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Soil biota response to raised water levels and reduced nutrient inputs in agricultural peat meadows

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ABSTRACT

In the Netherlands, peatlands are drained for agricultural purposes, resulting in CO_2 emissions, soil subsidence and biodiversity loss. Raising groundwater levels and reducing nutrient inputs are potential solutions, but their effects on soil biota in agricultural peat soils remain unclear. Therefore, we conducted a mesocosm experiment in which we exposed 40 intact fen meadow peat cores (80 cm, 20 cm in diameter) to four different water levels (0, 20, 40 and 60 cm below peat surface) and two nutrient application levels (50 and 250 kg N ha^{-1} year^{-1} with a N:P_2O_5:K_2O of 16:5:12), mimicking various rewetting degrees and land use options. After 15 months, we determined the bacterial, fungal and protozoan PLFA, and abundance and community composition of nematodes and earthworms. Our results show a significantly higher bacterial and saprophytic fungal PLFA abundance at high water levels (20 and 0 cm respectively) compared to the lower water levels, whereas nematodes and earthworms were significantly more abundant under lower water levels (60 and 40 cm respectively). Overall, water level influenced soil biota more strongly than nutrient levels, although nutrient effects became more prominent with increasing water levels. In the fully water saturated treatment with high nutrient application levels, no earthworms and fewer nematode taxa were found than under low nutrient levels. We conclude that wet conditions combined with a high nutrient application negatively affect soil food web stability. Furthermore, raising the water level results in a different soil biota composition, with potential implications for ecosystem functioning.

1. Introduction

Peatlands are valuable ecosystems that hold a high biodiversity and provide ecosystem services including nutrient cycling, carbon sequestration and water storage (Verhoeven and Setter, 2010). However, globally the functioning of these ecosystems and the services they provide are threatened as a consequence of land use change (Joosten and Clarke, 2002). In the Netherlands, most peatlands have been reclaimed for agricultural use, and have been primarily converted to grassland for dairy farming (Van den Born et al., 2016). To make dairy farming on these peatlands feasible, the peatlands are drained, leading to peat oxidation which in turn leads to soil subsidence and significant CO_2 emissions (Fenner and Freeman, 2011). Additionally, these agricultural grasslands receive high nutrient inputs of up to 250–300 kg N ha^{-1}, via slurry manure and synthetic fertilizer, to increase grass production (Kleijn et al., 2001). The combination of drainage and high nutrient input has not only caused soil subsidence and significant CO_2 emissions but has also negatively impacted above-and belowground biodiversity, mostly in a negative way. For example, agricultural intensification is linked to a lower food web complexity as well as a reduced total biomass of the soil biota (Tsiafouli et al., 2015).

Given the problems related to agriculturally used grasslands on peat soils (i.e. CO_2 emissions, soil subsidence and biodiversity loss), there is an increasing societal and political demand for solutions (Van den Born et al., 2016). These are mainly focused on raising the groundwater table and reducing nutrient inputs into the system (Emsens et al., 2020) thereby transitioning to a less intensive agricultural system. This significantly alters the abiotic conditions of the system which in turn can be expected to affect biodiversity in these peatlands. Soil biota play a crucial role in processes supporting peatland ecosystem functioning, such as nutrient cycling, maintenance of soil structure and water regulating capacity (De Vries et al., 2013; Wagg et al., 2014), and are sensitive to changes in groundwater level and nutrient availability (Ausden et al., 2001; Bobuľská et al., 2020; Deru et al., 2023; Van Dijk et al.,

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2009). To understand the response of peat meadow ecosystems to a shift to low-intensity farming with higher groundwater tables, it is therefore pivotal to first understand how changes in water and nutrient level affect soil biota. Especially since agriculture on peat grasslands shapes the belowground biodiversity via these two principal factors, i.e. (i) nutrient application level and (ii) water level.

First, fertilization directly increases resource availability via C and N input and indirectly via increased mineralization rates and grass production (Biederman et al., 2008; De Goede et al., 2003; Onrust and Piersma, 2019). The type of fertilizer that is used plays a major role in this which is for example shown by earthworms responding more positively to the addition of organic than inorganic fertilizers (Deru et al., 2023, 2018; Van Eekeren et al., 2009). Further, although bacteria are generally associated with nutrient-rich environments and fungi with low nutrient conditions (Bittman et al., 2005), within these groups different functional groups and species of bacteria and fungi respond differently to fertilization (Carey et al., 2016; Lori et al., 2023).

Second, groundwater level and its associated effects on soil moisture, oxygen availability and pH, also affect soil biota. Especially earthworms are found to be sensitive to a low pH (Curry, 2004) and optimum soil moisture percentages vary largely per soil biota group. Earlier studies have found a negative effect of high water levels on earthworm (Ausden et al., 2001; Wu et al., 2017; Zorn et al., 2005) and nematode abundance (Bobuľská et al., 2020; Deru et al., 2018; Wei et al., 2018), whereas results on microbial communities are less consistent (Mentzer et al., 2006; Van Dijk et al., 2009; Xu et al., 2023). Moreover, soil biota are not only directly affected by abiotic conditions but the activity of the soil community also feeds back to the abiotic conditions. Hence, raised water levels and reduced nutrient inputs are likely to alter soil biota composition, but to what extent remains unclear.

In Dutch agricultural peat meadows, the soil has been exposed to drainage (low water level) and high nutrient application levels for decades (Van den Born et al., 2016). This implies that current soil biota have adapted to these conditions over time and are probably very different from natural peatlands (Emsens et al., 2020). Moreover, studies addressing the response of soil organisms to the effect of raised groundwater levels and reduced nutrient application levels on agricultural peat grasslands are scarce (but see Van Dijk et al., 2009; Deru et al., 2018). To date, the effects of water and nutrient level have mostly been studied for only one or a few soil biota groups (Bobuľská et al., 2020; Brouns et al., 2016; Mentzer et al., 2006) and/or took into account only the drainage level (Emsens et al., 2020; Wei et al., 2018; Zorn et al., 2008) or nutrient application level (Deru et al., 2023; Onrust and Piersma, 2019; Van Vliet and De Goede, 2006). While the effect of nutrient application on soil biota in grasslands is relatively well studied (e.g. De Vries et al., 2013; Tsiafouli et al., 2015) the combined effect with a raised water level is far less understood.

The aim of this study was to better understand how soil biota in an intensively managed and drained agricultural peat soil respond to raised water levels and reduced nutrient application levels. Therefore we performed a mesocosm study in which we exposed 40 peat cores from an agricultural peat grassland to four different water levels and two nutrient application levels for a period of 15 months. After this period, soil samples were taken from which we analysed the following (functional) soil biota groups: bacteria, saprophytic fungi, actinomycetes, protozoa, nematodes (bacterivorous, fungivorous, herbivorous, omnivorous and predaceous) and earthworms.

2. Methods

2.1. Site description and peat core collection

The peat cores used in this mesocosm experiment were collected from the peat meadow area Zegveld polder (52°08′40.2″N 4°50′08.0″E, WGS-84 coordinate system). The peat soil (Terric Histosol; (FAO, 2015) on the sampling location is drained and in use for agricultural dairy farming. The vegetation on the field was dominated by the grass species *Lolium perenne*.

The peat cores were extracted close to each other, in three parallel rows on 11 February 2021 at a site where a future ditch was planned. For the extraction, sharpened PVC pipes were pushed carefully into the soil with a crane till a depth of 80 cm. When all the PVC pipes were in the soil, a trench was dug along the peat cores to lift the peat cores out of the soil. To prevent any peat from falling out of the PVC pipe during transport, they were closed with a bottom lid. The peat cores were then transported to the botanical garden of Utrecht University and placed in the treatment tanks (see Section 2.2).

Soil samples to characterize initial soil conditions in the field were taken after peat core collection from the top 10 cm. Samples were analysed for soil organic matter, moisture content and total nutrient concentrations (Table 1). Further, a soil sample of 450 g was taken at 0–10 cm depth and analysed for nematodes as described in Section 2.4.2. Last, two samples of 20 × 20 × 20 cm were taken from which earthworms were hand sorted and identified to species level (see Section 2.4.3). Results from these initial field conditions are shown in Table 1.

2.2. Experimental set-up

Cores were placed in tanks (85 cm, 60 cm Ø) in the botanical garden of Utrecht University from 11 February 2021 to 26 April 2022. The tanks were installed in the soil and the experiment took place in the open air to mimic field conditions. Every treatment consisted of one tank with five peat cores. In total, we included eight treatments in a 4 × 2 full factorial design, consisting of four different water levels (0, 20, 40 and 60 cm below peat surface) and two nutrient application levels (high and low). To allow water exchange between the peat cores and the tank we drilled eight holes (Ø 2–3 mm) in the bottom of the PVC cores. The 40 peat cores and 8 corresponding treatments in this study were a subset of a larger study on nutrient dynamics in rewetted peat meadows, more details about the study design can be found in Van der Laan et al. (2024).

Tanks were filled with groundwater from a 12 m deep well in the same polder area as where the peat cores were taken (see Supplementary Table S1 for chemical composition of the groundwater). To create the

Table 1
Peat abiotic and biotic conditions at the peat core collection site.

Parameter	Unit	
Soil (*n* = 5)		
Total C	g C kg^{-1} dry wt	286.4 ± 20.3
Total N	g N kg^{-1} dry wt	22.8 ± 1.5
Total P	g P kg^{-1} dry wt	1.9 ± 0.08
Total K	g K kg^{-1} dry wt	6.7 ± 0.1
Total S	g S kg^{-1} dry wt	9.5 ± 0.7
pH-KCl		5.1
Soil organic matter	%	56.8 ± 4.9
Moisture	%	62.6 ± 1.9
Nematodes (*n* = 1)		
Nr of species	Nr	38
Abundance	individuals 100 g^{-1}	4198
Bacterivores	%	38.6
Fungivores	%	8.2
Herbivores	%	47.2
Omnivores	%	4.6
Predators	%	1.3
Earthworms (*n* = 2)		
Total biomass	g m^{-2}	346
Abundance	individuals m^{-2}	729
Adults	individuals m^{-2}	425
Juvenile	individuals m^{-2}	287
Epigeic adults	individuals m^{-2}	100
Endogeic adults	individuals m^{-2}	325
Anecic adults	individuals m^{-2}	0

four different water table treatments, holes with a diameter of 25 mm were drilled in all tanks at the desired water level as an overflow to guarantee that excess water could leave the tank. In dry periods, groundwater was added manually to all tanks every few days to make sure water levels were kept constant. During the experiment, all water in the tanks was refreshed monthly for the period February 2021 to October 2021. During the winter period we did not replenish the water. The water was refreshed one last time on 25 March 2022. To refresh the water, we pumped all water out of the tanks and immediately refilled the tanks with groundwater that was collected and transported from the Zegveld polder area that same day.

A high and a low nutrient application treatment was created by fertilizing the columns with Osmocote® flower N (ICL Specialty Fertilizers, Geldermalsen, the Netherlands) slow release (2–3 months) granules with a N:P_2O_5:K_2O of 16:5:12, which translates to a N:P:K ratio of approximately 14:2:9. The N component consisted of NO_3-N (7.6 %) and NH_4-N (8.3 %) and next to P_2O_5 and K_2O the granules further contained MgO (2.5 %) and trace elements (Cu, Fe, Mn, Zn). Application levels resembled a total N input of 50 or 250 kg N ha^{-1} yr^{-1}, representative for low-intensity (Schippers et al., 2014) and high-intensity (Kleijn et al., 2001) agricultural use respectively. This means that next to N, the low nutrient application treatment received 6.8 kg P ha^{-1} $yr^{-1}$and 31.1 kg K ha^{-1} yr^{-1} whereas the high nutrient application level received 34.1 kg P ha^{-1} yr^{-1} and 155.6 kg K ha^{-1} yr^{-1}. Granules were added on 3 April 2021 (20 vs. 100 kg N ha^{-1}), 24 June 2021 (20 vs. 100 kg N ha^{-1}), 31 August 2021 (10 vs. 50 kg N ha^{-1}) and 9 March 2022 (20 vs. 100 kg N ha^{-1}).

2.3. Soil abiotic and vegetation measurements

Above-ground biomass was clipped six times throughout the whole experiment: five times during the growing season on day 78, 126, 167, 201 and 245 of the experiment (April–October 2021) and during the final week of the experiment, on 22 April 2022. Dry weight was measured after drying the plant material for 48 h at 70 °C. We further analysed the dried samples of the last harvest for total C, N, P and K concentrations.

At the end of the experiment (26 April 2022) soil samples were taken from the top 10 cm of the center of each peat core with an auger (Ø 2 cm). On the day of sampling, part of the sample (about 15 g) was weighed and freeze-dried for 48 h after which the sample was weighed again. Moisture percentage was calculated from the weight loss. C and N concentrations of the freeze-dried soil samples as well as the dried vegetation samples were measured using a CN-analyser (NA1500, Fisons Instruments, Milan, Italy). P and K concentrations were measured using total reflection X-ray fluorescence (S2 Picofox, Bruker Nano GmbH, Karlsruhe, Germany).

Within three days after sampling, plant available nitrogen was measured on a fresh field-moist soil sample. Ammonium and nitrate were extracted using a 1 M KCl solution. For each sample, the KCl extract was divided over three subsamples. One subsample was used to measure pH-KCl. The second subsample was used to colorimetrically measure NH_4-N using the indophenol blue method (Koreleff, 1976) and on the last subsample NO_3-N was measured following the vanadium chloride (TON-V) method and on a discrete analyser (Gallery, Thermo Fisher Scientific, Waltham, USA). Soil organic matter was determined by loss-on-ignition at 450 °C (Ball, 1964).

2.4. Soil biota measurements

Microbial and nematode samples were taken from the top 10 cm of the peat cores. Earthworms were sampled from the top 20 cm. Thereto the peat cores were taken from their tanks at the end of the experiment and the top 20 cm was cut off and sliced into two (0–10 and 10–20 cm depth). First, all earthworms were carefully removed from both halves. Then 450 g of field-moist soil from the upper half was put aside for nematode analyses and 4 g of field-moist soil was taken of the top 10 cm for the PLFA analysis.

2.4.1. Bacteria, fungi and protozoa

Phospholipid fatty acid (PLFA) analysis was used to determine bacterial, fungal, actinomycete and protozoan PLFA biomass. The analysis was performed by Eurofins (Wageningen, The Netherlands). A temperature gradient was used to separate the PLFA on a GC Trace 1300 and analysed on a TSQ 8000 mass spectrometer (20 m × 0.15 mm ID, 0.30 μm VF-5MS Agilent (Agilent, Santa Clare, United States); 1 μl injection; helium; full scan 50–300 *m/z*). For determination of the phospholipids, both the retention time and the mass spectrum of the various lipids were used. The analysis was performed according to standard methods NPR-CEN-ISO/TS 29843-1 and NPR-CEN ISO/TS 29843-2. For bacteria, the PLFA 10Me-16:0, 10Me-17:0, 10Me-18:0, 12Me-18:0, i15:0, ai15:0, i16:0, ai16:0, 16:1ω7c, 17:1w8c, i17:0, i17:0 ω7c, ai17:0, cy17:0, 18:1ω7c, 18:1ω9t, 18:1ω12t, cy19:0ω7c and cy19:0ω9c were chosen. PLFA 18:2ω6 was used as a marker of saprotrophic fungi. PLFA 10Me16:0, 10Me17:0 and 10Me18:0 were used to represent actinomycetes and for protozoa PLFA 20:4ω6c was used.

2.4.2. Nematodes

From the nematode sub-sample, about 100 g was used for free-living nematode extraction by using an Oostenbrink elutriator (Oostenbrink, 1960). Total nematode numbers were counted and expressed per 100 g of fresh soil. Then, the nematodes were fixed in hot formaldehyde and at least 150 individual nematodes were picked randomly from every sample and identified to species level. Nematode genus and species were assigned to trophic groups following Yeates et al. (1993) and allocated to the colonizer persister groups (cp-groups) following Bongers (1990) and Bongers et al. (1995). The Maturity Index was calculated as the weighted mean of the individual cp-values, in accordance with Bongers (1990). The Maturity Index is an ecological measure, which indicates the condition of the soil based on nematode species composition. Additionally, the nematode channel, enrichment and structure indices were calculated following Ferris et al. (2001) and using NINJA (Sieriebriennikov et al., 2014). The Channel Index provides information on the dominant decomposition pathway by bacteria or fungi via comparing the relative importance of specific fungal feeding nematodes to bacteria and other types of fungi feeding nematodes (Du Preez et al., 2022; Ferris et al., 2001). The Enrichment Index is calculated based on nematode groups that respond to increased food availability and nutrient enrichment and hence provides information about resource availability (Du Preez et al., 2022). Last, the Structure Index informs about the soil food web complexity and stability. It is calculated based on nematode guilds that need a stable environment in order to do well (Ferris et al., 2001). Low values indicate a low food web complexity and perturbed soil food web, and high values indicate a high food web complexity. Together the Enrichment Index and the Structure Index can be plotted in a diagram, the so called faunal analysis diagram (Ferris et al., 2001). This diagram consists of four quadrants, each describing a different state of the nematode community.

2.4.3. Earthworms

Earthworms were carefully removed from both soil layers (0–10 and 10–20 cm) by hand after which they were immediately counted, weighed and fixed in 70 % alcohol until further identification. Both earthworm abundance and biomass were expressed per m^2. All individuals, were identified to species level (Sims and Gerard, 1985; Stöp-Bowitz, 1969) and a distinction was made between juveniles and adults. Further, the earthworms were also classified into their respective functional groups; epigeic, endogeic or anecic earthworm species (Bouché, 1977).

2.5. Data analysis

All data analyses were performed in Rstudio version 4.1.2 (R Core Team, 2021). A two-way analysis of variance (ANOVA) was used to test the effect of water level and nutrient application level on all abiotic and biotic variables. Prior to data analysis, homogeneity of variance was checked for using Levene's test. Normality was visually assessed using residual plots. Data were log-transformed if homogeneity of variances was not met. The *emmeans* package with multivariate t-distribution adjustment was used to perform post-hoc tests (Russell et al., 2023). Pearson sample correlations were calculated and their significance were tested between all parameters. Correspondence analysis (CA) plots were used to visualize the variation in community composition of nematodes and earthworms between the various treatments. The relationship between the overall soil community composition and environmental variables was analysed with canonical correspondence analysis (CCA). Both CA and CCA plots were made using the *vegan* package (Oksanen et al., 2024). Differences in community composition between the treatments were tested for with permutational multivariate analysis of variance (PERMANOVA) with 999 iterations, by using the 'adonis2' function in the *vegan* package followed by a pairwise PERMANOVA. To test the overall significance of the CCA model and the significant effect of environmental variables on the CCA, permutation tests with 999 iterations were performed by using the 'cca.anova' function in the *vegan* package.

3. Results

3.1. Abiotic soil and vegetation parameters

Moisture content was significantly higher in WL0 and significantly lower in WL60 than in all other water level treatments (Fig. 1A). This shows that the water level treatments indeed resulted in different moisture contents in the top 10 cm of the cores (Table 2). pH (KCl) did not differ significantly between water level treatments (Table 2). Within the highest water level treatment (WL0) pH was significantly lower in the high nutrient treatment than in the low nutrient treatment ($P = 0.04$, Fig. 1B). Soil organic matter also only significantly differed between the

Table 2
ANOVA results of the effect of water table and nutrient application on soil biotic parameters.

	Water table	Nutrient application	Water table * nutrient application
Abiotic and vegetation parameters			
Moisture	<0.001***	0.873	0.819
pH-KCl	0.071	0.004**	0.222
Soil organic matter	0.335	0.025*	0.037*
Above-ground biomass	<0.001***	<0.001***	0.893
Total N	0.009**	0.002**	0.208
NH_4-N	0.013*	0.941	0.013*
Microbial parameters (PLFA abundance)			
Bacteria	<0.001***	0.282	0.915
Saprophytic fungi	0.002**	0.231	0.145
Actinomycetes	0.001**	0.379	0.733
Protozoa	0.057	0.198	0.158
Nematodes			
Abundance (individuals 100 g^{-1})	<0.001***	0.115	0.707
Taxonomic richness (nr of taxa)	<0.001***	0.038*	0.540
Earthworms			
Biomass	<0.001***	0.003**	0.172
Abundance	<0.001***	0.006**	0.059

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

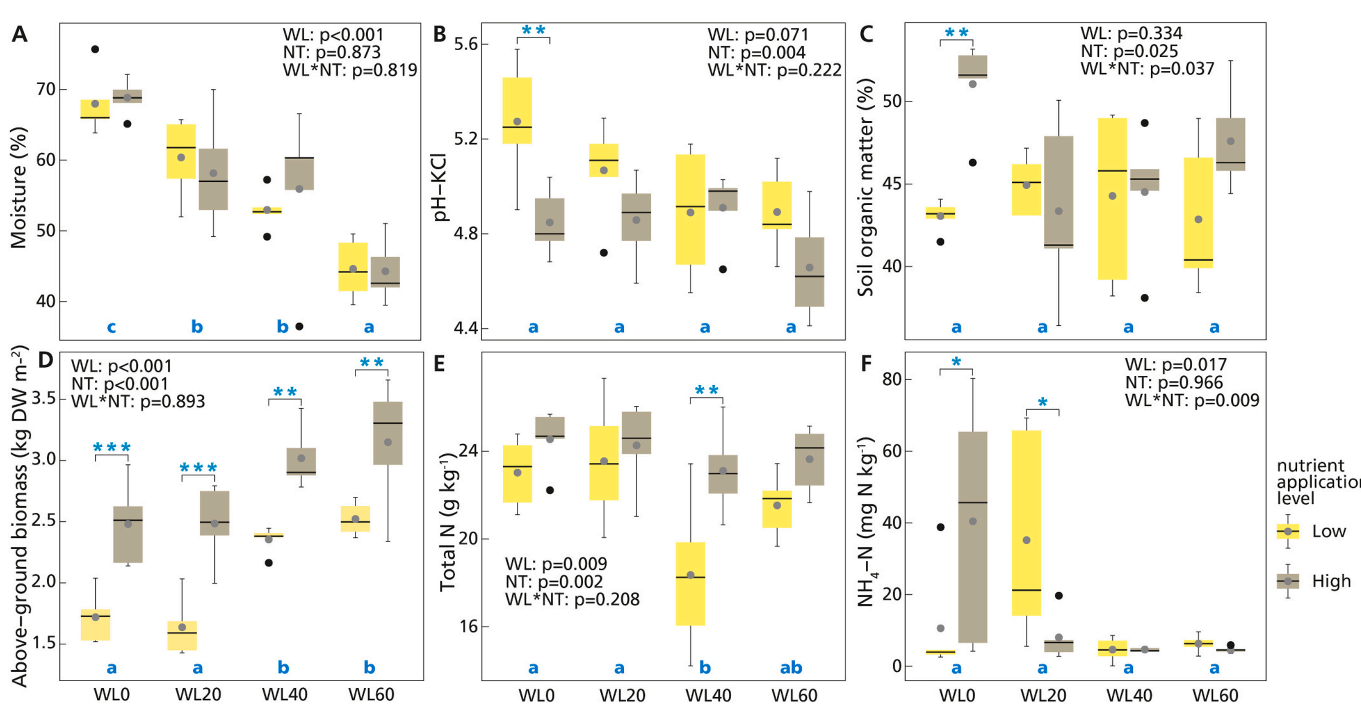

Fig. 1. Abiotic conditions in the top 10 cm of the peat cores in the various treatments. A: moisture content, B: pH-KCl, C: soil organic matter content, D: cumulative above-ground biomass production, DW = dry weight, E: total N concentration in the soil, F: Plant available N in the form of NH_4. Black lines represent the median, grey dots indicate the mean, black dots are outliers. $N = 5$ for each treatment combination. The blue letters denote the significant differences between the water table treatments; treatments sharing a letter do not significantly differ. Within every water table treatment the significant differences between the low and high nutrient application level are shown (if applicable): $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. WL0, WL20, WL40 and WL60 refer to water level (cm below surface).

nutrient application levels within the WL0 treatment ($P = 0.002$, Fig. 1C). Total carbon (Supplementary Fig. S1A) and N (Fig. 1E) concentrations were significantly higher in treatments that received a high nutrient application than those receiving a lower nutrient application ($P < 0.001$ and $P = 0.002$ respectively). Cumulative above-ground biomass production (i.e. total yield) throughout the experiment was significantly higher in the treatments receiving a high nutrient concentration compared to the treatments receiving a low nutrient application and was significantly higher in WL40 and WL60 than in WL20 and WL0 (Fig. 1D).

3.2. Microbial community

Bacterial PLFA made up the bulk of the total PLFA analysed. Bacterial PLFA was significantly higher in WL0 and WL20 than in WL40 ($P = 0.020$ and $P < 0.001$ respectively), while only WL20 was significantly higher than WL60 ($P = 0.013$, Fig. 2A). No significant nutrient effects were found on bacterial PLFA (Table 2, Fig. 2A). Saprophytic fungal PLFA was significantly higher in WL0 than in WL40 and WL60 and highest in the WL0 cores receiving a low nutrient application (Fig. 2B). Actinomycete PLFA was positively affected by high water levels although the post-hoc results show that actinomycete PLFA was only significantly higher in WL20 than in WL40 and WL60 and WL0 did not significantly differ from any of the other WL treatments (Fig. 2C). No significant differences between treatments were found for protozoan PLFA (Table 2, Fig. 2D).

3.3. Nematodes

Nematode abundance was significantly higher in WL60 compared to WL20 and WL0 ($P < 0.001$, Fig. 3A). With 3809 individuals 100 g^{-1}, the nematode abundance in the WL60 and low nutrient level treatment approximately resembled the nematode abundance at the start of the experiment (4198 individuals 100 g^{-1}, Fig. 3A). Taxonomic richness was lowest in WL0 compared to all other water table treatments ($P < 0.006$) and was within WL0 significantly higher in the low nutrient treatment than in the high nutrient treatment ($P = 0.046$, Supplementary Fig. S2). No significant differences between the treatments were found in the abundance of bacterivorous and fungivorous nematodes (Fig. 3A, Supplementary Table S2). Within the WL0 treatment the relative abundance for both bacterivorous and fungivorous nematodes was significantly higher in the high nutrient application level than in the low nutrient application level ($P = 0.001$ & $P < 0.001$ respectively, Fig. 3B, Supplementary Table S2). Herbivores were significantly more abundant in WL60 than in all other water table treatments ($P < 0.005$). Overall, a significantly higher percentage of omnivores and predators was found in low nutrient treatments than in high nutrient treatments, post-hoc tests revealed this was only significant within the WL0 water table treatment ($P = 0.004$ & $P = 0.005$ respectively, Fig. 3B, Supplementary Table S2).

The CA plot of the nematode community shows that the first axis explains 22.45 % of the variation in community composition and the second axis explains 13.48 % (Fig. 4). The nematode community composition was significantly affected by water level treatment (PERMANOVA, $P < 0.001$) and to a lesser extent by nutrient application levels (PERMANOVA, $P = 0.020$). Further, there was a significant interaction effect between water level and nutrient application (PERMANOVA, $P = 0.012$). The community composition in WL60 and WL40 were significantly different from each other and from the community composition in WL0 and WL20, while WL0 and WL20 did not significantly differ from each other. When looking at the nutrient application effect within every water level treatment, a significant effect of nutrient application level on nematode community composition was only found within the WL0 treatment (PERMANOVA, $P = 0.009$).

The faunal analysis diagram in Fig. 5 shows that most treatments are located in quadrant C, which is indicative of a maturing soil food web with a low food and nutrient availability. The Structure Index was significantly lower in peat cores that received a high nutrient input in the WL0 treatment ($P < 0.001$), indicative of a low food web complexity and/or disturbed system (Fig. 5, Du Preez et al., 2022). The Enrichment Index was significantly higher in WL60 than in WL20 and WL0 ($P = 0.003$ & $P = 0.037$ respectively, Supplementary Table S2). Within WL60 the Enrichment Index was significantly higher in the low nutrient treatment than in the high nutrient treatment ($P = 0.021$). The Maturity Index was within WL0 significantly lower in the higher nutrient level than in the low nutrient level ($P < 0.001$, Supplementary Table S2).

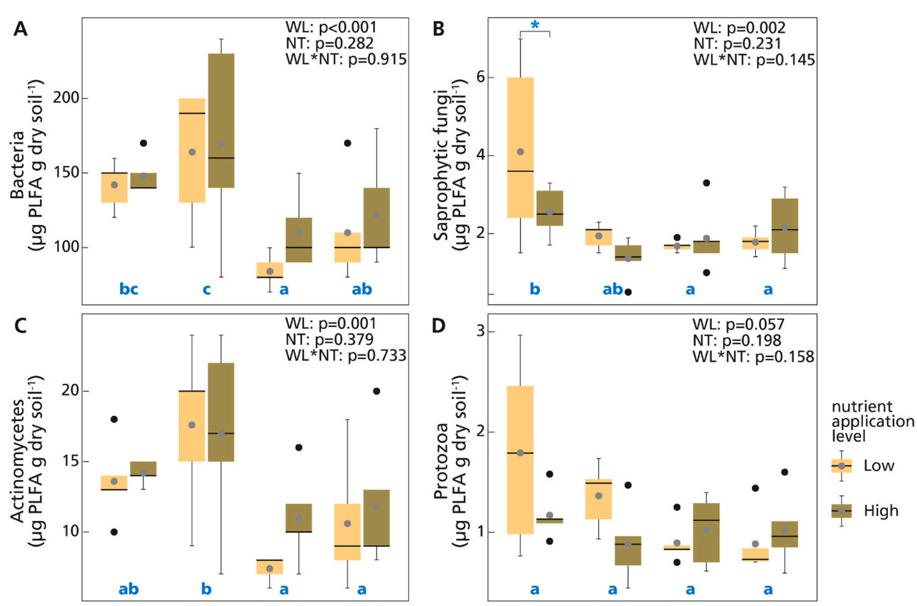

Fig. 2. Effects of water level and nutrient application on microbial PLFAs. A: bacteria, B: saprophytic fungi, C: actinomycetes, and D: protozoa. Black lines represent the median, grey dots indicate the mean, black dots are outliers. N = 5 for each treatment combination. The blue letters denote the significant differences between the water table treatments; treatments sharing a letter do not significantly differ. Within every water table treatment the significant differences between the low and high nutrient application level are shown (if applicable): *$p < 0.05$. WL0, WL20, WL40 and WL60 refer to water level (cm below surface).

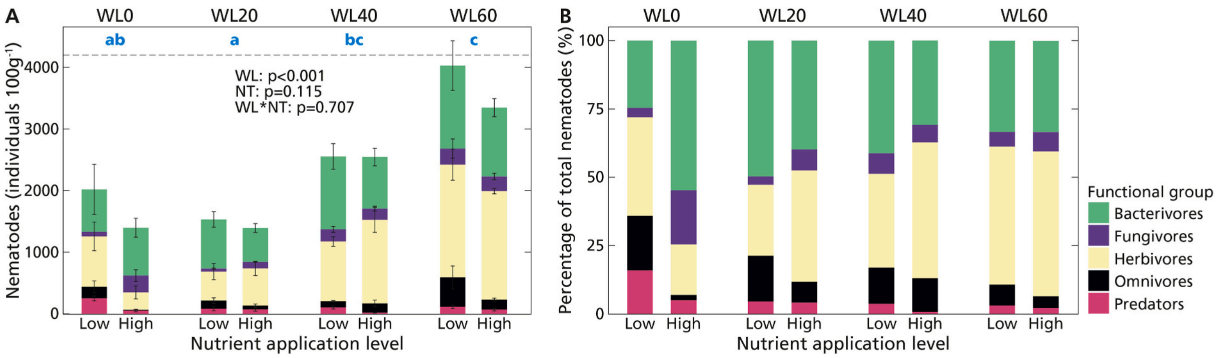

Fig. 3. Nematode abundance and relative abundance per treatment group, $n = 5$. WL0, WL20, WL40 and WL60 refer to water level (cm below surface). Blue letters denote the significant differences between the water table treatments; treatments sharing a letter do not significantly differ. The horizontal dotted line shows the nematode abundance measured in the field during peat core collection (4198 nematodes).

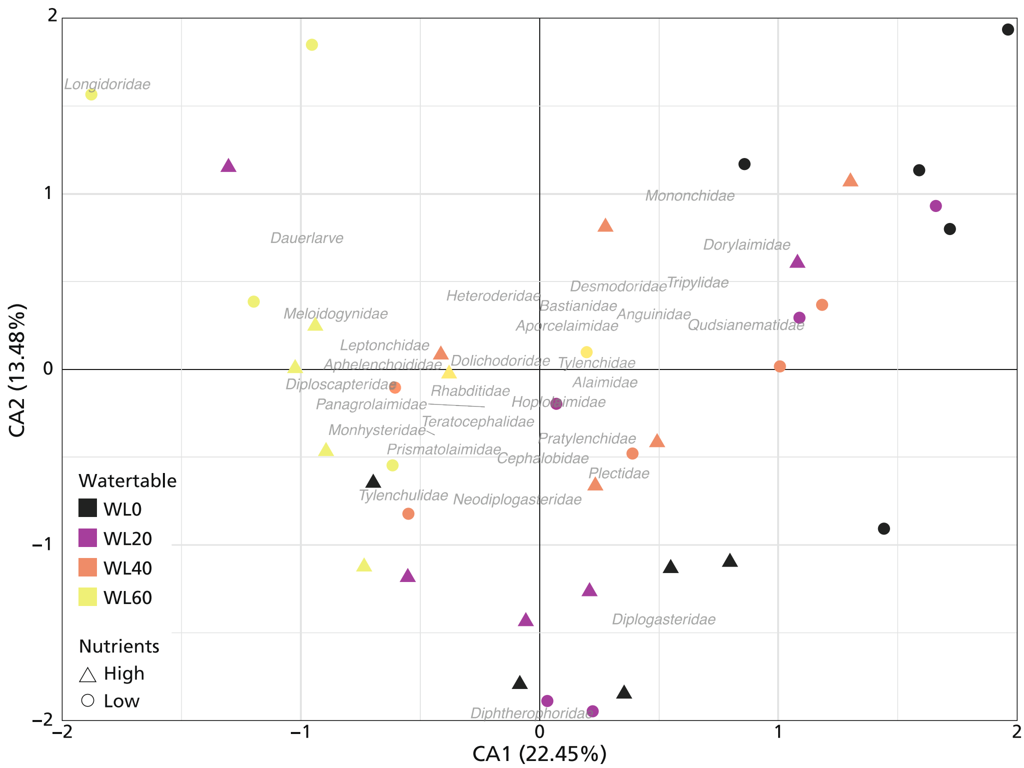

Fig. 4. Correspondence analysis (CA) plot of the nematode community composition (family level) in the various treatments. The different colors correspond to the four different water level treatments (0, 20, 40 and 60 cm below soil surface) whereas the different symbols refer to the high and low nutrient application level treatments.

3.4. Earthworms

Averaged over all water level treatments, earthworm biomass was significantly lower in the high nutrient treatments than in the low nutrient treatments (Table 2, Fig. 6A). Within water level treatments this difference was only significant in the high water level treatments (WL0 and WL20) (Fig. 6A). Both earthworm biomass and abundance were significantly lower in WL0 than in WL40 and WL60. Earthworm abundance was also significantly lower in the high nutrient treatments of the WL0 and WL20 treatments compared to their respective low nutrient treatments (Fig. 6B). The number of adult earthworms was significantly lower in the high nutrient treatments ($P = 0.015$) and was significantly higher in WL60 and WL40 than in WL0 (Supplementary Table S3). No earthworms were found in the WL0 treatment receiving a high nutrient application (Fig. 6B).

Fig. 7 shows a CA plot of the earthworm community. The first axis explained 43.64 % of the variation in earthworm community composition and the second axis 28.93 %. Community composition did not significantly differ between the various water level treatments (PERMANOVA, $P = 0.164$) and nutrient application treatments (PERMANOVA, $P = 0.853$). Further, no significant interaction effect between water and nutrient application level was found (PERMANOVA, $P = 0.506$). Note that no earthworms were found in the WL0 high nutrient application so this treatment is not included in the CA plot.

3.5. Response of the soil food web

Fig. 8 shows the relation of the soil community with environmental parameters. The first two CCA axes explain 56.20 % and 31.85 % of the constrained variance in species composition respectively. The overall CCA model was significant (pseudo-F = 2.818, P < 0.001), indicating that the environmental variables explained a significant part of the

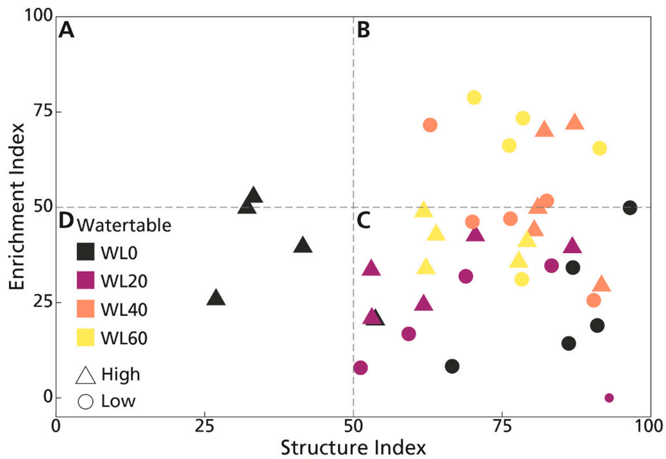

Fig. 5. Nematode faunal analysis diagram. The various colors depict the different water level (WL) treatments. Dots: low nutrient application level, triangles: high nutrient application level. The four quadrants indicate the following: A: degraded and N-enriched, B: maturing and N-enriched, C: maturing and depleted, D: degraded and depleted.

variation in the soil community composition. Of the environmental variables that were included in the CCA plot, soil moisture ($P = 0.004$), plant available NH_4-N ($P = 0.025$) and total above ground biomass ($P = 0.015$) showed a significant effect on soil community composition. All microbial groups are more associated with moist conditions (Fig. 8) and positively correlated with moisture content (Supplementary Table S4) whereas nematode and earthworm abundance correlated negatively with soil moisture (Supplementary Table S4) and are located in the left half of the CCA plot (Fig. 8). Community composition significantly differed between water level treatments (PERMANOVA, $P < 0.001$) and nutrient application treatments (PERMANOVA, $P = 0.027$) although the latter was only significant within the WL0 treatment ($P = 0.01$).

4. Discussion

4.1. Effect of water level

Our results show that all soil biotic groups that were included in this study (bacteria, fungi, protozoa, nematodes and earthworms) overall showed a strong response to changes in groundwater level. The water level in the WL60 treatment resembled the groundwater level in the field where the peat cores were collected. Not surprisingly, in this treatment the nematode and earthworm numbers and community composition differed least from the field situation at the start of the experiment. PLFA abundance was not measured in the field at the start of the experiment but does show differences between the water level treatments. Our results show that raising the water level to ≤20 cm below peat surface leads to differences in abiotic conditions of the system, which also resulted in a large shift in composition of the soil faunal community.

In general, peat soils are dominated by the bacterial part of the food web (Deru et al., 2018; Myers et al., 2012; Van Dijk et al., 2009), which was clearly reflected in our results (Fig. 2). We found a higher bacterial PLFA abundance under wet conditions compared to dry conditions. Earlier studies also showed an increased importance of bacteria upon rewetting (Mentzer et al., 2006; Van Dijk et al., 2009; Xu et al., 2023), which is attributed to a higher pH and a high nutrient environment because of the (previous) agricultural use (Van Dijk et al., 2009; Xu et al., 2023). Protozoan PLFA did not significantly differ between the various treatments (Fig. 2D). However, protozoan PLFA was positively correlated with pH, moisture and plant available NH_4 (Supplementary Table S4). This suggests that protozoa do indirectly respond to water levels which has also been found in earlier studies (e.g. Van Dijk et al., 2009). It is likely that the shift from aerobic (drained) to anaerobic (rewetted) conditions led to a different microbial community composition, towards species that thrive in low-oxygen environments (Edwards et al., 2023; Kitson and Bell, 2020). However, this cannot be confirmed by our study due to the limitations of PLFA analysis, such as an overlap in PLFA biomarkers between groups (Joergensen, 2022) and the fact that microbial community was not measured in the soil before treatment.

Nematode abundance was negatively affected by raised water levels as is shown by the lower nematode abundances in treatments with a high water level (Fig. 3A) and the negative correlation of nematode abundance with moisture content (Fig. 8, Supplementary Table S4). This difference was mainly caused by a strong drop in herbivorous nematodes under wet conditions (Fig. 3A, Supplementary Table S4), which is in agreement with earlier studies (Bobuľská et al., 2020; Cesarz et al., 2017; Wasilewska, 2006; Wei et al., 2018). We assume this decline is related to plant biomass production, which was also lower in high water level treatments (Fig. 1D), as we found a positive relationship between herbivorous nematodes and above-ground plant biomass (Fig. 8,

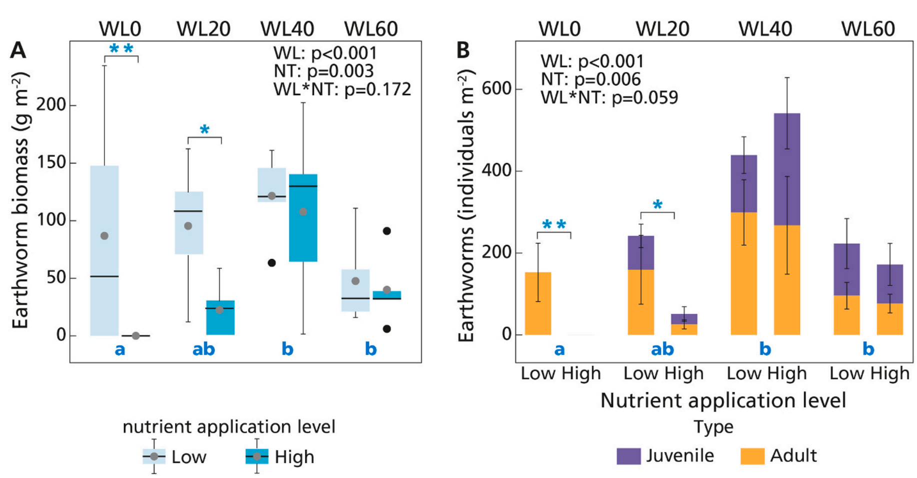

Fig. 6. Earthworm biomass (A) and abundance (B) in the different water table and nutrient application treatments, n = 5. WL0, WL20, WL40 and WL60 refer to water level (cm below surface). The blue letters denote the significant differences between the water table treatments; treatments sharing a letter do not significantly differ. Within every water table treatment the significant differences between the low and high nutrient application level are shown (if applicable): $*p < 0.05$, $**p < 0.01$. Earthworm biomass in the field was 346 g m^{-2} and earthworm abundance was 729 (Table 1). A: black horizontal lines represent the median, grey dots indicate the mean, black dots are outliers.

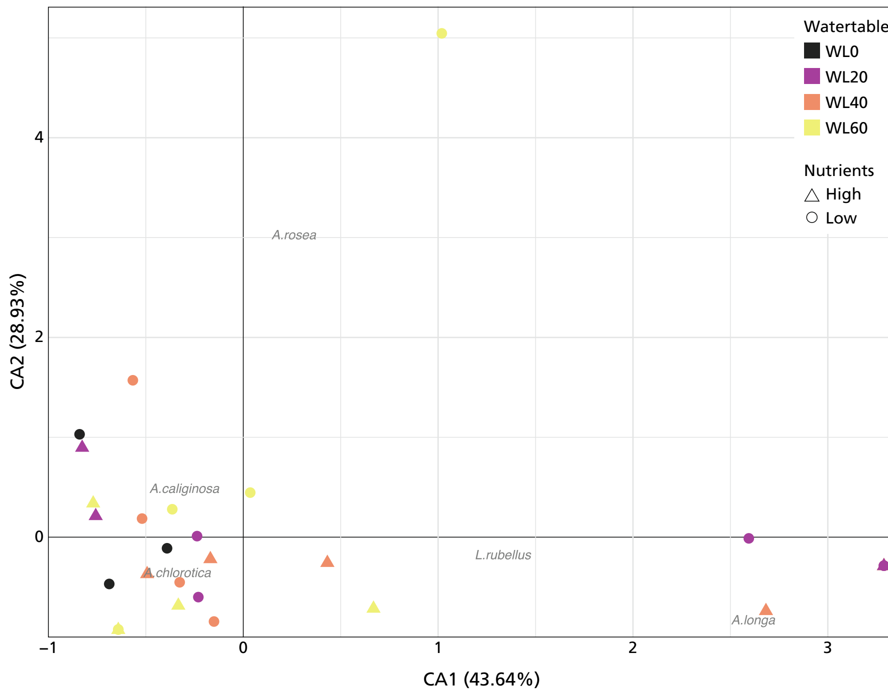

Fig. 7. Correspondence analysis (CA) plot of the earthworm community composition (species level) in the various treatments. The different colors correspond to the four different water level treatments (0, 20, 40 and 60 cm below soil surface) whereas the different symbols refer to the high and low nutrient application level treatments.

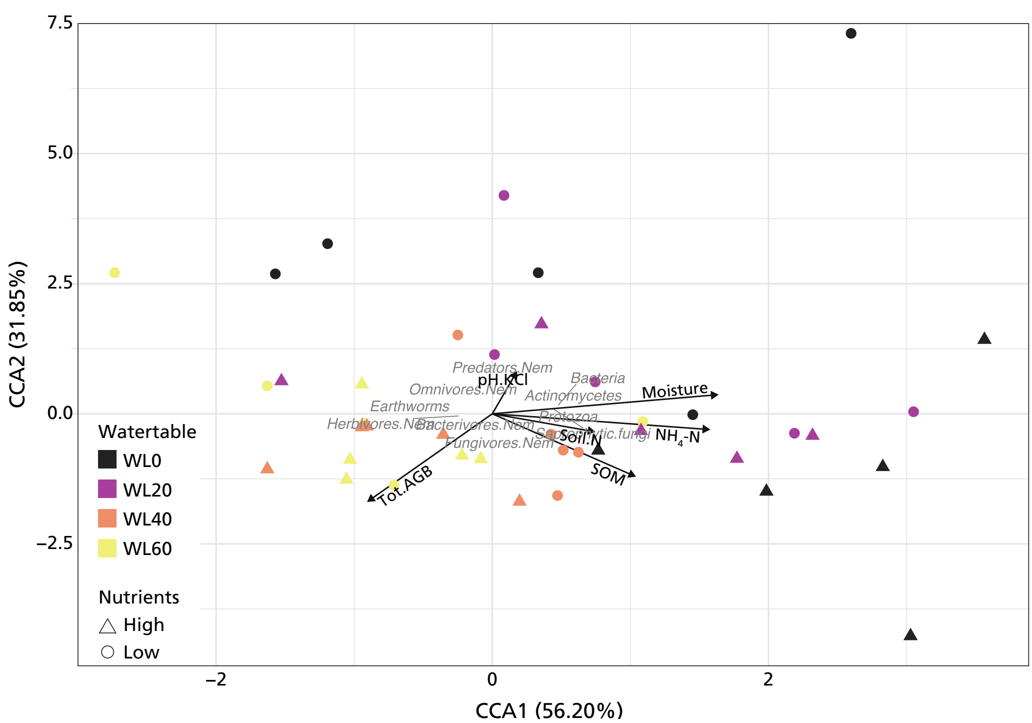

Fig. 8. Canonical correspondence analysis (CCA) plot of the soil community composition of the main soil biota groups in the different treatments. The different colors correspond to the four different water level treatments (0, 20, 40 and 60 cm below soil surface) whereas the different symbols refer to the high and low nutrient application level treatments. The arrows show different soil and vegetation parameters: total above-ground biomass (tot.AGB), pH-KCl in the soil (pH-KCl), moisture content in the soil (Moisture), plant available ammonium in the soil (NH_4-N), total N concentration in the soil (soil.N) and soil organic matter (SOM). Arrows are scaled with a scaling factor of 2 to increase plot readability.

Supplementary Table S4). Nematode abundance was very similar between the WL60 treatments and the field situation (Fig. 3A). Earlier studies on Dutch peat grasslands used for dairy farming reported nematode abundances that were twice as high as our field situation (Deru et al., 2018; Rutgers et al., 2009). However, nematode abundance and community composition can vary considerably over time (Biederman et al., 2008; Ferris et al., 2001). In general, it is assumed that as long as all nematode species from the original community are present, the nematode community can recover after disturbances in the environment (Postma-Blaauw et al., 2010). In our study, taxonomic richness was lowest in the WL0 treatment (Supplementary Fig. S2, Supplementary Table S2) with an average of 26 taxa and a lower abundance of herbivorous nematodes but a higher percentage of predaceous nematodes compared to the WL60 treatment (Fig. 3, Supplementary Table S2), resulting in a significantly different community composition in the different water level treatments (Fig. 4). In the other WL treatments, taxonomic richness ranged from 30 to 34 which is lower than the 38 taxa found in the field during peat core collection but similar to the average of 31 taxa found in other studies that looked at similar peat systems (Deru et al., 2018; Rutgers et al., 2009). Hence, upon fully rewetting some nematode species are potentially lost from the system.

We observed a strong negative effect of high water levels on earthworm biomass and abundance (Fig. 7B) with no juvenile earthworms found in the water saturated (WL0) treatments. The lack of juvenile earthworms implies that under very wet conditions the original earthworm population is unlikely to recover. We did not count earthworm cocoons and hence the potential for more earthworms in the WL0 treatment if the experiment ran for a longer period. However, hatching and early larval development have been shown to be negatively affected under moist conditions (Ausden et al., 2001). We also found a lower earthworm abundance under partially rewetted conditions (WL20) compared to WL40, indicating that even in not fully water saturated peat a negative effect of high water tables on earthworms exists. The ideal soil moisture range for earthworms is between 30 and 35 % (Onrust et al., 2019) which is well below our measured average soil moisture contents of 59 and 68 % in the WL20 and WL0 treatments, respectively (Fig. 1A). While earthworms can endure very wet conditions for a number of months (Ausden et al., 2001; Zorn et al., 2005), a soil moisture content of 65 % has a detrimental effect on earthworm health (Zorn et al., 2008). In this study, we think the negative effect of high water levels on earthworm biomass and abundance is a combination of a decreased oxygen availability (Wu et al., 2017; Zorn et al., 2005) and the cold winter weather from which earthworms could not escape by migrating downwards to warmer soil layers due to the confinement in our mesocosm systems (Timmerman et al., 2006). While earthworm abundance was lower in the WL0 treatment, individual earthworms were on average larger and heavier compared to the other WL treatments (Fig. 6). Larger earthworms are generally more resistant to environmental stressors such as low temperatures (Singh et al., 2019). It may further be possible that earthworms vacated the high WL treatment peat cores, although this has not been observed in this study and we therefore consider this unlikely.

4.2. Effect of nutrient application level

The effect of nutrient application level was overall less strong than the effect of water level. Nutrient level effects were mainly observed in high water level treatments (WL20 and WL0) and were mostly absent in low water level treatments. These results are in line with what could be expected given the similarity of these treatments with the conditions in the field where the peat cores were sampled. It is likely that the soil community in this field was adapted to low water levels and high nutrient input, which could explain the lack of response. Alternatively, the lack of response in the low water level treatments could be a result of the high mineralization rates under dry conditions (Olde Venterink et al., 2002), thereby partly masking the nutrient application effect in these treatments. With high water levels, mineralization rates are lower and therefore fertilization might show a stronger effect in these treatments.

None of the measured microbial groups showed a significant effect of nutrient application level (Fig. 2). An exception was the saprophytic fungal PLFA, which was higher in the low nutrient application treatment of the WL0 treatment. However, although no large effects of nutrient application level on overall PLFA groups were observed, it is not clear whether species composition within these groups differs between the low and high nutrient application treatments. Previous research has shown that microbial composition and activity within groups are altered after nutrient addition (Carey et al., 2016; Lori et al., 2023). Often nutrient addition benefits bacterial groups more than fungal groups (De Vries et al., 2006). Although bacterial PLFA showed no differences between the nutrient treatments, it was positively correlated to total N and plant available NH_4-N in the soil (Supplementary Table S4). The overall lack of response of bacteria to high nutrient application suggests there is still a large amount of easily degradable material available in the peat cores and that bacterial growth was not limited by mineral N (Bardgett and McAlister, 1999). Extra nutrient addition therefore likely did not lead to extra bacterial growth.

In contrast to the lack of a nutrient application effect on the microbial community, nematode and earthworms were much more sensitive to a high nutrient application level, especially under high water levels. The nematode taxonomic richness consisted of on average 24 taxa per core in the WL0 high nutrient treatment which was significantly lower than in the low nutrient treatment of that same water level (28 taxa) and lower than in all other treatments (Supplementary Fig. S2). A lower taxonomic richness is likely to reduce functional redundancy in the nematode community, which may also result in a lower food web stability (Neutel et al., 2002). The nematode based-indices confirm a low food web stability in the high nutrient WL0 treatment. For example, the Structure Index was lowest in this treatment, placing the WL0 high nutrient treatment in quadrant D of the faunal analysis diagram (Fig. 4) indicative of a more disturbed environment and degraded food web (Du Preez et al., 2022). Further, the Maturity Index of about 2 in this treatment also represents a simple soil food web structure and a high level of disturbance (Du Preez et al., 2022).

We found a negative effect of high nutrient application on earthworm abundance and biomass in the wettest treatments (WL0 and WL20). Moreover, when combined with a high nutrient application level no earthworms were found at all in WL0. In our study, we used inorganic fertilizer, which, along with slurry, is the most commonly used fertilizer on dairy grasslands in the Netherlands. Inorganic fertilizers are often found to have a less positive effect (if any) on earthworms compared to organic fertilizers (Deru et al., 2023; Van Eekeren et al., 2009; Whalen et al., 1998) and even negative effects have been reported (De Goede et al., 2003). This is attributed to the lower organic matter content and hence lower nutritional value of inorganic fertilizers (Deru et al., 2023; Whalen et al., 1998) or a lower pH after application (Ma et al., 1990). In our study, a significant pH reduction was observed only in the wettest treatment (WL0, Fig. 1B). The significantly lower pH in the WL0 high nutrient level compared to the WL0 low nutrient application level could explain the observed changes in soil biota composition such as the lower nematode taxonomic richness, lack of earthworms and higher saprophytic fungal PLFA. However, the pH in this treatment was not lower than in the other water level treatments receiving a high nutrient application level (Fig. 1B). Possibly, changes within one soil biota group in turn altered the diversity and composition in other soil biota groups (Neutel et al., 2002). To what extent this occurred in our experiment is not clear as the current treatment set-up does not allow for food web correlation tests. Future research could explore whether high moisture levels combined with high nutrient levels contribute to a decline in higher trophic groups (earthworms) and subsequently affect other trophic levels of the soil food web via top-down effects. It is important to note that since on most dairy grassland also slurry is used, the nutrient effects found in our study should be interpreted with some caution and might not fully reflect in situ conditions.

4.3. Potential implications for ecosystem functioning

Our results show that especially nematodes and earthworms were sensitive to changes in the environmental conditions in our mesocosms. This confirms other studies that found higher organisms within a food web to be more sensitive to disturbance compared to lower trophic groups (Cesarz et al., 2017; Postma-Blaauw et al., 2010; Voigt et al., 2007). In contrast to the nematodes and earthworms, the microbial community (bacteria, fungi and protozoa) was relatively more abundant under high water levels compared to low water levels. Microbes play a major role in nutrient cycling (Van der Heijden et al., 2008). In our case, as is the case in most peatlands (Deru et al., 2018; Gutknecht et al., 2006; Myers et al., 2012; Van Dijk et al., 2009), bacteria dominated processes are prevailing. A bacteria dominated microbial food web is generally associated with increased N losses and lower C sequestration compared to fungal dominated microbial communities (De Vries et al., 2012; Six et al., 2006). Emsens et al. (2020) showed that rewetting can result in a microbial community similar to natural peat systems, especially when the peat properties have not been altered too much and SOM is above 70 %. Since the peat used in this study has been exposed to drainage for several decades and was hence heavily degraded with a SOM of 40–50 % (Fig. 1C), it cannot be assumed that microbial community composition in our high water level treatments is similar to that of natural fens. Further, the agricultural nutrient legacy in the peat soil (especially phosphorous) after decades of nutrient application altered the system drastically (Van der Laan et al., 2024). Together, the degraded state of the peat and the nutrient legacy in peat meadows similar to the one we studied here (Fig. 1C, E, Supplementary Fig. S1) hamper the return to a natural peat fen and it may therefore be expected that decomposition rates will continue to be high or even increase upon rewetting of former agricultural fen meadows (Emsens et al., 2020; Laiho, 2006; Van Dijk et al., 2009). This may have implications for soil carbon dynamics and CO_2 emissions coming from these peatlands, potentially counteracting some of the intended benefits of raising water levels (Van Dijk et al., 2009).

For most groups, the effect of nutrient addition was small or absent under drained conditions. In contrast, the effect of nutrient application was very prominent in the fully water saturated treatments. Here, high nutrient levels created unfavorable conditions for earthworms and nematodes, which may have consequences for the soil food web structure. The strong interaction effect between water and nutrient level shows that changes in soil biota composition are complex. Especially changes in community composition resulting in a loss of functional diversity is considered problematic for ecosystem stability and functioning (De Vries et al., 2013; Heemsbergen et al., 2004). Earthworms play a key role in the soil food web by affecting lower trophic groups by their activity (Postma-Blaauw et al., 2006) but also play a major role in the ecosystem as a whole as they are an important food source for meadow birds (Onrust et al., 2019) which are the focus in most conservation schemes in Dutch agricultural peat meadows. Further, a lower soil biodiversity and stability is linked to a lower plant biodiversity, increased nutrient leaching and N_2O emissions (Lubbers et al., 2019; Wagg et al., 2014). Given the drastic changes in the soil community upon rewetting in combination with high nutrient application levels observed in our study, we suggest that nutrient inputs should be reduced when water levels are raised to 0–20 cm below peat surface. In practice (partial) rewetting does not always go hand in hand with a reduction in the nutrient application dose (Hoekstra et al., 2020; Wichtmann et al., 2016). Since agricultural production on wet peat soils is relatively new it is important to monitor these situations in the field to better understand the potential negative side-effects of for example nutrient leaching and emissions coming from these peatlands. Rewetting of agricultural peatlands is a measure that is widely considered in order to reduce anthropogenic greenhouse gas emissions globally. To make sure rewetting measures deliver on the intended outcomes without negative side effects, a better understanding of the soil community and its functioning is essential.

CRediT authorship contribution statement

Annick van der Laan: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nick van Eekeren:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Martin J. Wassen:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Karin T. Rebel:** Writing – review & editing, Conceptualization. **Jerry van Dijk:** Writing – review & editing, Methodology, 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.

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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.2025.105932.

Data availability

All data used in this study is available via doi:10.24416/UU01-WSYMAF.

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