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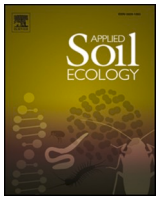

Research paper

More than a decade of irrigation alters soil nematode communities in a drought-prone Scots pine forest

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ABSTRACT

With their various feeding types, soil nematodes play a crucial role in the soil food web. Here, we investigated if and how soil nematodes responded to a long-term irrigation in a drought-prone Scots pine (*Pinus sylvestris*) forest in southern Switzerland, applied to study whether greater soil water availability would improve tree health and reduce tree mortality. After 14 years of irrigation, tree vitality and soil development had improved significantly. However, morphological observations of the soil nematodes revealed a decrease in their total number in the irrigated plots. Overall, the irrigated plots had a lower nematode richness compared with the dry control plots, but the Shannon index did not differ between the two treatments. In addition, the nematode community shifted significantly as a result of the irrigation. Soil physical parameters, such as sand and silt contents and bulk density, were significantly positively correlated with the nematode community in the irrigation treatment. According to a DNA marker sequence analysis, a total of 43 genera of nematodes were assigned. Predatory nematodes were significantly less abundant in the irrigated plots than in the dry control plots, as the average number decreased to 74 in the irrigated plots compared to 3579 in the dry control plots, while non-predators were not significantly affected. A differential abundance analysis revealed that the genera *Tripyla* and *Anatonchus* were the predators that declined the most. Overall, marker sequence analysis of forest soil nematodes appears to be a suitable tool for assessing changes in nematode communities and taxa. The disappearance of predatory nematodes under irrigation, however, can perhaps only be explained if other predatory animal groups, such as predatory mites or millipedes, are also analyzed at the same time.

1. Introduction

Nematodes are the most abundant animals on Earth, constituting a critical component within the soil microfauna (van den Hoogen et al., 2019). Nematodes are active at various trophic levels in the soil food web; they feed on plants as primary consumers (herbivores), on microorganisms as secondary consumers (bacterivores, fungivores), and on microfauna as tertiary consumers (predators, omnivores) (Briones, 2014). Some nematodes live inside other organisms, for example in plant roots, and can cause damage as parasites (Briones, 2014). Nematodes also play a crucial role in the mineralization of organic matter, especially in the carbon (C) and nitrogen (N) cycles, as they feed on various food sources and release nutrients through their excrements, and they leave behind a N-rich body when they die (Osler and Sommerkorn, 2007). The longevity of nematodes is estimated at three days to several years (Gems, 2000). In recent years, attention has also been paid to the composition of nematodes in the soil, as they provide information on soil complexity, nutrient flows and decomposition pathways, which in turn reflect soil quality (Bongiorno et al., 2019).

As soil nematodes are semiaquatic animals, they are highly dependent on water films and water-filled soil pores for their movement (Neher, 2010; Bristol et al., 2023). It is therefore assumed that nematodes respond to changes in precipitation and soil moisture conditions (Blankinship et al., 2011; van den Hoogen et al., 2019), e.g. with changes in their movements in the soil matrix and thus their access to food sources (Martin et al., 2024). Changes in soil water conditions are likely to affect their activity in the short term and their abundance and community composition in the long term (Bristol et al., 2023).

The Pfynwald forest is a Scots pine (*Pinus sylvestris* L.) forest in the southern part of Switzerland (canton of Valais). Since the end of the last

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century, water-limited forests in this transition zone between continental and Mediterranean climates in the Swiss Rhône valley, as well as in other valleys in the Central Alps in Italy and Austria, have been suffering from increasing Scots pine mortality, due to prolonged drought periods and elevated temperatures (Rigling et al., 2013; Bose et al., 2022; Hunziker et al., 2022, 2024). It was therefore hypothesized that reducing stress from water limitation would improve tree vitality and reduce mortality. Thus, about 20 years ago, an irrigation system was installed that doubled the natural rainfall in this Pfynwald forest. Rapid positive responses to increased water availability were recorded for Scots pine needle length and tree ring with (Bose et al., 2022). Belowground responses such as fine root biomass were slow, however, and a significant increase was observed only after about one decade (Brunner et al., 2009, 2019). The microbial communities in the soil and in the root litter responded with a shift from an oligotrophic to a more copiotrophic character (Hartmann et al., 2017; Herzog et al., 2019). Along with the long-term mitigation of drought, the functional potential and life strategies of the soil microbiome, which is involved in the decomposition of organic matter, changed with irrigation (Hartmann et al., 2023). The soil fauna in particular was found to be involved in the vertical redistribution of soil organic C (Guidi et al., 2022), as earthworms were mainly observed in the irrigated plots of the Scots pine forest. Mesofaunal community compositions, in particular mites, springtails and centipedes, shifted as well, with a greater presence of drought-sensitive species in irrigated soils. Overall, Guidi et al. (2022) concluded that the soil fauna is highly sensitive to drought and that drought can result in a reduced C transfer from organic layers to the mineral soil.

The Pfynwald forest site was one of a total of 29 study sites in a European latitudinal gradient from Norway to Portugal, where the diversity, community structure and abundance of soil nematodes in woody vegetation were analyzed (Donhauser et al., 2023). In the present study we aimed to shed light on the long-term effects of the irrigation treatment on the soil nematode communities in the drought-prone Pfynwald forest site through an analysis using the soil nematode isolation method. We specifically aimed to: (a) document changes in alpha- and beta-diversity caused by the irrigation treatment, (b) determine taxonomic groups that benefit or suffer from irrigation, and (c) identify indicator taxa.

2. Materials and methods

2.1. Study site and experimental setup

The study site is located in a drought-proned forest in the Rhone Valley (Pfynwald, Valais, Switzerland, 46°18′N, 7°37′E, 615 m a.s.l.). The forest stand is almost a pure Scots pine forest that is about 120 years old, with a few occasional interspersed pubescent oaks (*Quercus pubescens* Willd.). The density of the stand is about 730 stems ha^{-1} (Brunner et al., 2009). The climate of the study area is moderately continental, with a mean annual temperature of 10.3 °C and mean annual precipitation of 594 mm (from 1981 to 2016; Herzog et al., 2019). The terrain is flat and the soil consists of a shallow Pararendzina with an approximately 20 cm thick topsoil developed on an alluvial fan, with a high contents of skeletal material below 20 cm depth (Herzog et al., 2019).

For the long-term irrigation treatment, which started in 2003, a 1.23 ha area along a water channel was subdivided into eight plots of 1000 m^2 (25 m × 40 m) each, aligned side by side along the channel, with 5 m buffer areas around each plot. Water is taken from the water channel, which is fed by the Rhone River, and spread with sprinklers at night during the vegetation period (May to October). Four plots are irrigated (hereafter referred to as "irrigated"), and four plots are left untreated as control plots (hereafter referred to as "dry"). The amount of water added was about doubled from around 600 to 1200 mm $year^{-1}$. The volumetric soil water content was continuously monitored by time domain reflectometry at a soil depth of 10 cm, and irrigation resulted in a significantly increase from around 28 % in the dry plots to about 34 % in the irrigated plots (Herzog et al., 2014).

2.2. Soil and nematode sampling

Soil and nematode sampling were carried out in June 2017 according to the standardized protocol of Stone et al. (2016). In each of the eight plots, one square area of 1 m^2 was selected. In order to minimize heterogeneity among the plots, the squares were chosen at sites without understory vegetation. Before soil cores were taken, the litter of the square area was removed. In total, five soil cores (5 cm diameter × 10 cm height) were taken in each square, with three cores for soil physico-chemical parameters and two cores for nematode isolation. The use of separate soil cores for different analyses that require different processing is a common approach (George et al., 2017, 2019). The cores were then placed in cool boxes, transported to the lab, and stored at 4 °C for further processing. The three cores for soil properties were pooled per plot and analyzed for soil physico-chemical parameters, as described in Donhauser et al. (2023). Briefly, immediately upon arrival in the laboratory, the three cores for soil physico-chemical analyses were homogenized, sieved (mesh size 4 mm), and dried for 48 h at 60 °C. Soil pH was measured potentiometrically in 0.01 M $CaCl_2$ (soil:solution ratio = 1:2; 30 min equilibration time). Total C and N concentrations were analyzed by dry combustion for fine-ground samples, using a CN analyzer NC 2500 (CE Instruments, Wigan, United Kingdom). In order to quantify the organic C in the samples, inorganic C in the samples was removed with acid vapor when soils had a pH >6.5 (Frey et al., 2021). Bulk density was calculated by dividing the weight of the dried fine fraction (< 2 mm) by the volume of the full soil sample. Soil particles were fractionated into sand, silt and clay using the pipette method (Gee and Bauder, 1986).

2.3. Nematode isolation and morphological classification

The two soil cores per plot for the nematode isolation were treated using the Oostenbrink dish method, proposed by the European and Mediterranean Plant Protection Organization (EPPO) for isolating nematodes from soils prior to DNA metabarcoding for community analysis (Oostenbrink, 1960; EPPO, 2013). For each soil core, the soil was homogenized and then divided into two soil parts (approximately 200 g of soil per part). Each soil part was placed on a double cotton milk filter (FT25 Sana Vliesstoff-Filter, Zeltner Systemtechnik AG, Fulenbach, Switzerland) on a sieve in a dish containing regular tap water (see Donhauser et al., 2023). The nematodes were then allowed to migrate through the filter into the water at 23 °C. After 72 h, the water in the dishes containing the nematodes was collected, the suspensions were passed over a 20-μm sieve to reduce the volume of water and transferred to centrifugation tubes, and then water was added to make 10 mL suspensions. Three 1 mL subsamples from each 10 mL suspension were visually inspected under a dissecting microscope at 40× magnification; the nematodes in each subsample were counted and their mouthparts were morphologically classified according to Yeates et al. (1993), Coleman et al. (2024) and https://soil.evs.buffalo.edu/index.php/Nematodes into bacteria feeders ("bacterivores"), fungus feeders ("fungivores"), plant feeders ("herbivores"), and "predators-omnivores". After the nematodes were counted and classified, the subsamples were returned to the 10 ml suspensions. Counts of all nematode taxa were extrapolated to the entire sample (two soil cores per plot, two parts per soil core) and expressed per 100 g dry soil. The suspensions were then used for DNA extraction.

2.4. DNA extraction of nematodes and amplicon sequencing

After the application of the Oostenbrink dish method, DNA was extracted from the nematodes using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, except for the incubation step, which was run at 56 °C for 4 h (see also Resch et al., 2022). DNA was quantified using a high-sensitivity Qubit

assay (Thermo Fisher Scientific, Reinach, Switzerland) and diluted to 2.69 ng μL^{-1} for polymerase chain reaction (PCRs) (Donhauser et al., 2023). PCR amplification was performed on the V6–V8 region of the eukaryotic small subunit (18S) with the forward primer NF1 (5′-GCCTCCCTCGCGCCATCAGGGTGGTGCATGGCCGTTCTTAGTT-3′) and the reverse primer 18Sr2b (5′-GCCTTGCCAGCCCGCTCAGTACAAAGGGCAGGGACGTAAT-3′; Porazinska et al., 2009). These primers cover soil nematodes well and are widely used, but they are not nematode-specific, which is why they also amplify other eukaryotes. The 5′ ends of the primers were tagged with the CS1 (forward) and CS2 (reverse) adapters required for multiplexing samples using the Fluidigm Access Array System (Fluidigm, South San Francisco, CA, USA). The PCR steps consisted of an initial denaturation at 95 °C for 2 min, 35 cycles of denaturation at 95 °C for 40 s, annealing at 58 °C for 40 s, elongation at 72 °C for 1 min, and a final elongation at 72 °C for 10 min (Resch et al., 2022). Each sample was amplified in triplicate and pooled, then purified with Agencourt AMPure XP beads (Beckman Coulter, Berea, CA, USA) and quantified with the Qubit 2.0 fluorometric system (Life Technologies, Paisley, UK). Amplicon pools were then sent to the Génome Québec Innovation Centre at McGill University (Montréal, Canada) for library preparation, and paired-end sequencing 2 × 250 base pairs (bp) was performed on the Illumina MiSeq v3 platform (Illumina Inc., San Diego, CA, USA).

2.5. Bioinformatic analyses

The demultiplexed raw paired-end *FASTQ* files were imported into the *QIIME2* (Quantitative Insights Into Microbial Ecology 2) software (v.2022.2.0; Bolyen et al., 2019) through *miniconda* (v.4.14.0; Anaconda Software Distribution, 2020; https://www.anaconda.com/). Subsequently, primers and adaptors were removed using the protocol described by Martin (2011) with a minimum length threshold of 250 bp. Sequences were denoised using the Divisive Amplicon Denoising Algorithm 2 (DADA2; Callahan et al., 2016) to determine amplicon sequence variants (ASVs; Callahan et al., 2017). To remove low quality reads, forward reads were truncated at 240 bp and reverse reads at 215 bp, and reads not fulfilling quality criteria were discarded (maxN = 0, maxEE = 2). The taxonomic classification was performed using 18S-NemaBase (https://github.com/WormsEtAl/18SNemaBase; accessed January 24, 2024) with the "classify-sklearn" plugin with default parameters (Gattoni et al., 2023). Genera that could not be classified were labeled as "unclassified". Following classification, singletons were removed from the dataset.

2.6. Statistical analysis

All statistical analyses were conducted in R (v.4.3.3; R Core Team, 2024). Most of the analyses were carried out using the *microeco* package (v.1.5.0; Liu et al., 2021). To calculate alpha-diversity (richness, Shannon index), rarefaction was performed at the lowest sequencing depth of 13,852 reads. Alpha-diversity was calculated using the "trans_alpha" function from the *microeco* package. Meanwhile, differences in beta-diversity, assessed based on Bray-Curtis dissimilarities, were tested using permutational multivariate analysis of variance (PERMANOVA) with a Monte Carlo correction and were represented using principal coordinates analysis (PCoA). Both analyses were conducted with the raw data using PRIMER7 (Clarke and Gorley, 2005). Due to the characteristics of the data, differences in alpha-diversity among groups were assessed using one-way analysis of variance (ANOVA) after confirming that the assumptions of normality and homoscedasticity were met. If significant, this test was followed by pairwise Tukey tests. The remaining tests were conducted using the non-parametrical Kruskal-Wallis test, followed by pairwise comparisons using the Dunn test. Relative abundance was calculated based on the raw data using the "trans_abund" function from the *microeco* package. Differences were considered significant at $P < 0.05$. To assess the effect of soil physico-chemical parameters on the nematode communities, a non-metric multi-dimensional scaling (NMDS) ordination based on Bray–Curtis dissimilarities was performed on Hellinger-transformed raw data using the "metaMDS" and the "envfit" functions from the *vegan* package (v.2.6–4; Oksanen et al., 2022).

Feeding types of the marker sequence abundances were manually assigned from the genera using the Nemaplex (http://nemaplex.ucdavis.edu) database as a reference (Ferris, 2001), utilizing the raw counts (absolute abundances) to evaluate differences between treatments: bacteria feeders ("bacterivore"), fungus feeders ("fungivore"), plant feeders ("herbivore"), and animal feeders ("predator"). Other feeding types, such as animal parasites, marine parasites, or omnivores were not recorded. Unassigned sequences were excluded from this analysis. To evaluate the relationship between feeding types assigned based on morphology and those based on metabarcoding, Pearson correlation analyses were performed. The results were visualized through scatterplots. All the plots were generated using the *ggplot2* (v.3.5.0; Wickham, 2016) and *ggpubr* packages (v.0.6.0; Kassambara, 2023).

To identify indicator taxa, a differential abundance analysis of count data was performed using the *DESeq2* package (v.1.42.1; Love et al., 2014). This analysis was based on raw counts after unreliable assignments had been excluded (i.e. keeping ASVs that appeared in at least three samples per treatment). These low-confidence assignments were removed using the "filter_taxa" function from the *phyloseq* package (v.1.46.0; McMurdie and Holmes, 2013).

3. Results

3.1. Forest site characteristics and nematode abundance

Long-term irrigation increased the pH of the topsoil at the Pfynwald forest site, with values of about 6.6 in irrigated plots and 5.5 in control plots after 14 years of treatment (Table 1). In contrast, organic matter, C and N contents decreased over the years in the irrigated plots due to increased mineralization, with significant differences from the dry controls emerging for the latter two parameters. However, the C:N ratio was not altered by irrigation. Among the physical parameters, the clay content was significantly lower, and the silt content was significantly higher in the irrigated plots (Table 1). Soil moisture was higher in samples from the irrigated plots than in those from the dry control plots, but not significantly so, as this parameter is very much dependent on the weather conditions at the time of soil sampling. As mentioned in Subsection 2.1, however, the mean annual soil water content is significantly higher under the irrigation treatment.

Morphological observations of the isolated nematodes revealed a higher, but not significantly so, total number of nematodes in the dry

Table 1
Effect of irrigation on soil chemical and physical parameters (means±SE; $n = 4$).

Soil parameters	Dry	Irrigated	$F^{\dagger}$	$P^{\dagger}$
Chemical				
pH	5.54 ± 0.34	6.63 ± 0.15	8.46	**0.027**
Carbon (C) (%)	14.7 ± 1.50	9.68 ± 1.09	7.22	**0.036**
Nitrogen (N) (%)	0.54 ± 0.05	0.36 ± 0.04	7.95	**0.030**
C:N ratio (C:N)	27.1 ± 1.53	26.7 ± 1.09	0.03	0.86
Organic matter (OM) (%)	23.2 ± 1.78	18.0 ± 3.09	2.08	0.19
Physical				
Soil moisture (SM) (%)	21.1 ± 0.57	23.5 ± 1.18	3.41	0.11
Bulk density (BD) (g cm^{-3})	0.46 ± 0.02	0.56 ± 0.04	5.58	0.05
Clay (%)	13.2 ± 0.28	11.2 ± 0.26	24.7	**0.002**
Silt (%)	37.4 ± 0.46	39.1 ± 0.42	7.41	**0.034**
Sand (%)	49.4 ± 0.22	49.6 ± 0.19	0.60	0.47

† Means were tested with one-way analysis of variance (ANOVA) and are displayed with the F-ratio (F) and the level of significance (P). Significant values ($P < 0.05$) are given in bold.

plots, with about 3300 individuals per 100 g dry soil, compared with about 2800 individuals in the irrigated plots (Table 2). When the feeding types were assessed based on morphological assignment, bacterial feeders emerged as the most abundant feeding type, followed by fungivores and herbivores. The predator-omnivore feeding type was least abundant. Neither the abundances of individual feeding types nor the abundances of all feeding types together were significantly influenced by the irrigation treatment (Table 2).

3.2. Diversity, taxonomic classification and indicator taxa

Regarding nematode alpha-diversity, the marker sequence data revealed a significantly lower richness in the irrigated plots ($F = 6.26$; $P = 0.046$), with a mean of 61.8 ± 9.93 (mean ± S.E.) compared with 89.3 ± 8.44 in the dry control plots (Fig. 1A). However, the Shannon index did not differ significantly between the two treatments, with values of 2.48 ± 0.39 in the irrigated plots and 2.77 ± 0.50 in the dry control plots (Fig. 1B). For nematode beta-diversity, the PERMANOVA indicated a significant difference (*pseudoF* = 3.11; $P = 0.028$) in nematode community structure as result of the irrigation treatment, showing that the two nematode communities differed from each other (Fig. 1C).

Effects NMDS plot generated to assess the effects of soil parameters on the nematode communities pointed to a clear separation of the dry control and the irrigated plot (Fig. 2). All of the considered soil physical parameters were significantly linked to the community change: a high clay content was significantly associated with the dry control plots, whereas high sand and silt contents and high bulk density were significantly associated with the irrigation treatment (Fig. 2). None of the considered soil chemical parameters were significantly linked to the treatments (see also Supplementary Table S1).

The taxonomic classification grouped the nematodes into the two classes Enoplea and Chromadorea (Fig. 3A), with a total number of twelve orders (Fig. 3B). In particular, the irrigation treatment led to a strong increase in Plectida and a strong decrease in Mononchida and Dorylaimida. In total, 43 genera could be assigned from the marker sequences (Supplementary Table S2). The dominant genera were *Rhabditis* and *Miculenchus*, followed by *Prismatolaimus*, *Tripyla, Anatonchus, Filenchus,* and *Coomansus* (Fig. 3C). However, between 37 % and 53 % remained unclassified. The irrigation treatment led to strong a decrease in *Rhabditis, Tripyla, Anatonchus, Filenchus,* and *Coomansus,* whereas *Miculenchus* and *Prismatolaimus,* increased considerably (Fig. 3C).

The differential abundance analysis highlighted several taxa as significant indicators (Fig. 4). Among them, *Miculenchus* and *Anaplectus*, both had $\log_2$-fold change values clearly higher than +4 (indicator taxa for the irrigation treatment), while *Tripyla*, *Anatonchus*, *Filenchus*, and *Metateratocephalus* had values clearly lower than −4 (indicator taxa for the dry control; Fig. 4).

Table 2
Effect of irrigation on the abundances of nematode feeding types assessed by morphological assignment (number of individuals per 100 g dry soil) and marker sequence abundances of genera according to the Nemaplex database (mean ± SE; n = 4).

Feeding types	Dry	Irrigated	Chi^2†	P†
Morphologically assigned abundance				
Bacterivores	1950 ± 829	1238 ± 224	0.00	1.00
Fungivores	595 ± 180	600 ± 50	1.33	0.25
Herbivores	444 ± 230	620 ± 226	1.33	0.29
Predators-omnivores	357 ± 137	348 ± 76	0.02	0.89
All feeding types	3346 ± 1301	2807 ± 468	0.08	0.77
Marker sequence abundance				
Bacterivores	8501 ± 3530	5892 ± 3822	2.08	0.15
Fungivores	1428 ± 607	386 ± 113	1.33	0.26
Herbivores	585 ± 193	1952 ± 919	0.75	0.39
Predators	3579 ± 1083	74 ± 49	5.33	**0.018**
All feeding types	14,092 ± 2052	8303 ± 3371	2.10	0.15

† Means were tested with the non-parametric Kruskal-Wallis one-way analyses of variance and are displayed with the corresponding Chi-squared value (Chi^2) and the level of significance (P). Significant values ($P < 0.05$) are given in bold.

3.3. Feeding types

Marker sequence abundances assigned to feeding types showed, in contrast to the morphological assignment, a significant decrease in predators with irrigation ($F = 5.33$, $P = 0.018$), with abundances almost 50 times lower than in control plots (Table 2). However, all other feeding types, as well as all feeding types assessed together, were not significantly influenced by the irrigation treatment (Table 2). A correlation analysis of the feeding types between morphological and metabarcoding assignment did not show any significant relationships, except for all feeding types together ($R = 0.96$; $P = 0.038$) in the irrigation treatment (Supplementary Fig. S1).

The analysis of individual genera showed that several predators were significantly less abundant under the irrigation treatment than in the dry control plots, among them *Anatonchus, Clarkus, Pristionchus,* and *Tripyla* (Supplementary Table S2). Significant decreases with irrigation also emerged for the bacterivores *Metateratocephalus* and *Rhabditophanes,* the fungivore *Filenchus,* and the herbivore *Malenchus.* The only increase due to the irrigation treatment was observed for the bacterivore *Anaplectus* and the herbivore *Ditylenchus* (Supplementary Table S2).

4. Discussion

4.1. Responses of nematode abundance, diversity and community structure to irrigation

An average decrease of number of nematodes in our long-term irrigation was found in the irrigated plots compared with dry control plots. Reports on how soil nematodes respond to increased water availability are mixed. This controversy is probably due to the different durations of irrigation, ecosystem types and soil conditions (Bristol et al., 2023). Some authors have reported an increase in nematode abundance with increased water availability (Blankinship et al., 2011; Goncharov et al., 2023), while others have observed limited effects or even a decline (Sylvain et al., 2014; Vandegehuchte et al., 2015; Ankrom et al., 2020; Peng et al., 2022). It should be noted that most studies dealing with water conditions have been carried out in non-forested areas, such as deserts, grasslands or heathlands.

A recent meta-analysis on the response of soil nematodes to altered precipitation regimes covered studies from various ecosystems (Bristol et al., 2023). In that study, the authors analyzed data from 46 independent observations from 37 field studies, seven of which were from temperate and boreal forests, and one from a subtropical forest. However, the durations of the irrigation treatment in forests were between one month and eight years. Only two studies, Sohlenius and Wasilewska (1984) and Liu et al. (2020), where irrigation treatments were implemented for more than three years, were considered to be suitable for comparison with the Pfynwald Scots pine forest, where irrigation has been applied for more than a decade. Sohlenius and Wasilewska (1984), who irrigated and/or fertilized a pine forest on podzolic soil in central Sweden for eight years, classified the nematodes morphologically and found that nematode abundance did not vary strongly with irrigation. Liu et al. (2020) performed a study with water and N addition in a *Quercus-Liquidambar* forest in China over three years and found that water addition significantly reduced nematode richness, but not the Shannon index, similar to our observations. In particular, the authors observed a suppression of fungivores and predators due to the irrigation. The authors concluded, that the fungivores were possibly negatively affected by a reduced fungal biomass under irrigation, as observed in another study in the same region (Shi et al., 2018).

A metabarcoding survey of nematodes from 787 sites in different

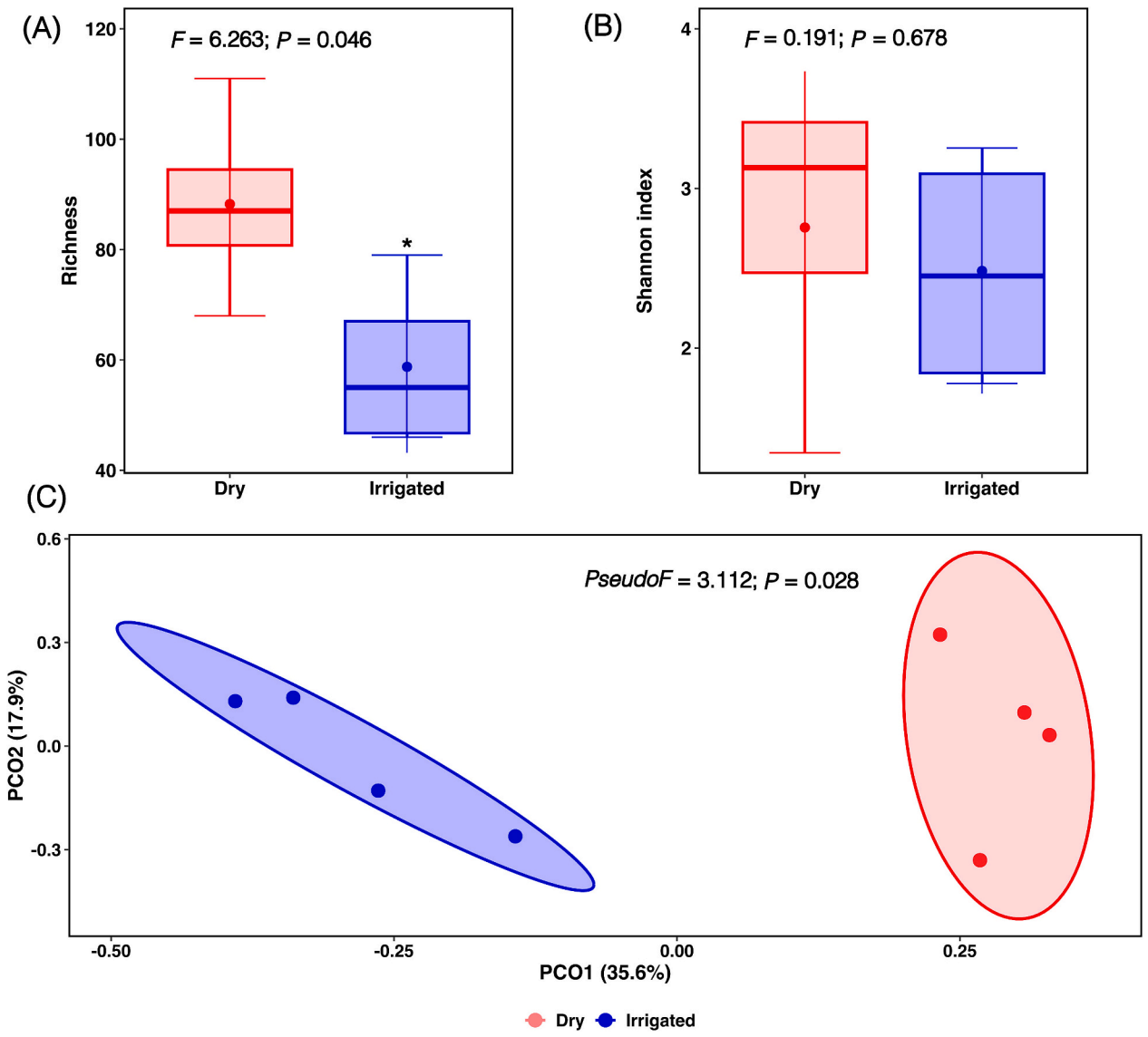

Fig. 1. Effects of the irrigation treatment on alpha-diversity; (A) richness and (B) Shannon index, assessed by one-way analysis of variance (ANOVA); and (C) effects on beta-diversity based on Bray-Curtis dissimilarities, represented with a principal coordinate analysis (PCoA) with 95 % confidence intervals and assessed by multivariate permutational analysis of variance (PERMANOVA) with a Monte Carlo correction.

European vegetation types, in which a fragment of the 18S rDNA gene obtained by eukaryote-specific primers was sequenced directly from DNA extracted from the soil, revealed a mean richness of 29 and a Shannon index of 2.1 for coniferous forests and values of 37 and 2.3 for deciduous forests, respectively (Köninger et al., 2023). Our richness (89 in the dry control vs. 62 in the irrigated plots) and Shannon index data (2.8 in dry control vs. 2.5 in the irrigated plots) are both above these values, indicating an intact soil ecosystem status. A similarly high richness in forests as in our study, namely about 90, was found by Gong et al. (2023) when they used the same nematode-specific primers of the 18S rDNA gene from Porazinska et al. (2009) as in our study and sequenced DNA directly from forest soils from sites in and around 12 cities in China. Notably, the extraction of DNA directly from the soil has some disadvantages in terms of richness detection compared with DNA extraction directly from nematodes after their isolation from soils using the Oostenbrink dish method (Donhauser et al., 2023). The Oostenbrink dish method primarily selects the active nematode community, as the nematodes must actively migrate through a cotton wool filter, and therefore eggs and nematodes in immobile resting stages are not assessed (Donhauser et al., 2023).

The most important finding in our study was that 14 years of irrigation significantly shifted the nematode community structure of the Scots pine forest. Ewald et al. (2022) similarly observed a shift in the nematode community structure when they irrigated arable land with potatoes. However, we could not find any other metabarcoding studies on the beta-diversity of nematodes under an irrigation treatment. Further, soil parameters were significantly linked to the community changes in our study, high sand and silt contents and high bulk density correlated with irrigation and with high clay content significantly correlated with the dry control. We observed significant decreases in C and N with the irrigation treatment, but these variables were not significantly linked to community shifts. Köninger et al. (2023) observed that ecosystem parameters significantly affected the beta-diversity of nematodes. In particular, aridity was the best predictor of nematode diversity. However, among the variables describing soil parameters, the soil C:N ratio was more important than soil physical parameters, such as sand content or bulk density. Considering beta-diversity, Gong et al. (2023) observed a wide overlap of the nematode community composition among land-use types such as forest, farmland, parks, and residential areas. In forests, the main environmental drivers were annual

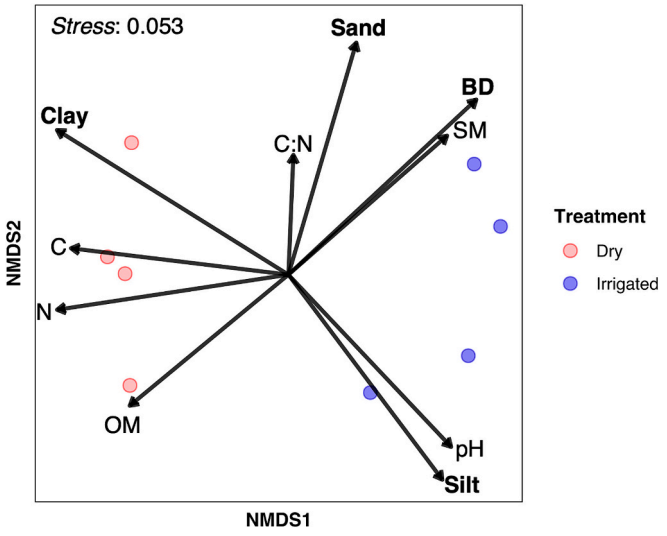

Fig. 2. Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarities for the nematode communities in the dry control and irrigated plots. Soil parameters are projected as arrow vectors onto the NMDS ordinations. Vector arrowheads show the direction of the effects of the soil parameters, and vector lengths reflect the strengths of the correlations of the variables with the NMDS axes. Soil parameters in bold letters differ significantly ($P < 0.05$; see also Supplementary Table S1). BD: soil bulk density, C:N: soil carbon to nitrogen ratio, OM: soil organic matter, SM: soil moisture.

precipitation and soil total N. Soil moisture was the only soil physical parameter considered but was not a key factor.

4.2. Nematode taxa and feeding types that benefit from or suffer under irrigation

Miculenchus, an herbivorous genus, might have profited from an increased availability of plant roots under irrigation. Over more than a decade of irrigation, the biomass of the fine roots of Scots pine has increased significantly (Brunner et al., 2019), as has the total cover of understory plants (Herzog et al., 2014). Members of the Tylenchidae family, in the order Rhabditida to which *Miculenchus* belongs, are primarily feeders of epidermal cells and root hairs (Cesarz et al., 2015). Moreover, *Miculenchus* abundance seems to be positively correlated with the soil water content, as observed by Li et al. (2024).

Filenchus is a genus that decreased strongly with water addition in our experiment. In the Nemaplex database, this species is considered a fungivore, although the database indicates algae, mosses, lichens and plant roots as food sources. Cesarz et al. (2015), considered *Filenchus* a plant feeder, similarly to *Miculenchus* within the Tylenchidae family. But some species clearly feed on fungal sources as demonstrated by Okada et al. (2005). We detected *Filenchus discepans* in our study, one of the *Filenchus* species found to feed on fungi by Okada et al. (2005). Interestingly, Liu et al. (2020) mentioned that irrigation can lead to a decrease in fungal biomass, which then would result in a decrease in fungivores. However, we were unable to confirm this result in our study.

Two predator genera that decreased strongly with irrigation in our study were *Tripyla* and *Anatonchus*. *Tripyla* belongs to the Tripylidae family of the Enoplida order. According to the Nemaplex database, they are generalist predators of small organisms, including nematodes. Observations of intestinal contents suggest that all genera of this family generally consume protozoa, small nematodes, tardigrades and rotifers (Asghari et al., 2017). *Anatonchus* belongs to the Mononchida order, and they feed by cutting, sucking and engulfing an intact prey (Bilgram, 2008). Explaining why these two genera have disappeared due to irrigation would be pure speculation. Too little is known about their ecology and their behavior in dry and moist conditions.

Similar to in our study, Sohlenius and Wasilewska (1984) observed that bacterivores were the most abundant and predators the least abundant feeders in a Swedish Scots pine forest. Interestingly, these authors observed a decrease in bacterivores and herbivores in the upper humus horizon under irrigation, but an increase in the deeper mineral horizons. This was most likely related to a shift in soil parameters due to allocation and leaching processes, as we have observed in our Scots pine forest. Overall, these authors reported a decrease in predators under irrigation. This is in accordance with our metabarcoding results but not with our finding based on the morphological assessment. By contrast, strong increases in the abundances of the non-predators *Rhabditis*, *Tylencholaimus* and *Prismatolaimus* with irrigation were reported by Sohlenius and Wasilewska (1984). Except for *Rhabditis*, where we observed a decrease, positive trends with irrigation also emerged for these genera in our experiment. Decreasing genera as listed in Sohlenius and Wasilewska (1984), such as the non-predators *Wilsonema*, *Rhabdolaimus* and *Diphterophora,* were not detected in our experiment. Sohlenius and Wasilewska (1984) concluded that bacterial feeders were favored under irrigation, but mainly in the treatment was combined with fertilization. Similarly, Ferris (2001) observed a positive correlation between bacterial decomposition rate and soil water content.

We did not find significant effects of the irrigation treatment on the abundances of the different feeding types, except for a decrease in predators according to the metabarcoding assessment. Despite the fact that root biomass increased significantly over a decade of irrigation in our Scots pine forest, total bacterial and fungal biomass was not affected (Hartmann et al., 2017). This could explain why bacterivore and fungivore nematode abundances did not change in our experiment. We only observed a strong increase in the abundance of the root feeder *Miculenchus*. It could be that competition among herbivores favors this genus or that predators omit it or prefer the bacterial feeders. The strong decrease in predators with irrigation could be a consequence of the food web, in that predators and omnivores have a relatively large body size compared with fungivores and bacterivores (Sechi et al., 2018) and thus are easier for nematode-feeding predatory mites or centipedes to catch. Guidi et al. (2022), who studied several soil animal groups in the same experimental Scots pine forest, observed an increase in mites and centipedes in the irrigated plots, although no distinction was made between predatory and litter-feeding mites.

4.3. Use of high-throughput sequencing for soil nematode assessment

DNA metabarcoding provides information on entire nematode communities, which can potentially be used to compute nematode-based indices (Du Preez et al., 2022). However, caution is required here, as different applications may result in different values of the indices, and that these indices may not be suitable for calculation from DNA data (Griffiths et al., 2018). Therefore, in the present study, it was decided not to use nematode-based indices based on marker gene data. Information on soil nematode assemblages generated in this way may differ from that obtained via conventional morphotyping, as shown by Kitagami et al. (2022) in a study on Mount Ibuki in Japan, although these authors did find that the nematode community responses to altitudinal gradients were comparable with the two approaches. In a subsequent study, Kitagami and Matsuda (2022) concluded that high-throughput sequencing covers a greater nematode diversity than conventional morphotyping and is useful for forming a comprehensive overview of the nematode communities, also because amplicon sequencing analysis is an effective tool for rapid and objective nematode identification. In addition, new sequencing tools, such as PacBio, Oxford Nanopore and LoopSeq, could enable the sequencing of long reads and thus allow a taxonomic resolution at the nematode species level (Mulder and Vonk, 2011; Du Preez et al., 2022).

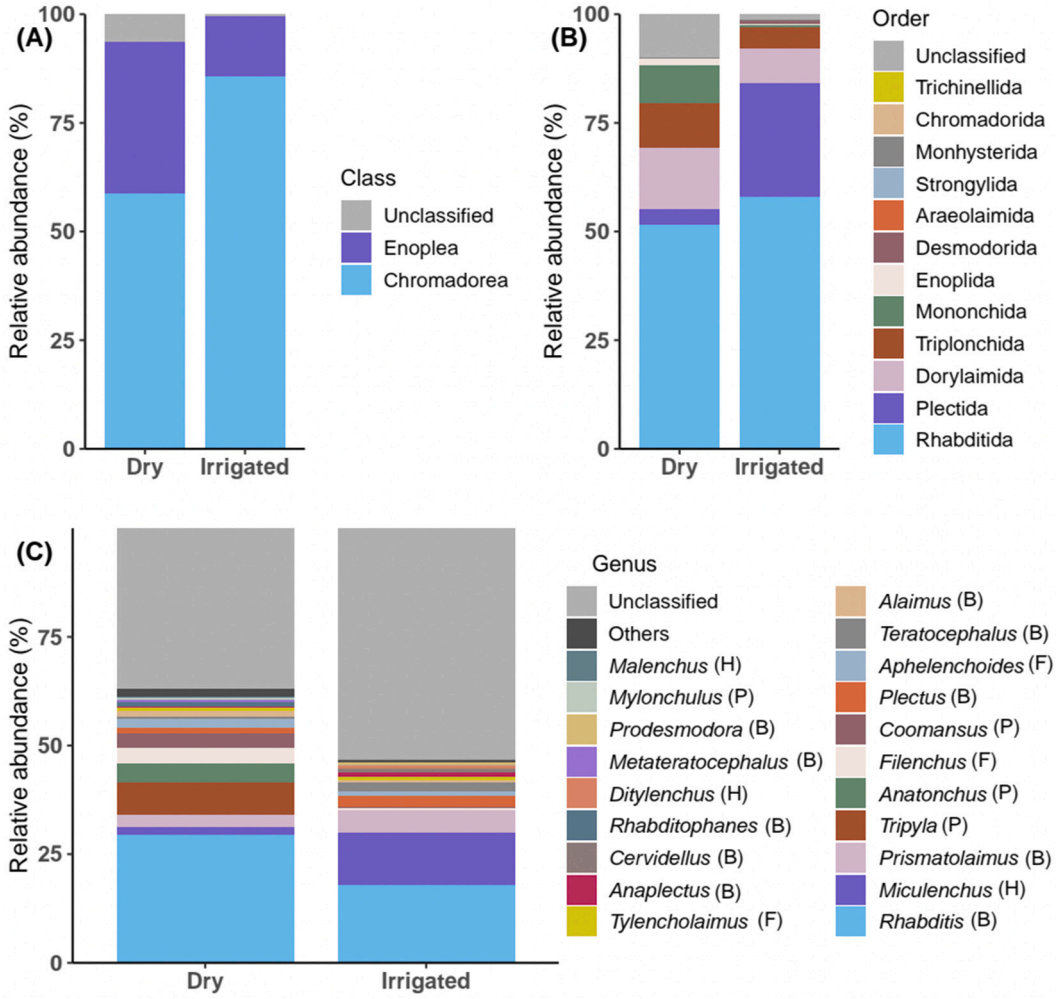

Fig. 3. Relative abundances of (A) classes, (B) orders and (C) the 20 most abundant genera in the dry control and irrigated plots, based on the taxonomical assignment of amplicons. Letters in brackets represent the feeding types according to the Nemaplex database: B: bacterivores, F: fungivores, H: herbivores, and P: predators.

5. Conclusions

In our experimental drought-prone Scots pine forest, nematode richness decreased with irrigation, whereas the Shannon index did not change. The decrease in richness was mainly due to a strong reduction in predators. This result is difficult to understand, as the potential prey, i.e. non-predatory nematodes, did not differ significantly. The main predators that declined were *Tripyla* and *Anatonchus*, both of which feed on nematodes, but most likely also on other microfauna, such as protozoa, small nematodes, tardigrades and rotifers. Their decline therefore can hardly be a consequence of a food shortage, but rather may be related to an increase with other predators within the mesofauna, e.g. predatory mites, which feed on insect larvae and on larger nematodes. We therefore suggest that not only nematodes but also other soil animal groups within the food web should be investigated to obtain a more complete picture of the changes in forest soil biodiversity, especially when irrigation treatments are investigated.

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CRediT authorship contribution statement

Jessica Cuartero: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Beat Frey:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Reinhard Eder:** Writing – review & editing, Validation, Methodology. **Ivano Brunner:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Investigation, Formal analysis, Conceptualization.

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

The sequence data have been submitted to the National Center for Biotechnology Information (NCBI) BioProject databank under the

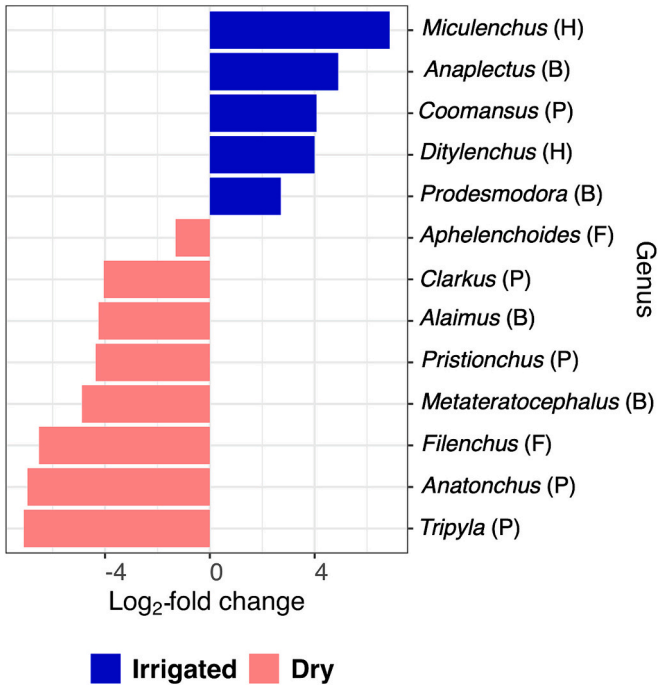

Fig. 4. Differentially abundant nematode genera in the dry control vs. irrigated treatment according to the *Deseq2* analysis. Only genera with significant log_2-fold changes are shown ($P < 0.05$). Letters in brackets represent the feeding types according to the Nemaplex database: B: bacterivores, F: fungivores, H: herbivores, and P: predators.

accession identifier PRJNA1089647 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1089647).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105621.

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