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Temporal monitoring of free-living nematode communities for evaluation of soil health in an arable crop rotation

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ABSTRACT

There is a growing interest in finding reliable methods for monitoring soil health using bioindicators. Free-living nematodes are an ideal indicator group because of their rapid response to changes in soil conditions. This UK study aims to assess their efficacy as bioindicators using two field experiments. In Experiment-1, the treatments included Farmyard Manure, Green Manure consisting of a mix of *Raphanus sativus* and *Vicia* sp., and Standard Practice serving as the control receiving N-fertiliser only. The same treatments, except Farmyard Manure, were compared in Experiment-2, which was on a sloping site with a different textured soil. Soil samples were collected twice during each crop season, in Spring and Autumn, for Experiment-1, and only in Autumn for Experiment-2. Ecological indices that categorise nematodes by feeding preference using morphological differences and life strategies (i.e. functional guilds) were calculated. Indices were compared with the abundance of nematode trophic groups to evaluate their use as soil indicators for understanding crop management practices and their legacy effects. Results showed that identification to trophic groups alone was not a sufficiently sensitive approach for assessing changes in the selected management practices. The variations among trophic groups and treatments within the same sampling period were significantly different for bacterivores, fungivores, predators, omnivores, and herbivores. These differences did not always cooccur within the same sampling period, with bacterivores and plant-parasites of economic importance showing greater responses. The food web analyses, calculated by applying the Enrichment Index and Structure Index, and Plant Parasite Index, provided a more sensitive indicator and allowed more effective diachronic monitoring. While using the composition of trophic groups appears to be an attractive solution, their application is best linked to quantifying short-term changes in soil condition and were not as well suited to longer-term soil health monitoring.

1. Introduction

Soil is a vital resource that supports all terrestrial life, and as such, must be managed appropriately to ensure sustainable crop production (Orgiazzi, et al., 2016; FAO, 2021). The significance of soil health is widely recognised; leading to a growing interest in management practices which enhance soil functions and can be assessed using both physicochemical and biological measurements (Defra, 2020; FAO et al., 2020; EC, 2021). Soil health is defined in this study as the soil's capability to deliver successful agronomic outcomes under the management system being practiced (Powlson, 2020; Giller et al., 2021). Population densities of different soil fauna such as earthworms, collembola and nematodes can provide useful insights into soil's structure and pest pressure (Griffiths et al., 2016; Neher, 2001; Sechi et al., 2017; Yeates et al., 2009; Yeates and Bongers, 1999; Huber et al., 2008). However, the exact approach for quantifying and assessing the status of many of these soil organisms remains under debate (Jeffery and Verheijen, 2020). Furthermore, it is unclear how these can be used to assess soil health and be translated into agronomic decisions (Powlson, 2020; Giller et al., 2021).

The morphologically distinct head structures of free-living soil nematodes can easily be used to determine their feeding preferences, i.e. whether they are bacterivores, fungivores, omnivores, herbivores, and predators (Yeates et al., 1993; Bongers and Ferris, 1999; Ferris and

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Bongers, 2006). Nematode abundance and community structure may respond rapidly due to environmental changes brought by management practices. They respond distinctively to organic fertilisation and disturbances, and are sensitive to pollutants (Bongers, 1990; Bongers and Bongers, 1998). However, nematode diversity and function are complex and remain to be fully explored and understood within the context of soil health in agricultural systems. These responses have been used to develop the Maturity Index by classifying nematodes into a coloniser-persister (cp) series ranging from 1 to 5 (Bongers, 1990; Bongers and Bongers, 1998). The classification is based on trophic groups and corresponding life strategy associated with each family or genus, i.e. functional guilds; specifically referring to r- and K-strategists (MacArthur and Wilson, 1967). Nematodes with low cp values are considered opportunistic colonisers, while those with high cp values are regarded as persisters. Basal nematodes are categorised as cp-2 along the enrichment and structure axis, whereas cp-1 bacterivores and cp-2 fungivores indicate enrichment and all cp-3–5 nematodes relate to community structure (Ferris et al., 2001). The abundance of different functional guilds indicates the level of stability within the system, where disturbances and enrichment can reverse succession. Lower Maturity Index values signify an environment that has experienced disturbance and/or fertilisation, whereas higher values indicate a more stable environment (Bongers, 1990). Subsequently, the Enrichment and Structure Indices were formulated to describe food web conditions. They are based on functional redundancy utilising the weighted relative abundance of functional guilds (Bongers, 1990; Yeates et al., 1993; Bongers and Bongers, 1998).

While there is increasing interest in using soil bioindicators to determine the health status of a soil, the cost of analysis and lack of taxonomic expertise makes nematodes less accessible as a bioindicator. Taxonomic identification of plant parasitic nematodes (no other nematode feeding types included) to genera level starts at £70/sample (Fera, 2021). In comparison, standard soil chemical analysis (P, K, Mg and pH) can cost ~£10 per sample (NRM Laboratories, 2021). To circumvent setbacks associated with costs and lack of widespread expertise, relatively simple tests using the abundance of nematode trophic groups are commercially available (e.g. Laverstock Park Farm, 2021). These include the ratio of fungal to bacterial feeders with/without predators. The use of simpler morphological analysis speeds up the assessment process and reduces the need for scarce expert knowledge, thus potentially decreasing costs and increasing accessibility (Griffiths et al., 2018).

The aim of this study was to investigate whether the application of trophic groups alone differentiates impacts of management practices sufficiently to inform decisions, or whether it is necessary to use a higher taxonomic resolution, i.e. family/genus, and nematode community indices. To examine this, we explored if treatment effects, N-fertiliser, FYM and cover crops, were apparent over a two-year period, in large-scale field experiments. Both higher and lower nematode taxonomic classifications were tested to assess their response, thus their role as indicators of soil health, and to elucidate the application of nematode abundance and community structure in agronomic decisions by providing a case study of an arable system within the UK.

2. Methods and materials

2.1. Experimental design

The experimental site, situated in Norbury Park, Staffordshire, UK (52°48'20.9"N, 2°17'49.9"W), was established in April 2017 (Fig. 1) after a fallow period during the preceding autumn and winter months. The soil texture in the field ranged from clay loam to sandy loam, as indicated in Table 1. Experiment-1 (area of 2 ha) included three treatments and six replicate plots, each measuring 200 × 6 m, arranged in a completely randomised design. Treatments consisted of: Control, representing the farmer's "Standard Practice"; Farmyard Manure (FYM), which received 14 Mg/plot of farmyard manure (equivalent to 40 Mg ha^{-1}, spread on April 13, 2017); and Cover crops (Green Manure), consisting of a combination of fodder radish (*Raphanus sativus*) and vetch (*Vicia* sp.) in a 50:50 (w/w) ratio, direct drilled at a rate of 29 kg ha^{-1} on April 14, 2017, and used as green manure during the following crop season. The Standard Practice plots were fertilised on May 4, 2017, with 150 kg ha^{-1} of N-fertiliser (Nitram, 34.5% N), while the FYM plots received 125 kg ha^{-1} of N-fertiliser to achieve an equivalent level of available nitrogen. Spring wheat (*Triticum aestivum* var. Mulika) was directly drilled on May 14, 2017, at a rate of 150 kg ha^{-1} on all plots except the Green Manure plots. Experiment-2 (area of 0.3 ha) consisted of 17 plots measuring 25 × 6 m, randomly assigned to two treatments, and established in the same period as Experiment-1: Green Manure (8 plots) and Standard Practice (9 plots), following a completely randomised design too and was conducted on a sloping side of the field. In the subsequent crop season in Year 2, winter oats (*Avena sativa* var. Mascani) with Beret Gold (Fludioxonil) seed treatment were directly drilled across all plots in both experiments on October 18, 2017. For a detailed management plan, please refer to Natalio et al. (2024).

2.2. Nematode extraction and identification

The experiments commenced on April 7, 2017, with the initial soil sampling for Experiment-1 taking place on May 3, 2017. Post-harvest soil sampling occurred on October 12, 2017, for Experiment-1 and on November 15, 2017, for Experiment-2. In the subsequent crop season, soil sampling for Experiment-1 was conducted on April 30, 2018, in spring and on October 2, 2018, in autumn. For Experiment-2, the soil sampling was carried out on November 1, 2018. Unfortunately, sampling of Experiment-2 during the spring season was not feasible due to time constraints. Two topsoil sub-samples were collected from random

Table 1
Soil texture classes identified in each experiment investigating the effects of soil amendments in no-till arable system on free-living nematodes.

Texture Class	Experiment-1 (%)	Experiment-2 (%)
Loamy sand	2.4	73.5
Sandy loam	48.4	26.5
Sandy clay loam	45.2	0.0
Clay loam	4.0	0.0

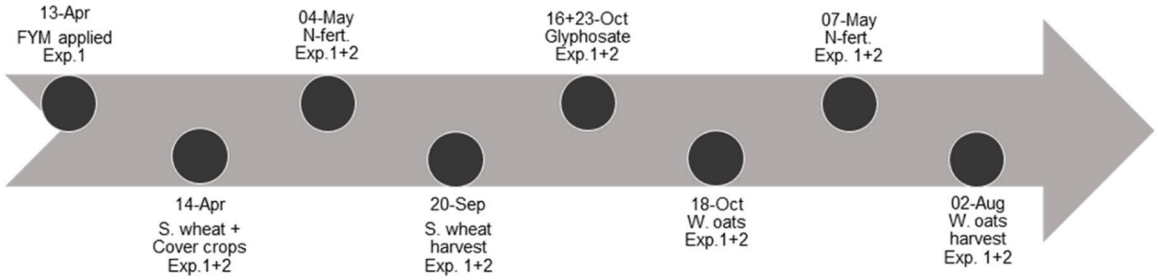

Fig. 1. Timeline of experimental inputs and harvest periods for both Experiment 1 and 2.

points in each plot using an auger (10 cm depth; 2 cm diameter). For each sub-sample, 10 cores were taken and pooled together. Samples were kept at 4°C in press-grip bags until extraction. Nematodes were extracted from 200 g of fresh soil homogenised by hand-mixing. A modified version of the Whitehead and Hemming (1965) tray method of nematode extraction was used. A plastic tray (45 x 35 x 10 cm) was lined with wire support and 1 mm plastic mesh covered with 1-ply tissue paper (Kleenex original facial white tissue). Hand crumbled soil was laid on the tissue, tap-water was added until the soil was immersed and left for 48 h at room temperature (15–21°C). After, the wire tray holding mesh, tissue and soil was gently drained. The tray suspension was sieved (38 μm mesh) and nematodes collected in 50 ml sample bottles in clean tap water suspensions. The suspension was left to settle for 2 h and reduced to 10 ml volume using a pipette to extract volume below meniscus line (Bell and Watson, 2001). Nematodes (100 randomly selected individuals per 1 ml from each sample suspension) were identified to family or genus level using an inverted microscope, up to 400x magnification (Leica DMi8 M/C/A). Trophic groups were determined from family/genus taxonomic identifications in accordance with Yeates et al., (1993), Bongers and Bongers, (1998) and Ferris et al., (2001).

2.3. Statistical analysis

Statistical analyses were conducted in R-Studio (R version 4.2.1 (2022–06–23 ucrt) – "Funny-Looking Kid"; R version 4.3.2 (2023–10–31 ucrt) – "Eye Holes"; R Core Team), and the packages "lattice" "lme4", "effects", "tidyverse", "ggpubr", "rcompanion", "stats", and "Matrix" (Sarkar, 2015; Bates *et al.*, 2015; Fox and Weisberg, 2018, Wickham et al., 2019; Kassambara, 2020; Mangiafico, 2021; R Core Team, 2013; Bates et al., 2023).

Generalised Linear Models (GLM) were applied on individual sampling periods to determine whether there were differences in individual trophic group mean abundances between treatments for each experiment. GLM log transforms the response variable (trophic groups, count data) in accordance with Poisson family using discrete probability distributions. A Quasi-Poisson model was applied to calculate the dispersion when the assumption required by Poisson that the variance equals the mean was not met. Thus, compensating for underdispersion or overdispersion, i.e. dispersion parameter $\neq 1$ (Zuur, et al., 2009). Mean comparisons of feeding groups were done between all sampling periods for each experiment by fitting a Generalised Linear Mixed-Effects Model (GLMM). The treatment and sampling factors were combined to create a new variable and applied to the model formula as the fixed effect. Treatment and sampling period were added as individual random effects. The same family parameter and compensation for underdispersion or overdispersion were applied as per the GLMs.

Ninja software (Sieriebriennikov et al., 2014) was used to calculate the Maturity Index (MI), Enrichment Index (EI), Structure Index (SI), and Plant Parasitic Index (PPI). MI assesses nematode communities (excluding herbivores) using the coloniser-persister (cp) scale and values are expressed in 1–5 units. Calculation uses the weighted relative abundance of functional guilds. Whereas EI and SI calculates the weighted faunal components of functional guilds sharing a common cp value (Ferris et al., 2001). A comprehensive list of genera and respective cp values, and stepwise calculations applied in Ninja can be found in Bongers (1990), Yeates et al. (1993) and Bongers and Bongers, (1998). Food web analysis was visually represented using the EI and SI. The weighted abundance of nematode guilds was used to calculate each individual index. Nematode functional guilds are: Bac_x = bacterivores; $Fung_x$ = fungivores; $Pred_x$ = predators; Omn_x = omnivores (Ferris et al., 2001) where x indicates their coloniser-persister assignments. Differences between treatments and sampling periods were assessed using two-way ANOVA and Tukey's HSD *post-hoc* test was used for pairwise comparison of means ($p < 0.05$).

Ratios have been used in ecological studies to determine Energy Channel and food source availability. The Energy Channel is calculated using the ratio of fungal to bacterial feeders (F/B), while food source availability is calculated using the ratio of dauers (RD) to actively feeding (R) Rhabditids (Ferris and Bongers, 2006).

The Shannon-Weiner diversity index was applied across different time periods using the identified families/genera from each sampling period. This helped ascertain the richness and evenness of the species present. The index, denoted as H, is calculated using the formula H = -sum*p*i*log(10)pi, where p*i* represents the proportional abundance of species *i*. Two-way ANOVA was used to assess the impact of treatments and sampling period (May, October, and November) on the Shannon-Weiner diversity index.

3. Results

3.1. Nematode community composition

A total of 83 taxa, a combination of both families and genera, were identified across all sampling periods for both experiments: Experiment-1 (May and October, 2017–2018) and Experiment-2 (November 2017–2018). In Experiment-1, the number of identified taxa were: 47 (May-2017), 62 (October- 2017), 65 (May-2018) and 44 (October-2018). Experiment-2 exhibited less variation in the number of taxa identified with 47 in November-2017 and 41 in November-2018.

The Rhabditid bacterial feeders (cp-1) were the most abundant taxa, with their feeding stage making up 11% of the counts and the remaining 89% consisted of dauers (juvenile stage, motile but non-feeding) (Fig. 1). Three different taxa dominated the Rhabditids: Cephalobidae (25%, cp-2; which 19% were *Acrobeloides*), *Rhabditis* (18%) and *Panagrolaimus* (12%, cp-1) (Fig. 1a-c). There were three dominant taxa within the fungivores, with *Aphelenchoides* (cp-2) making up 35% of the total abundance followed by *Filenchus* (cp-2) with 29% and *Ditylenchus* (cp-2) with 19%. The Aporcelaimidae (cp-5) and Qudsianematidae (cp-4) families had greater abundance within the omnivores, 38% and 43% respectively, with the *Dorylaimids* (cp-4) making up 18% of the omnivore numbers (Fig. 2a-c). Most predators were in the Mononchidae family (63%, cp-4), and had a considerably greater abundance in May-2018 where they account for 75% of the individuals encountered (Fig. 3). Herbivores were dominated by plant-parasitic nematodes (48%, those of economic importance), followed by root-feeders (40%) and algal-feeders (11%). The genus *Tylenchorhynchus* (pp-3) made up 40% of the plant parasitic taxa, while *Helicotylenchus* (pp-3) and *Pratylenchus* (pp-3) made up 21% of the total numbers. The root-feeders were dominated by the family Tylenchidae (pp-2, 62%). Plant-parasitic nematodes include root feeders, but this category was kept separate to differentiate from the former one which are of economic importance. The genus *Tylenchus* (pp-2) made up 99.8% of the algal-feeders.

3.2. Diversity

In Experiment-1, the Shannon-Wiener diversity index was highest in May-2018 (6.1), followed by October-2017 (5.9), October-2018 (5.4) and May-2017 (4.6). In Experiment-2, taxonomic diversity decreased from 4.9 in November-2017 to 4.5 in November-2018.

3.3. Temporal and treatment comparisons

A rise in the total number of nematodes from when sampling first started was observed (Fig. 5). In Experiment-1, where sampling started in spring 2017, nematode abundance was higher in October 2017, and in May and October 2018 (by 71%, 64% and 62%, respectively) than in May 2017. In Experiment-2, with November sampling only, 20% more nematodes were found in 2017 than in 2018. During the autumnal sampling periods (i.e. October and November), 15% and 47% more nematodes were extracted in Experiment-2 than in Experiment-1 in 2017 and 2018, respectively. A linear relationship ($R^2 = 0.54$) between nematode abundance and soil texture was observed, where Experiment-

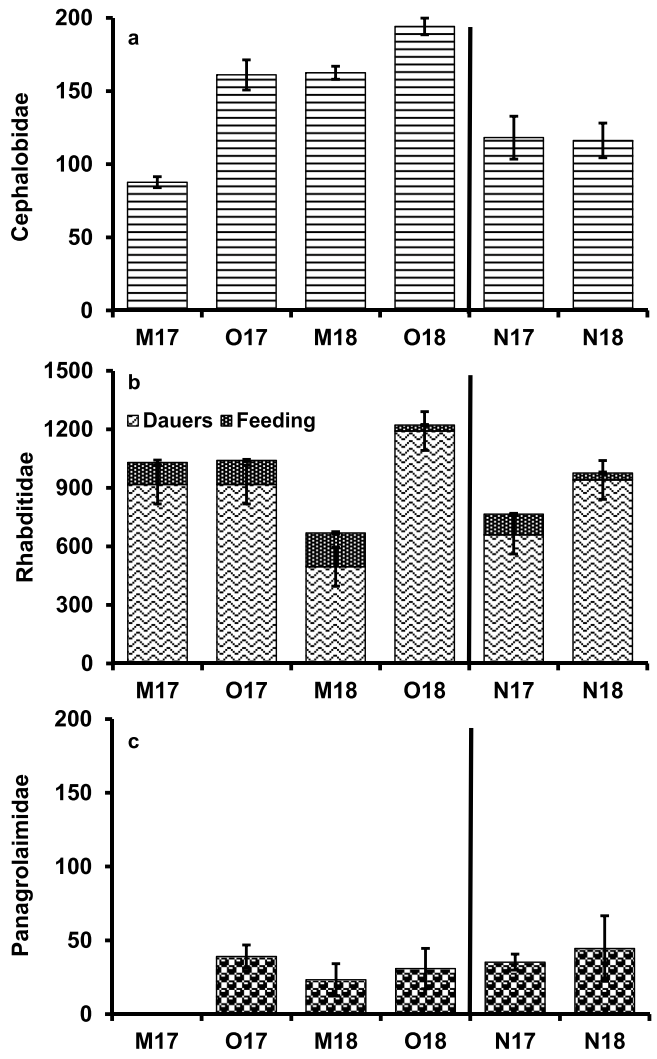

Fig. 2. Abundance of bacterivores within the order Rhabditida: Cephalobidae (a), Rhabditidae and dauers (non-feeding Rhabditidae) (b), and Panagrolaimidae (c) per sampling period, ±SEM. Experiment-1 = May and October, 2017–2018 (M17, O17, M18 and O18); E2 = November, 2017–2018 (N17 and N18).

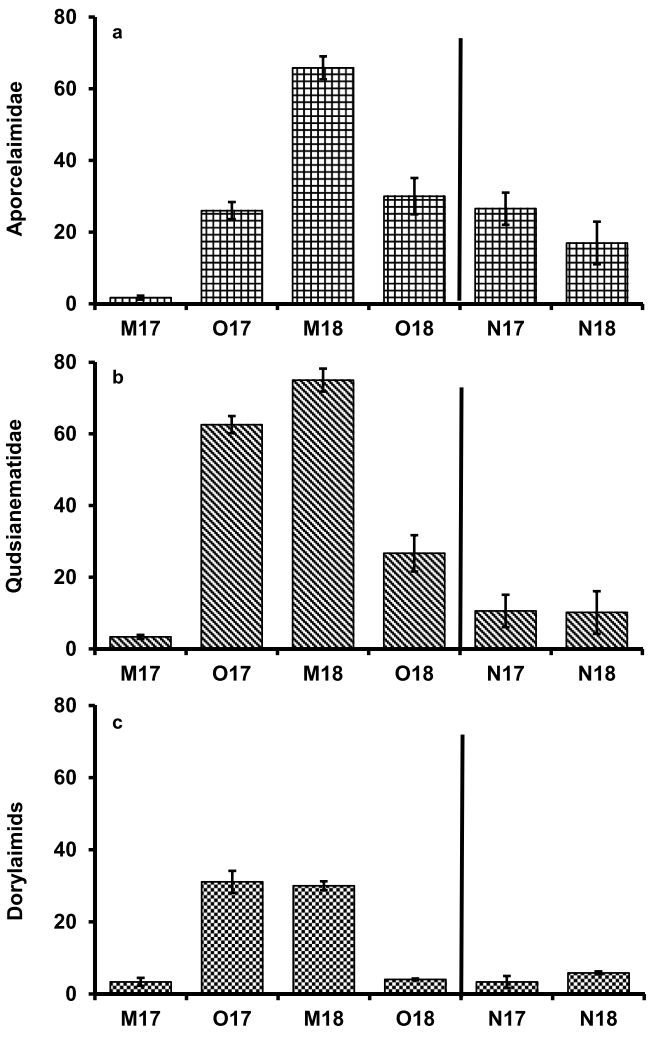

Fig. 3. Abundance of omnivores: Aporcelaimidae (a), Qudsianematidae (b) and Dorylaimids (c) per sampling session, ±SEM. Experiment-1 = May and October, 2017–2018 (M17, O17, M18 and O18); Experiment2 = November, 2017–2018 (N17 and N18).

2 with ~80% sand content had greater numbers than Experiment-1 with <70% sand content (Fig. 5).

3.4. Trophic groups - comparison between treatments

Significant differences in the number of individual trophic groups were observed among treatments on par with respective sampling period (Fig. 6a and b). In Experiment-1, 36% higher mean numbers of bacterivores were observed following incorporation of FYM in spring 2017 than in the Standard Practice treatment, but not to a significant level. Significant differences were observed in the Green Manure treatment in comparison to the Standard Practice treatment in May 2018 (p = 0.01) (Fig. 6a). In Experiment-2, bacterivores displayed significant distinctions in mean abundances between Green Manure and Standard Practice in November 2018 (p = 0.04) (Fig. 6b).

No significant treatment differences (α set at 0.05) were observed in the number of fungivores and omnivores of either Experiment 1 or 2 (Fig. 6c and d). Only the Standard Practice treatment of Experiment-1 displayed significantly greater number of predators (p = 0.01) in May 2018 than Green Manure (Fig. 6e).

In Experiment-1, greater abundance of plant-parasitic nematodes (May-2017 p = 0.04) were seen in the FYM treatment than Standard

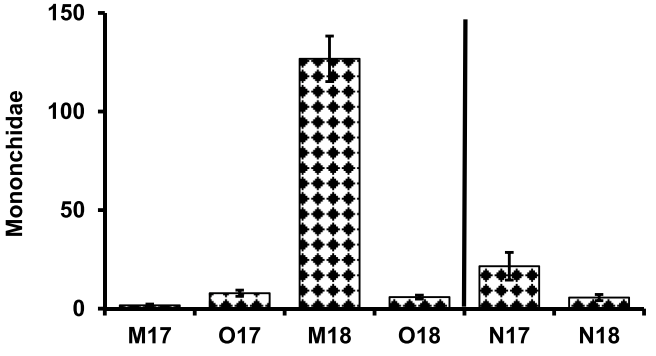

Fig. 4. Abundance of Mononchidae predators per sampling session, ±SEM. Experiment-1 = May and October, 2017–2018 (M17, O17, M18 and O18); Experiment2 = November, 2017–2018 (N17 and N18).

Practice, but lower abundance of root-feeders between the two treatments (p = 0.04) in October-2017. Whereas more plant-parasitics were found in the Green Manure treatment (Oct-2017 p = 0.03) than in Standard Practice, but the reverse occurred with the number of rootivores in October-2017 and May-2018 (p = 0.04 and 0.01, respectively) (Fig. 7a and e). May-2018 saw more algivores in Standard Practice than

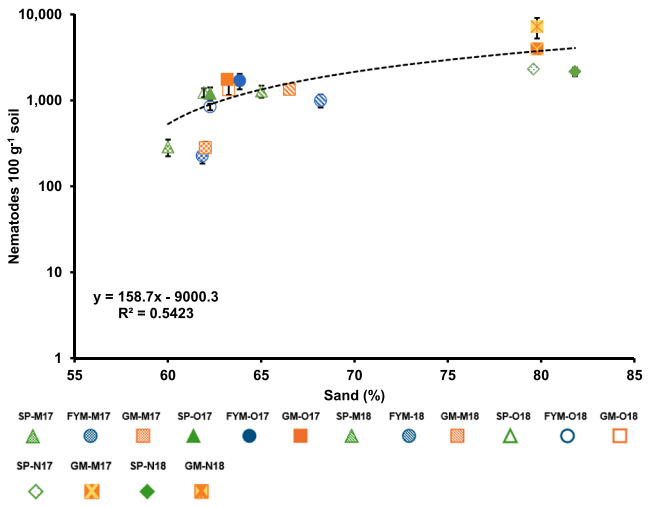

Fig. 5. Relationship between the total abundance of nematodes (Log_{10} 100 g^{-1} fresh soil) and the percentage of sand in Experiment-1 (May and October, 2017–2018 (M17, O17, M18 and O18), n = 6) and Experiment-2 (November, 2017–2018 (N17 and N18), GM n = 8; SP n = 9). Treatments are represented by different colours: Farmyard Manure (FYM) is shown in blue, Green Manure (GM) in orange, and Standard Practice (SP) in green.

in the treatments of FYM (p = 0.04) and Green Manure (p = 0.03) (Fig. 7c and d). No significant treatment differences (α set at 0.05), either in November 2017 or 2018, were observed in Experiment-2 (Fig. 7b, d and f).

3.5. Relative abundance of coloniser-persister nematodes and plant-parasitic index values

The abundance of cp-4 nematodes significantly dropped from May-2018 to October-2018 (*Diff.* = −11.0%, p = 0.02). However, no significant treatment effects were observed.

There were more plant parasitic nematodes with pp-2 index value in May-2017 than in October-2017 (*Diff.* = 33.5%, p = 0.01), May-2018 (*Diff.* = 32.6%, p = 0.01) and October-2018 (*Diff.* = 39.6%, p = 0.003), and November-2017 (*Diff.* = 62.6%, $p < 0.001$) and November-2018 (*Diff.* = 53.7%, p = 0.001). Numbers were lower in November-2017 than May-2018 (*Diff.* = −30.0%, p = 0.03), but higher than October-2017 (*Diff.* = 29.1%, p = 0.04). Treatment effects were seen with greater pp-2 abundance in the FYM treatment compared to Green Manure (*Diff.* = 23.8%, p = 0.005). Conversely, the abundance of pp-3 nematodes was lower in May-2017 compared to May-2018 (*Diff.* = −32.3%, p = 0.01), October-2017 (*Diff.* = −33.5%, p = 0.01) and 2018 (*Diff.* = −39.5%, p = 0.003), and November-2017 (*Diff.* = −62.4%, $p <$ 0.001) and 2018 (*Diff.* = 53.4%, p = 0.001). Treatments significant differences were seen between Green Manure and FYM (*Diff.* =23.9%, p = 0.01).

3.6. Fungal:bacterial feeder ratios and indices

Significant differences in Fungal:Bacterial feeder ratios were found between sampling periods, but no treatment effect was detected. Ratios were lower in May-2018 (*Diff.* = −0.4, $p < 0.001$), October-2017 (*Diff.* = −0.3, p = 0.003) and 2018 (*Diff.* = −0.3, p = 0.003), and November-2017 (*Diff.* = −0.5, $p < 0.001$) and 2018 (*Diff.* = −0.5, $p < 0.001$) in comparison to May-2017. Whereas greater ratios were seen in October-2017 and 2018 in comparison to November-2017 (*Diff.* = 0.2, p = 0.03; *Diff.* = 0.2, p = 0.03, respectively) and 2018 (*Diff.* = 0.3, p = 0.01; *Diff.* = 0.3, p = 0.01, respectively).

Differences in Structure Index values were found between sampling periods (p = 0.004) but not between treatments (p = 0.2) (Fig. 8). Values were lower in May-2017 in comparison with October-2017 (*Diff.* = −35.8%, p = 0.02) and 2018 (*Diff.* = −32.0%, p = 0.04), and May-2018 (*Diff.* = −54.8%, p = 0.002). Maturity Indices were dominated by cp-1 and cp-2 nematodes on the coloniser-persisters scale throughout the course of two-years for both Experiment-1 and Experiment-2. In Experiment-1, the numbers of cp-4 significantly decreased in October-2018 in comparison with May-2018 (*Diff.* = −11.0%, p = 0.02). The Maturity Index (MI) ranged from 1.6 to 2.5, but neither treatment (p = 0.1) nor sampling session (p = 0.07) had a significant impact on MI values.

The Plant-Parasite Index (PPI), which compares to MI but includes herbivores instead, was not significantly different between treatments (p = 0.3) and sampling times ($F_{5,8}$ = 1.07, p = 0.4). Nematodes in the pp-2 and pp-3 scale dominated, with greater number of pp-2 herbivores in the FYM treatment in comparison with Green Manure (*Diff.* = 23.8%, p = 0.005), and pp-3 in Green Manure compared to FYM (*Diff.* = 23.9%, p = 0.005). Significantly greater pp-2 s was observed in May-2017 compared to October-2017 (*Diff.* = 33.5%, p = 0.01) or 2018 (*Diff.* = 39.6%, p = 0.003), May-2018 (*Diff.* = 32.6, p = 0.01), and November-2017 (*Diff.* = 62.6%, $p < 0.001$) or 2018 (*Diff.* = 53.7%, p = 0.001). November-2017 had lower pp-2 numbers than October-2017 (*Diff.* = −29.1%, p = 0.04) or May-2018 (*Diff.* = 30.0%, p = 0.03). In contrast, a lower density of pp-3 was seen in May-2017 than October-2017 (*Diff.* = −33.5%, p = 0.01) or 2018 (*Diff.* = −39.5%, p = 0.003), May-2018 (*Diff.* = −32.3%, p = 0.01), and November-2017 (*Diff.* = −62.4%, $p < 0.001$) or 2018 (*Diff.* = 53.4%, p = 0.001). November-2017 had greater pp-3 numbers than October-2017 (*Diff.* = 29.0%, p = 0.04) or May-2018 (*Diff.* = 30.1%, p = 0.03).

4. Discussion

Morphological differences used to categorise nematodes into trophic groups, i.e. differences in mouthparts, offers only limited insights that do not adequately capture their ecological functions and reproductive capabilities (Bongers and Ferris, 1999). While no significant variances were detected when comparing benchmark data to subsequent sampling periods, notable differences emerged among bacterivores, fungivores, predators, omnivores, and herbivores when comparing different treatments within the same sampling periods in both experiments. These differences likely occurred due to substrate enrichment through the application of FYM and N-fertiliser. This agrees with the findings of Cesarz et al. (2015), who reported an increase in the abundance of opportunistic nematodes such as bacterivores with cp 1–2 values under elevated N. Significant changes in nematodes communities in response to the treatments of FYM and/or N-fertiliser (Standard Practice) and Green Manure were not conclusively observed over a two-year period. Moreover, treatment legacy effects were not seen one year after initial experimental setup, likely caused by responses to N-fertilisation in the second year, which was consistent across all treatments (Cesarz et al., 2015). However, significant temporal differences were detected in Fungal:Bacterial feeder ratios, Structure Index (SI) and Plant-Parasitic Index (PPI).

In general, this study highlights that nematode trophic groups did not function as a sensitive bioindicator for monitoring change in management practices in a UK arable system. Instead, the application of indices based on a higher taxonomic resolution proved to be a more sensitive indicator of changes within the conditions of this study. Bhusal et al. (2014), reported similar results, stated that trophic groups alone are less efficient at discriminating land use when compared to the higher taxonomic approach as applied in indices.

Morphological adaptations (i.e. mouthparts) permit nematodes to explore different food sources and facilitates distinction between trophic groups (Bongers and Ferris, 1999). Trophic group categorisation is appealing because it reduces the expertise required and could be considerably easier and quicker to learn than higher taxonomic classification (Bongers and Ferris, 1999). However, feeding preferences might

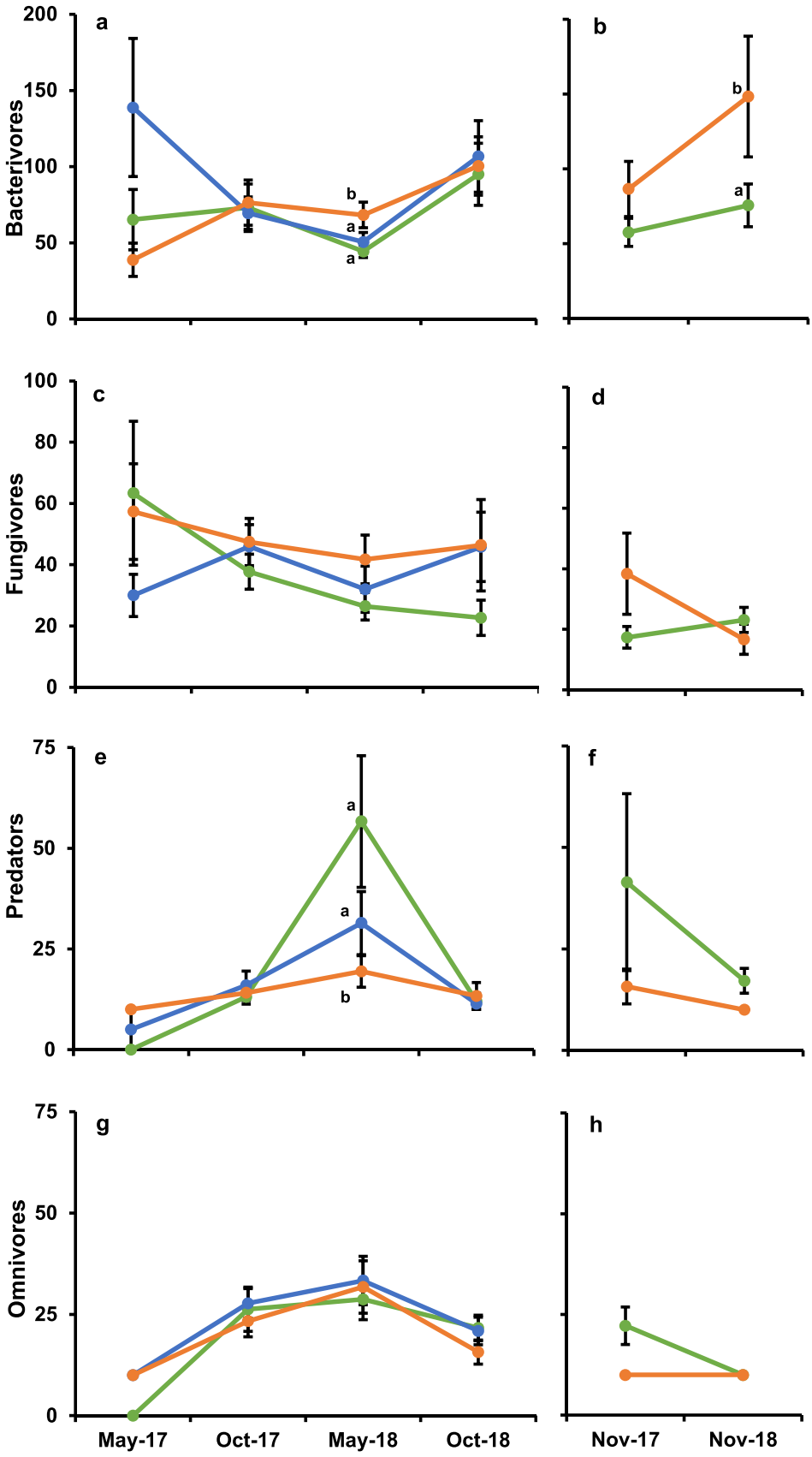

Fig. 6. Temporal changes of nematodes mean abundance per trophic group (bacterivores, fungivores, predators and omnivores) over a period of two cropping seasons. Experiment-1 (panels a, c, e, and g), with four sampling periods in May and October 2017 and 2018, n = 6. Experiment-2 (panels b, d, f, and h) with two sampling periods in November 2017 and 2018, GM n = 8 and SP n = 9. Treatments: Farmyard Manure (FYM, blue); Green Manure (GM, orange); Standard Practice

(SP, green). Error bars show the standard error of the mean. Letters (a-b) symbolise significant ($\alpha = 0.05$) differences between treatments on a per sampling time basis.

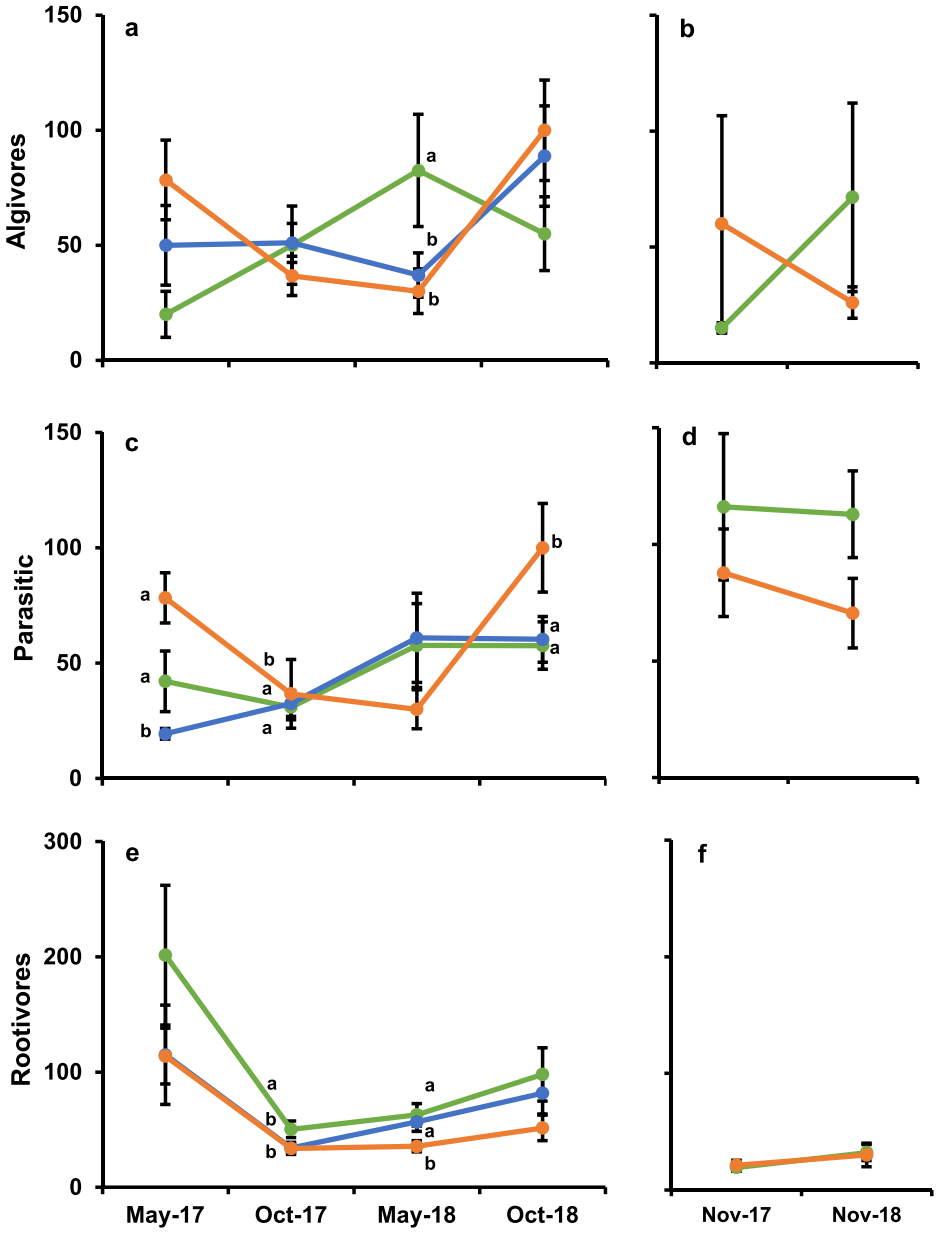

Fig. 7. Temporal changes of herbivores mean abundance grouped by whether of economic importance (i.e. parasitic) or feeding preference (i.e. algae and root feeders) over a period of two cropping seasons. Experiment-1 (panels a, c, and e), with four sampling periods in May and October 2017 and 2018, n = 6. Experiment-2 (panels b, d, and f) with two sampling periods in November 2017 and 2018, GM n = 8 and SP n = 9. Treatments: Farmyard Manure (FYM, blue); Green Manure (GM, orange); Standard Practice (SP, green). Error bars show the standard error of the mean. Letters (a-b) symbolise significant ($\alpha = 0.05$) differences between treatments on a per sampling time basis.

not be strict to specific food sources if alternatives are abundant in comparison to their recognised food choice. For example, nematodes in the family Cephalobidae (cp-2, which includes members of Acrobeloides) and genus *Chiloplacus* (cp-2) are categorised as bacterivores but can be cultured on fungi, and the species *Caenorhabditis elegans* and *Distolabrellus veechi* will feed on a less favourable diet when under stress conditions (Marlin et al., 2019; Liu et al., 2021). This means that some genera are able to explore different substrates, consequently potentially misleading the interpretation of Fungal:Bacterial feeder ratios, which are expected to be fungal dominated in systems with lower levels of disturbance and N-enrichment (Malik et al., 2016).

Ratios based on trophic groups of free-living nematodes are used commercially for bioindicator analysis of soil health (Bennett, n.d). The most used is the Energy Channel (EC) analysis, a measure of the ratio of Fungal:Bacterial feeders. Like other studies (Scharroba et al., 2016; Song et al., 2016; Shaw et al., 2019), it was found that EC, when applied to this study, was largely influenced by bacterial feeders (ratios < 1), at all sampling times, and consistently across all the treatments. A significant difference was found between sampling periods; ratios significantly dropped after May-2017 in response to enrichment regardless of

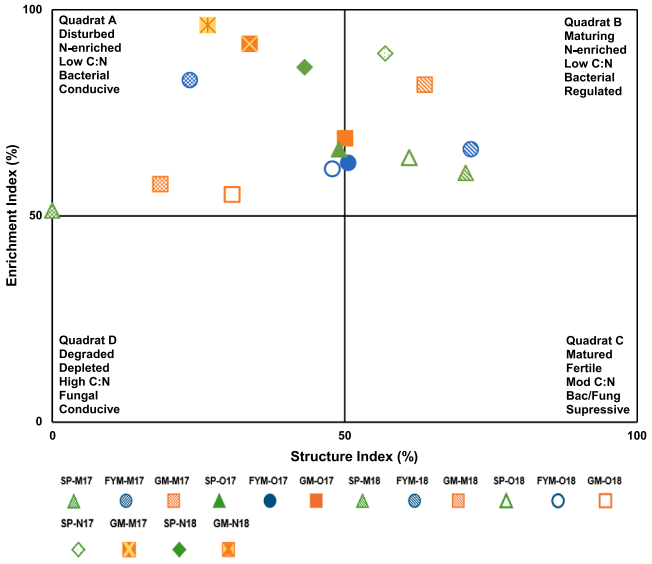

Fig. 8. The Structure (SI) and Enrichment (EI) Indices, showing nematode community profiles, organised into trajectory plots based on treatments and sampling periods. The plots utilised different colours to indicate treatments, shapes years, and fills seasons. In Experiment-1, there were four sampling sessions conducted in May and October of both 2017 and 2018, with a total of 18 samples per session (n=6 per treatment). Experiment-2 involved two sampling sessions in November of 2017 and 2018, with Green Manure (n=8) and Standard Practice (n=9) treatments. Treatments were Standard Practice (SP) represented by the colour green, Green Manure (GM) in orange, and Farmyard Manure (FYM) in blue.

whether it was from fertiliser inputs (FYM and/or N-fertiliser) or cover-crop residues. Conditions that promote microbial growth can have a chain effect, leading to an increase in the abundance and diversity of bacterivores and fungivorous nematodes (Ferris *et. al.*, 2001; Ferris and Bongers, 2006). Arable rotations usually receive regular N-fertiliser applications to boost crop productivity. This is conducive for bacterial growth, and so are likely to result in systems dominated by bacterivores (Marschner et al., 2003; Liang et al., 2009; Scharroba et al., 2016; Song et al., 2016; Shaw et al., 2019). The bacterivores in greatest abundance were the *Acrobeloides* (cp-2), *Rhabditis* (cp-1) and *Panagrolaimus* (cp-1), which have also been found in other studies to be the taxa that dominate disturbed or enriched soil samples (Háněl, 2008; Sánchez-Moreno et al., 2010; Wu et al., 2017). Thus, the potential of moving towards a fungal dominated decomposition pathway might require a sizable reduction or elimination of N-fertiliser inputs potentially reducing crop productivity. Yang and colleagues (2021) also reported that EC was bacterial dominated in both conventional and organic farming systems but not in the lower input grassland system differentiating it from the other two. In this study, EC lacked applicability when attempting to differentiate the effects of FYM, N-fertiliser, or cover-crops residues or their legacy effects following conversion to no-till. It showed limitations in differentiating management practices as part of a soil health monitoring programme.

The disparity observed between Experiment-2 and Experiment-1 could potentially be attributed to variations in soil texture. Notably, the higher sand content in Experiment-2 may have created less favourable conditions for omnivores and predators in general. The spatial site of Experiment-1 had an average sand content of 63%, whereas Experiment-2 had 80%. The heterogeneity of soil provides diverse habitats for nematode communities, which in turn affects their diversity (Bongers and Ferris, 1999). Studies have suggested that soils with greater pore spaces, which can offer more connected habitat options, tend to be less restrictive for larger nematodes, such as those with higher cp values like omnivores and predators (Briar et al., 2011; Andriuzzi and Wall, 2018). Additionally, soil texture has been found to influence the distribution of nematode families such as Aporcelaimidae, Qudsianematidae, and Mononchidae (Hunt, 1993). However, factors like resource availability and the absence of continuous water films can also limit nematode densities (Briar et al., 2011; Andriuzzi and Wall, 2018). Experiment-2 exhibited higher overall nematode abundances, especially in the Green Manure treatment, indicating that resource availability was not a limiting factor. Although this study did not conclusively establish soil texture as a factor influencing the distribution and abundance of omnivores and predators, further exploration of the impact of soil texture on Aporcelaimidae (cp-5), Qudsianematidae (cp-4), and Mononchidae (cp-4) distributions holds promise for gaining insights into how indices may be affected by inherent soil characteristics (Erktan et al., 2020).

The lower abundance of omnivores and predators in the Green Manure treatment compared to Standard Practice may be linked to isothiocyanates produced by the *Raphanus sativus* cover-crop and its residues (Ngala et al., 2015a; Vervoort et al., 2014). Isothiocyanates are biocidal compounds that are released from the breakdown of glucosinolates from root exudates or damaged tissues of brassica plants. It is unclear why these specific trophic groups were affected. However, bacterivores are opportunistic and recolonise much faster than their predator and omnivore counterparts. Biofumigant brassica cover crops, such as *Brasica juncea* (Indian mustard) and *R. Sativus*, are often sowed for suppressing economically significant plant-parasitic nematodes and improve soil structure (Neher, 2010; Wada et al., 2011; Wang et al., 2014). However, the biocidal compunds of biofumigation affects non-target nematodes, reducing the complexity of nematode communities, as suggested by the present study (Neher, 2010; Ngala et al., 2015a). Furthermore, soil texture is recomended to be considered as it can influence the effectiveness of biofumigants (Neher, 2010). Other studies have also reported similar impacts on the abundance of omnivores and predators, with recovery times depending on the extent of the decline (Neher, 2010; Ngala et al., 2015a; Sánchez-Moreno et al., 2010; Wada et al., 2011; Wang et al., 2014).

Environmental disturbance and/or enrichment can be measured using the Maturity Index (MI), which is based on nematode community species composition. It can range from less than 2.0–4.0, where smaller values indicate disturbed environments and the larger represent environments of greater stability (Bongers, 1990; Neher et al., 2004). Dauers are excluded because they do not offer information on the current functioning of the soil food-web (Bongers, 1990). Lower values are associated with inputs that facilitate bacterial growth such as N-fertilisers and farmyard manure (Bongers and Ferris, 1999). This may explain the lowest MI value observed in May-2017 for the FYM treatment of Experiment-1 (MI = 1.6), which provided an early indication of enrichment.

The initial faunal status stimulated by microbial activity leads to a phase of succession in nematode communities (Háněl, 2008; Steel et al., 2010). This was reflected in Experiment-1 data where MI values were observed to increase seasonally from May-2017–2018, but then decreased again in October-2018 likely caused by N-fertilisation across all plots in Spring 2018 (Table 1). The succession setback following fertilisation has been previously reported (Bongers et al., 1997; Zhao and Neher, 2013; Bhusal et al., 2014). However, no treatment or temporal effect was significant in this study, even though MI values varied between 1.6 and 2.5 (Table 1) across both experiments. Similarly it was found in another study that crop or litter applications had no effect on MI values (Scharroba et al., 2016). The functioning of the soil food-web using MI is only observable because it looks at the assemblages of nematode taxa, meaning that identification to trophic groups alone would not be adequately applicable in the calculations used (Bongers, 1990; Neher *et. al.*, 2004).

The Plant-Parasite Index (PPI), which is comparable to Maturity Index (MI), infers a more stable nematode community when the values are low which is contrary to MI (Bongers *et. al.*, 1997). Under enriched conditions the PPI is higher than in samples from impoverished soils

(Bongers *et. al.*, 1997; Neher *et. al.*, 2004). It was observed that pp-3 nematodes increased in abundance with time which is often explained by greater plant vigour. This was also seen in other studies where crop productivity enhanced by enrichment determined assemblages of plant-parasitic nematodes mainly dominated by pp-3 to pp-5 (Yan et al., 2018; Shaw et al., 2019).

5. Conclusion

This study examined the impact of different management practices and their legacy effects on nematode communities in an arable system in the UK over a two-year period. Trophic groups and higher taxonomic resolution indices were used to assess changes in community structure. The quantification of nematode trophic groups alone did not provide a comprehensive indicator of changes in management practices as part of a diachronic study in an arable system; there was too great a loss of information on nematode communities when used at this taxonomic resolution. Temporal significant differences were detected in Fungal: Bacterial feeder ratios, Structure Index (SI), and Plant-Parasitic Index (PPI). The information obtained on specific plant-parasitic taxa is likely useful when making cropping decisions.

No approaches provided clarity as to how such measurements could be translated into agronomic decisions, and whether targeted taxa screening would be a more viable solution. When economically feasible, nematode analyses provide valuable data on the soil biota, and as integral parts of the soil food web will improve our comprehension of the role of soil fauna within the broader context of soil health as more data become available. The inherent heterogeneity of the soil likely exerted a strong influence on nematode abundances and community structure, and so such data are essential to be included in future studies to facilitate cross study comparisons. Additional long-term studies conducted at a local scale are necessary to gain a better understanding of how soil biological communities respond to different management practices and whether these biological assessments have practical applications in agriculture or remain primarily of scientific interest. Implementing long-term soil monitoring instead of isolated surveys can provide valuable insights into the effects of management practices that are meaningful and applicable for farmers and land managers. However, costs associated with such analyses can be prohibitive and make it less appealing than simpler tests. Other limitations in implementing nematodes commercially in soil health bioindicator analysis, particularly with higher taxonomic resolution, emerges from the lack of taxonomic expertise and the considerably lengthy periods required at the microscope.

CRediT authorship contribution statement

Ana I.M. Natalio: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Mohammed Ahmed:** Formal analysis, Visualization, Writing – review & editing. **Matthew Back:** Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing. **Andrew Richards:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Simon Jeffery:** Conceptualization, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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 uploaded to a EU repository.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.pedobi.2024.150959.

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