

A STUDY ON THE CORRELATIONS BETWEEN THE NEMATODE COMMUNITY AND PRIMARY DECOMPOSERS IN THE SOIL ECOSYSTEM OF THE NATURE AREA *DE MOSSEL*

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Abstract. In the last decade the use of nematodes as indicators for soil health has gained much popularity. Without a detailed understanding of the interactions nematodes have with their environment, the information nematode-based soil health assessments give is limited. Little is known on the food preference of these organisms and the knowledge we have is quite broad. To understand the relationships nematodes have with their environment, information on the environmental factors and organisms nematodes occur with would be useful. In this study we tried to find correlations between the microbial community and nematode community. Using a quantitative PCR-based method we determined the abundance of fungal, bacterial and nematode taxa in the nature area *de Mossel*. The results of this study indicate that the interaction nematodes have with their environment is possibly genera specific. The spatial abundances of the nematode taxa that correlate with the pH give rise to a division into two sub-communities. More research on the environmental drivers of the microbial and nematode community is needed to determine the biotic and abiotic factors driving this division. The spatial distribution of fungivorous nematodes and fungi suggest there is an interaction between them, while we found no significant correlations between the two groups. Our data demonstrates the complexity of the relationship between fungivorous nematodes and soil fungi.

INTRODUCTION

Ecosystems and their soils are essential to human life. They provide us with an invaluable amount of services such as food production, climate mitigation, carbon cycling, water purification and many more (Brussaard, 1997). The success of these services is not only dependent on the plants and animals we see above ground, it also relies on the life below our feet. Soil biota govern numerous important soil processes such as nutrient cycling and carbon sequestration and have a key role in ecosystem functioning (de Vries et al., 2013; Fortuna, 2012).

Soil biota

Soil biota consist of plants, soil animals and microorganisms that live in or on the soil (Fortuna, 2012). Plants are the primary producers of the soil system, converting inorganic compounds into organic compounds. They are responsible for the amount of carbon that enters the soil system and

provide nutrients through their roots or as detritus *i.e.* dead organic material. Roots are generally not permeable for organic compounds. In order for the nutrients to be available again for plants, the detritus has to be decomposed first (Swift et al., 1979).

Bacteria and fungi are the primary decomposers of the soil system. They are directly capable of breaking down detritus into simpler organic and inorganic molecules (Juma, 1998) and can mineralize the nutrients contained within the complex organic molecules. Fungi have a larger variety of enzymes than bacteria, making them better decomposers in soils that have a lower nutrient availability. Bacteria can decompose sugars, starches and simple proteins from fresh detritus faster than fungi. Bacteria are however not efficient in the decomposition of tough residues like lignin and fungi will have the advantage when there is an input of substrates with a lower degradability. Primary decomposers retain the

carbon and nutrients of the detritus for their metabolism and growth. The secondary decomposers, such as nematodes and protozoa (heterotrophic single-celled eukaryotes), graze on primary decomposers. Grazing (uptake of bacteria and fungi (or parts thereof) as food) results in the release of carbon as CO₂ and mineralizes nitrogen, phosphorus and sulphur incorporated in the bacteria's protoplasm, making these nutrients available for plants (Anderson et al., 1981; Benckinser, 1997). Furthermore, consumption decreases bacterial competition, regulates bacterial abundance and stimulates microbial productivity and turnover. Protozoa consume bacteria while nematodes are represented by numerous bacterivorous and fungivorous taxa. Fungal grazers can stimulate or reduce the decomposing processes depending on the grazing intensity and nutrient conditions. Grazing can increase nutrient release by disrupting the hyphae but it can also cause overcompensation of fungal growth, potentially resulting in net nutrient immobilization (Wardle, 2002). Predaceous soil organisms form the highest trophic level of the food web; they feed in their turn on the secondary decomposers (Verhoef and Brussaard, 1990).

Biodiversity and environmental indicators

The diversity of soil organisms is immense; soil communities are often even seen as one of the most species rich components of terrestrial ecosystems (Giller, 1996). This diversity is amongst others driven by the high heterogeneity of niches provided by a great variability of soil factors e.g. soil structure, pH, moisture and organic matter content (Killham, 1994). Environmental conditions are dynamic and some species are more tolerant to particular conditions than others. The disappearance of species executing an essential role can threaten the stability of an ecosystem and may even lead to ecosystem collapse. According to the redundancy hypothesis there are several species that can fulfil similar functions or even compensate for each other. The probability that a species can substitute another depends on the diversity of adapted species in an ecosystem

(Benckinser, 1997). Climate change, human disturbances and invasive species can however reduce the diversity of the soil ecosystem making it more vulnerable to changes.

In the last few decades the importance of biodiversity and the lack of diversity in modern agriculture have received much attention (Tsiafouli et al., 2014). Species diversity preserves ecosystems, enhances soil fertility, guarantees the recycling of waste products and ensures ground quality (Benckinser, 1997). Growing concerns have arisen questioning the strong dependence of modern farming on non-renewable resources, the use of chemical fertilizers and pesticides and the effect of agricultural practices on biodiversity, safety and soil-, food- and environmental quality (Altieri, 1999). To estimate the consequences of the human impact on ecosystems, a good understanding of soil system functioning is essential. Risk assessment of contaminated soils and soil quality tests of agricultural fields are traditionally based on organic and inorganic matter content and chemical analyses. This approach fails to cover the bioavailability of substances or providing insight in the condition of the soil community (Bierkens et al., 1998; Martinez-Solgado et al., 2010). Due to the great diversity and complexity of the soil measuring all the different components of the soil food web is not feasible. To overcome this problem soil faunal communities are frequently used as indicators for the soil status (Vervoort, 2013). Soil faunal communities reflect the state of their food sources, the primary decomposers (Vervoort, 2013). Changes in the faunal communities reflect disturbances affecting the primary decomposers and their food source.

Nematodes

When choosing an indicator that is suitable (e.g. reflects the soil status) several elements are important; suitable indicators reflect the status of the ecological processes in the soil, are fast-responding, sensitive to change and accurately and efficiently identified. Nematodes meet these requirements and have several other advantages making them suitable indicators (Neher, 2001).

Nematodes are roundworms belonging to the phylum Nematoda, present in terrestrial, freshwater and marine habitats (Ferris et al., 2001). They form a diverse, abundant and important group occurring in all soil types and fulfil an important role in the soil food web. Nematodes can be found in several trophic levels and show a great diversity in feeding strategies; they can feed upon bacteria, fungi, protozoa and algae, hunt on other nematodes or parasitize on plants or animals (Benckinser, 1997). Nematodes are more stable to monitor than microbes since they have longer generation times. Their easy extractability from the soil matrix makes monitoring also more facile (Neher et al., 2005; Schloter et al., 2003).

Research showed that Amoebae, protozoan grazers, can have distinct grazing preferences for specific bacterial taxa (Rosenberg et al., 2009). A detailed understanding of the specific interactions nematodes have with their environment is however still lacking. This information is crucial if we want to use nematodes to predict the composition and health of the soil community.

The relationship between nematodes and their prey is largely unknown. Previously bacterivorous and fungivorous nematodes were considered as indiscriminate grazers, but recent observations seem to point at more specific trophic relations (Quist et al., 2014; Vervoort, 2013). Most food preference studies conducted so far are performed on agar plates (Okada and Kadota, 2003) and not in an ecological setting. The use of molecular methods on field samples might provide information that is still lacking. Modern techniques make more in depth research on the complex soil food-web possible and new developments have increased the precision and speed with which the abundance and diversity of nematodes, bacteria and fungi can be tested. For the identification of nematodes we no longer rely solely on the morphological characters seen under the light microscope. The small subunit ribosomal DNA (SSU rDNA) is in nematodes relatively variable and can be used for the identification of different phylogenetic relationships. The development of a

phylum wide SSU and LSU rDNA sequence framework and the development of taxon-specific primers of nematodes, fungi and bacteria make the use of a qPCR-based approach possible.

The aim of this study was to get more insight in the niches of individual nematode taxa and a better understanding of taxon-specific responses of nematodes to the environment. We investigated whether correlations could be observed between the abundance of several bacterivorous and fungivorous nematode taxa, and main bacterial and fungal groups. Using qPCR-based methods we hoped to gain more information on the trophic interactions between these groups.

METHODS

Soil sampling

Sampling took place in De Planken Wambuis (Mossel, 52°06' N, 05°75' E), a nature reserve area that is part of the Southwest-Veluwe in the Netherlands. This area has been used as arable land since 1920. In 1995 Natuurmonumenten decided to restore this area to extensively grazed grassland (Korthals et al., 2001). According to the map presented by Figure 1 eight transects with each six samples were taken, resulting in 48 samples. Each sample was composed of three randomly taken soil cores (\varnothing 1.5 cm, depth: 25 cm). The cores were thoroughly mixed, homogenized and these composite samples were immediately stored at 4°C. The organic matter (OM) content (%) and the pH was measured per transect (a mixture of six samples). The nitrogen ($\text{mg N} \cdot \text{kg}^{-1}$ soil), phosphorus (P_2O_5 100g dry weight (DW) soil) and clay content (% lutum) was determined for the whole area, using a mixture of all 48 samples.

Nematode community analysis

Living nematodes were extracted using the Oostenbrink funnel elutriation method complemented by a sieving and cotton-wool extraction (Oostenbrink, 1960). This was done

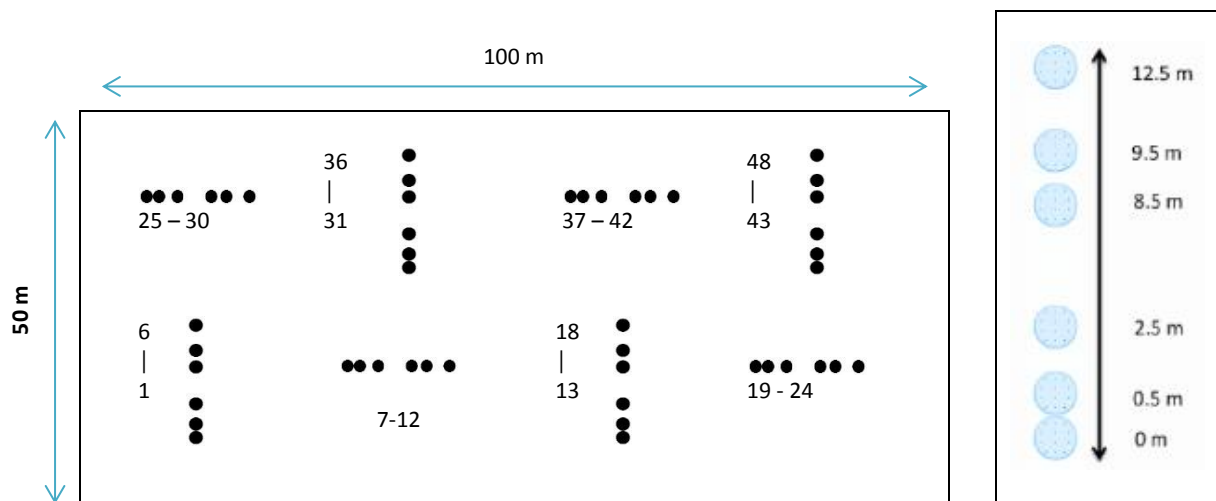


Figure 1. Overview of locations of the eight transects in De Planken Wambuis (Mossel, 52°06' N, 05°75' E) (left) and distances between the six plots of each transect (right)(Quist et al., Unpublished).

with a subsample of 100 gram of each composite sample. DNA extraction, lysate purification and subsequent qPCR reactions were performed as described by Vervoort et al. (2012). The selection of suitable taxon-specific primer sets was based on a nematode taxa biodiversity check. For this check, a mixture of all 48 composite samples was made and a qPCR reaction for all available primers was performed. 31 out of 61 nematode taxa were detected and the primer sets for these groups were used for further analysis., attached in the appendix shows an overview of the selected groups and their feeding habits. The abbreviations given in this table will be used for referring to the corresponding nematode taxa.

Bacterial and Fungal community analysis

The PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA USA) was used for the extraction process. After filtration of the large soil particles an internal standard (mammalian DNA) was added to the supernatants of each composite sample estimate the efficiency of the lysis and purification procedure. The abundance of ten taxa was determined by quantitative PCR reactions. Primers for seven bacterial taxa (Total Bacteria, Bacteroidetes, Firmicutes, Actinobacteria, Acidobacteria, Alpha-proteobacteria and Beta-proteobacteria) and three fungal taxa (Total fungi, Ascomycota and Basidiomycota) were used. A separate primer combination was used to quantify the internal standard in each sample. The quantitative PCR reactions were performed with a thermal cycler (Bio-Rad CFX), using the following

PCR protocol: 95°C for 15 minutes, 39 x (95°C, 30 sec; 60°C, 30 sec, 72°C, 30 sec) followed by a melting curve program (10 sec from 72 to 95°C with steps of 0.5°C). Reaction volume was 20 µL containing 3 µL of 100 times diluted template, 2 µL of a 5 µM primer set, 5 µL milliQ and 10 µL Absolute SYBR Green Fluorescein Mix (Thermo Fisher). Sample 42 was lost during processing.

The qPCR data, expressed in quantification cycles (Cq) was converted into abundance by conversion equations. The equations are unique for each taxon and based on calibration curves of variable concentrations of the corresponding taxon (Harkes et al. Unpublished). Conversion formulas do not match perfectly with reality; this can sometimes cause unlikely ratios between taxa.

Data Analysis

Data analysis was done with the R statistical software (R Development Core Team, n.d.). The R package visweb (Dormann et al., 2009) was used for the correlational network matrixes (Spearman's rank order correlation, critical value of spearman's rho (r_s) = 0.285). The shading of the boxes indicates the size of r_s ; the bigger r_s is, the darker the colour of the box. The redundancy analysis was done with the R package 'vegan' and the R packages 'rgdal', 'mapplots', 'sp', 'gstat', 'spcosa', 'ggplot2', 'geoR', 'lmtest', 'stats' and 'raster' where used for the spatial distribution analysis.

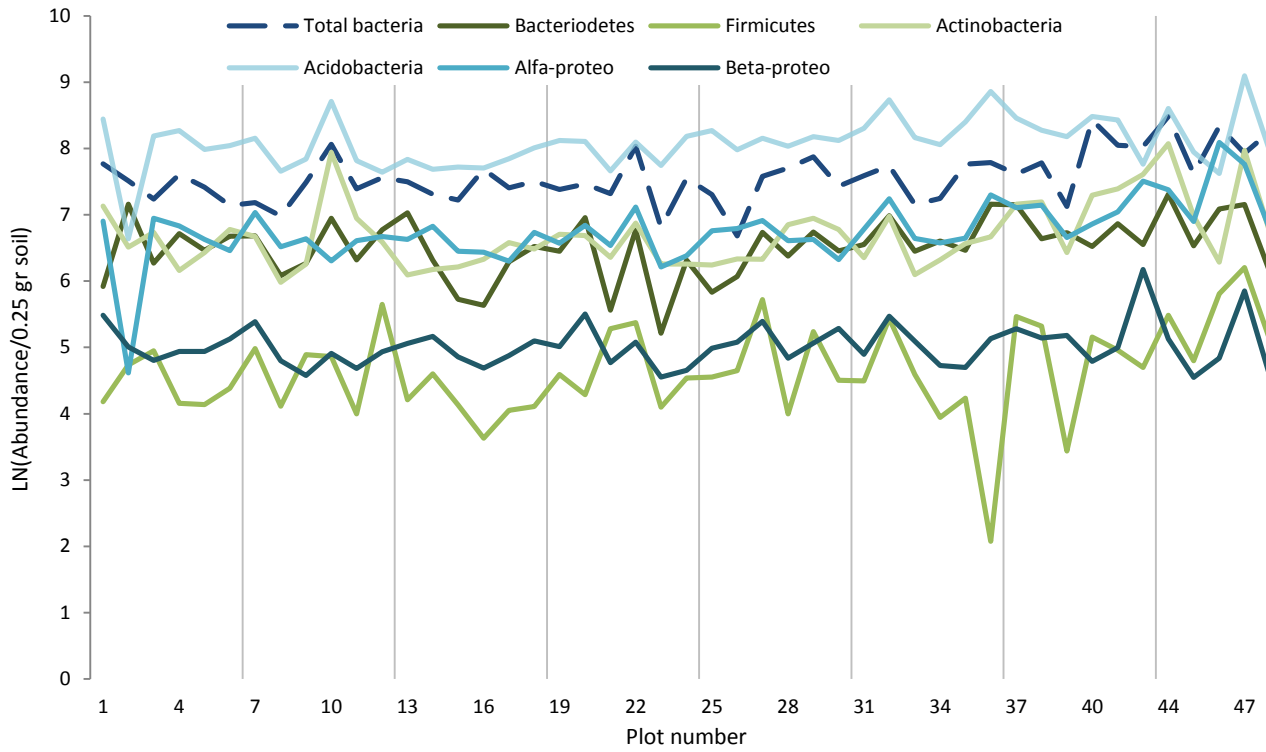


Figure 2. The abundance of bacterial groups per 0.25 grams of soil, on a natural logarithmic scale. Each gridline separates a transect of six samples.

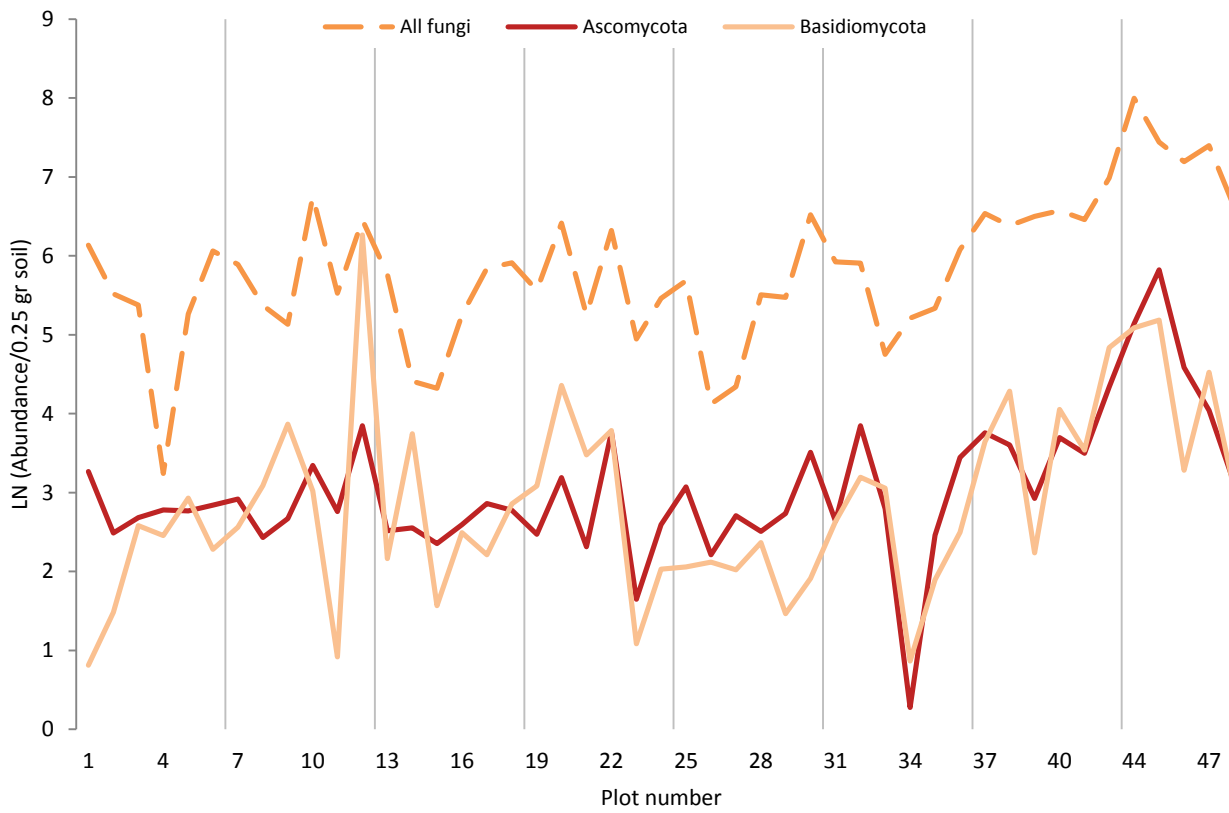


Figure 3. The abundance of fungal groups per 0.25 grams of soil, on a natural logarithmic scale. Each gridline separates a transect of six samples.

RESULTS

Overview of abiotic factors and nematode and microbial abundances

The nitrogen and phosphorus content of the area were respectively 950 N mg · kg⁻¹ soil and 120 P₂O₅/100g DW soil. The clay content of the soils was smaller than 5% (2%) and the soil is thus regarded as a sandy soil. The average pH was 5.2 and the average OM content was 3.3%. The pH and OM per transect can be found in the appendix in Table 7. Information on the abiotic factors of each sample.

In the measured area the Acidobacteria were the most abundant microbial taxa and the ACHR are the most abundant nematode taxa (Table 1). Most bacterial taxa show similarity in their pattern of abundance (Figure 2 and Figure 3) There are here and there however unique peaks and dips. The most deviant dip is that of the Firmicutes in plot 36. The fungal patterns also show a high degree of similarity. The Ascomycota have a unique peak in plot 11 and a dip in plot 2. The total fungi have a unique dip in plot 4. The abundances of nematodes, bacteria and fungi per plot can be found in Supplementary Table 5. Abundance of the bacterial taxa.

Correlations

Positive correlations between nematode taxa and microbial taxa

Four nematodes showed a significant positive correlation with a microbial group (Figure 4). The abundance of PRST was correlated with the most abundant group of bacteria, the Acidobacteria. ANAP occurred more in plots with a higher abundance of the total amount of bacteria, the same applies for the TCEP. TCEP also increased at higher densities of Actinobacteria and fungi. META showed a correlation with the Basidiomycota. META and TCEP are however not fungivorous nematodes.

None of the fungivorous nematodes showed a positive correlation with any of the microbial groups. Of the three omnivorous taxa only DOD3 showed a positive correlation, with the Actinobacteria.

Table 1. Microbial and nematode abundance (mean ± SD, n=47), PH and OM content (mean ± SD, n=8) and lutum, nitrogen and phosphorus content of the area.

Abundance per 0.25 gr soil		
Microbial community		
Acidobacteria	3517.0 ±	1446
Total Bacteria	2165.0 ±	1042
Alfa-proteobacteria	959.7 ±	506.0
Actinobacteria	936.7 ±	631.7
Bacteroidetes	737.8 ±	306.4
Beta-proteobacteria	162.2 ±	67.7
Firmicutes	129.6 ±	93.0
Total Fungi	502.3 ±	528.8
Basidiomycota	40.9 ±	82.2
Ascomycota	33.9 ±	53.2
Nematode community		
Total Nematodes	1511.0 ±	506
ACHR	1051.0 ±	1005
CEPH	507.3 ±	350.0
ANAP	252.9 ±	252.1
PRST	56.9 ±	48.5
MONH	45.3 ±	41.3
PLEC	32.2 ±	28.1
TCEP	25.1 ±	49.0
MESOR	16.0 ±	17.7
META	13.1 ±	24.2
CYLI	6.4 ±	13.9
GLA	0.9 ±	1.6
PRIM	0.7 ±	1.6
APHE	71.3 ±	42.9
ACHO	15.9 ±	17.3
DIPH	14.9 ±	20.4
TYLO	10.2 ±	13.5
DOPP2	164.2 ±	377.7
STEIN	80.2 ±	125.3
DOD9A	47.4 ±	45.4
PRACRE	42.8 ±	55.0
MYLO	30.3 ±	55.7
COS	17.8 ±	36.2
DOD3	11.3 ±	21.7
FIL3	11.3 ±	15.5
DOPP1	3.2 ±	5.3
HETER	3.0 ±	3.9
MONM3	1.8 ±	5.0
TYLH	0.8 ±	1.1
TRIP	0.5 ±	3.2
FIL2	0.2 ±	0.4
FIL1	0.0 ±	0.0
Abiotic factors		
pH	5.2 ±	0.2
OM (%)	3.3 ±	0.4
Lutum	2	%
Nitrogen	950	(mg·kg ⁻¹)
Phosphorus	120	(P ₂ O ₅ /100g DW soil)

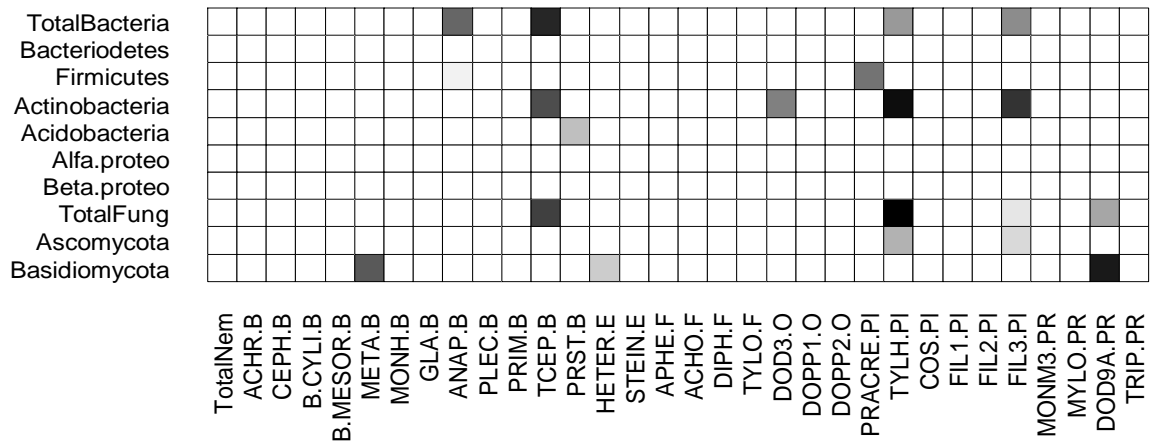


Figure 4. Network matrix of the significant positive correlations ($r_s > 0.285$) between the nematodes and the microbial community. Behind the abbreviations of the nematode groups a letter is placed referring to the feeding habit (The abbreviating ACHR.B thus means Achromadoridae.Bacterivorous. All abbreviations are displayed in Supplementary Table 3.)

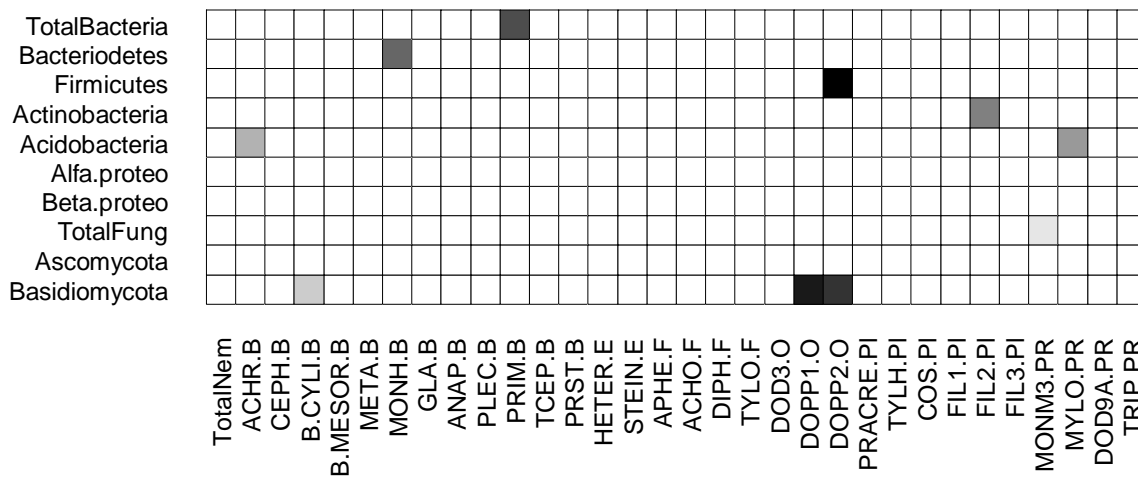


Figure 5. Network matrix of the significant negative correlations ($r_s > 0.285$) between the nematodes and the microbial community. Behind the abbreviations of the nematode groups a letter is placed referring to the feeding habit (All abbreviations are explained in Supplementary

Three positive correlations with plant parasitic nematodes were detected. PRACRE showed this with the firmicutes, TYLH and FIL3 with the total amount of bacteria, the Actinobacteria, the total amount of fungi and the Ascomycota. DOD9a was the only predatorous nematode showing a significant interaction with microbial groups. This was with the total amount of fungi and the Ascomycota.

Negative correlations between nematode taxa and microbial taxa

We observed a negative correlation for four bacterivorous nematodes (Figure 5); ACHR with the Acidobacteria, CYLI with the Basidiomycota, ONH

with the Bacterioidetes and PRIM with the total bacteria abundance.

No significant negative correlation was found for any of the fungivorous nematode groups.

Of the omnivorous nematodes both DOPP1 and DOPP2 showed a negative correlation with the Basidiomycota. The abundance of DOPP2 was also correlated with the Firmicutes. Only one negative correlation was found for the plant parasitic nematodes; for FIL2 and the Actinobacteria.

Of the predatorous nematodes MONM3 and MYLO showed a negative interaction; MONH3 with the total fungi and MYLO with the acidobacteria.

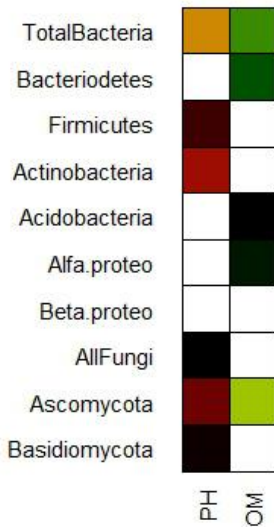


Figure 6. Correlation matrix for microbial groups with pH and OM. The correlations with pH are negative and coloured red, the correlations with OM are positive and coloured green.

Abiotic factors

All the fungal groups, the total bacterial abundance, the Firmicutes and the Actinobacteria showed a negative correlation with pH. The taxa that correlate negatively with the pH will from now on be called acidophilic. None of the microbial taxa correlated positively with pH (Figure 6).

Six nematodes taxa show a positive correlation with the pH and six taxa show a negative correlation. ACHR, GLA, TCEP, PRACRE, TYLH and FIL3 show a negative correlation with pH. They showed positive correlations with acidophilic microbial taxa (Figure 8) and each other. TYLH correlates with TCEP and FIL3 but there are no other positive connections between them. There are also no negative correlations between them.

CYLI, PRST, STEIN, DOPP1, DOPP2 and MONM3 correlated positively with pH. They show a negative correlation with the acidophilic microbial taxa (Figure 8) and the above mentioned nematodes (Figure 7). CYLI shows a negative correlation with ACHR, TCEP and TYLH, STEIN with FIL3, DOPP1 with GLA, DOPP2 with PRACRE and ACHR, MONM3 with TYLH and FIL3. There is only one exception: DOPP2 and FIL3 correlate positively.

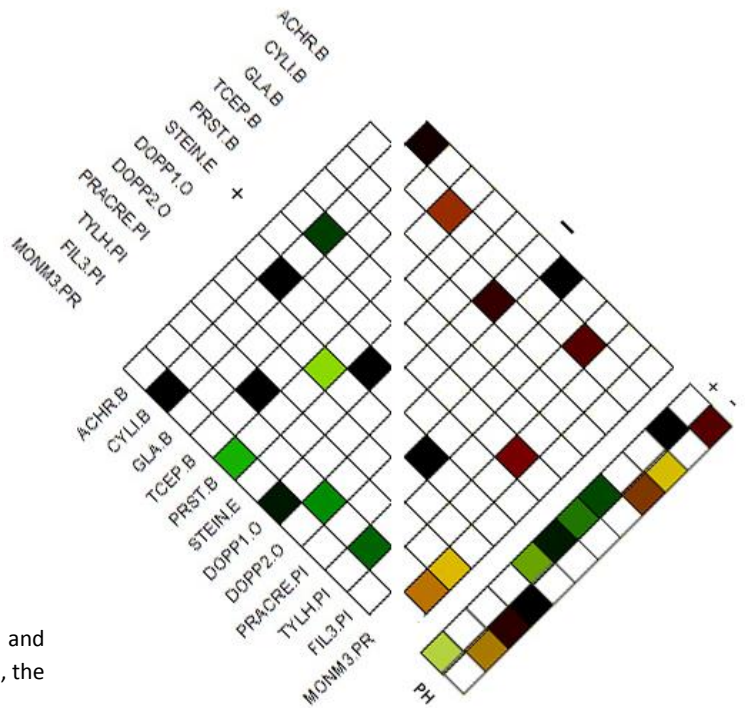


Figure 7. Correlation matrix for nematode taxa that showed a significant correlation with pH. The correlations of nematodes with the pH can be found at the right. The positive correlations the selected nematodes have with each other are depicted in the left, green-toned triangle and the negative correlations are in the middle, in the red-toned triangle.

The nematodes that correlate positively with the pH correlate positively with each other; CYLI is correlating positively with PRST, DOPP1 and MONM3, PRST with CYLI, DOPP2 and MONM3, MOMN3 with CYLI, PRST and DOPP1 and STEIN with DOPP1.

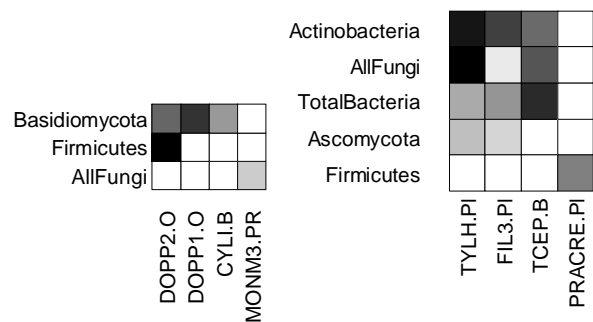


Figure 8. Acidophilic microbial groups and their correlations with nematodes of group 1 (8a, negative) or group 2 (8b, positive).

There were no significant negative correlations found for the microbial community with the OM content. There were however five taxa that correlated positively; total bacteria abundance, Bacteroidetes, Acidobacteria, Alfa-proteo bacteria and the Ascomycota (Figure 6). Only four nematode taxa showed a correlation with OM; CYLI, PRST and DOPP2. ACHR correlated negatively.



Figure 9. The significant correlations of nematodes with the organic matter. The green boxes are positive correlations and the red box is a negative correlation.

A redundancy analysis with the pH, the OM content, the acidophilic microbial groups and the nematodes that correlate with the pH can be found in the appendix (Figure 11).

Spatial distributions and visualisations

An overview of the pH and OM content per transect is shown in Figure 10. The spatial distribution of the nematodes correlating with the pH are displayed in Figure 12 and the spatial distribution of the fungal taxa and fungivorous nematodes are displayed in Figure 13. These figures can all be found in the appendix.

DISCUSSION

This discussion section will start with a short overview of explanations for the correlations found for the nematode and microbial taxa. This will be followed by a discussion on the resolution the bacterivorous primers are tested with. Here after we will introduce our proposition of the existence of two sub-communities. We will finish with a hypothesis regarding the interaction between fungivorous nematodes and fungi.

The total bacteria abundance was not higher than any other bacterial taxa while it should be the sum

of all bacterial taxa in the soil. The abundance of the Acidobacteria was higher, the difference on average 1300. This can be explained by the used conversion formulas. In the last decade molecular surveys of soils indicate a high abundance of Acidobacteria (Philippot et al., 2010) and this seems to be in accordance with our data. Earlier research also demonstrated the abundance of acidobacteria increases when the pH of the soil decreases. In the research Lauber et al. (2009) the relative abundance of the acidobacteria increased when the pH became lower. At a pH below five the relative abundance of Acidobacteria counted for the largest fraction of bacteria ($\pm 50-60\%$). At a pH between 5 and 6 the relative abundance was average $\pm 30\%$ and at a pH above 6 just $\pm 20\%$. In our research the Acidobacteria do not show a correlation with the pH. The differences in pH were possibly not low enough to observe a spatial distribution based on the pH of the Acidobacteria. The abundance of Fungi is also known to increase at a low pH (Bååth and Anderson, 2003) and our results confirm this. All fungal taxa showed a negative correlation with the pH (Figure 6).

Some microbial taxa did not show correlations with any of the measured nematode taxa. For the Betaproteobacteria a correlation with any of the measured nematode taxa, pH or OM content was absent. Also no correlations were found between the nematode community and Alphaproteobacteria or Bacteroidetes. The microbial groups might respond to factors we did not measure. Our measurements were performed at phylum or class level, the use of a smaller resolution might result in stronger correlations. The opinion by Philippot et al. (2010) suggests that bacterial habitat preference can be distinguished up to order level due to ecological coherence. The use of primers on order level might result in the detection of stronger and more correlations, but this could be phylum dependent; for some bacterial taxa measurements at phylum level could be sufficient.

In our experiment we see that the abundance of the Ascomycota and Basidiomycota vary throughout the spatial samples according to the same pattern, with some exceptions (Figure 3). It seems they are responding to common important factors (like

moisture content) while also having unique drivers of abundance. We for example expect that fungal taxa have specialised in decomposing specific substrates, causing a different niche preference (McGuire et al., 2010).

Primer resolution bacterivorous nematodes

Total nematode abundance did not show a positive or negative correlation with either total fungal and bacterial abundance or with the individual microbial taxa included in this study. These results show that there is not one microbial taxon influencing the abundance of the majority of nematodes. The response on the microbial taxa appears to be quite specific for each nematode taxon; in fact it even seems to be specific on genus level.

Four taxa of bacterivorous nematodes showed a significant positive correlation with bacterial or fungal taxa; META, TCEP, ANAP and PRST (Figure 4). Of these taxa only one genus is present in *the Mossel* (Supplementary Table 3), only the family META is presented by two genera. There were also four bacterivorous nematodes that showed a negative correlation: ACHR, CYLI, MONH and PRIM (Figure 5). Interestingly, MONH is presented by five genera, the other three by again just one.

Four of the twelve bacterivorous nematode taxa harbour more than two genera. Remarkably, three of them showed no correlations with microbial taxa; CEPH (9 genera), GLA (7 genera) and PLEC (4 genera). It thus seems it is less likely that correlations are found when primers are specific for several genera. Presumably the individual pattern of one genus becomes invisible when there are differences between the genera from one taxon. This might prevent the detection of correlations a genus has with microbial groups. For future research on correlations therefore advise to measure nematodes at genus level if possible.

Existence of sub-communities

Six nematode taxa appeared to correlate positively with the pH and six nematode taxa negatively. Based

on their correlation with the pH and their correlations with each other we can divide these nematodes in two groups; Group 1 (CYLI, PRST, STEIN, DOPP1, DOPP2 and MONM3) and Group 2 (ACHR, GLA, TCEP, PRACRE, TYLH and FIL3). In this section we will further illustrate the reasoning behind this division and introduce some additional hypotheses on factors that could support the division.

Group 1 correlated positively with the pH. These nematodes occur together and thus also show positive correlations with each other. Group 2 correlated negatively with the pH and the same is the case for the acidophilic microbial taxa (Total Bacteria Abundance, Actinobacteria, Firmicutes, Total Fungi Abundance, Ascomycota, Basidiomycota). Moreover did both Group 2 as the acidophilic microbial taxa show negative correlations with the nematodes of Group 1. Based on these correlations we can state that Group 1 and Group 2 do not occur together. We can divide the area in two sides: Side A and Side B (Table 2). The pH was of side A was higher than the pH of side B. Logically are the nematodes of Group 1 more abundant in Side A, the nematodes of Group 2 and the acidophilic microbes in side B. This can be seen in Supplementary Figure 12, where the spatial distributions of the nematode taxa of Group 1 and 2 are shown.

Table 2. Overview of the plots, transects and average pH of the Side A and Side B of the sampled area.

Side A - average pH = 5.3		Side B - average pH = 5	
Transect 5 Plot 25-30	Transect 6 Plot 31-36	Transect 7 Plot 37-41	Transect 8 Plot 43-48
Transect 1 Plot 1-6	Transect 2 Plot 7-12	Transect 3 Plot 13-18	Transect 4 Plot 19-24

The indication two sub-communities exist, gives rise to the hypothesis the biotic or/and biotic environment of these sub-communities differs. Differences in the measured OM content do not explain or invalidate this division. Our data shows the pH is different between side A and side B. These differences are however quite small (between 4.9 and 5.5). Observations based solely on pH should be

treated with caution. On the other hand, small differences in pH can cause big differences in the biochemical environment of the soil. The pH has a great influence on the bioavailability of for example phosphorus. The surface charge and surface potential of the soil is for example pH dependent and thereby also the adsorption and release of phosphorus. Furthermore, at a pH around 5.5 the speciation of phosphorus changes (Hinsinger, 2001). Hence, small changes in pH can already influence the soil system.

A change in the abiotic factors of the soil also affects the plant community. Small differences in pH can for example change the root exudates (Hinsinger et al., 2003). Big changes in pH can even alter the composition of the plant community. A change in pH can therefore also indirectly influence the soil community.

Perhaps the nematodes of Group 2 and the acidophilic microbial taxa are not only attracted by the lower pH but also, or more, by the environment the plants create. Plants that have more phosphorus to their disposal could for instance be less susceptible to plant parasites (Brennan, 1989). Our data corresponds with this hypothesis. The taxa of group 2 are more abundant in plots with a lower pH and thus lower phosphorus availability. There are also three plant parasitic nematodes in Group 2 (PRACRE, TYLH, FIL3), while there are none in Group 1. Another explanation could be that plant parasites have a close relationship with their host and might be attracted to some specific species that are more abundant in side B of the field.

The interaction between fungivorous nematodes and fungi

It was hypothesized that fungivorous nematodes would be attracted to their food source, resulting in a higher abundance in plots with high fungal abundance. Surprisingly, no significant correlations were observed between the abundance of fungal groups and fungivorous nematodes. We will try to explain this lack of correlation. We will also introduce a new hypothesis on the interaction

fungivorous nematodes have with their food source, based on the spatial distribution of the fungivorous nematodes and fungi.

There are big fluctuations in the fungal and fungivorous nematode abundance (Figure 3 and Table 3). These significant fluctuations cannot solely be explained by coincidence, indicating there is a response to some environmental factors. We thus believe the lack of correlation should be interpreted with considerable caution. It might for instance be the case that some fungivorous nematodes correlate with fungal taxa we did not measure, making some relationships possibly invisible. In plot 1 a peak of the total fungal abundance is visible, in plot 4 a big dip. There is however no significant change in the Ascomycota or Basidiomycota. This suggests a change in the abundance of different fungal taxa. Another explanation might lie in the spatial distribution of the fungivorous nematodes and fungi. When increases in nematode and fungal abundance are not at exactly the same place, no positive correlation can be found (Figure 2 and 3). The fact we found no correlation, does not mean it is not possible there is still an interaction between them.

The spatial distribution of the fungivorous nematode taxa and fungal taxa (Figure 13) indicate that the abundance of fungivorous nematodes is higher in plots that are located next to plots with a high fungal abundance. In other words, the fungivorous nematode abundance was not higher on locations with a high fungal abundance but next to it. Therefore we did not find correlations: the abundance of fungi and fungivorous nematodes is not higher in the same plots. They do not follow the same spatial pattern. The Basidiomycota abundance was for example high in plot 12 and much lower in plot 11 (Figure 3). Remarkably, there is no increase in fungivorous nematodes in plot 12. Instead there is, dependent on the fungivorous taxa, an increase in plot 9, 10 or 11. (Figure 13, Table 4). We can see the same in transect 8; the fungal abundance is high in plot 43-47 and lower in plot 48. On the other hand the abundance of the fungivorous nematodes increases in plot 48.

The fungal biomass could be smaller at places with high fungivorous nematodes abundance due to strong grazing. There are however some problems with this assumption. If this would be the case, you would expect to find negative correlations between fungal taxa and fungivorous taxa. We did not find negative correlations. Moreover, the fungal abundance is not always declining when the nematode abundance rise. Or in other words; high fungivorous nematode abundances are not located at the same place as fungal peaks, nor at fungal dips (Figure 13, Table 4). However, changes in fungi abundance and nematode abundance do occur close to each other indicating there is possibly a relationship.

The rise of fungivorous nematodes next to plots with a high fungal abundance could be explained by the cellular differentiation of fungal hyphae. The growth of hyphae takes place at the tips. Since growth requires nutrients, the tips tend to have a higher concentration of proteins and other substances (Gooday, 1971; Steele and Trinci, 1975; Zolotar, 1959). This biochemical differentiation could make the hyphal tips more attractive for nematode grazing. If fungivorous nematodes feed on the hyphae tips, their abundance would be higher in areas where hyphae will elongate. Unfortunately not all data necessary to research this statement was measured. It is unclear where the exact borders of a fungal community were located and whether these borders contained more fungal tips. Information on the spatial structure of the fungal community would enable us to make more underpinned conclusions.

RECOMMENDATIONS

Unfortunately it remains unknown what factors, apart from the pH, contribute to the emergence of the sub-communities we determined. For future research, we recommend a more in depth assessment of the abiotic factors of the soil in each plot as well as an elaborate analysis of the present plant community. For the abiotic factors we advise to include measurements such as the amount of

bioavailable phosphorus and nitrogen, the C:N ratio (Knorr et al., 2005), the pH, the moisture content and if possible the composition of the organic matter. The identification of the plant species that grow in each plot can contribute greatly to a better understanding of the soil environment. For future research this is advised.

To ensure the nematode and microbial abundances match the environmental factors we propose a different sampling method. It is quite common to mix cores taken in one plot. This excludes local differences, which are not in line with the general pattern, one is generally looking for. If we want to connect abiotic factors and plant community with soil biota, we should be certain this data is from the exact same place. For this purpose mixing of cores is not advised. The results of this study confirm that changes in the microbial community abundance can occur at very short distances (Ettema and Wardle, 2002). Spatial interpolation for the microbes appeared not possible. They did however manage to do this in earlier research (Philippot et al., 2009). To understand spatial distribution of microbes, sampling with shorter distances between plots might help. A change in the sampling design could also contribute to the research on the interaction between fungi and fungivorous nematodes. This study did not manage to obtain conclusive results on this relationship.

CONCLUSION

Our data highlights the specificity by which nematode taxa respond to their environment. Correlations between bacteria and bacterivorous nematodes even seem to be genera specific. We believe that the specific responses of nematode taxa lead to sub-communities in the soil biota where species with the same preferences for biotic or/and abiotic circumstances, occur in the same places. Future studies on these sub-communities are required to validate the division we made, to identify what biotic and abiotic factors further drive this division and to discover whether other soils also point at the existence of sub-communities.

An approach focussing solely on correlations seems to fall short when examining the nature of the interactions fungivorous nematodes have with fungi. We suggest that the interactions are more complex than initially thought and different strategies are needed to unravel this. Based on the spatial distribution of the fungivorous nematodes and fungi, we hypothesized that fungivorous nematodes might prefer the consumption of the nutrient rich hyphal tips.

We still have a rocky road to go before we can formulate conclusions on feeding behaviour of nematodes. Our work is a good step forward by contributing to the determination where nematode taxa are located and with whom. We hope that our recommendations are useful for further research on the interactions between the nematodes and primary decomposers of soil ecosystems.

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APPENDIX

Table 3. The selected nematode taxa and their feeding strategies.

Nematode taxa	Abbreviation	Number of genera present in the Mossel	Feeding strategy	Abbreviation
Achromadoridae	ACHR	1	Bacterivorous	B
Cephalobidae	CEPH	9	Bacterivorous	B
<i>Cylindrolaimus</i>	CYLI	1	Bacterivorous	B
<i>Mesorhabditis</i>	MESOR	1	Bacterivorous	B
Metateratocephalidae	META	2	Bacterivorous	B
Monhysteridae	MONH	5	Bacterivorous	B
Panagrolaimidae	PGLA	7	Bacterivorous	B
<i>Anaplectus</i>	ANAP	1	Bacterivorous	B
Plectidae minus <i>Anaplectus</i>	PLEC	4	Bacterivorous	B
<i>Prismatolaimus</i>	PRIM	1	Bacterivorous	B
<i>Teratocephalus</i>	TCEP	1	Bacterivorous	B
<i>Pristionchus</i>	PRST	1	Bacterivorous	B
Heterorhabditidae	HETER	1	Entomopathogenic	ENT
<i>Steinernema</i>	STEIN	1	Entomopathogenic	ENT
Aphelenchidae	APHE	2	Fungivorous	F
Aphelenchoididae	ACHO	2	Fungivorous	F
Diphtherophoridae	DIPH	1	Fungivorous	F
<i>Tyolaimophorus</i>	TYLO	1	Fungivorous	F
Dorylaimida D3	DOD3	5	Omnivorous	O
Dorylaimida PP1	DOPP1	3	Omnivorous	O
Dorylaimida PP2	DOPP2	1	Omnivorous	O
<i>Pratylenchus crenatus</i>	PRACRE	1	Plant parasite	PI
<i>Tylenchorhynchus</i>	TYLH	1	Plant parasite	PI
<i>Coslenchus</i>	COS	1	Plant parasite	PI
Filenchus group 1	FIL1	1	Plant parasite	PI
Filenchus group 2	FIL2	1	Plant parasite	PI
Filenchus group 3	FIL3	1	Plant parasite	PI
Mononchidae M3	MONM3	3	Predator	Pr
Mylonchulidae M1	MYLO	1	Predator	Pr
Dorylaimida D9A	DOD9A	4	Predator	Pr
Tripyla no Tripylella	TRIP	1	Predator	Pr
Total of nematodes	TotNem			

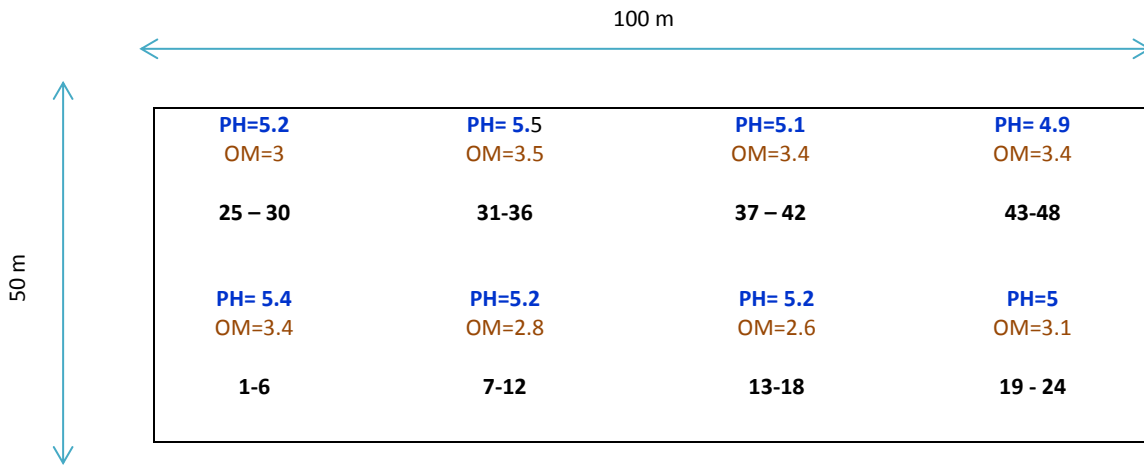


Figure 10. Visualisations of the pH and OM content for each transect.

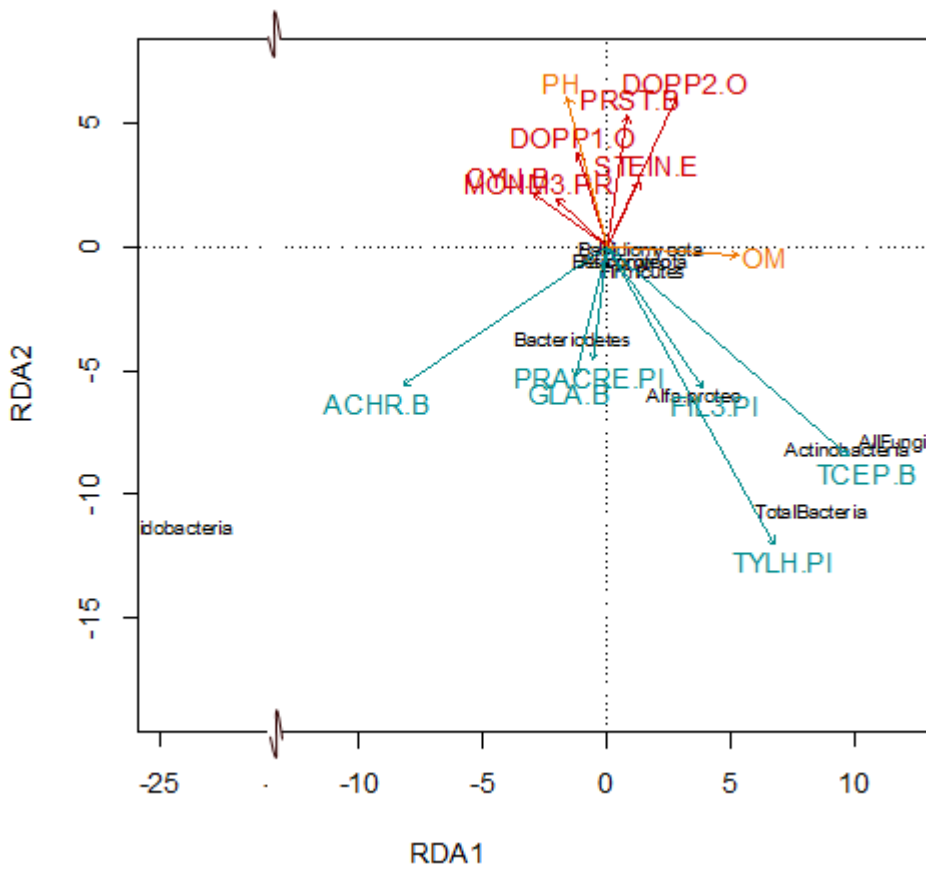


Figure 11. Redundancy analysis with the acidophilic microbial groups (black), group 1 (red), group 2 (blue), the OM content and the pH (orange).

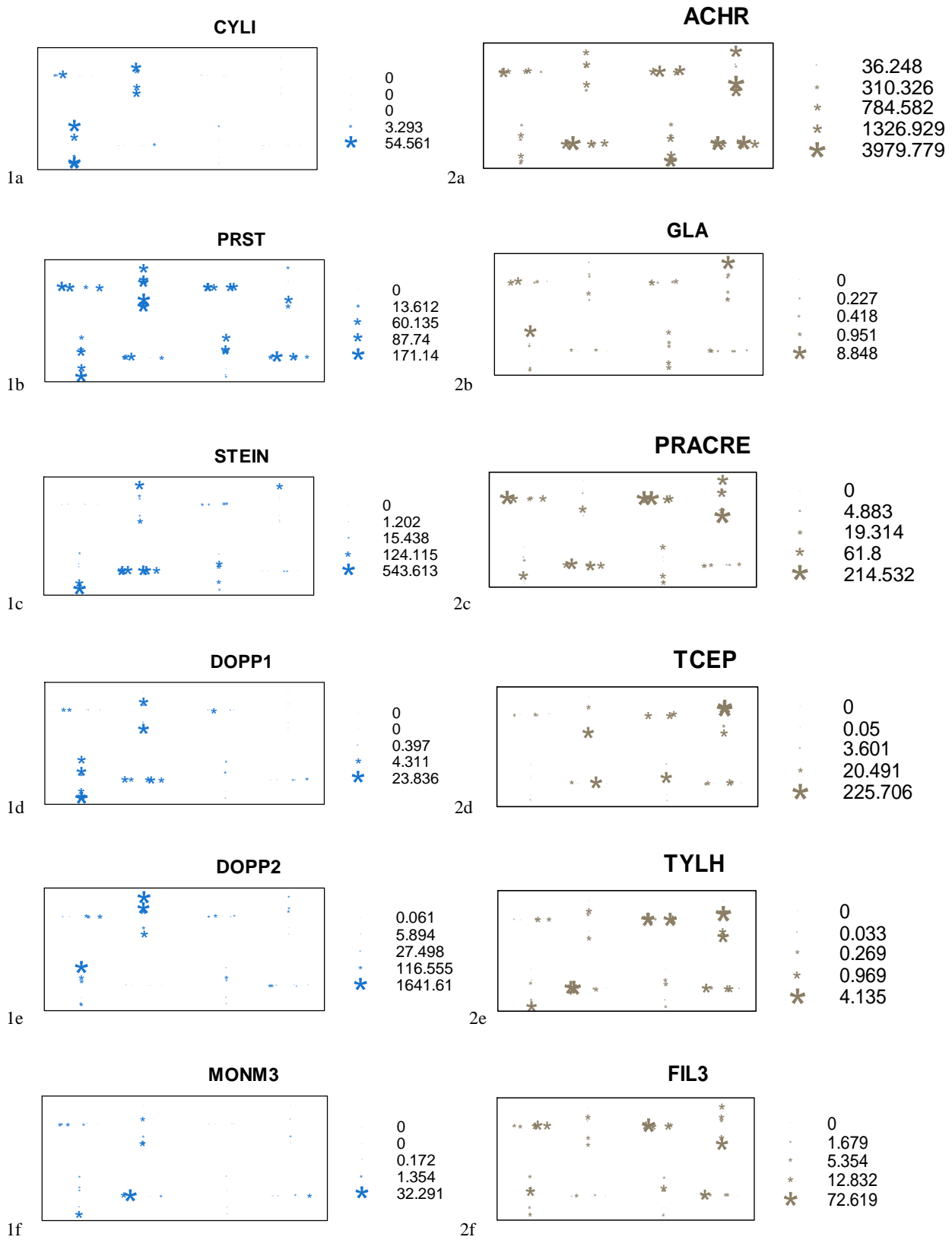
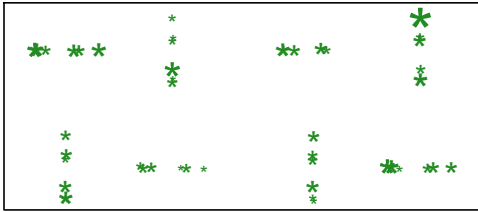


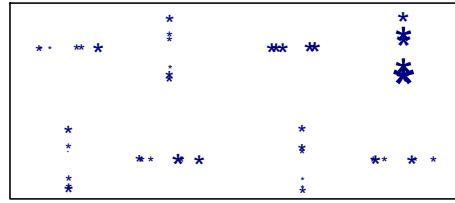
Figure 12. Spatial abundance distribution of the nematodes of group 1 (1a-1f) and group 2 (2a-2f). The values indicate the maximum value for corresponding star size.

APHE



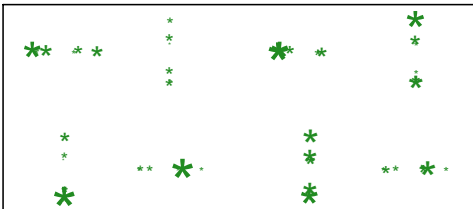
- 19.616
- 40.642
- * 60.509
- * 95.326
- * 257.895

Total Fungi



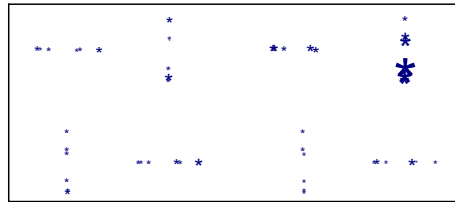
- 25.564
- 200.257
- * 344.541
- * 649.619
- * 2976.181

ACHO



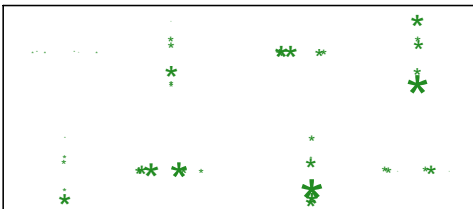
- 0.767
- 4.078
- * 7.82
- * 22.655
- * 65.534

Ascomycota



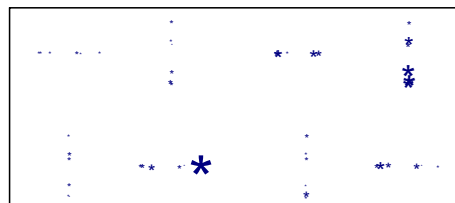
- 1.318
- 13.093
- 16.399
- * 34.58
- * 337.1

DIPH



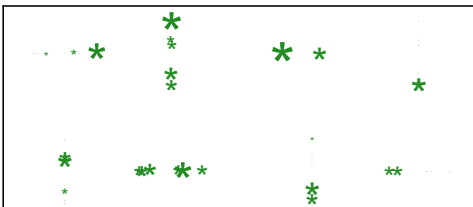
- 0
- 2.019
- * 6.524
- * 19.236
- * 93.607

Basidiomycota



- 2.254
- 8.053
- 13.906
- * 39.229
- * 525.467

TYLO



- 0
- 0
- * 3.278
- * 16.54
- * 56.698

Figure 13. Spatial distribution of the fungivorous nematodes and fungal taxa.

Table 4. Abundance of the nematode taxa

Sample	ACHR	CEPH	CYLI	MESOR	META	MONH	PGLA	ANAP	PLEC	PRIM	TCEP	PRST
1	191.36	403.24	43.06	0.43	0.00	73.88	0.72	196.31	78.74	0.00	0.00	165.55
2	312.98	766.68	54.25	25.00	0.08	82.49	0.29	78.08	24.65	0.22	1.40	15.34
3	535.90	506.59	3.31	39.75	1.40	65.67	0.00	69.17	43.37	0.00	0.00	72.90
4	200.68	276.24	0.00	1.10	2.86	15.71	0.48	178.13	31.70	2.11	0.09	87.95
5	583.22	271.89	21.34	2.62	0.10	36.71	0.10	43.33	12.95	0.25	0.05	35.90
6	152.71	286.24	54.56	24.07	0.46	34.67	7.37	111.50	19.75	0.08	0.00	28.71
7	1483.60	527.23	0.00	6.42	50.68	20.33	0.61	79.00	34.24	0.23	12.53	26.81
8	785.03	492.42	0.00	6.05	14.44	34.02	0.10	86.02	8.64	0.95	4.39	37.64
9	3583.42	530.62	0.00	12.07	148.81	266.45	0.00	1528.76	25.61	1.70	0.05	79.31
10	1149.09	288.70	0.00	23.10	5.82	33.16	0.05	348.85	42.02	0.16	5.21	0.00
11	413.74	541.37	0.00	30.63	8.83	44.24	0.53	329.87	66.37	0.13	132.96	0.00
12	1025.92	561.43	3.28	5.83	10.99	15.10	0.00	250.43	28.32	0.22	0.00	23.03
13	1669.93	1125.16	0.00	5.47	16.02	10.06	0.03	145.98	9.06	0.02	0.76	6.78
14	2594.57	1602.11	0.00	5.31	6.37	1.83	1.38	55.78	7.95	0.00	0.00	2.58
15	1068.40	1704.60	0.00	4.62	5.87	24.32	1.33	290.01	39.40	0.30	0.00	0.73
16	838.28	353.13	0.00	22.49	1.64	66.13	0.74	73.58	63.25	0.38	119.14	60.13
17	347.98	247.97	0.00	9.16	8.64	69.23	0.68	127.22	22.24	4.14	6.54	87.40
18	307.68	247.58	1.52	25.79	13.90	28.80	1.19	112.72	34.17	2.54	1.69	82.72
19	1054.04	515.39	0.00	19.19	12.06	41.97	1.12	262.56	15.65	0.14	10.75	4.01
20	3433.72	1522.69	0.00	30.42	1.06	75.15	0.89	486.97	57.08	0.43	23.65	27.91
21	898.43	211.71	0.00	14.88	5.33	31.04	0.32	248.30	7.39	0.00	0.19	141.24
22	1170.26	260.77	0.00	7.54	58.87	44.06	0.39	183.86	19.37	0.00	45.45	78.90
23	3286.44	309.96	0.00	24.95	9.90	56.88	0.40	112.73	39.99	1.08	16.26	20.01
24	1116.03	297.41	0.00	6.69	2.03	46.55	0.24	158.47	23.09	0.07	0.04	18.49
25	462.08	325.61	3.52	53.46	8.14	74.85	2.06	117.90	136.08	0.00	8.06	113.26
26	1523.40	174.42	1.31	1.41	0.80	46.11	0.47	48.71	8.50	0.63	1.68	7.82
27	497.43	578.95	24.80	1.46	9.73	33.72	2.98	320.26	80.85	0.08	0.00	94.74
28	484.80	152.80	0.00	3.42	0.02	18.33	0.32	188.62	14.55	0.91	13.69	22.36
29	677.83	375.39	0.00	8.60	0.01	19.34	0.67	536.54	7.85	0.03	3.85	0.00
30	88.91	293.96	0.00	6.33	0.04	11.64	0.08	258.78	18.22	0.00	1.07	65.81
31	68.57	472.55	22.69	36.98	19.47	85.75	0.21	983.36	28.37	0.23	115.13	162.26
32	156.19	283.53	3.41	7.30	0.86	21.70	0.00	157.47	6.39	0.56	21.28	87.53
33	784.58	486.30	16.42	100.67	4.70	96.21	1.08	350.30	16.50	0.53	0.00	171.14
34	208.87	457.20	10.90	8.05	4.02	6.23	0.28	88.30	6.24	0.00	1.71	121.86
35	754.32	536.72	35.26	20.41	1.31	24.11	0.20	152.77	13.51	0.00	19.70	92.64
36	618.34	365.68	0.00	11.48	26.73	32.19	0.11	140.22	5.07	0.00	0.00	95.12
37	129.10	247.66	0.00	1.75	27.26	19.87	0.40	288.91	36.37	6.60	47.62	92.03
38	1028.39	456.40	0.31	20.23	2.69	37.83	2.02	470.91	12.56	0.96	3.60	89.18
39	2170.32	473.62	0.00	9.33	0.18	46.36	0.27	202.28	33.03	7.94	0.00	62.63
40	799.90	388.52	0.00	6.42	11.61	24.79	0.38	395.07	23.62	0.07	52.44	87.37
41	1788.06	523.45	0.00	4.11	14.65	16.09	1.28	395.85	41.17	0.00	36.42	84.45
43	2512.64	1142.02	0.00	20.27	7.18	54.41	1.02	165.80	29.01	0.00	52.32	28.22
44	165.91	282.67	0.00	3.72	0.27	2.36	0.42	85.05	5.21	0.00	3.45	0.00
45	3979.78	329.19	0.00	37.21	54.28	113.70	0.54	145.27	50.16	0.00	10.60	81.57
46	89.91	535.37	0.00	35.64	9.34	47.07	0.41	460.78	42.64	0.12	225.71	0.00
47	36.25	403.17	0.00	1.38	3.64	25.36	0.81	176.63	14.85	0.00	181.58	0.00
48	2163.21	708.21	0.00	0.90	23.66	53.29	8.85	198.47	126.21	0.00	0.08	11.88

Sample	HETER	STEIN	APHE	ACHO	DIPH	TYLO	DOD3	DOPP1	DOPP2
1	0.00	543.61	30.58	5.16	5.43	0.00	39.82	23.84	92.48
2	0.17	160.60	99.95	65.53	25.15	0.00	18.19	10.38	74.65
3	3.16	222.45	94.98	6.41	1.85	4.66	3.35	5.60	1.20
4	2.41	3.94	37.92	0.77	4.55	23.37	1.42	1.98	155.82
5	1.20	6.21	69.82	5.14	2.18	16.08	5.83	14.98	260.55
6	3.73	20.08	60.51	11.76	0.49	0.00	7.76	10.15	1641.61
7	1.50	213.33	39.70	2.47	11.81	12.20	38.73	7.85	0.77
8	2.75	299.62	57.39	5.08	18.74	16.36	136.16	0.27	5.22
9	12.63	287.57	66.90	4.82	45.24	20.38	37.40	3.66	0.11
10	0.68	376.69	22.70	2.65	55.59	13.35	15.29	6.30	0.06
11	2.45	315.98	51.73	64.13	9.16	37.33	4.61	9.39	1.66
12	0.73	191.51	25.48	2.37	4.83	13.83	11.67	5.27	3.29
13	1.35	21.78	26.36	1.49	19.64	0.00	0.38	0.16	7.27
14	0.57	0.00	32.35	42.46	31.00	16.90	0.00	0.00	4.33
15	0.31	68.53	91.08	24.70	93.61	24.11	0.00	0.00	10.37
16	2.08	65.73	58.39	13.23	20.25	0.00	0.00	0.00	36.16
17	0.26	149.28	53.92	24.84	1.31	0.00	0.53	1.25	118.97
18	11.48	6.46	70.68	31.27	6.83	1.13	0.40	0.81	17.70
19	4.76	0.00	135.16	2.13	5.48	0.00	0.00	0.00	111.40
20	0.45	1.19	119.09	9.19	7.12	11.32	11.19	0.00	100.20
21	0.64	0.00	24.68	5.06	0.00	11.93	0.62	0.09	17.03
22	2.81	16.04	56.87	10.92	5.58	0.00	9.90	0.78	9.28
23	1.31	15.44	70.82	32.44	17.74	0.00	0.00	0.26	27.50
24	1.30	0.00	72.75	3.33	0.00	0.00	0.00	1.59	4.14
25	0.55	0.00	136.05	42.63	1.08	0.00	2.09	0.00	6.56
26	2.43	0.00	106.06	5.27	0.31	0.00	3.05	2.88	15.49
27	0.83	2.54	53.34	20.61	1.00	1.96	5.63	2.81	8.81
28	6.53	1.36	104.49	2.24	0.39	3.28	2.02	0.14	144.32
29	3.90	0.17	73.24	11.47	0.00	0.00	38.19	0.16	151.62
30	0.98	0.00	126.48	19.43	0.61	37.01	12.76	0.13	234.79
31	1.60	13.20	63.40	7.39	3.15	16.72	31.10	18.33	492.16
32	1.11	98.95	19.62	1.87	3.40	0.00	3.37	2.08	25.54
33	3.25	11.24	131.32	7.82	28.97	23.08	0.52	0.43	114.14
34	1.16	13.01	34.93	1.23	6.61	10.32	1.31	0.51	366.92
35	1.32	33.18	27.15	7.25	5.75	6.20	19.42	12.92	1405.40
36	0.28	315.07	29.78	5.11	0.00	45.88	0.00	0.00	1622.74
37	3.00	8.84	111.88	56.93	13.64	0.00	2.86	0.00	55.61
38	6.23	23.52	60.38	43.36	31.57	56.70	0.09	0.28	10.10
39	0.00	18.23	71.80	10.40	33.74	0.00	2.42	4.96	130.38
40	8.33	46.63	95.67	6.26	14.28	22.97	10.29	0.40	10.22
41	2.01	35.14	41.89	14.32	6.52	0.00	26.67	0.07	35.91
43	2.38	2.30	111.38	28.31	87.47	26.61	13.67	0.00	0.98
44	0.17	1.21	41.58	10.85	2.79	4.90	0.48	0.00	0.08
45	19.59	0.00	51.28	2.34	10.15	0.00	4.45	0.00	2.98
46	3.78	0.00	88.16	2.42	18.83	0.00	0.00	0.00	85.61
47	12.42	0.00	43.92	15.25	5.93	0.00	2.73	0.00	58.06
48	0.89	157.99	257.89	45.68	31.55	0.00	2.91	0.00	36.09

Sample	PRACRE	TYLH	COS	FIL1	FIL2	FIL3	MONM3	MYLO	DOD9A	TRIP	TotNem
1	0.00	1.88	157.07	0.00	0.00	6.32	11.36	3.91	16.21	0.00	2188
2	1.28	0.32	65.24	0.01	0.00	1.37	1.45	1.33	28.25	1.28	1585
3	73.00	0.08	31.75	0.02	0.00	3.71	0.46	5.71	24.05	73.00	1249
4	8.06	0.20	34.16	0.00	0.00	34.26	0.82	0.00	9.83	8.06	1812
5	2.50	0.02	0.00	0.00	0.04	5.23	0.80	7.87	35.56	2.50	2214
6	0.00	0.00	7.74	0.02	0.00	4.37	0.82	24.89	40.09	0.00	2172
7	40.59	3.00	0.00	0.00	0.00	0.00	6.39	5.40	19.34	40.59	1118
8	7.58	3.40	0.00	0.00	0.00	0.71	0.00	0.00	108.28	7.58	1522
9	108.54	0.06	15.01	0.02	1.49	5.68	32.29	0.00	180.24	108.54	1988
10	17.68	0.10	0.00	0.00	0.00	0.07	0.00	89.10	8.35	17.68	1325
11	87.81	0.41	0.00	0.00	0.77	1.99	0.00	1.20	53.50	87.81	1988
12	44.84	0.00	0.00	0.00	0.00	0.00	1.48	234.57	38.52	44.84	1486
13	22.57	0.13	0.25	0.00	0.56	3.42	0.08	114.82	10.25	22.57	1482
14	8.67	0.00	0.00	0.00	0.00	0.10	0.00	92.98	5.24	8.67	1914
15	28.07	0.03	4.83	0.00	1.18	0.00	0.13	244.19	10.49	28.07	2105
16	0.00	0.38	9.95	0.00	0.00	4.16	0.00	59.81	13.45	0.00	1744
17	0.00	0.27	0.00	0.00	0.00	18.98	0.00	11.77	10.79	0.00	1230
18	28.90	0.00	2.71	0.00	0.00	2.51	0.31	0.47	106.88	28.90	1283
19	22.12	0.28	0.00	0.00	0.00	8.44	0.00	20.06	47.18	22.12	1488
20	8.07	1.39	0.00	0.00	0.83	42.19	0.13	20.46	226.94	8.07	1835
21	17.68	0.23	5.33	0.01	0.00	4.50	0.22	8.05	23.08	17.68	856
22	3.69	0.83	10.31	0.00	0.00	4.88	0.72	15.90	30.10	3.69	1281
23	6.08	0.96	0.00	0.00	0.00	4.67	0.00	94.83	28.51	6.08	2436
24	13.78	0.03	0.00	0.00	0.00	0.00	3.58	86.52	36.21	13.78	793
25	179.15	0.03	153.25	0.00	1.44	0.92	3.06	5.79	21.63	179.15	2361
26	76.40	0.00	4.83	0.00	0.00	4.38	0.90	2.53	18.70	76.40	961
27	67.37	0.00	4.01	0.00	0.00	7.60	3.07	6.67	37.87	67.37	1727
28	19.31	0.13	2.88	0.02	0.00	18.21	1.47	0.03	25.76	19.31	874
29	12.82	0.60	2.55	0.04	0.00	34.64	0.17	0.00	23.38	12.82	1078
30	42.52	0.43	0.00	0.02	0.00	26.72	0.00	0.00	38.41	42.52	1218
31	1.22	0.38	0.03	0.00	0.00	6.67	6.78	3.07	61.31	1.22	2243
32	0.00	0.07	37.08	0.00	0.43	0.00	2.92	1.63	28.01	0.00	726
33	51.41	0.00	86.84	0.06	0.00	6.92	1.26	11.88	49.16	51.41	1502
34	3.28	0.18	0.00	0.00	1.17	0.48	4.84	19.48	18.14	3.28	767
35	0.00	0.72	0.00	0.01	0.00	11.32	0.00	13.22	23.69	0.00	1489
36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.05	54.54	0.00	1082
37	194.23	3.26	0.00	0.00	0.00	26.82	0.00	0.63	49.27	194.23	1281
38	62.68	2.15	37.34	0.00	0.00	72.62	0.00	0.42	87.35	62.68	1610
39	194.81	0.14	83.16	0.04	0.00	16.12	0.00	2.41	21.78	194.81	1113
40	60.92	3.06	0.00	0.01	0.00	20.81	0.12	0.25	31.74	60.92	965
41	48.49	1.14	19.52	0.03	0.00	8.60	0.00	5.36	118.85	48.49	2205
43	214.53	2.41	0.00	0.00	0.00	61.63	0.00	131.95	30.85	214.53	2501
44	12.47	0.98	0.58	0.00	0.00	5.98	0.00	0.28	3.65	12.47	334
45	29.82	0.91	0.00	0.03	0.00	11.43	0.94	5.05	65.63	29.82	1280
46	73.07	4.14	59.80	0.00	0.54	5.35	0.33	1.75	133.88	73.07	1791
47	14.21	1.20	0.00	0.01	0.00	12.31	0.00	0.60	75.31	14.21	1451
48	101.35	0.00	0.00	0.01	0.00	13.36	0.00	62.86	95.85	101.35	1387

Table 5. Abundance of the bacterial taxa.

Sample	Bacteria (Total)	Bacterioidetes	Firmicutes	Actinobacteria	Acidobacteria	Alfa-Proteo	Beta-Proteo
1	2363.43	372.90	65.30	1249.44	4637.95	997.00	240.73
2	1831.40	1289.38	113.93	672.83	764.05	100.87	149.43
3	1388.67	530.27	141.30	840.66	3604.90	1041.05	122.29
4	2017.78	825.03	63.87	473.32	3911.51	928.59	139.87
5	1659.13	640.44	62.82	620.59	2933.00	754.62	139.60
6	1262.60	795.26	80.22	877.29	3110.03	638.64	168.93
7	1319.04	797.99	146.09	790.60	3474.75	1134.51	218.67
8	1074.36	437.88	61.25	395.41	2118.29	678.18	121.65
9	1787.37	527.42	132.96	521.02	2545.33	765.29	97.18
10	3176.65	1042.59	129.56	2811.11	6062.33	548.23	135.44
11	1625.69	555.77	54.51	1039.35	2478.37	740.40	108.04
12	1918.98	876.37	284.31	727.93	2085.91	789.30	139.13
13	1805.65	1126.61	67.43	442.73	2528.07	756.91	157.22
14	1496.44	552.41	99.43	480.98	2182.12	921.49	175.25
15	1370.61	306.02	62.25	498.37	2248.04	632.60	128.82
16	2175.26	279.58	37.68	559.75	2221.94	622.33	108.48
17	1648.98	538.42	57.54	721.28	2551.15	544.56	131.17
18	1813.70	680.03	60.90	653.59	3003.49	842.40	163.92
19	1606.65	631.25	98.47	818.89	3365.62	713.21	150.28
20	1740.77	1050.45	72.70	802.95	3318.75	936.73	245.76
21	1508.73	260.71	196.36	577.33	2129.37	689.34	118.13
22	3082.35	875.09	216.03	965.89	3288.49	1234.46	160.90
23	907.98	183.58	60.23	520.46	2306.94	498.32	94.87
24	1905.43	553.08	93.48	520.36	3587.88	590.77	105.02
25	1484.18	340.68	94.76	514.50	3904.18	860.07	146.83
26	797.07	432.56	104.38	562.28	2918.73	892.58	160.39
27	1953.72	840.03	304.53	561.29	3472.07	1004.51	220.10
28	2220.70	587.69	54.60	945.80	3077.50	742.13	126.49
29	2633.30	845.06	188.15	1038.72	3557.45	757.57	158.62
30	1687.58	636.08	90.31	879.68	3365.13	558.35	198.07
31	1977.12	699.09	89.36	573.40	4047.76	882.77	133.61
32	2292.01	1083.43	229.97	1072.69	6205.00	1399.12	236.93
33	1282.47	633.44	98.63	444.59	3503.71	765.78	163.06
34	1402.74	737.11	51.63	555.95	3158.66	716.96	113.07
35	2348.03	643.14	68.88	709.46	4440.03	772.25	109.60
36	2410.79	1284.40	7.96	785.69	7019.26	1479.54	169.02
37	2025.94	1276.37	235.95	1286.76	4713.78	1221.77	196.93
38	2397.94	763.71	204.72	1327.64	3930.80	1266.11	171.17
39	1246.04	836.48	31.15	621.08	3559.69	780.23	177.94
40	4570.78	679.49	173.84	1470.59	4822.68	954.30	120.52
41	5850.26	808.29	137.20	1537.71	4750.02	1046.12	187.61
43	3071.41	699.08	109.71	2013.07	2346.79	1822.47	479.38
44	4819.40	1496.01	240.96	3198.73	5454.63	1594.81	168.34
45	2032.74	687.95	121.13	1075.54	2827.03	993.33	94.62
46	4157.61	1194.72	332.93	536.71	2040.75	3268.52	126.26
47	2766.51	1280.62	494.42	2889.54	8891.58	2348.91	347.02
48	3857.18	461.96	165.82	841.15	2856.63	878.29	96.11

Table 6. Abundance of the fungal taxa.

Sample	Fungi (Total)	Ascomycota	Basidiomycota
1	461.11	26.23	2.25
2	249.18	12.03	4.39
3	216.20	14.59	13.21
4	25.56	16.10	11.65
5	192.67	15.93	18.68
6	428.49	17.12	9.76
7	361.42	18.47	12.96
8	215.29	11.35	21.93
9	169.63	14.42	47.83
10	853.85	28.32	20.43
11	250.83	15.75	2.50
12	634.83	46.72	525.47
13	318.86	12.36	8.68
14	82.14	12.81	42.38
15	75.21	10.50	4.78
16	190.86	13.38	12.10
17	344.54	17.46	9.13
18	369.66	16.02	17.41
19	260.60	11.83	21.79
20	609.57	24.25	78.13
21	190.44	10.10	32.39
22	555.08	43.09	44.10
23	140.35	5.20	2.95
24	235.76	13.38	7.59
25	293.73	21.57	7.81
26	61.76	9.12	8.30
27	76.66	14.97	7.55
28	245.60	12.26	10.66
29	237.94	15.38	4.33
30	678.37	33.43	6.75
31	372.95	13.96	13.91
32	367.77	46.75	24.39
33	115.72	16.40	21.23
34	182.83	1.32	2.38
35	207.84	11.70	6.64
36	436.20	31.38	12.13
37	688.51	42.78	38.14
38	589.76	36.77	72.59
39	664.41	18.62	9.35
40	716.25	40.30	57.53
41	759.53	35.73	40.31
43	1081.89	76.39	125.94
44	2976.18	170.54	161.84
45	1706.79	337.10	178.76
46	1328.85	97.99	26.64
47	1627.78	56.79	92.27
48	760.67	22.97	21.37

Table 7. Information on the abiotic factors of each sample.

Sample	X m	Y m	PH	N mg · kg ⁻¹	Phosphorus mg · 100 g DW	OM %	LUTUM %
1	12.5	5.75	5.4	950	120	3.4	0.02
2	12.5	6.25	5.4	950	120	3.4	0.02
3	12.5	8.25	5.4	950	120	3.4	0.02
4	12.5	14.25	5.4	950	120	3.4	0.02
5	12.5	15.25	5.4	950	120	3.4	0.02
6	12.5	19.25	5.4	950	120	3.4	0.02
7	28.25	12.5	5.2	950	120	2.8	0.02
8	28.75	12.5	5.2	950	120	2.8	0.02
9	30.75	12.5	5.2	950	120	2.8	0.02
10	36.75	12.5	5.2	950	120	2.8	0.02
11	37.75	12.5	5.2	950	120	2.8	0.02
12	41.75	12.5	5.2	950	120	2.8	0.02
13	65	5.75	5.2	950	120	2.6	0.02
14	65	6.25	5.2	950	120	2.6	0.02
15	65	8.25	5.2	950	120	2.6	0.02
16	65	14.25	5.2	950	120	2.6	0.02
17	65	15.25	5.2	950	120	2.6	0.02
18	65	19.25	5.2	950	120	2.6	0.02
19	80.75	12.5	5	950	120	3.1	0.02
20	81.25	12.5	5	950	120	3.1	0.02
21	83.25	12.5	5	950	120	3.1	0.02
22	89.25	12.5	5	950	120	3.1	0.02
23	90.25	12.5	5	950	120	3.1	0.02
24	94.25	12.5	5	950	120	3.1	0.02
25	5.75	37.5	5.2	950	120	3	0.02
26	6.25	37.5	5.2	950	120	3	0.02
27	8.25	37.5	5.2	950	120	3	0.02
28	14.25	37.5	5.2	950	120	3	0.02
29	15.25	37.5	5.2	950	120	3	0.02
30	19.25	37.5	5.2	950	120	3	0.02
31	35	30.75	5.5	950	120	3.5	0.02
32	35	31.25	5.5	950	120	3.5	0.02
33	35	33.25	5.5	950	120	3.5	0.02
34	35	39.25	5.5	950	120	3.5	0.02
35	35	40.25	5.5	950	120	3.5	0.02
36	35	44.25	5.5	950	120	3.5	0.02
37	58.25	37.5	5.1	950	120	3.4	0.02
38	58.75	37.5	5.1	950	120	3.4	0.02
39	60.75	37.5	5.1	950	120	3.4	0.02
40	66.75	37.5	5.1	950	120	3.4	0.02
41	67.75	37.5	5.1	950	120	3.4	0.02
43	87.5	30.75	4.9	950	120	3.4	0.02
44	87.5	31.25	4.9	950	120	3.4	0.02
45	87.5	33.25	4.9	950	120	3.4	0.02
46	87.5	39.25	4.9	950	120	3.4	0.02
47	87.5	40.25	4.9	950	120	3.4	0.02
48	87.5	44.25	4.9	950	120	3.4	0.02

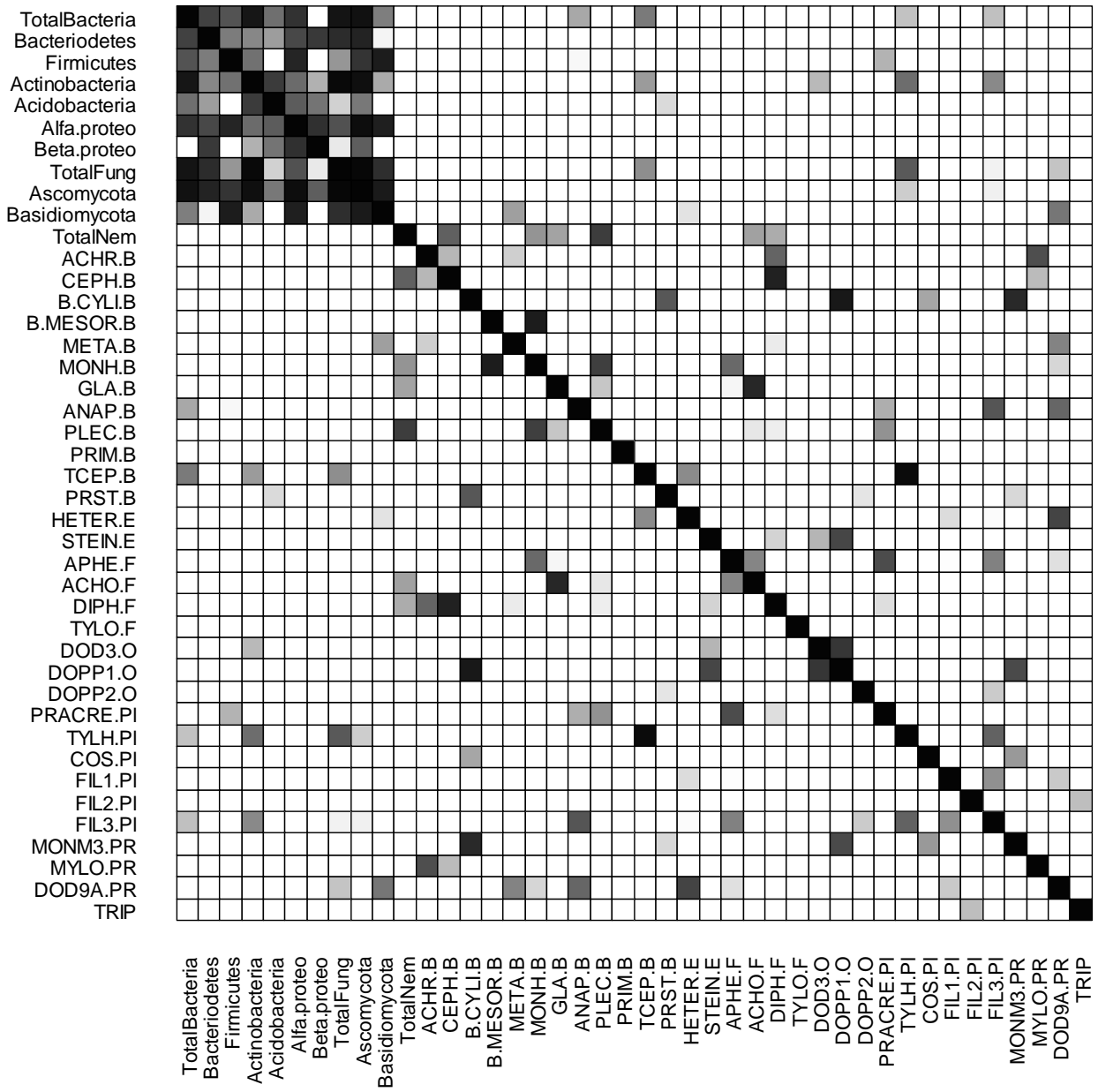


Figure 14. Network matrix of all positive correlations between the nematodes and primary decomposers.

