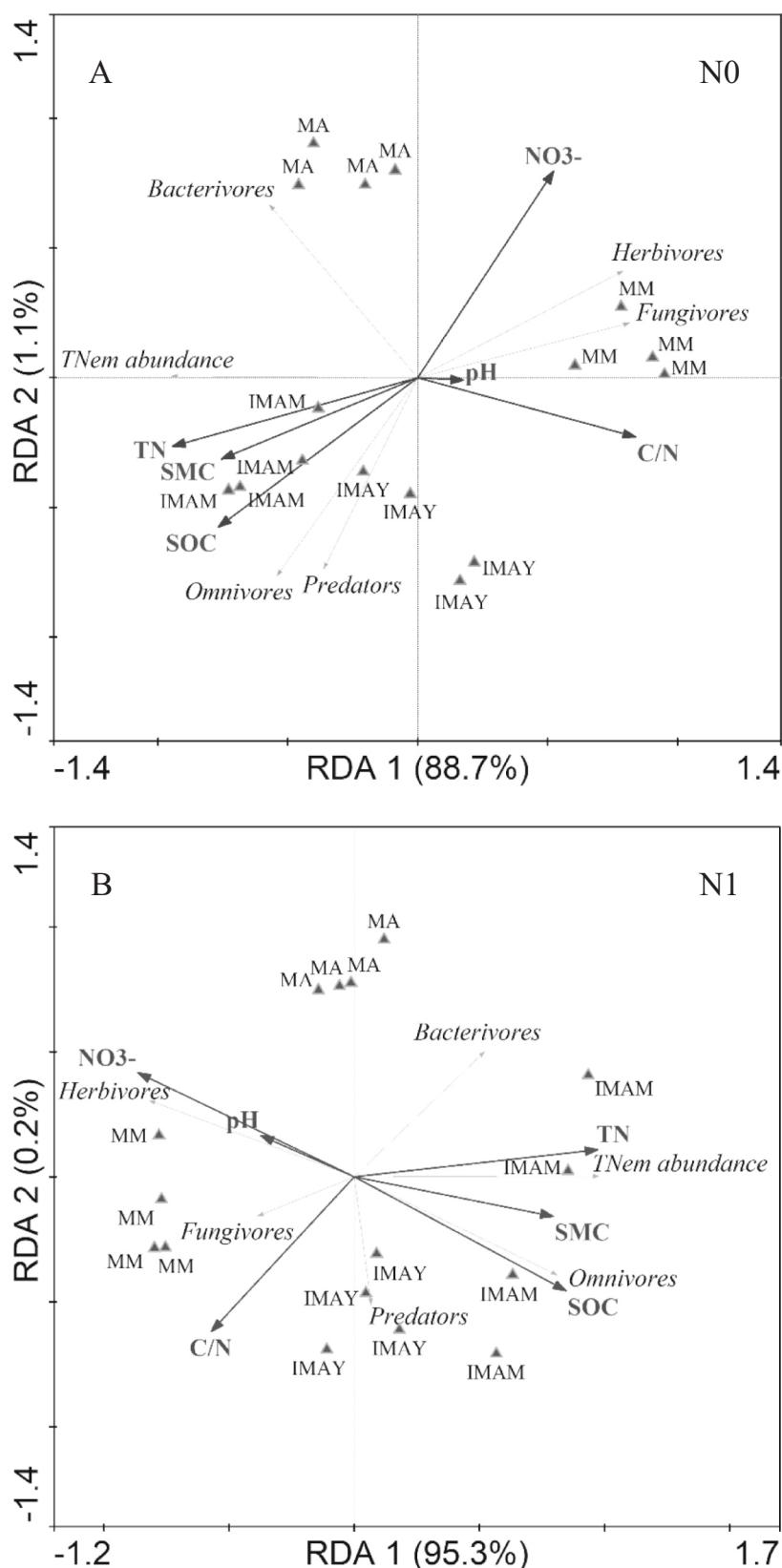


Fig. 5. Redundancy analysis (RDA) of the relationship between soil physicochemical parameters including soil moisture content (SMC); soil total nitrogen (TN); soil organic carbon (SOC); nitrate concentration ($\text{NO}_3\text{-N}$), and soil carbon-nitrogen ratio (C:N) and TNem and the relative abundance of nematode trophic groups in the maize/alfalfa intercropping under (a) no N fertilization (N0) and (b) N fertilization (N1). MA: monoculture alfalfa, MM: monoculture maize, IMAM: intercropped alfalfa, IMAY: intercropped maize. See Fig. 1 for other abbreviations.

(2006) that fungivorous nematodes such as members of aphelenchid taxa increased in abundance following enrichment with high-C:N organic material, the findings of the present study revealed that *Aphelenchus* and *Aphelenchooides* (identified as aphelenchid groups) were more

abundant in the soil in the MM treatment with a higher C:N ratio than in that in the IMAY treatment (Tables S1 and S2).

Like previous researchers (Azpilicueta et al., 2014; Hu et al., 2017; Liu et al., 2016a; Song et al., 2015), we found a significant increase in

the abundance of TNem but a strong decrease in the abundance of omnivores and predators, nematode diversity, and SR in response to N fertilization (Fig. 2a–b, d–e). Given that N is a limiting nutrient in most agricultural soils and is critical for plant growth, its input often leads to high root biomass and root exudation (Zhang et al., 2019). The positive effects of N fertilization on root biomass and rhizodeposition could contribute to the increase in TNem abundance. This is because N fertilization can influence nematode community structure through bottom-up control of their food resources (plant roots and soil microbes) or through effects on soil pH and soil moisture (Liu et al., 2016b). N fertilization led to a decline in soil pH, but the soil remained slightly alkaline. Although the effect of soil pH remained nonsignificant, the abundance of omnivores and predators was negatively related to the increase in soil NO_3^- -N concentration (Table S5). These findings are in agreement with those of Song et al. (2016) and Azpilicueta et al. (2014), who found that increased N fertilization increased the availability of soil NO_3^- -N, which in turn led to the suppression of omnivorous and predatory nematodes. Considering the important role of omnivores and predators in reconstructing the soil food web and enhancing nutrient recycling, a decrease in the abundance of such groups may weaken the soil food web complex (Song et al., 2015).

4.2. Effects of cropping mode and N fertilization on the soil food web

Given the ecological services provided by soil biodiversity, increases in soil biota abundance and diversity are crucial for the sustainability of agroecosystems. Specifically, agricultural practices that increase the belowground input of C and N through incorporation, for instance, planting leguminous crop plants, increase the abundance and activity of microbes compared with those in conventional systems (Altieri, 1999). In this study, intercropping of alfalfa and maize increased nematode diversity and SR and the MI, SI, and EI values under both N levels, but the effect was greater in the absence of N fertilization. The increases in the MI and SI in the intercropping systems were associated with an increased abundance of *Aporcelaimus*, *Discolaimus*, *Dorylaimus* and *Paravulvulus* (K-strategist nematodes with high sensitivity to disturbance). An increased EI value indicates an abundance of resources available for plants. Previous studies have indicated that plant diversity is an important driver of changes in soil biota, and thus, diversification of cropping systems increases soil biodiversity and associated ecosystem functions (Bedoussac et al., 2015; Hufnagel et al., 2020; Tamburini et al., 2020).

In accordance with the first hypothesis, N fertilization significantly decreased the MI, SI, and EI values in all the treatments, and the effects were more pronounced in the monoculture systems. In a meta-analysis of data from 54 studies (Liu et al., 2016a), nematode MI and SI values were found to have decreased with an increased application rate of N fertilizers. Nematode MIs or SIs reflect the weighted abundance of taxa including cp3-5 (omnivores and predators), which are highly sensitive to environmental disturbances (Ferris et al., 2001). Hence, the reduction in these functional groups of nematodes was attributed to the lower MI and SI values recorded in the N fertilization treatment (Ferris et al., 2001). For instance, nematode faunal analysis revealed that the MM treatment presented the lowest EI and SI values and was mapped to quadrant D in the faunal analysis plot following N fertilization, representing resource-depleted soil food web conditions. In general, in the absence of N fertilization, all cropping modes were mapped to quadrat B in the EI vs. SI plot (Fig. 3); however, after N fertilization, they tended to clearly shift toward quadrat D. This shift in the soil food web was from the higher EI and SI values under no N fertilization to the lower EI and SI values under N fertilization. These findings indicated a shift from an enriched and complex food web to a depleted and simple food web, the latter of which is typical of high-input conventional agroecosystems. On the other hand, the BI and CI values were higher in the monoculture treatments, and the effect was more obvious with N fertilization. Higher EI and SI values and lower BI and CI values were also found in low-input and organic

management systems compared with high-input and conventional management systems (Berkelmans et al., 2003; Sánchez-Moreno et al., 2009).

The metabolic footprint of soil nematodes is related to C and nutrient cycling in the soil food web. Ferris (2010b) proposed the use of nematode metabolic footprints as indices that reflect the magnitude of ecosystem services and functions, including the amount of C used for nematode metabolic activity. In this study, the metabolic footprint of omnivores and predators and TNem biomass increased significantly in the intercropping system (Table 4), indicating that intercropping may increase the metabolic activity and biomass of nematodes, thus improving soil food web function. Previous studies have shown that the responses of soil nematode communities to management practices could be explained by bottom-up control resulting from changes in food resources and/or changes in the soil environment (Liu et al., 2016b). The results of the RDA in this study indicated that some cropping modes, particularly the intercropping systems, were positively associated with total and omnivore and predator nematode abundance (Fig. 5). In addition, SEM revealed a direct and positive correlation between cropping mode and the metabolic footprint of omnivores and predators. This may be attributed to changes in the soil environment since the path coefficients via food resources (i.e., bacterivore and fungivore footprints) were nonsignificant (Fig. 4). In contrast to cropping mode, N fertilization negatively affected the metabolic footprint of omnivores and predators, indicating that N fertilizer reduced the otherwise positive effect of intercropping on the activity of higher-trophic level nematodes, which is in line with the second hypothesis. The SEM results further showed that the flow of resources was only positive and significant from herbivores to predators (explaining 7–56 % of the total variation in the metabolic footprints of herbivores and predators). This may indicate that herbivores made a significantly greater contribution to nematode-regulated resource flow to predators than did other nematode trophic groups, which could be attributed to feeding by herbivores maybe having induced the release of plant-derived C into the soil. For instance, Bardgett et al. (1999) demonstrated that a small amount of root herbivory by clover cyst nematodes increased clover root growth by 141 %. The increase in clover root growth was associated with an increased supply of C and other nutrients since root infestation by nematodes had previously shown no effect on white clover root biomass, which also led to increased microbial activity and biomass (Denton et al., 1999). Furthermore, Ferris (2010a) stated that, when feeding on plants at low population densities, by enhancing root exudation or by serving as prey for predators, herbivorous nematodes serve as resources that prime the beneficial services of other organisms. In contrast, the nonsignificant or negative correlation of the microbivore and omnivore–predator footprints may be indicative of the top-down control of bacterivorous and fungivorous nematodes (Neilson et al., 2020).

4.3. Conclusion

The results of this study clearly showed that alfalfa/maize intercropping increased TNem abundance and biomass, the density of omnivores and predators, and nematode metabolic footprints in the absence of N fertilization. In addition, nematode faunal analysis revealed that intercropping was associated with increased EI and SI values, reflecting improved nematode structure and food web complexity as a result of intercropping. Therefore, intercropping alfalfa with maize in low-N soil is a promising approach for improving soil health and enhancing food web development, which would be beneficial for agricultural sustainability and would further contribute to ensuring food security.

Declaration of competing interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work

submitted.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2023.104809>.

References

- Albrecht, A., Cadisch, G., Blanchart, E., Sitompul, S.M., Vanlauwe, B., 2004. Below-ground inputs: relationships with soil quality, soil C storage and soil structure. In: Noordwijk, M.V., Cadisch, G., Ong, C.K. (Eds.), *Below-ground Interactions in Tropical Agroecosystems: Concepts and Models with Multiple Plant Components*. CAB International, Wallingford, United Kingdom, pp. 193–207.
- Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. *Agric. Ecosyst. Environ.* 74, 19–31. [https://doi.org/10.1016/S0167-8809\(99\)00028-6](https://doi.org/10.1016/S0167-8809(99)00028-6).
- Atandi, J.G., Haukeland, S., Karuki, G.M., Coyne, D.L., Karanja, E.N., Musyoka, M.W., Fiaboe, K.K.M., Bautze, D., Adamtey, N., 2017. Organic farming provides improved management of plant parasitic nematodes in maize and bean cropping systems. *Agric. Ecosyst. Environ.* 247, 265–272. <https://doi.org/10.1016/j.agee.2017.07.002>.
- Azpilicueta, C.V., Cristina Aruani, M., Chaves, E., Reeb, P.D., 2014. Soil nematode responses to fertilization with ammonium nitrate after six years of unfertilized apple orchard. *Span. J. Agric. Res.* 12, 353–363. <https://doi.org/10.5424/sjar/201412-4634>.
- Bardgett, R.D., Denton, C.S., Cook, R., 1999. Below-ground herbivory promotes soil nutrient transfer and root growth in grassland. *Ecol. Lett.* 2, 357–360. <https://doi.org/10.1046/j.1461-0248.1999.00001.x>.
- Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. *Nature* 515, 505–511. <https://doi.org/10.1038/nature13855>.
- Barillot, C.D.C., Sarde, C.O., Bert, V., Tarnaud, E., Cochet, N., 2013. A standardized method for the sampling of rhizosphere and rhizoplane soil bacteria associated to a herbaceous root system. *Ann. Microbiol.* 63, 471–476. <https://doi.org/10.1007/s13213-012-0491-y>.
- Bedoussac, L., Journet, E.P., Hauggaard-Nielsen, H., Naudin, C., Corre-Hellou, G., Jensen, E., Prieur, L., Justes, E., 2015. Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming: a review. *Agron. Sustain. Dev.* 35, 911–935. <https://doi.org/10.1007/s13593-014-0277-7>.
- Berkelmans, R., Ferris, H., Tenuta, M., van Bruggen, A.H.C., 2003. Effects of long-term crop management on nematode trophic levels other than plant feeders disappear after 1 year of disruptive soil management. *Appl. Soil Ecol.* 23, 223–235. [https://doi.org/10.1016/S0929-1393\(03\)00047-7](https://doi.org/10.1016/S0929-1393(03)00047-7).
- Bongers, T., 1988. De nematoden van Nederland. Stichting Uitgeverij Koninklijke Nederlandse Natuurhistorische Vereniging, Utrecht.
- Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* 83, 14–19. <https://doi.org/10.1007/BF00324627>.
- Bonkowski, M., Villenave, C., Griffiths, B., 2009. Rhizosphere fauna: the functional and structural diversity of intimate interactions of soil fauna with plant roots. *Plant Soil* 321, 213–233. <https://doi.org/10.1007/s11104-009-0013-2>.
- Cai, S.M., Wang, J.J., Lv, W.G., Xu, S.X., Zhu, H.T., 2020. Nitrogen fertilization alters the effects of earthworms on soil physicochemical properties and bacterial community structure. *Appl. Soil Ecol.* 150, 103478 <https://doi.org/10.1016/j.apsoil.2019.103478>.
- De Graaff, M.-A.A., Hornstein, N., Throop, H.L., Kardol, P., Van Diepen, L.T.A.A., 2019. Effects of agricultural intensification on soil biodiversity and implications for ecosystem functioning: a meta-analysis. *Adv. Agron.* 155, 1–44. <https://doi.org/10.1016/bs.agron.2019.01.001>.
- Denton, C.S., Bardgett, R.D., Cook, R., Hobbs, P.J., 1999. Low amounts of root herbivory positively influence the rhizosphere microbial community in a temperate grassland soil. *Soil Biol. Biochem.* 31, 155–165. [https://doi.org/10.1016/S0038-0717\(98\)00118-7](https://doi.org/10.1016/S0038-0717(98)00118-7).
- Djigal, D., Chabrier, C., Duyck, P.-F., Achard, R., Quénéhervé, P., Tixier, P., 2012. Cover crops alter the soil nematode food web in banana agroecosystems. *Soil Biol. Biochem.* 48, 142–150. <https://doi.org/10.1016/j.soilbio.2012.01.026>.
- FAO, 2001. Lecture notes on the major soils of the world. In: Food and Agriculture Organization (FAO), Italy, Rome.
- Ferris, H., 2010a. Contribution of nematodes to the structure and function of the soil food web. *J. Nematol.* 42, 63–67.
- Ferris, H., 2010b. Form and function: metabolic footprints of nematodes in the soil food web. *Eur. J. Soil Biol.* 46, 97–104. <https://doi.org/10.1016/j.ejsobi.2010.01.003>.
- Ferris, H., Bongers, T., 2006. Nematode indicators of organic enrichment. *J. Nematol.* 38, 3–12.
- Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Appl. Soil Ecol.* 18, 13–29. [https://doi.org/10.1016/S0929-1393\(01\)00152-4](https://doi.org/10.1016/S0929-1393(01)00152-4).
- Ferris, H., Bongers, T., de Goede, R.G.M., 2004. Nematode faunal analyses to assess food web enrichment and connectance. In: Cook, R.C., DJ (Eds.), *Proceedings of the Fourth International Congress of Nematology. Nematology Monographs and Perspectives*. Brill, Netherlands, p. 866.
- Ferris, H., Matute, M.M., 2003. Structural and functional succession in the nematode fauna of a soil food web. *Appl. Soil Ecol.* 23, 93–110. [https://doi.org/10.1016/S0929-1393\(03\)00044-1](https://doi.org/10.1016/S0929-1393(03)00044-1).
- Freckman, D.W., 1988. Bacterivorous nematodes and organic-matter decomposition. *Agric. Ecosyst. Environ.* 24, 195–217. [https://doi.org/10.1016/0167-8809\(88\)90066-7](https://doi.org/10.1016/0167-8809(88)90066-7).
- Griffiths, B.S., 1994. Microbial-feeding nematodes and protozoa in soil: their effects on microbial activity and nitrogen mineralization in decomposition hotspots and the rhizosphere. *Plant Soil* 164, 25–33. <https://doi.org/10.1007/Bf00010107>.
- Hodson, A.K., Ferris, H., Hollander, A.D., Jackson, L.E., 2014. Nematode food webs associated with native perennial plant species and soil nutrient pools in California riparian oak woodlands. *Geoderma* 228–229, 182–191. <https://doi.org/10.1016/j.geoderma.2013.07.021>.
- Hooks, C.R.R., Wang, K.H., Ploeg, A., Mc Sorley, R., 2010. Using marigold (*Tagetes spp.*) as a cover crop to protect crops from plant-parasitic nematodes. *Appl. Soil Ecol.* 46, 307–320. <https://doi.org/10.1016/j.apsoil.2010.09.005>.
- Hooper, D.J., Hallmann, J., Subbotin, S.A., 2005. Methods for extraction, processing and detection of plant and soil nematodes. In: Luc, M., Sikora, R.A., Bridge, J. (Eds.), *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, 2nd ed. CAB International, Wallingford, p. 917 pp.
- Hooper, D.U., Bignell, D.E., Brown, V.K., Brussard, L., Dangerfield, J.M., Wall, D.H., Wardle, D.A., Coleman, D.C., Giller, K.E., Lavelle, P., Putten, V.D., HW, Ruiter, D., CP, Rusek, J., Silver, W.L., Tiedje, J.M., Wolters, V., 2000. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. *BioScience* 50, 1049–1061. [https://doi.org/10.1641/0006-3568\(2000\)050\[1049:IBAABB\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2000)050[1049:IBAABB]2.0.CO;2).
- Hu, J., Chen, G.R., Hassan, W.M., Chen, H., Li, J.Y., Du, G.Z., 2017. Fertilization influences the nematode community through changing the plant community in the Tibetan Plateau. *Eur. J. Soil Biol.* 78, 7–16. <https://doi.org/10.1016/j.ejsobi.2016.11.001>.
- Hu, L.T., Bentler, P.M., 1999. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. *Struct. Equ. Modeling* 6, 1–55. <https://doi.org/10.1080/10705519909540118>.
- Hufnagel, J., Reckling, M., Ewert, F., 2020. Diverse approaches to crop diversification in agricultural research: a review. *Agron. Sustain. Dev.* 40, 14. <https://doi.org/10.1007/s13593-020-00617-4>.
- Jackson, L.E., Brussard, L., de Ruiter, P.C., Pascual, U., Perrings, C., Bawa, K., 2013. Agrobiodiversity. In: Levin, S.A. (Ed.), *Encyclopedia of Biodiversity*, (Second Edition). Academic Press, Waltham, pp. 126–135. <https://doi.org/10.1016/B978-0-12-384719-5.00233-1>.
- Jensen, E.S., Carlsson, G., Hauggaard-Nielsen, H., 2020. Intercropping of grain legumes and cereals improves the use of soil N resources and reduces the requirement for synthetic fertilizer N: a global-scale analysis. *Agron. Sustain. Dev.* 40, 5. <https://doi.org/10.1007/s13593-020-0607-x>.
- Knörzer, H., Graeff-Hönninger, S., Guo, B., Wang, P., Claupein, W., 2009. The rediscovery of intercropping in China: a traditional cropping system for future Chinese agriculture - a review. In: Lichtfouse, E. (Ed.), *Climate Change, Intercropping, Pest Control and Beneficial Microorganisms*. Springer, Dordrecht, London, p. 514. <https://doi.org/10.1007/978-90-481-2716-0>.
- Kou, X., Su, T., Ma, N., Li, Q., Wang, P., Wu, Z., Liang, W., Cheng, W., 2018. Soil micro-food web interactions and rhizosphere priming effect. *Plant Soil* 432, 129–142. <https://doi.org/10.1007/s11104-018-3782-7>.
- Lavelle, P., Moreira, F., Spain, A., 2014. Biodiversity: conserving biodiversity in agroecosystems. In: Van Alfen, N.K. (Ed.), *Encyclopedia of Agriculture and Food Systems*. Academic Press, Oxford, p. 2848. <https://doi.org/10.1016/B978-0-444-52512-3.00019-X>.
- Lei, P.W., Wu, Q., 2007. Introduction to structural equation modeling: issues and practical considerations. *Educ. Meas. Issues Pract.* 26, 33–43. <https://doi.org/10.1111/j.1745-3992.2007.00099.x>.
- Lenoir, L., Persson, T., Bengtsson, J., Wallander, H., Wirén, A., 2007. Bottom-up or top-down control in forest soil microcosms? Effects of soil fauna on fungal biomass and C/N mineralisation. *Biol. Fertil. Soils* 43, 281–294. <https://doi.org/10.1007/s00374-006-0103-8>.
- Li, C., Hoffland, E., Kuyper, T.W., Yu, Y., Zhang, C., Li, H., Zhang, F., van der Werf, W., 2020. Syndromes of production in intercropping impact yield gains. *Nat. Plants* 6, 653–660. <https://doi.org/10.1038/s41477-020-0680-9>.
- Li, Q., Liang, W., Zhang, X., Mahamood, M., 2017. Nematode genera and species description along the transect. In: Li, Q., Liang, W., Zhang, X., Mahamood, M. (Eds.), *Soil Nematodes of Grasslands in Northern China*, 1st ed. Elsevier Inc., San Diego, p. 254 pp. <https://doi.org/10.1016/B978-0-12-813274-6.00003-X>.
- Liu, T., Chen, X.Y., Hu, F., Ran, W., Shen, Q.R., Li, H.X., Whalen, J.K., 2016a. Carbon-rich organic fertilizers to increase soil biodiversity: evidence from a meta-analysis of nematode communities. *Agric. Ecosyst. Environ.* 232, 199–207. <https://doi.org/10.1016/j.agee.2016.07.015>.

- Liu, T., Whalen, J.K., Ran, W., Shen, Q.R., Li, H.X., 2016b. Bottom-up control of fertilization on soil nematode communities differs between crop management regimes. *Soil Biol. Biochem.* 95, 198–201. <https://doi.org/10.1016/j.soilbio.2016.01.005>.
- Liu, Y.B., Li, X.Y., Liu, Q.Z., 2016c. Soil nematode communities in jujube (*Ziziphus jujuba* mill.) rhizosphere soil under monoculture and jujube/wheat (*Triticum aestivum* Linn.) intercropping systems: a case study in Xinjiang arid region, northwest of China. *Eur. J. Soil Biol.* 74, 52–59. <https://doi.org/10.1016/j.ejsobi.2016.02.001>.
- Lynch, M.J., Mulvaney, M.J., Hodges, S.C., Thompson, T.L., Thomason, W.E., 2016. Decomposition, nitrogen and carbon mineralization from food and cover crop residues in the central plateau of Haiti. *Springerplus* 5, 973. <https://doi.org/10.1186/s40064-016-2651-1>.
- Matson, P.A., Parton, W.J., Power, A.G., Swift, M.J., 1997. Agricultural intensification and ecosystem properties. *Science* 277, 504–509. <https://doi.org/10.1126/science.277.5325.504>.
- Neilson, R., Caul, S., Fraser, F.C., King, D., Mitchell, S.M., Roberts, D.M., Giles, M.E., 2020. Microbial community size is a potential predictor of nematode functional group in limed grasslands. *Appl. Soil Ecol.* 156, 103702 <https://doi.org/10.1016/j.apsoil.2020.103702>.
- Postma-Blaauw, M.B., de Goede, R.G.M., Bloem, J., Faber, J.H., Brussaard, L., 2012. Agricultural intensification and de-intensification differentially affect taxonomic diversity of predatory mites, earthworms, enchytraeids, nematodes and bacteria. *Appl. Soil Ecol.* 57, 39–49. <https://doi.org/10.1016/j.apsoil.2012.02.011>.
- Ritz, K., Trudgill, D.L., 1999. Utility of nematode community analysis as an integrated measure of the functional state of soils: perspectives and challenges. *Plant Soil* 212, 1–11. <https://doi.org/10.1023/A:1004673027625>.
- Rousk, J., Baath, E., 2007. Fungal and bacterial growth in soil with plant materials of different C/N ratios. *FEMS Microbiol. Ecol.* 62, 258–267. <https://doi.org/10.1111/j.1574-6941.2007.00398.x>.
- Sánchez-Moreno, S., Minoshima, H., Ferris, H., Jackson, L.E., 2006. Linking soil properties and nematode community composition: effects of soil management on soil food webs. *Nematology* 8, 703–715.
- Sánchez-Moreno, S., Nicola, N.L., Ferris, H., Zalom, F.G., 2009. Effects of agricultural management on nematode-mite assemblages: soil food web indices as predictors of mite community composition. *Appl. Soil Ecol.* 41, 107–117. <https://doi.org/10.1016/j.apsoil.2008.09.004>.
- Santiago, D.C., Arieira, G.D.O., de Almeida, E., Guimarães, M.D.F., 2012. Responses of soil nematode communities to agroecological crop management systems. *Nematology* 14, 209–221. <https://doi.org/10.1163/138855411x587103>.
- Scheunemann, N., Scheu, S., Butenschoen, O., 2010. Incorporation of decade old soil carbon into the soil animal food web of an arable system. *Appl. Soil Ecol.* 46, 59–63. <https://doi.org/10.1016/j.apsoil.2010.06.014>.
- Shaw, E.A., Boot, C.M., Moore, J.C., Wall, D.H., Barone, J.S., 2019. Long-term nitrogen addition shifts the soil nematode community to bacterivore-dominated and reduces its ecological maturity in a subalpine forest. *Soil Biol. Biochem.* 130, 177–184. <https://doi.org/10.1016/j.soilbio.2018.12.007>.
- Sieriebriennikov, B., Ferris, H., de Goede, R.G.M., 2014. NINJA: an automated calculation system for nematode-based biological monitoring. *Eur. J. Soil Biol.* 61, 90–93. <https://doi.org/10.1016/j.ejsobi.2014.02.004>.
- Smilauer, P., Lepš, J., 2014. Multivariate Analysis of Ecological Data Using CANOCO 5, 2nd ed. Cambridge University Press, Cambridge.
- Song, M., Jing, S., Zhou, Y., Hui, Y., Zhu, L., Wang, F., Hui, D., Jiang, L., Wan, S., 2015. Dynamics of soil nematode communities in wheat fields under different nitrogen management in northern China plain. *Eur. J. Soil Biol.* 71, 13–20. <https://doi.org/10.1016/j.ejsobi.2015.09.002>.
- Song, M., Li, X.M., Jing, S.S., Lei, L.J., Wang, J.L., Wan, S.Q., 2016. Responses of soil nematodes to water and nitrogen addition in an old-field grassland. *Appl. Soil Ecol.* 102, 53–60. <https://doi.org/10.1016/j.apsoil.2016.02.011>.
- Sun, B., Peng, Y., Yang, H., Li, Z., Gao, Y., Wang, C., Yan, Y., Liu, Y., 2014. Alfalfa (*Medicago sativa* L.)/maize (*Zea mays* L.) intercropping provides a feasible way to improve yield and economic incomes in farming and pastoral areas of northeast China. *PLoS ONE* 9. <https://doi.org/10.1371/journal.pone.0110556>.
- Tamburini, G., Bommarco, R., Wanger, T.C., Kremen, C., van der Heijden, M.G.A., Liebman, M., Hallin, S., 2020. Agricultural diversification promotes multiple ecosystem services without compromising yield. *Sci. Adv.* 6, eaba1715 <https://doi.org/10.1126/sciadv.aba1715>.
- Tiemann, L.K., Grandy, A.S., Atkinson, E.E., Marin-Spiotta, E., McDaniel, M.D., 2015. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecol. Lett.* 18, 761–771. <https://doi.org/10.1111/ele.12453>.
- van den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D.A., de Goede, R.G.M., Adams, B.J., Ahmad, W., Andriuzzi, W.S., Bardgett, R.D., Bonkowski, M., Campos-Herrera, R., Cares, J.E., Caruso, T., de Brito Caixeta, L., Chen, X., Costa, S.R., Creamer, R., da Cunha, Mauro, Castro, J., Dam, M., Djigal, D., Escuer, M., Griffiths, B.S., Gutierrez, C., Hoberg, K., Kalinkina, D., Kardol, P., Kergunteuil, A., Korthals, G., Krashevskaya, V., Kudrín, A.A., Li, Q., Liang, W., Magilton, M., Marais, M., Martin, J.A.R., Matveeva, E., Mayad, E.H., Mulder, C., Mullin, P., Neilson, R., Nguyen, T.A.D., Nielsen, U.N., Okada, H., Riis, J.E.P., Pan, K., Peneva, V., Pellissier, L., Pereira, Carlos, da Silva, J., Pitteloud, C., Powers, T.O., Powers, K., Quist, C.W., Rasmann, S., Moreno, S.S., Scheu, S., Setala, H., Sushchuk, A., Tiunov, A.V., Trap, J., van der Putten, W., Vestergaard, M., Villenave, C., Waeyenberge, L., Wall, D.H., Wilschut, R., Wright, D.G., Yang, J.I., Crowther, T.W., 2019. Soil nematode abundance and functional group composition at a global scale. *Nature* 572, 194–198. <https://doi.org/10.1038/s41586-019-1418-6>.
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707. [https://doi.org/10.1016/0038-0717\(87\)90052-6](https://doi.org/10.1016/0038-0717(87)90052-6).
- Velthof, G.L., Barot, S., Bloem, J., Butterbach-Bahl, K., de Vries, W., Kros, J., Lavelle, P., Olesen, J.E., Oenema, O., 2011. Nitrogen as a threat to European soil quality. In: Sutton, M.A., Howard, C.M., Erisman, J.W., Billen, G., Bleeker, A., Grennfelt, P., Grinsven, H.V., Grizzetti, B. (Eds.), The European Nitrogen Assessment: Sources, Effects and Policy Perspectives. Cambridge University Press, Cambridge, United Kingdom, pp. 495–510. <https://doi.org/10.1017/CBO9780511976988.024>.
- Viketoff, M., Bengtsson, J., Sohlenius, B., Berg, M.P., Petzsch, O., Palmborg, C., Huss-Danell, K., 2009. Long-term effects of plant diversity and composition on soil nematode communities in model grasslands. *Ecology* 90, 90–99. <https://doi.org/10.1890/08-0382.1>.
- Viketoff, M., Palmborg, C., Sohlenius, B., Huss-Danell, K., Bengtsson, J., 2005. Plant species effects on soil nematode communities in experimental grasslands. *Appl. Soil Ecol.* 30, 90–103. <https://doi.org/10.1016/j.apsoil.2005.02.007>.
- Wagg, C., Bender, S.F., Widmer, F., Van Der Heijden, M.G.A.A., Heijden, M.G.A.V.D., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proc. Natl. Acad. Sci. USA* 111, 5266–5270. <https://doi.org/10.1073/pnas.1320054111>.
- Wang, X., Gao, Y., Zhang, H., Shao, Z., Sun, B., Gao, Q., 2019. Enhancement of rhizosphere citric acid and decrease of NO₃⁻/NH₄⁺ ratio by root interactions facilitate N fixation and transfer. *Plant Soil* 447, 169–182. <https://doi.org/10.1007/s11104-018-03918-6>.
- Weih, M., Minguez, M.I., Tavoletti, S., 2022. Intercropping systems for sustainable agriculture. *Agriculture* 12, 291. <https://doi.org/10.3390/agriculture12020291>.
- Ye, Y.Y., Rui, Y.C., Zeng, Z.X., He, X.Y., Wang, K.L., Zhao, J., 2020. Responses of soil nematode community to monoculture or mixed culture of a grass and a legume forage species in China. *Pedosphere* 30, 791–800. [https://doi.org/10.1016/S1002-0160\(20\)60039-X](https://doi.org/10.1016/S1002-0160(20)60039-X).
- Yeates, G.W., Bongers, T., De Goede, R.G., Freckman, D.W., Georgieva, S.S., 1993. Feeding habits in soil nematode families and genera: an outline for soil ecologists. *J. Nematol.* 25, 315–331.
- Zhang, H., Wang, X., Gao, Y., Sun, B., 2019. Short-term N transfer from alfalfa to maize is dependent more on arbuscular mycorrhizal fungi than root exudates in N deficient soil. *Plant Soil* 446, 23–41. <https://doi.org/10.1007/s11104-019-04333-1>.
- Zhang, M.M., Wang, N., Hu, Y.B., Sun, G.Y., 2018. Changes in soil physicochemical properties and soil bacterial community in mulberry (*Morus alba* L.)/alfalfa (*Medicago sativa* L.) intercropping system. *MicrobiologyOpen* 7, e00555. <https://doi.org/10.1002/mbo3.555>.
- Zhao, J., Neher, D.A., 2014. Soil energy pathways of different ecosystems using nematode trophic group analysis: a meta analysis. *Nematology* 16, 379–385. <https://doi.org/10.1163/15685411-00002771>.
- Zhao, J., Wang, X.L., Wang, X.L., Fu, S.L., 2014. Legume-soil interactions: legume addition enhances the complexity of the soil food web. *Plant Soil* 385, 273–286. <https://doi.org/10.1007/s11104-014-2234-2>.
- Zhong, S., Zeng, H., Jin, Z., 2015. Responses of soil nematode abundance and diversity to long-term crop rotations in tropical China. *Pedosphere* 25, 844–852. [https://doi.org/10.1016/S1002-0160\(15\)30065-5](https://doi.org/10.1016/S1002-0160(15)30065-5).