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# Unseen population build-up of *Pratylenchus* in organically grown clover grass leys

Jan H. Schmidt[1*], Stephan König[2], Morten Möller[3], Miriam Athmann[3], Christian Bruns[3]

[1]Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn Institute (JKI)—Federal Research Centre for Cultivated Plants, Braunschweig, 38104, Germany
[2]Institute for National and International Plant Health, Julius Kühn Institute (JKI)—Federal Research Centre for Cultivated Plants, Braunschweig, 38104, Germany
[3]Organic Farming and Cropping Systems, University of Kassel, Witzenhausen, 37213, Germany

*Correspondence to*: Jan H. Schmidt (jan-henrik.schmidt@julius-kuehn.de)

**Abstract.** Legumes constitute up to 50% of all crops in organic rotations surrendering these susceptible towards a range of soil-borne pests and diseases, such as plant-parasitic nematodes of the genera *Meloidogyne* and *Pratylenchus.* Here, we ask how plant-parasitic and other free-living trophic nematode guilds are affected by four diverse organic farming strategies, i.e. three stockless organic farm types "Cash Crop", "Soil Fertility", "Vegan" Farm type and one "Mixed" Farm type which includes cattle, each with individually designed legume cropping frequencies.

These farm types have been set up with individual fertilization strategies in four replicates in 2017 in an organically managed long-term experiment. Soils were always sampled in spring between 2022 and 2024 (not all farm types were sampled each year). Nematodes were extracted from soils using Oostenbrink elutriators, enumerated and eventually identified to the genus and/or family level. Morphology and molecular analysis of the 18S region helped to identify *Pratylenchus* to the species level. Average over all years, the Mixed Farm type harbored 3356 nematodes 100 ml soil$^{-1}$, between 37 and 52% more than the other farm types. Plant-parasitic nematodes were dominant in all farm types constituting 40 % (Soil Fertility Farm type) to 80 % (Mixed Farm type) of the total nematode community. *Pratylenchus* was the most abundant genus with up to 1,300 specimen 100 ml soil$^{-1}$ within the treatments of the Mixed Farm type. Organic fertilizer had inconsistent impacts on nematode trophic groups but negatively affected the herbivorous family of Tylenchidae. A strong positive correlation ($R^2 = 0.78$, $p = 0.004$) of clover grass biomass production and numbers of *Pratylenchus* in the Cash Crop Farm type was observed in the last sampling year. Such a hidden population build up emphasizes further monitoring of the population dynamic of involved *Pratylenchus* spp. over the course of the rotation.

## 1 Introduction

The development of more sustainable agroecosystems is indispensably linked with higher cropping frequencies and/or durations of legumes. Their capability to fix atmospheric nitrogen in symbiosis with rhizobia as well as their use in animal nutrition allows for increased nutrient cycling on the farm level and decreased dependency on external fertilizer supply and pesticides (Finckh et al. 2015; Böhm et al. 2020). In such systems, ecological complementarity of perennial legume-grass leys enables deep root networks that mobilize nutrients from subsoil extending farm internal nutrient cycles (Han et al., 2021). However, long-lived soil-borne pests and diseases including plant-parasitic nematodes (PPN) can seriously compromise legume root health and concomitantly nitrogen fixation (Le May et al. 2014; Šišić, Baćanović-Šišić, Karlovsky, et al. 2018; Baćanović-Šišić et al. 2018). Although PPN are important drivers amongst the manifold pathogens involved in the legume root-rot complex, their significance in organic land-use systems is largely unknown. Reasons may be that farmers are not aware of nematode damage in their fields or because crop damage is overlooked or attributed to other pests and diseases (Hallmann and Kiewnick, 2015). Furthermore, the area under organic management is about 10% in Europe and Germany (Eurostat, 2022) and the oftentimes long and diverse rotations in organic systems harbor less specialized PPN (e.g. cyst nematodes). Consequently, organic farms were not in the focus of PPN research so far. Few available studies on PPN in organic farming systems indicate that *Meloidogyne* spp. and *Pratylenchus* spp. occur frequently in considerable densities (Schmidt et al., 2017a; Hallmann et al., 2007). Besides both genera, ecto-parasites of the genera *Trichodorus, Helicotylenchus, Paratylenchus* and members of the family Dolichodoridae occur in organic rotations, particular after legumes and clover grass ley (Hallmann et al., 2007; Schmidt et al., 2017a). All of these PPN have a broad host range and are potentially detrimental to upcoming cash crops in the rotation, such as carrots and potatoes (Hallmann and Kiewnick, 2015).

On the other hand, perennial clover grass leys mimic permanent pastures and grasslands which harbor a great diversity of beneficial nematodes that include bacterivorous, fungivorous, omnivorous and predacious nematode trophic groups (Pothula et al., 2019). In such permanent systems, beneficial nematodes are generally more abundant than in annual cropping systems (Li et al., 2022). In the latter study, the normalized difference vegetation index, soil organic carbon, and a low (no) management intensity were responsible for the positive impact on beneficial nematode communities in pastures. Organic matter management can further improve the first two parameters. Organic matter applied as composts, mulches, manure, and cover crops supports the abundance and activity of microbes that serve as prey for bacterivorous and fungivorous nematodes (Schmidt et al., 2020). Both, in turn, are themselves prey for omnivorous and predacious nematodes that are indicative for a structured and suppressive soil food web far beyond the phylum Nematoda. That's probably why organic matter management is a promising strategy to regulate PPN (Stirling, 2014), demonstrated for *Pratylenchus* in several studies (Schmidt et al., 2017b; Zhao et al., 2022; El Titi and Ipach, 1989; Barros et al., 2017; Dias-Arieira et al., 2021). Cover crops, however, can have ambiguous effects on PPN (Neupane and Yan, 2023). While tagetes and sun hemp showed antagonistic effects against *Pratylenchus*, other herbaceous and leguminous crops also serve as additional hosts for less specialized PPN (e.g. *Pratylenchus*), and thus build a "green bridge" during periods where otherwise fallows cause a natural decline of PPN populations (Neupane and Yan, 2023).

The purpose of the study is to identify organic cropping systems and organic matter management strategies that suppress potentially harmful PPN while simultaneously fostering beneficial nematode trophic groups. Both effects should endure over the course of the crop rotation.

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We investigated the soil PPN population dynamic in an organic long-term field experiment (LTFE) over three consecutive years (2022-2024). Treatments studied were four diverse organic farming strategies each with six-year rotations, i.e. three stockless organic farm types "Cash Crop", "Soil Fertility", "Vegan" Farm type and one "Mixed" Farm type which includes cattle. Hence, rotations had a distinct intensity of legumes (33-50%), cereals (16-50%), and root-/tuber crops (16-33%). Each system had an individual fertilization strategy to make use of the clover grass ley that was the corner stone of each rotation, each one of which was compared to a control treatment where the clover grass ley was cut and left on the field (mulching) without specific use.

We hypothesized that i) a high legume abundance in rotations and long legume grass ley periods foster all nematodes trophic groups, ii) the high abundance of (perennial) legumes and cover crops in the rotation supports PPN with a broad host range (*Pratylenchus*, ecto-parasites), and iii) added composts and other organic fertilizer increase trophic links (higher number of omnivorous and predatory nematodes) and thereby decrease PPN populations compared to the mulched control.

## 2 Material and Methods

The long-term field experiment originally started in 2017 with a one-year period of clover grass followed by potatoes (2018) in all treatments (Tab. 1). During the course of the rotations, crops and fertilizer varied between individual main (farm type) and subordinated (fertilizer) treatments. Within all farm types, the control treatments were managed identically, with utilization of the legume grass mixtures as green manure and no other fertilization measures, thereby enabling the comparison of the rotation as such. The field is located on the organic experimental farm (certified since 1998) of the University of Kassel (Germany) in Frankenhausen (51°24'35.4"N, 9° 26'03.2"E, 250 m ASL with an average annual temperature of 10.0°C and mean annual precipitation of 553 mm in the period from 2014 to 2024. The soil type is a Haplic Luvisol with 1.5% sand, 80% silt and 18.5% clay (USDA classification Zc). Soil organic carbon and pH were 1.04% and 6.75, respectively, in 2020. The crop rotation until start of the long-term field experiment in 2017 consisted of 50% legumes (2/3 fodder legumes), 40% tuber crops, and 10% cereals. The experiment is fully described in Möller et al. (2025, in revision).

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**Table 1. Crop rotations of the four organic farm types and individual fertilizer applied to the respective fertilizer sub-treatments between 2017 and 2024. Soil samples for nematode diagnostics marked by an "X" were taken in spring 2022, 2023, and 2024 (prior to the fertilization and planting of potatoes). Clover and lucerne grass were tilled in November 2023 via shallow rotary cultivation and again in March 2024 via moldboard ploughing (long fallow period). Abbreviations are BR: Biogas residues; GWC: green waste compost; CGC: clover grass compost; MC: manure compost; CS: cattle slurry; TM: tofu residues; PP: plant pellets; F: Fallow; Po: Potato; and CC: Cover crop (for details, see 2.1).**

| | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 2024 |
|---|---|---|---|---|---|---|---|---|
| **Cash Crop Farm** | Clover grass | F \| Po \| CC | Carrot \| CC | Field bean \| F | Wheat \| F | Spelt | Clover grass \| F | Po |
| Fertilization | | BR | | BR | BR | | | BR |
| **Soil Fertility Farm** | Clover grass | F \| Po \| F | Wheat \| CC | Field bean \| F | Oats \| CC | Spelt | Lucerne grass \| F | Po |
| Fertilization | GWC | CGC | | GWC | | | GWC | CGC |
| **Mixed Farm** | Clover grass | F \| Po \| F | Wheat \| CC | Field bean \| Green rye | Clover grass | | \| F | Po |
| Fertilization | | MC, CS | | MC | CS | MC | | MC |
| **Vegan Farm** | Clover grass | F \| Po \| CC | Carrot \| CC | Field bean \| F | Spelt \| CC | Oats | Clover grass \| F | Po |
| Fertilization | | TM | PP | | | | | TM |
| **Nematode sampling** | | | | | | X | X | X |

## 2.1 Experimental design, investigated farm types and organic fertilizer strategies

The experiment consists of a strip-plot design with four replicates (each 76 x 36 m) with farm type as main factor (76 x 9 m) and fertilizer strategy as subordinated factor (15 x 9 m = plot size) randomly nested within the farm type strips. In total, 16 plots (2 farm types x 2 fertilizer strategies x 4 replicates), 24 plots (3 farm types x 2 fertilizer strategies x 4 replicates), and 32 plots (4 farm types x 2 fertilizer strategies x 4 replicates) were investigated in 2022, 2023, and 2024, respectively (Tab. 1). The first six-year crop rotation cycle (Tab. 1) started with perennial clover grass (20 months) followed by potatoes in all farm types. Thereafter, the crop rotations were individually designed for the specific intentions of the respective farms.

### 2.1.1 Cash Crop Farm

The Cash Crop Farm type simulates a production strategy (crop rotation and fertilization) that seeks for optimizing economic performance. Economically attractive root crops in the rotation (33 %) are key elements of the Cash Crop Farm type. Cover crops (mustard) were sown after potatoes and after carrots in the crop rotation during the study period. For this study, winter spelt (variety 'Zollernspelz') sown in October 2021, clover grass (50% *Trifolium pratense,* 50% *Lolium perenne*) sown in September 2022 and cultivated until November 2023 (fallow period until April 2024) were of major importance.

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The two fertilizer treatments in the Cash Crop Farm type were either no fertilizer (control) or application of biogas residues (from clover grass) in 2018 (38 kg N $ha^{-1}$), 2021 (133 kg N $ha^{-1}$), 2022 (93 kg N $ha^{-1}$).

### 2.1.2 Soil Fertility Farm

The Soil Fertility Farm type aims at enhancing soil organic matter and other soil fertility parameters with a lower focus on economic performance than in the Cash Crop Farm type. A lower proportion of root crops (potato, 17 %) than for any other stockless farm types tested in this study is accompanied by an increased share of cereals. The cover crop sown before spring-sown field beans was mustard while before spelt, a mixture consisting of 50% *Fagopyrum*, 37.5% *Helianthus annuus,* and 12% Phacelia was sown. Unlike other farm types, the clover grass alternates with lucerne grass over the 12-year (2 x 6 years) rotation (Tab. 1). For this study, winter spelt (variety 'Zollernspelz') sown in October 2021, lucerne grass (50% *Medicago sativa, 25% Festuca* spec. *x Lolium* spec., 15% *Festuca rubra*, 10% *Festuca pratensis*) sown in September 2022 and cultivated until November 2023 (fallow period until April 2024) were standing crops during the sampling period.
The two fertilizer treatments in Soil Fertility Farm type were either no fertilizer (control) or clover grass compost applied in 2018 (112 kg N $ha^{-1}$) and 2022 (96 kg N $ha^{-1}$) as well as green waste compost in 2020 (214 kg N $ha^{-1}$) and 2023 (292 kg N $ha^{-1}$).

### 2.1.3 Mixed Farm

The Mixed Farm type corresponds to the idea of largely closed nutrient cycles with obligate integration of livestock. Therefore, the crop rotation consists of a larger proportion of fodder legumes (50%) compared to other farm types. Standing crop over the sampling period in this study was clover grass (50% *Trifolium pratense,* 50% *Lolium perenne*) grown about 29 months from June 2021 until November 2023 (fallow period until April 2024).
The two fertilizer treatments in the Mixed Farm type were either no fertilization (control, no cattle) and composted farmyard manure applied in 2018 (112 kg N $ha^{-1}$), 2020 (187 kg N $ha^{-1}$) and 2022 (63 kg N $ha^{-1}$) as well as cattle slurry applied in 2018 (92 kg N $ha^{-1}$) and 2021 (89 kg N $ha^{-1}$). The application rate reflects the manure and slurry produced by two dairy cows $ha^{-1}$ over the course of the 1st rotation (Tab. 1).

### 2.1.4 Vegan Farm

The Vegan Farm type is free of any livestock derived products throughout the entire production cycle. The objective is to produce as much food as possible for human nutrition and to minimize periods of fallow while maximizing the benefits of cover crops to compensate for the lack of animal-based manures. Cover crops sown before field beans in 2020 and winter oat in 2021 are identical with the Soil Fertility Farm type. During the sampling period, winter oats (October 2021 until July 2022) and clover grass (September 2022 until November 2023 following a fallow period until April 2024) composed of 50% *Trifolium pratense* and 50% *Lolium perenne* were standing crops.

The two fertilizer treatments in the Vegan Farm type were either no fertilizer (control) or application of tofu residues in 2018 (40 kg N ha$^{-1}$) as well as application of plant pellets ('phytopearls') in 2019 (77 kg N ha$^{-1}$).

## 2.2 Nematode assessment, soil sampling, nematode extraction, morphological and molecular characterization

In spring 2022-2024, about 10 soil cores (4 replicates per treatment) were taken randomly from each plot center (30 cm soil depth). Hence, soils were sampled during winter spelt (Cash Crop Farm) and clover grass (Mixed Farm) cultivation in 2022, clover grass cultivation (both farm types) as well as lucerne grass cultivation (Soil Fertility Farm) in 2023, and 4.5 months after clover grass/ lucerne grass tillage in 2024 in all treatments listed in Tab. 1. Soils were stored at 4-6°C in plastic bags until processing.

To investigate the active soil nematode community, 100 ml soil aliquots of each plot were processed in an Oostenbrink elutriator (Hallmann and Subbotin 2018). Nematodes were collected on three mounted 45 µm sieves, washed into beakers, and transferred onto Oostenbrink dishes to get clean samples. After incubation at room temperature for 48 h, the nematodes in the Oostenbrink dishes were collected on 20 µm sieves and transferred to measuring cylinders. The water in the cylinders was reduced to 10 ml after nematodes had settled overnight. Nematodes were resuspended and their total densities were counted from 1 ml aliquot (= 10 % of the total suspension) at 63-fold magnification under an inverse microscope. After further reducing the suspension to 3-4 ml, an aliquot of the nematode suspension was transferred to 5 x 8 cm microscope slides equipped with a paraffin ring along its margins. After adding a cover slide, nematodes were heat killed (70°C) on a heating block and sealed between both slides (mass slide). Nematode populations on mass slides were identified to the genus level and in rare case to the family level (on average 109, 165, and 137 individuals per sample in 2022, 2023, and 2024, respectively) by using the keys of Bongers (1994), Brzeski (1998) and Andrássy (2005, 2007, 2009).

Nematodes were assigned to trophic groups by using the nematode indicator joint analysis web tool (Sieriebriennikov et al. 2014)(https://shiny.wur.nl/ninja/). Automated calculations for each plot followed the standard settings of the web tool, which retrieves information from the NEMAPLEX data base of the UC Davis (http://nemaplex.ucdavis.edu/). In our study, we use the terms "herbivorous nematodes" when referring to the trophic group as a whole and "plant-parasitic nematodes (PPN)" when referring to individual genera and species of the herbivorous nematode trophic group.

In 2023, 10 specimens of the genus *Pratylenchus* were randomly picked and identified to the species level using morphological traits (key for German *Pratylenchus* spp. developed by Dieter Sturhan). For molecular diagnostics in 2024, eight individuals out of the field study soils were randomly picked and accompanied by five additional samples (positive controls) out of the JKI's living nematode collection (*P. neglectus, P. penetrans, P. thornei, Ditylenchus dipsaci, Meloidogyne hapla*). Nematode DNA was extracted using the QIAamp DNA Micro Kit (Qiagen, Hilden, DE) following the manufacturers instruction with an overnight lysis of the nematodes incubated in 180 µl ATL buffer with 20 µl of Proteinase K at 56°C. PCR was conducted using primer pairs 988F/1912R and 1813F/2646R (adapted from Holterman et al., 2006) for Small Subunit rDNA, and primer pair JB3/JB5 for amplification of Cytochrome Oxydase Subunit I (adapted from Hu et al., 2002) as described in EPPO protocol PM7/129(2) (EPPO, 2021). PCR products were purified using ExoSAP-IT Express PCR Product Cleanup (ThermoFisher

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Scientific, Frankfurt a.M., DE) following the manufacturers protocol. Sanger Sequencing was externally conducted by Microsynth SeqLab GmbH (Göttingen, DE). Sequence editing was examined using SEQUENCHER software for windows vers. 5.0 (Gene Codes Corporation, Ann Arbor MI). Closest matches to all specimen were determined using BLASTN sequence similarity search tool in the National Centre for Biotechnology Information (NCBI) database. Phylogenetic relationships were tested by Neighbour joining Maximum Likelihood analysis coupled with 1000 replicates of non-parameter bootstrapping using Kimura two parameter (K2P) distances as implemented in software MEGA 7.0.26 (Kumar et al., 2016).

### 2.3 Statistics

Statistical analyses were performed with R version 4.0.2 (R Core Team 2020) using the packages “nlme” (Pinheiro et al. 2020) for analyses via linear mixed models and “emmeans” (Lenth 2020) for linear contrasts. The “car” package was used for calculating Levene tests (Fox and Weisberg 2019) and boxplots of model residuals were used to test the applied models for their variance homogeneity. In case of violations of variance homogeneity, linear mixed-effects models (lme) were adjusted with the weighting function “varIdent” in order to use individual standard errors for each factor level (Zuur et al. 2009).

To test for temporal plant–parasitic nematode (PPN) dynamic, each farm type was analyzed individually with experimental year as the main variable and replicates and plots as random variables to account for the repeated measures. In case of autocorrelations between sampling years, a first-order autoregression structure was used (Crawley, 2007).

For the comparison of the farming system and fertilization strategy effects on nematodes, the three experimental years were analyzed separately due to the increasing number of treatments over time. In this case, field replicates served as random factor. The fixed factor “system” was used as second random factor nested in the field replicate to account for the strip plot design. Estimated marginal means and pairwise comparison were used to identify significant differences amongst factor levels. Tests were performed within the factor level “control” of fertilizer treatments (farm type effects) and within factor levels of farm types (fertilizer effects compared to the “control”). Alpha error inflation due to multiple testing of farm type levels was corrected by using the “mvt” adjustment method. Means, standard errors (SE) visualized in the figures derived from the emmeans model outputs.

Parametric Pearson correlations were used to study the dependence of clover grass and lucerne yields on the number of *Pratylenchus.*

## 3 Results

Total numbers of 2750, 2340, and 2930 nematodes 100 ml soil$^{-1}$ were extracted in 2022, 2023, and 2024 respectively, averaged over all treatments (Fig. 1 F). Herbivorous, bacterivorous, fungivorous, omnivorous, and predacious nematodes accounted for 68.4 %, 23.1 %, 4.2 %, 2.0 %, and 2.3 %, respectively, of all nematodes averaged over all treatments and years. The average number of taxa per sample distinguished by the genus and in rare cases by the family level (Dolichodoridae, Tylenchidae,

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Criconematidae as well as rhabditid dauer forms) was 20.6, 21.7, and 21.5 in 2022, 2023, and 2024, respectively (data not shown).

### 3.1 Nematode trophic groups affected by farm type in unfertilized controls

Within the unfertilized control treatments of the farm types (= effect of crop rotations), herbivorous nematodes accounted for 72-79 % of the total nematode community in the Mixed Farm type in the first two years (Fig. 1 A-F). In the same years, herbivorous nematodes constituted 57 to 61 % of the total nematode fauna in the Cash Crop and Soil Fertility Farm type. In 2023, the total number of herbivorous nematodes was significantly higher in the Mixed Farm type than in the Cash Crop Farm type and the Soil Fertility Farm type ($F_{2,6}$ = 16.7, p = 0.0035, Fig. 1 A). Until the last sampling year after clover grass (2024), herbivorous nematodes had significantly increased in the Cash Crop Farm type (Tab. 2) and accounted for between 69 % and 76 % of the total nematode community throughout all farm types, while farm type effects were similar than in 2023 ($F_{3,8}$ = 12.1, p = 0.0024, Fig. 1 A).

Bacterivorous nematodes accounted for between 31 % and 34 % of the total nematode fauna in the Cash Crop and Soil Fertility Farm types in 2022 and 2023, and 21 % and 14 % in the Mixed Farm type in 2022 and 2023, respectively (Fig. 1 B). The numbers of bacterivorous nematodes decreased significantly ($t_6$ < -4.06, p < *0.03)* from 2023 to 2024 in the Cash Crop and Soil Fertility Farm types (Tab. 2). Thus, bacterivorous nematodes accounted for 13.9-17.1 % of the total nematodes in all farm types in 2024. The differences among farm types were generally low and insignificant.

Summarized over all investigated farm types, the relative abundance of fungivorous nematodes varied between 1.8 % (Soil Fertility Farm type 2023) and 8.7 % (Soil Fertility Farm type 2024). The numbers of fungivorous nematodes increased significantly from 2023 to 2024 in the Soil Fertility Farm type ($t_3$ = 4.3, p = *0.02*) and by trend in the Cash Crop Farm ($t_6$ = 2.37, p = *0.056*) and Mixed Farm ($t_6$ = 2.3, p = *0.06*, Fig. 1 C, Tab. 2) types. Differences among farm types were low throughout all sampling intervals.

Omnivorous and predacious nematodes generally constituted less than 5 % of the total nematode community (Fig. 1D and E). The abundance of predacious nematodes gradually increased over the course of the clover grass period in the Mixed Farm since 2022 with a significant increase from 2023 to 2024 (Tab. 2). Although unconfirmed statistically, this resulted in the highest numbers of predacious nematodes in the Mixed farm compared to all other farm types in 2024 (Fig. 1 D and E).

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**Table 2 T- ratios following linear mixed effects models and estimated marginal means comparing the consecutive temporal dynamic of nematode trophic groups from 2022 to 2024 in the control treatments of the Cash Crop Farm, Mixed Farm and Soil Fertility Farm types. Bold t-ratios indicate significant increase (positive t-ratio) or decrease (negative t-ratio) of nematode taxa with n.s. and *, **, *** indicating *p* –values >0.05, <0.05, <0.01, < 0.001, respectively. Compare also Fig. 1.**

| Farm type (df) | Years | Herbivores | Bacterivores | Fungivores | Omnivores | Predators | Total |
|---|---|---|---|---|---|---|---|
| | | t- ratio | | | | | |
| Cash Crop Farm (6) | 2022 - 2023 | 0.67 n.s. | -0.202 n.s. | -1.712 n.s. | 0.488 n.s. | 1.087 n.s. | 0.309 n.s. |
| | 2023 - 2024 | **6.723 ***** | **-4.301 **** | 2.368 n.s. | 0.340 n.s. | -0.109 n.s. | **3.067 *** |
| Mixed Farm (6) | 2022 - 2023 | 0.367 n.s. | -1.170 n.s. | -1.121 n.s. | 1.301 n.s. | 0.956 n.s. | -0.140 n.s. |
| | 2023 - 2024 | 1.094 n.s. | 0.630 n.s. | 2.322 n.s. | -0.610 n.s. | **2.559 *** | 1.610 n.s. |
| Soil Fertility Farm (3) | 2022 - 2023 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| | 2023 - 2024 | 0.873 n.s. | **-4.016 *** | **4.323 *** | -0.121 n.s. | -0.035 n.s. | 0.532 s. |

### 3.2 Fertilizer with inconsistent effects on nematode trophic groups

In general, the number of nematodes in the fertilization treatments in the four farm types followed the same annual (crop specific) trend (chapter 3.2) that was observed for their respective control treatments. In detail, a population decline or increase over time always occurred simultaneously in both treatments. Overall, the different utilization approaches of the clover grass, i.e. biogas fermentation (Cash Crop Farm type), cattle fodder and subsequent composting of the cattle manure (Mixed Farm type), direct composting of clover grass (Soil Fertility Farm type), and silage production from clover grass (Vegan Farm type) (all termed fertilizers hereafter), had only few and inconsistent effects on the nematode trophic group abundance compared to the clover grass that was mulched and left in the field (hereafter called control) (Fig. 1 A-F) and depended on the sampling year (i.e., standing crop).

In the Cash Crop Farm type, one year after application of biogas slurry, herbivorous ($t_6 = -2.1$, $p = 0.08$) and predacious ($t_6 = 2.1$, $p = 0.08$) nematodes were by trend higher compared to the control in 2022 (Fig. 1 A and E). For predacious nematodes, this trend was stable in all study years. In 2023, one year after the biogas slurry application in 2022, the number of herbivorous (-28 %, $t_9 = -5.9$, $p < 0.001$) and omnivorous (-13 %, $t_9 = -2.4$, $p = 0.04$) nematodes were significantly lower in the biogas slurry treatment compared to the control (Fig. 1 A, D). Other nematode trophic groups had similar abundances in both treatments. The year 2024 reflected the first experimental year by showing significantly higher numbers of herbivorous nematodes (33%, $t_6 = 2.9$, $p < 0.015$) in the fertilizer treatment compared to the control.

The Mixed Farm type revealed significantly lower numbers of herbivorous nematodes (-9%, $t_6 = 8$, $p < 0.001$) in 2022, one year after cattle slurry application to spelt in 2021, in the cattle manure/cattle slurry treatment compared to the control. This trend also occurred in 2023 one year after cattle manure compost application, in three of four plots (-28 to -42 % number of

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nematodes compared with the control). However, an outlier in the fourth repetition (+149% number of nematodes compared with the control) obscured these results (see high standard error in Fig 1 A). Vice versa, exceptionally high numbers of herbivorous nematodes were recorded in the fertilization treatment compared to the control (+38 %, 3538 nematodes 100 ml soil$^{-1}$, $t_{11}$ = 1.64, p > *0.05*) in 2024, two years after the last organic fertilizer application. Fertilization did not affect other nematode trophic groups.

In the Soil Fertility Farm type, the clover grass compost (applied in spring 2018 and 2022) and green waste compost (applied in 2020 and 2023) had inconsistent effects on omnivorous nematodes (+122 %, $t_9$ = 1.7, *p = 0.12* for 2023*;* -50 %, $t_{11}$ = -2.3, *p = 0.045* for 2024) as well as herbivorous, bacterivorous, and fungivorous nematodes. Predacious nematodes (+19 % and +48% in 2023 and 2024, respectively) were by trend higher in the fertilizer treatment than in the control.

In the Vegan Farm type that was only sampled in 2024, fungivorous nematodes were significantly lower (-21 %, $t_{11}$ = 2.705, *p* = 0.020) in the fertilizer treatment (three years after the last application of phytopearls) compared to the control.

Regarding the total number of nematodes, biogas slurry application in the Cash Crop Farm type had a significantly negative impact ($t_9$ = 3.66, *p = 0.0053*) one year after application and a significantly positive impact ($t_{11}$ = 2.6, *p = 0.027*) two years after application compared with the unfertilized control. The latter mirrored the trends occurring for herbivorous, bacterivorous, omnivorous, and predacious nematodes.

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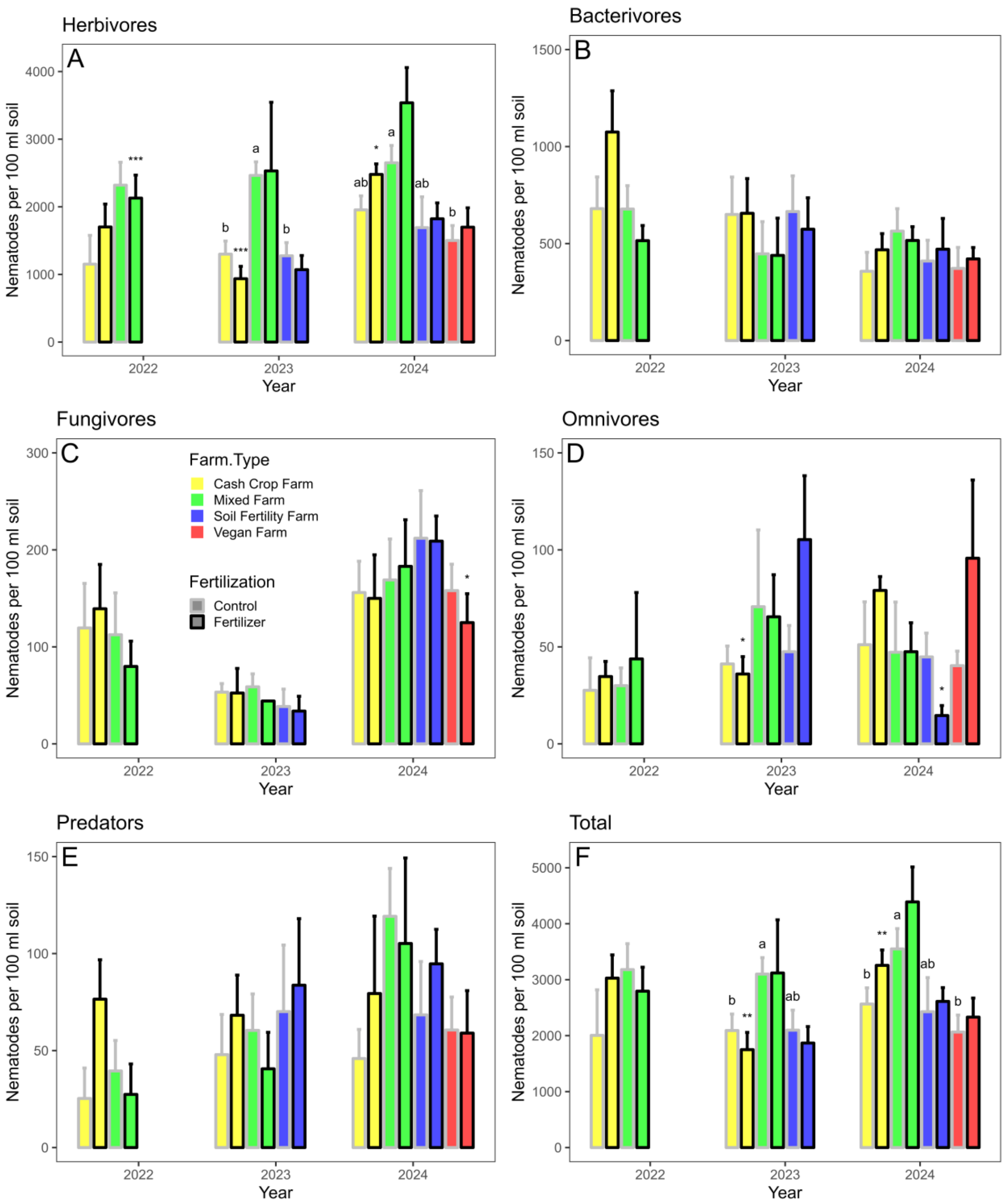

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**Figure 1 Estimated marginal means (emmeans + se) for the number of nematodes per 100 ml soil for the five trophic groups (A-E) and the total number of nematodes (F) separated by year, farm type (colors), and fertilization (grey and black borders around (error) bars indicate the Control and Fertilizer treatments, respectively). The Soil Fertility Farm type was sampled in 2023 and 2024, the Vegan Farm type in 2024 only. Farm types of the control treatment (grey bordered bars) that do not share a common lower letter are statistically different at $p < 0.05$ (emmeans contrasts); * and *** indicate whether the fertilizer treatment within a farm type is significantly different from the control treatment at $p < 0.05$ and $p < 0.001$, respectively (emmeans contrasts). For optical reasons, y-axes were scaled individually for the respective nematode trophic groups.**

### 3.3 Plant-parasitic nematodes affected by farm type in unfertilized controls

In total, we differentiated 15 taxa of plant-parasitic nematodes (PPN), of which the six most abundant taxa are shown in detail (Fig. 2). The abundances of specimen within individual genera in the family Tylenchidae were summed but the most prevalent genera in 2024 were *Boleodorus, Neopsilenchus* and *Coslenchus,* with 286, 121, and 60 nematodes 100 ml soil$^{-1}$, respectively (data not shown).

*Pratylenchus* was by far the most dominant taxa in the Mixed Farm type (50 % of all PPN) with stable populations over all three years (1150-1350 nematodes 100 ml soil$^{-1}$). In comparison, the numbers of *Pratylenchus* were lower in the other farm types (234-780 nematodes 100 ml soil$^{-1}$) but this could not be confirmed statistically when analyzing the years individually (Fig. 2 E). When using a repeated measurements linear mixed effect model for the years 2023 and 2024 (statistics not shown), the Mixed Farm type had significantly more individuals of *Pratylenchus* than the Soil Fertility Farm type ($t_6 = 3.6$, $p = 0.023$) and the Cash Crop Farm type ($t_6 = 3.0$, $p = 0.0496$). In the latter two farm types, a significant increase of *Pratylenchus* was recorded between 2023 and 2024 (4.5 months after clover grass tillage, Tab. 3).

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**Table 3 T- ratios following linear mixed effects models and estimated marginal means comparing the temporal dynamic of plant-parasitic nematode taxa from 2022 to 2024 for the Cash Crop Farm, Mixed Farm and Soil Fertility Farm types. Bold t-ratios indicate significant increase (positive t-ratio) or decrease (negative t-ratio) of nematode taxa with n.s. and * indicating *p* –values >0.05 and <0.05, respectively.**

| Farm Type (df) | Years | Doli-chodoridae | Helico-tylenchus | Meloi-dogyne | Para-tylenchus | Pra-tylenchus | Tylen-chidae |
|---|---|---|---|---|---|---|---|
| | | t- ratio | | | | | |
| Cash Crop (6) | 2022 - 2023 | 0.404 n.s. | 0.722 n.s. | 0.0 n.s. | 1.328 n.s. | -1.265 n.s. | 1.891 n.s. |
| | 2023 - 2024 | 0.366 n.s. | 2.276 n.s. | 1.225 n.s. | -1.064 n.s. | **2.955 *** | 1.643 n.s. |
| Mixed Farm (6) | 2022 - 2023 | -1.974 n.s. | -0.087 n.s. | 1.000 n.s. | 2.156 n.s. | 0.346 n.s. | 1.857 n.s. |
| | 2023 - 2024 | 0.400 n.s. | 1.708 n.s. | -0.987 n.s. | -0.361 n.s. | -0.213 n.s. | 1.622 n.s. |
| Soil Fertility (3) | 2022 - 2023 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| | 2023 - 2024 | -2.036 n.s. | 2.623 n.s. | 1.200 n.s. | 0.578 n.s. | **4.549 *** | -0.062 n.s. |

The nematode family Tylenchidae was the most abundant taxa in the Cash Crop, Soil Fertility, and Vegan Farm types with 36, 55, and 47% of the total PPN population, respectively, averaged across all sampling intervals. While the number of Tylenchidae was by trend higher in the Soil Fertility Farm type than in the Mixed and Cash Crop Farm types in 2023 (Fig. 2 F), numbers were similar (712-817 nematodes 100 ml soil$^{-1}$) in all farm types in 2024.

Dolichodoridae and *Helicotylenchus* made 9.5% and 6.7% of the total number of PPN averaged over all years and farm types. While the Dolichodoridae showed inconstant numbers of nematodes among the farm types, the numbers of *Helicotylenchus* increased over time in all farm types. The Mixed Farm type harbored significantly higher numbers of *Helicotylenchus* compared to the Soil Fertility Farm type in 2023 and 2024 and to Vegan Farm type in 2024 (Fig. 2 B and E). In all farm types, the highest number of *Helicotylenchus* was found in the last sampling year after clover grass.

In general, *Paratylenchus* and *Meloidogyne* occurred at low numbers (5- 138 nematodes 100 ml soil$^{-1}$, Fig. 2 C and D). In addition, the numbers of nematodes of both taxa were highly variable depicted by high standard errors in all farm types.

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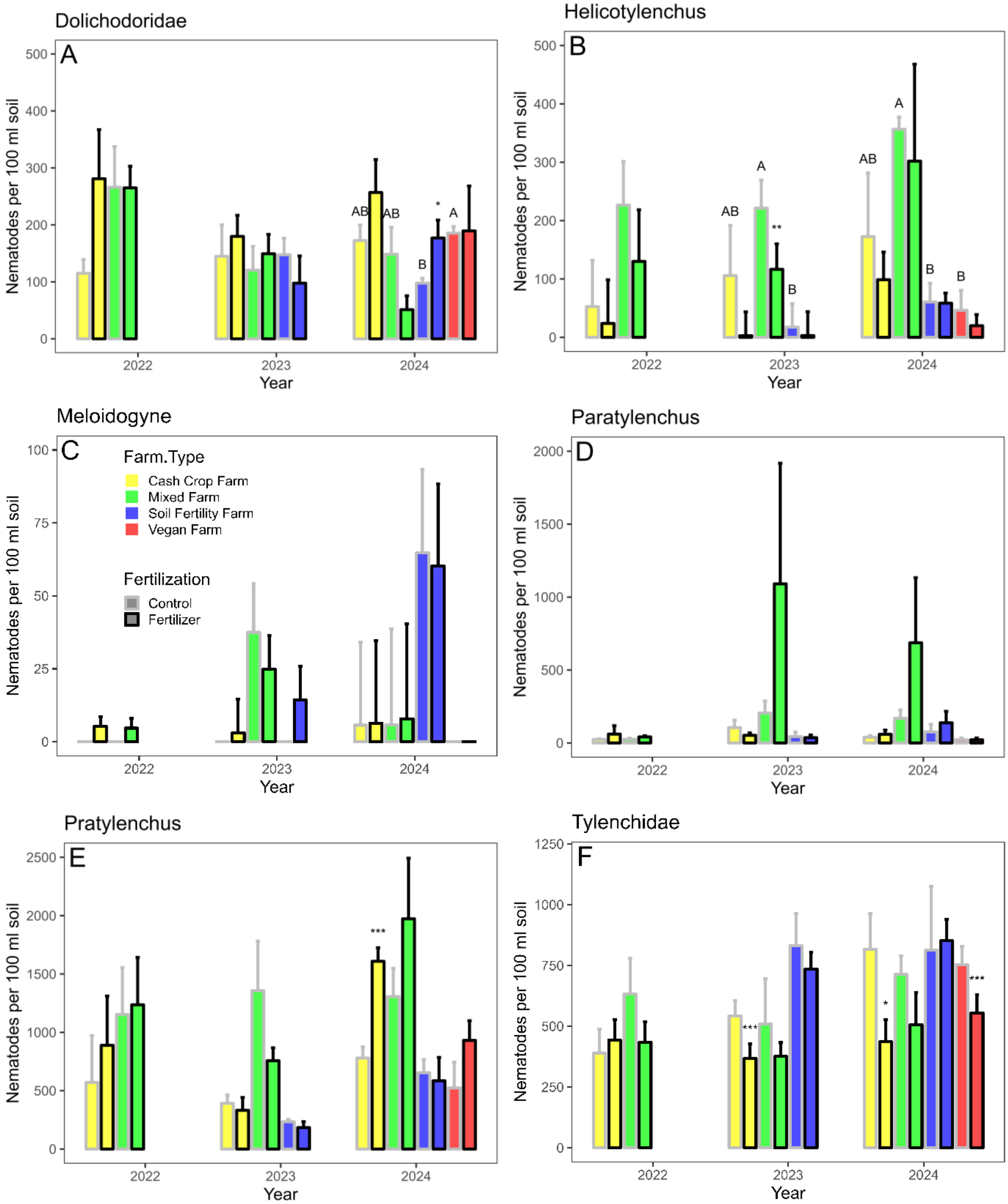

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**Figure 2. Estimated marginal means (emmeans + se) for the number of nematodes per 100 ml soil for the six most abundant plant-parasitic nematodes (A-F) separated by year, farm type (colors), and fertilization (grey and black borders around bars and se indicate the Control and Fertilizer treatments, respectively). The Soil Fertility Farm type was sampled in 2023 and 2024, the Vegan Farm type in 2024 only. Farm types of the control treatment (grey bordered bars) that do not share a common letter are statistically different at $p < 0.05$ (emmeans contrasts); *, **, and *** indicate whether the fertilizer treatment within a Farm type is significantly different from the control treatment at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively (emmeans contrasts). For optical reasons, y-axes were scaled individually for the respective plant-parasitic nematode genera/ families.**

### 3.4 Plant-parasitic nematode response towards farm type inherent organic fertilizer

The overall effect of the farm types' inherent fertilizer on PPN ranged from low (e.g., *Meloidogyne*, all years and farm types) to very high (e.g., *Paratylenchus* and *Pratylenchus* in 2023 and 2024 in the Mixed Farm type). Some of the peaks were affected by outliers that occurred in the Mixed Farm type for *Pratylenchus* in 2024 (3505 nematodes 100 ml soil$^{-1}$, replicate three) and for *Paratylenchus* in replicate four in 2023 (3594 nematodes 100 ml soil$^{-1}$) and in 2024 (2016 nematodes 100 ml soil$^{-1}$). Out of all detected nematode species, *Tylenchidae* responded most consistently on the different fertilizer applications: fertilizer application in all farm types caused a reduction of this nematode family compared to the control. Opposed effects occurred only twice, in the Cash Crop Farm type in 2022 and in the Soil Fertility Farm type in 2024 (Fig. 2 F). With respect to *Pratylenchus,* biogas slurry (Cash Crop Farm type) applied in 2022 and 2023 increased the number of this nematode genus in 2024 compared with the control (+829 nematodes 100 ml soil$^{-1}$, $t_{11} = 9.7$, $p < 0.001$, Fig. 2 E). A similar trend for this genus showed up for the fertilizer treatments in the Mixed Farm type and the Vegan Farm type while a neutral effect occurred in the Soil Fertility Farm type. *Helicotylenchus* was significantly affected one year after the biogas slurry application (2022) in the Cash Crop Farm type (-206 nematodes 100 ml soil$^{-1}$) and one year after the composted cattle manure application (2022) in the Mixed Farm type (-256 nematodes 100 ml soil$^{-1}$).

### 3.5 Diagnostics of Pratylenchus species

Two out of the ten specimen of *Pratylenchus* spp. that were identified via morphology in 2023 were identified as *P. neglectus* due to the characteristic two lip rings and empty female spermathecas. The remaining eight specimen had three lip rings; however, their general features were similar to but not unique for *P. penetrans*, *P. vulnus,* and *P. pseudopratensis.* In particular, the form of the spermatheca was intermediate between "round" and "elongated" and therefore, the diagnostics needed molecular support. For the eight single individuals (6 females, 2 males) that were picked for molecular diagnostics (field samples), we failed to obtain a complete 447 bp fragment of the COI gene in PCR. For two females and both males, a subclade of *Pratylenchus* spp. clade a (following Subbotin et al. 2008) closely related to *P. pratensis* emerged when using the SSU region of the 18S rDNA with fragment length between 1660 and 1704 bp (NCBI accession numbers PX578225- PX578228, Fig. 3). The controls of *P. neglectus*, *P. penetrans*, *P. thornei*, *Meloidogyne hapla*, and *D. dipsaci* matched by 100% with their clades (Fig. 3 A). In 2024 after potatoes (data not shown in this study), a detailed morphological analysis revealed about 40% *P. neglectus and 45% P. pratensis* (Fig. 3 B) of the total *Pratylenchus* population averaged over all treatments.

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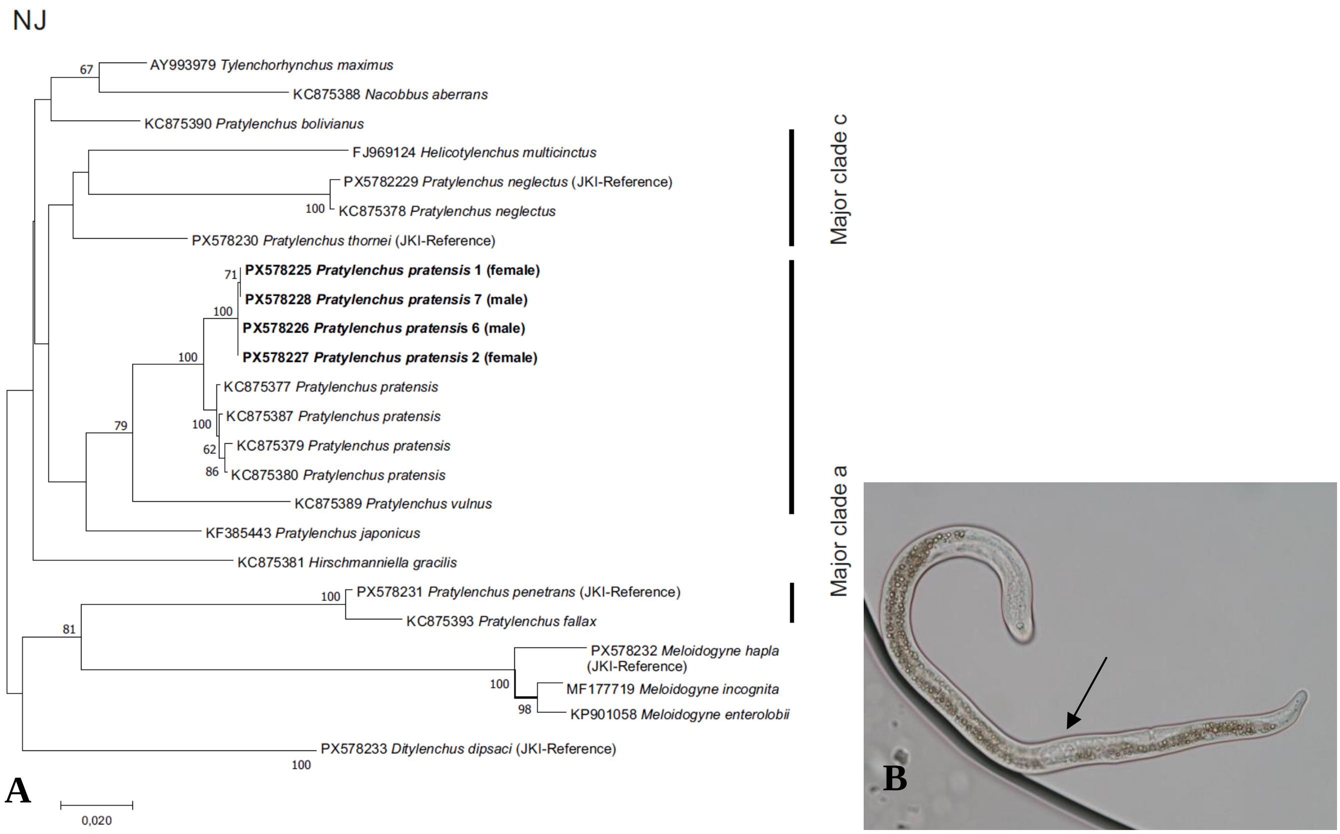


**Figure 3 A: Neighbour Joining Maximum Likelihood phylogenetic tree of partial regions of the SSU 18S rDNA of *Pratylenchus* spp. individuals randomly picked from field samples (Accession numbers PX578225- PX578228) and selected reference taxa (JKI) in comparison with available NCBI data. Samples with JKI in brackets represent reference specimen from the living nematode collection of the JKI and refer to *P. neglectus, P. thornei, P. penetrans, Ditylenchus dipsaci, and Meloidogyne hapla*. B: Picture of a female *P. pratensis* relative with large and oval spermatheca (arrow).**

### 3.6 Impact of *Pratylenchus* spp. on lucerne and clover grass yield

There was no negative correlation between the number of *Pratylenchus* (100 ml soil$^{-1}$) and the lucerne grass (Soil Fertility Farm type) or clover grass (Cash Crop and Mixed Farm type) dry matter yield (dt ha$^{-1}$) in 2023. In contrast, there was a significant positive correlation of the number of *Pratylenchus* 100 ml soil$^{-1}$ in 2024 and clover grass yields in 2023 in the Cash Crop Farm type (Fig. 4) with a very high multiplication of *Pratylenchus* in the high yielding plots (> 90 dt ha$^{-1}$). *Pratylenchus* also multiplied in all plots in the lucerne grass stand of the Soil Fertility Farm type and in six out of eight plots in the already strongly with *Pratylenchus* infested clover grass stands of the Mixed Farm type (Fig. 4).

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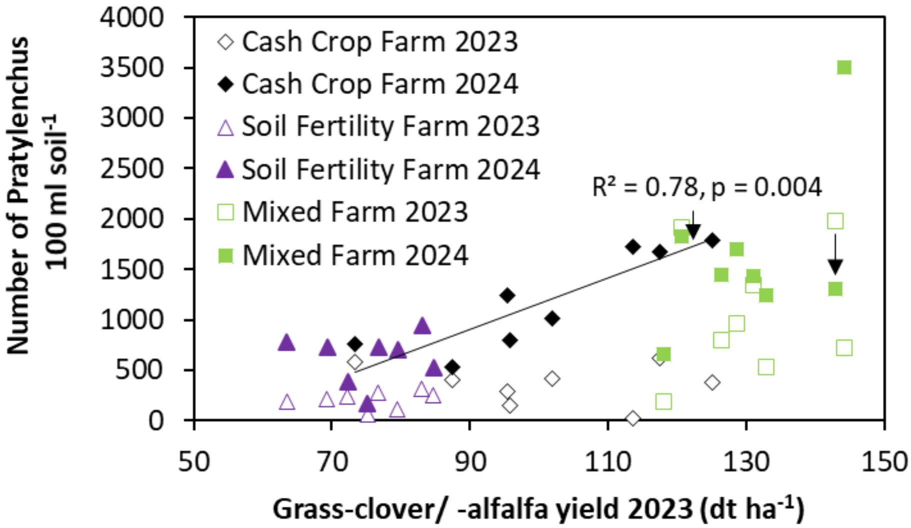


**Figure 4 Relevance of clover grass and lucerne grass dry matter yields in 2023 for *Pratylenchus* spp. multiplication between spring 2023 and spring 2024 separated by farm types (the Vegan Farm type was sampled in 2024 only and therefore, not shown here) and without separation of fertilizer treatments. Empty symbols indicate the status quo population of *Pratylenchus* in 2023; filled symbols indicate the *Pratylenchus* population after break down of the clover grass/ alfalfa stand in 2024. Each pair of a horizontally arranged solid and filled symbol of one type (squares, diamonds, triangles) represent one plot in the experiment (both fertilized and control plots were used without differentiation). Solid lines indicate significant correlations of yields in 2023 and the number of *Pratylenchus* in 2024 (Pearson correlation). The black arrow indicates the plots with a decline of *Pratylenchus* from 2023 to 2024.**

## 4 Discussion

Legumes, in particular perennial legume grass mixtures play a fundamental role in nutrient mobilization in organic farming systems. Our study is the first that highlights the impact of organic legume grass leys on nematode trophic groups and the most important taxa of PPN in soil. The total number of nematodes quantified in our study are much in line with the extrapolated total number of nematodes for Central and Northern Germany (van den Hoogen et al., 2019). The trophic nematode community composition was dominated by herbivorous nematodes which is in contrast to the study by Schmidt et al. (2020) who found equal numbers of herbivorous and bacterivorous nematodes in another organically managed field nearby. The historical crop rotation of the field in this study consisted of 50% legumes, which might explain the high numbers of unspecialized ecto-parasitic and migratory endo-parasitic nematode taxa. Juveniles of specialized sedentary nematodes of the family Heteroderidae were not found in the investigated soils, reasonably due to the long and diverse rotations that inhibit the establishment of these taxa.

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### 4.1 Herbivorous nematodes benefit from rotational crops, predacious nematodes from long clover grass ley periods in the Mixed Farm type

The high number of herbivorous nematodes in the first sampling year in the Mixed Farm type suggested that these had multiplied already in the preceding crops faba bean and winter rye. The consistent large numbers of herbivorous nematodes in the Mixed Farm type during all sampling years and the significant increase of herbivores in the Cash Crop Farm type after clover grass highlighted the good host status of the plant species in the clover grass mixture for the resident nematode population. Schmidt et al. (2017a) showed that herbivorous nematodes further increased after clover grass during the subsequent winter wheat cropping season. The fact that only endo-parasitic nematodes increased during this period and that the authors investigated herbivorous nematodes in the soil under clover grass before tillage emphasizes that the authors likely underestimated the numbers of nematodes after grass clover. Interestingly, the 29 months ley period in the control of the Mixed Farm type only maintained the number of herbivorous nematodes while, after 14 months ley period in the controls of the Cash Crop and Soil Fertility Farm type, the population of this trophic group had increased. Reasons for this appeared to be strongly genus specific and are therefore discussed further below.

In contrast to herbivorous nematodes, predacious nematodes constantly increased in the Mixed Farm type over time while they remained stable or slightly decreased in the Cash Crop and Soil Fertility Farm types. This is in line with our first hypothesis that an overall increase of nematodes is supported by long-term ley cropping. While adult predacious nematodes prey non-selectively on diverse nematode trophic groups (including cannibalism), juvenile predators predominantly use bacteria for their diet (Bilgrami and Brey, 2005). The fact that bacterivorous nematodes did not follow the same trend as the predacious nematodes indicates that the accessible bacterial prey was not the driving factor for the increase in predacious nematodes. Hence, the longer period of no-tillage during the 29-month clover grass cropping in the Mixed Farm type likely fostered the predacious nematodes more than during the 14-month clover or lucerne grass period in all other farm types. Similarly, in a study conducted in Western Germany, any kind of tillage significantly reduced the nematode maturity index, an indicator that positively correlates with omnivorous and predacious nematodes (Lenz and Eisenbeis, 2000).

However, omnivorous nematodes remained unaffected by any of the farming systems nor increased over the ley period which is in contrast to the findings by Lenz and Eisenbeis (2000) as well as our first hypothesis. This emphasized that even the 29 months clover grass ley period in the Mixed Farm treatment was insufficient to support this trophic group. Another reason may be of technical nature. The Oostenbrink elutriator approach for nematode extraction in this study is especially developed for small to median sized nematodes (250 µm to 800 µm length) (Goede and Verschoor, 2000). The oftentimes larger taxa of the omnivorous dorylaimid nematodes equipped with a low motility (Bongers and Bongers, 1998) have an up to 80 % lower recovery rate than herbivorous nematodes after passing the 1 mm sieve in the Oostenbrink elutriator and the subsequent filters during the 48 h Oostenbrink dish extraction (Goede and Verschoor, 2000). Whether higher trophic level organisms, such as predatory mites, spring tails and others (Creamer et al., 2022), affected the number of this trophic crop was not investigated here but is worth considering in future studies.

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The abundance of fungivorous nematodes was similar in all farm types and peaked in 2024, 4.5 months after termination of the ley. The shallow termination of the clover grass ley via rotary tilling likely prevented the disruption of mycorrhizal networks which are generally supported by long periods of clover and lucerne grass leys without soil perturbation (Gosling et al., 2006; Oehl et al., 2003). By the time of nematode sampling, the quick degradation of nitrogen-rich organic residues through predominantly (gram negative) bacteria alters into a slower degradation of organic residues with lower nitrogen contents by fungi (Wang and Kuzyakov, 2024). Both biological processes increase the prey for the fungivorous nematodes and would explain the high numbers of this trophic group in the last sampling year.

### 4.2 Legumes but not cover cropping supported *Pratylenchus* and *Helicotylenchus*

Amongst the individual herbivorous nematode taxa, the genus *Pratylenchus* and the family of Tylenchidae were most abundant in all sampling intervals. *Pratylenchus* has a very broad host range with over 400 plant species, ranging from cereals over vegetables to grassland species, and is omnipresent in organic fields in Germany (Hallmann et al., 2007). Likewise, members of the Tylenchidae occur in almost any soil; however, knowledge regarding their feeding habits is poor. Reasons for this are their economic unimportance as well as their lack of clear characters for morphological diagnosis (Qing and Bert, 2019). The growing numbers of Tylenchidae over the course of the rotation in this study suggest that this family benefited from clover/ lucerne grass leys grown in all treatments from 2023 to 2024. Although some members of this large group of nematodes are considered fungivorous (Okada et al., 2005), we assume that the vast and dense root structure developed in the 14 to 29 months ley period fostered the predominantly root hair feeding taxa of this family. This is in line with a field study in Ireland where Tylenchidae was the dominant nematode family after clover and ryegrass cultivation (Ikoyi et al., 2023).

Regarding the four organic farm types, the Mixed Farm type with its high legume frequency (50 %) and long clover grass ley period in the rotation harbored the highest number of *Pratylenchus* and *Helicotylenchus* of all farm types. Both species can multiply rapidly under red clover and perennial ryegrass leys (Stevens et al., 2018; Ikoyi et al., 2023) which confirms the capability of clover grass leys to build up large PPN populations without suffering any obvious damage (Schmidt et al., 2017a). While the number of *Helicotylenchus* particularly increased in the last sampling year, the number of *Pratylenchus* remained constant in all three sampling intervals. Clover and lucerne grass produce deep root networks, especially when growing for two years or longer, and likely contributed to a vertical transport of *Pratylenchus* in the Mixed Farm type. This is in line with a Dutch study, in which the authors suggested an optimum sampling depth of 45 cm for the detection of *Pratylenchus penetrans* in maize (Pudasaini et al., 2006). In a Dutch hay field dominated by perennial ryegrass (*Lolium perenne*), the vertical distribution of herbivorous nematode strongly depended on the taxa and plant root length (Verschoor et al., 2001), which emphasizes that a more “in depth” analysis of the vertical distribution of herbivorous nematodes along the soil horizon is necessary. Finally, we extracted the nematodes only from the mineral soil fraction via Oostenbrink elutriator and subsequent 48 hours extraction in Oostenbrink dishes. Hence, we cannot exclude that a large part of the endo-parasitic nematode population remained undetected in the organic soil fraction (fine roots) and that the number of *Pratylenchus* spp. shown in this study may be underestimated.

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In contrast, the lucerne grass ley in the Soil Fertility Farm type harbored lower numbers of *Pratylenchus* and *Helicotylenchus* compared with the Mixed Farm type. Whether this can be attributed to a weaker host status of lucerne than clover to both resident PPN genera (Neupane and Yan, 2023) remained unclear due to the substantial increase of both specimen between 2023 and 2024 in the Soil Fertility Farm type.

While the economic impact of the semi-endoparasite *Helicotylenchus* can be neglected in this study, the large number of *Pratylenchus* deserves further attention. For example, *Pratylenchus crenatus* and *P. penetrans* are capable to cause yield losses in grasses (Mercer et al., 2008) and can already damage potatoes and carrots at densities between 100-200 specimen 100 ml soil$^{-1}$ (Hallmann and Kiewnick, 2015). The fact that both crops will follow on the ley periods in most farm types and that the above-mentioned damage threshold level is far below those densities of *Pratylenchus* found in our study is alarming. Moreover, as discussed above, the population of *Pratylenchus* may be underestimated in our study which highlights the need for the quantification of individual *Pratylenchus* species to address the damage potential for subsequent crops. Meanwhile, a re-thinking of the crop rotational design could be a first step to reduce the number of PPN in the field. The addition of *Plantago* and *Cichorium* to the clover grass components appeared helpful in one study (Ikoyi et al., 2023). Another idea could be the introduction of spelt or winter oat in the rotation of the Mixed Farm type. Both crops grew in the Soil Fertility Farm and Vegan Farm types in this study and kept *Pratylenchus* and *Helicotylenchus* at low levels.

In contrast to our hypothesis, cover cropping did not result in population build-up of PPN with a broad host range. Cover cropping was intensively used in the Soil Fertility and Vegan Farm types. Both had amongst the lowest number of PPN after termination of the leys. Cover crops can act as a "green bridge" that supports PPN, such as *Pratylenchus* and *Helicotylenchus*, both with a broad host range, in periods where otherwise no crop is grown (Neupane and Yan, 2023; Desaeger and Rao, 2000). Mustard pure stands as well as cover crop mixtures consisting of *Helianthus, Fagopyrum,* and *Phacelia* did not show this in our study. Hence, the right choice of cover crops can result in several agro-ecosystem services, such as organic carbon sequestration, nitrogen storage and protection from soil erosion without worrying about disservices such as the population build-up of damaging PPN. Besides the right choice of cover crops, knowledge regarding the indigenous population of plant-parasitic nematode taxa is of major importance. The most damaging genera in organic farming systems are *Pratylenchus* and *Meloidogyne*, both with a different spectrum of host plants also between species (Hallmann and Kiewnick, 2015). The Northern Root-knot nematode *Meloidogyne hapla* but not the Southern Root-knot nematode *Meloidogyne incognita* can multiply on *Phacelia* as well as on mustard (Uthoff et al., 2023; Gardner and Caswell-Chen, 1994), while the multiplication of *P. penetrans* on *Phacelia* was variety depending (Taning et al., 2024). Therefore, it may occur that the control of one genus or even species results in the multiplication of another taxa. Although the slight increase of *Meloidogyne* in the Soil Fertility Farm type is not alarming, further monitoring of this genus is recommended due to its high damage potential on crops that are cultivated in this system (Hallmann and Kiewnick, 2015).

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### 4.3 Green waste composts and clover grass composts prevent build-up of *Pratylenchus* populations in the Soil Fertility Farm type

The organic fertilizer produced from lucerne and clover grass leys had only minor and highly variable effects on nematode trophic groups. Most evident were the positive effects of fertilizer on bacterivorous (exception: the Mixed Farm type) and herbivorous nematodes after termination of the leys in 2024. In addition, the application of biogas slurry in the Cash Crop treatment had a continuously positive effect on the numbers of bacterivorous and also predacious nematodes. Biogas slurry has a low carbon:nitrogen ratio which fosters bacterial breakdown of soil inherent organic matter, also known as priming effect (Kumar et al., 2022). Of the bacterial blooming generally follows an increase of bacterivorous nematodes, which may explain the high amplitude of bacterivorous nematodes in 2022. Similarly, predacious nematodes prey on bacteria during their juvenile development, and therefore likely benefited from higher bacterial densities. For 2024, all farm types except the Mixed Farm type had higher numbers of bacterivorous nematodes after fertilization compared to the control. The larger clover and lucerne grass biomass production in the fertilizer treatments (+4 % to +26 %) and the respective larger amount of food for microbiota after termination of the ley in fall 2023 (indirect effect) compared with the control might explain this.

Herbivorous nematodes showed a variable response towards organic fertilizer between years. The Cash Crop and the Mixed Farm types had a lower number of herbivorous nematodes after fertilization in the first year of the clover grass ley (Mixed Farm type: 2022; Cash Crop Farm type: 2023) compared to the control without fertilizer. However, 6 months after termination of the ley in 2024, the opposite effect occurred. As mentioned above, all fertilizer treatments increased the aboveground biomass production of clover or lucerne grass and potentially also the belowground root formation. Hence, an extended root system could harbor more endo-parasitic nematodes of the dominant genus *Pratylenchus* as demonstrated by the positive correlation of grass clover biomass and *Pratylenchus* population (Fig. 4) as well as by the increased number of *Pratylenchus* in the fertilization treatment compared to the control in 2024 (Fig. 2 E). If so, these nematodes were removed from the mineral soil fraction during the ley period (2023) and could not be detected with the Oostenbrink elutriator extraction procedure used in this study (Hallmann and Subbotin, 2018).

Interestingly, fertilization with green waste and clover grass composts did not increase the number *Pratylenchus* in the Soil Fertility Farm type, which was in strong contrast to all other farm types and their respective fertilizer systems. However, this potential suppressive effect on *Pratylenchus* could not be attributed to increased trophic links in the nematode food web (increased number of omnivorous and predacious nematodes) which was in contrast to our last hypothesis. According to Neher and Hoitink (2022), there are manifold modes of action that are responsible for pest and disease suppression by composts also against endo-parasitic nematodes. These range from induced resistance over inoculation of microbial antagonists towards chemical and physical changes in the soil matrix. Overall, the combination of crop rotation and fertilizer in the Soil Fertility Farm type appeared promising for the regulation of most PPN in our study.

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In contrast to *Pratylenchus,* the number of nematodes belonging to the family Tylenchidae was continuously lower in the fertilizer treatments in the Cash Crop, Mixed, and Vegan Farm types when compared to the control. Interpreting this fact appeared difficult due to the unclear feeding preference of this highly diverse family with ecto-parasitic feeding on root hairs or fungi. We can only speculate whether byproducts of the compost, i.e. substances released during the decaying process, introduced rhizosphere competent microorganisms, nutrients, etc., had a negative impact on this group of nematodes or, vice versa, a positive impact on plant resistance (Neher et al., 2022).

### 4.4 Unknown significance of *Pratylenchus* spp. for subsequent crops

The fact that both diagnostic approaches failed to describe the species of *Pratylenchus* correctly in the first approach indicate that the present taxa is rather uncommon. The second molecular approach aiming for the small subunit (SSU) of the 18S mitochondrial region gave more evidence that species in question is closely related to *P. pratensis.* Although the SSU lacks some genetic resolution due to the variability of individual nematodes in this region (Bogale et al., 2020), the simultaneous morphological analysis of this nematode supported this outcome. This species has been rarely found in agricultural fields and studies reporting this species were published long ago (Oostenbrink, 1956; Sturhan, 1984). Although both authors agree that *P. pratensis* predominantly occurs on grasses, they disagree regarding the economic relevance for arable systems. While Sturhan (1984) describes this species from wet and salty grasslands and thus being irrelevant for arable systems, Oostenbrink (1956) could show that *P. pratensis* can multiply rapidly on oats and rye while it was also capable to penetrate potato roots. However, in his study, Oostenbrink found declining populations of *P. pratensis* after potato cultivation. Potatoes, which are following on the clover grass and lucerne leys in our long-term experiment, should be therefore monitored carefully as extreme damage on crops may occur despite the nematode's incapability to multiply on the host. Such a connection exists for example for *P. penetrans* and onion (Pang et al., 2009).

## 5 Outlook

Our study clearly highlighted the usefulness of long-term experiments with focus on crop rotations and fertilizer sources to study soil nematode dynamics. We identified legume grass leys as important sources for fungivorous, predacious and herbivorous nematode enrichment in soil. On the one hand, these trophic groups will serve as prey for the next higher levels in the trophic cascade of soil organisms, such as mites, spring tails, beetles, and spiders and therefore, contribute to an overall greater soil biodiversity. On the other hand, the enrichment of herbivorous nematodes and especially the genus *Pratylenchus* in legume grass leys can negatively affect agricultural production. Although the species complex of *Pratylenchus* did not affect clover grass or lucerne grass yields in the respective farm types and in one farm type even a positive correlation between ley biomass and the number of *Pratylenchus* occurred, there is no all-clear signal for succeeding crops. It rather suggests that the *Pratylenchus* population was still far below the damage threshold level. In general, the damage threshold level for any PPN species depends on environmental and laboratory factors. For example, the PPN damage threshold level is higher on loamy

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than on sandy soils while the extraction efficiency that depends on the individual methods used in a given nematode diagnostic laboratory can vary greatly. This is why damage threshold levels often comprise a wide range of nematode densities per soil volume or weight (Hallmann and Kiewnick, 2015). In the course of the further crop rotations applied to the four farm types, potatoes, carrots, winter wheat and pea/ faba bean will follow in the next three years. Except for potatoes (Oostenbrink, 1956), none of these crops has been tested for their hosting ability for *P. pratensis*. This requires further nematode monitoring to study the susceptibility of the subsequent crops for this PPN and its population development over time. Getting a clean culture of this *P. pratensis* population is highly recommended to conduct additional host range tests in order to highlight the significance of this pest for agricultural crops and to develop applicable rotation schemes with non- or weak hosts aiming for a sustainable suppression of this species.

## 6 Conflict of Interest

*The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.*

## 7 Author Contributions

Conceptualization, CB, JHS; methodology, JHS, SK; investigation, JHS, SK, MM; formal analysis, JHS; writing—original draft, JHS, MA; writing—review and editing, all authors; funding acquisition, CB; resources, JHS, MA. All authors have read and agreed to the published version of the manuscript.

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## 9 Data Availability Statement

Upon acceptance, the datasets for this study will be uploaded into the Open Agrar repository [https://www.openagrar.de].

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