Research article

Characterizing nematode communities in various types of environmental samples using Oxford Nanopore sequencing

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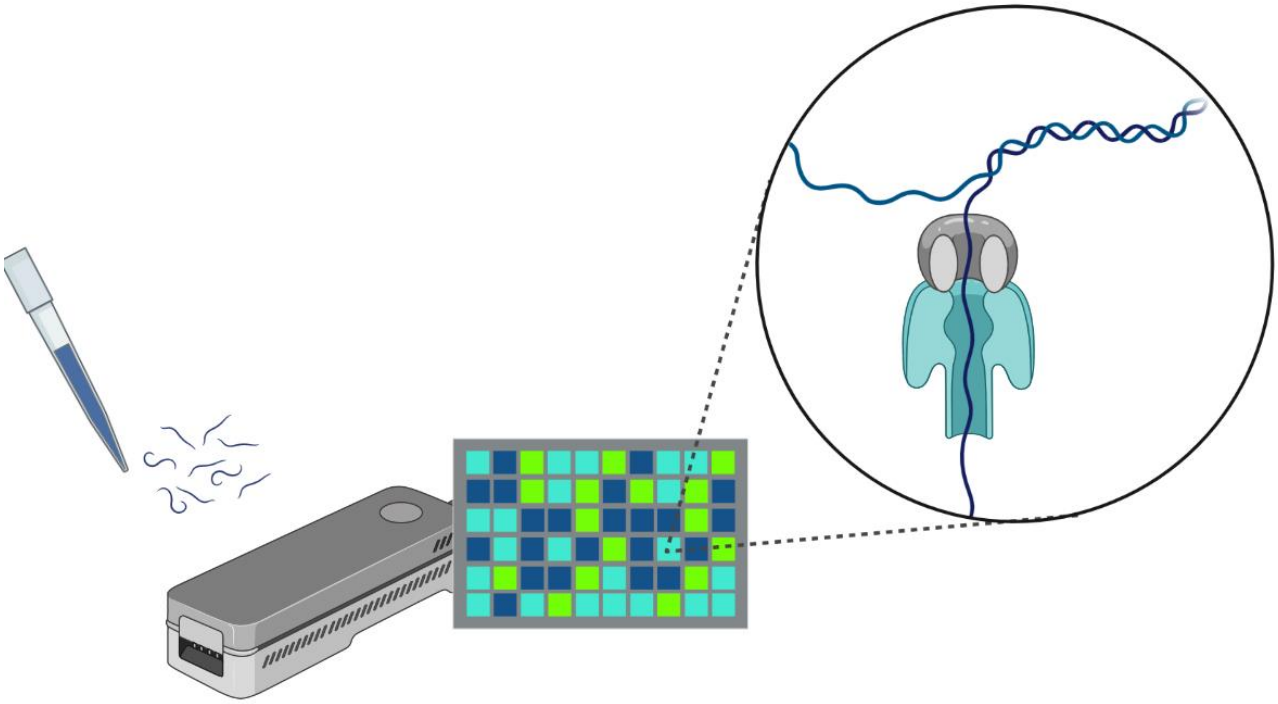

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Abstract

Nematodes are a key component of the soil microbiome, as they greatly contribute to soil nutrient cycling and the upkeep of soil fertility. Accurate characterization of nematode communities is pivotal to any researcher in the field. Current metabarcoding techniques mainly focus on relatively small regions within the small subunit ribosomal DNA, which results in resolution till (at best) genus level. As species within a genus can still be very different from each other, the lack of resolution hampers a researcher's ability to analyse complex nematode communities. This report evaluates the use of Oxford Nanopore MinION sequencing to characterize nematode communities. Using two primers to amplify the nearly complete 18S small-subunit rDNA fragment, the Nanopore sequencing method, and a bioinformatics pipeline called Decona, 75 community samples were analysed and 94 unique nematodes species were characterized up to species level. This report also gives a direct application by analysing nematode communities that were collected in the surroundings of Wageningen from habitats that meet the ecological requirements of the bacterivorous nematode *Caenorhabditis elegans*. This study identified several differences in nematode community composition in various *C. elegans*-friendly micro-habitats and it is expected that Oxford Nanopore sequencing could be instrumental for nematode community analysis in general.

Introduction:

In the past, most attention was paid to parasitic nematodes which might be detrimental to plant or animal health and agricultural production. However, other nematodes have been shown to greatly contribute to soil nutrient cycling and thus nutrient availability of plants (1,2). Estimations have been made that, up to 40% of nutrient mineralisation is due to nematodes and the microbial populations they feed on (3). As the understanding of the significance of nematodes increases, nematodes have been used more and more as, for example, indicators of soil health (4,5). In current research, there is still no accurate way of characterization of nematode communities, up to species level or even up to genus level(6). Therefore it would be highly useful to develop a method to accurately characterize complex nematode communities, as community composition information can be essential for many kinds of research.

Multiple high throughput sequencing techniques can be used to characterize nematode communities. Currently, the most common way is using the Illumina sequencing platform (7), which uses different fluorescently labelled terminators and a laser to analyse the DNA sequence base-by-base with a relatively short read length (Table 1). This technique has the advantage of having the highest throughput, read accuracy, and the lowest per-base cost (8). However, because of these short reads, the taxonomic resolution level is often too low for analysing soil communities. In metabarcoding, which allows for simultaneous characterization of multiple taxa in one sample, a higher read length can result in more information to achieve a species level resolution. Table 1 shows several other different commonly used sequencing techniques for characterising the soil microbiome. Of these techniques, Oxford Nanopore sequencing seems very promising for metabarcoding purposes. This technique uses a flow cell that contains tiny holes, called nanopores. When a molecule passes through this pore, the current in an associated electrode is disrupted in a specific way. This can then be used to determine a DNA or RNA sequence in real-time. The study of Nygaard et al. (9), found that between the Illumina and nanopore sequencing methods, at the level of family and above, no significant

difference between the bacterial compositions was revealed. However, at the genus level and more specifically the species level, the use of Oxford Nanopore MinION sequencing showed a greater taxonomic resolution than using Illumina sequencing. Using nanopore sequencing, the problem of low taxonomic resolution might be resolved. Oxford Nanopore sequencing to characterize nematode communities has never been documented in scientific literature, which shows the knowledge gap covered by this project. A reliable and robust way of characterizing nematode communities accurately up to species level will also greatly benefit the scientific community when investigating the role of the microbiome in an ecological or agricultural context.

Table 1: Overview of different commonly used sequencing techniques for characterizing soil microbiome.(10,11)

Platform	**Sequencing technology**	**Read length**	**Output/run**	**Error rate**	**Example of use**	**Type of instrument and run time**
Illumina	Sequencing by synthesis	Short reads 1x36 bp – 2x300 bp	0.3-1000 Gb	Low	Variant calling	Benchtop 2 - 29 h
Ion Torrent	Sequencing by synthesis	Short reads 200-400 bp	0.6-15 Gb	Low	Variant calling	Benchtop 2 - 4 h
PacBio	Single molecule sequencing by synthesis	Long reads up to 60 kb	0.5-10 Gb	High	De novo assembly of small bacterial genomes and large genome finishing	Large scale 0.5 - 4 h
Oxford Nanopore	Single molecule	Long reads up to 100 kb	0.1-20 Gb	High	Complete genome of isolates and metagenomics	Portable 1min - 72 h

The goal of this study is to evaluate if Oxford Nanopore MinION sequencing can be reliably used to characterize nematode communities in a qualitative and (semi-)quantitative manner. As it is highly conserved and can be very species-specific, the small-subunit (SSU) rDNA (appr 1,700bp) is found to be a promising region with enough resolution to be able to characterize a wide range of parasitic and nonparasitic nematodes (12). To evaluate the use of nanopore sequencing we used 75 nematode community samples. These community samples were collected from different sites around Wageningen (The Netherlands) and originated from different rotting substrate materials, such as fruit, vegetable, plant and soil material. All samples were taken from model organism *C. elegans* friendly environments. The DNA from these communities was isolated and amplified, sequenced using the Oxford Nanopore MinION Mk1C device, characterized with the use of a pipeline called Decona, and a multivariate analysis was done to analyse the effects of abiotic factors on community composition. This study evaluates if MinION sequencing can be reliably used to characterize nematode communities up to species level in ecological context.

Materials and Methods

Sampling

The collection of sample material and the following steps described in this paragraph were done by L. van Sluijs at the Wageningen University Laboratory of Nematology. In the summer and fall of 2021, 210 nematode communities from different locations around Wageningen (NL) were sampled. These samples originated from different rotting substrate materials, being fruit, vegetable, nutty (walnut), and plant material. Adding to this, soil samples were also taken. As L. van Sluijs is mainly interested in *C. elegans*, all samples were taken from *C. elegans* friendly environments. Furthermore, the temperature, pH, and humidity were recorded during sampling. The nematode populations were separated from their substrate material using the blender nematode filter method (13) and the number of nematode species present were subsequently determined using a light microscope. In preparation for further analysis, DNA was extracted from the nematode communities (14). This resulted in 80 samples from which DNA was successfully extracted.

DNA amplification

To amplify the nearly complete 18S rRNA gene, the primer 988F (5'- ctcaaagattaagccatgc-3') was used in combination with primer 2646R (5'-gctaccttgttacgactttt-3') (12). An initial pilot experiment including samples with a known nematode composition showed that these primer combination yields reliable results (results not shown). Primer pairs were barcoded with barcode sequences of the EXP-NBD196 kit (Oxford Nanopore Technologies ltd., UK). LongAMP Taq 2x MM DNA polymerase was used and the PCR cycle conditions were as follows: one cycle of 94°C for 3 min., followed by 30 cycles of 94°C for 30 s + annealing temperature of 57°C for 30 s + 65°C for 2 min., and finally one cycle of 65°C for 10min. After DNA amplification, 5µl of product was loaded on a 1,5% agarose gel to verify the amplification. Of the 80 samples, 78 were successfully amplified. Due to time constraints, the remaining two were omitted. The concentration of all PCR products was measured using a broad range dsDNA Qubit assay with a Qubit 4 Fluorometer (15).

Library preparation and sequencing

For practical reasons, the 78 samples were divided into 4 sequencing libraries and in each library samples were pooled in equimolar ratios. To remove unwanted small fragments (<600bp), each library was bead cleaned using NucleoMag NGS beads (16) with a ratio of 0,5(beads):1(sample)

For sequencing, the MinION MK1C, flowcell R9.4.1, and Ligation Sequencing Kit SQK-LSK112 were used. Following the ligation sequencing of amplicons protocol SQK-LSK112 (17), for the first three libraries, around 180 fmol of the pooled library was used for the DNA repair, end-prep, adapter ligation and clean-up steps. 80fmol of pooled library was used for the fourth library run, as this library lacked a sufficient DNA concentration. The first library was sequenced for 5 hours, while the other three were sequenced for more than 40 hours. This was to make sure enough reads were generated. Basecalling was not done on the MinION device, but using the program Guppy (v 6.2.1) (Oxford Nanopore Technologies ltd., UK), in super accuracy mode (./guppy_basecaller -c dna_r9.4.1_e8.1_sup.cfg -x cuda:0).

Bioinformatics

The basecalled reads were demultiplexed using Guppy barcoder(v 6.2.1.) (guppy_barcoder –barcode_kits “EXP-NBD196 --trim-adapters --trim_barcodes). Very few reads were generated with samples LvS26, LvS81, and LvS148, so these three were excluded from the analysis. The basecalling quality was determined using the program NanoPlot (v.1.40.0) (mean Phred quality score >15). The pipeline called Decona (v0.1.3.) (18) was used to filter read length (1400-2600bp) with quality (q 15), cluster at 95% and build Medaka consensus sequences from each cluster larger than 100 sequences (decona -f -l 1400 -m 2600 -q 15 -n 100 -g 1 -c 0,95 -M -B). NCBI’s BLAST+ command line tool is integrated in Decona, and an in-house reference database was used for taxonomic identification.

Data analysis

All medaka summary BLAST files were merged using a custom python script and identifications with a ID percentage below 97% were excluded. From the resulting Decona output, data such as the total number of species per sample, average number of species per sample, and most abundant species per location/type was extracted.

Furthermore, the resulting Decona output was processed using R studio (v.4.1.2). A pseudo count of +1 was added to all data points to deal with all 0s. A centred log-ratio transformation was performed on the sequence data to deal with the compositional nature (19). As this makes the data symmetrical and linear, a redundancy analysis (RDA) was used to compare the relationships between environmental properties and nematode community composition.

Results

In total, 94 different species were characterized up to species level (Appendix 2). For some nematodes, only the genus name could be noted. This is due to the database lacking specific species information. Compared to the species identification using microscopy, the number of species found after sequencing was substantially higher. In only 2 of 75 cases, both species counts were the same (Appendix 3). The average species count per sample when identified morphologically was 4. While the average species count per sample after sequencing is 9.

Table 2 shows a more elaborate overview of the nematode community composition based on the number of times a specific species was present. The number of community samples which are part of a specific location and/or substrate type is shown, together with the average nr of species per community sample. Furthermore, the total number of unique species that were found per location and/or substrate is shown. The average number of species per sample is around 8 species per sample. Some outliers are present at the locations 'Heelsum – Plant & Soil'. Which both have an average of 11 species per sample. What is notable, is that the fruit and soil communities are a lot less diverse than the communities at other locations. Contrary to that, the plant material communities have the highest total number of unique species found, excluding the low amount found at 'Joost – Plant'. The two vegetable-type communities have a comparable number of unique species found.

Table 2: Overview of nematode community distribution at different locations and substrate types. The three main locations and individual substrate type components are given.

Location	Total nr of community samples	Average nr of species per sample	Total nr of unique species
Heelsum	37	9.7	81
Beekdal	15	8.1	57
Garden Joost	23	8.2	63

Type/location	Total nr of community samples	Average nr of species per sample	Total nr of unique species
Heelsum			
Vegetable	16	7.7	44
Plant	17	11.9	64
Fruit	3	7.7	13
Soil	1	11	11
Beekdal			
Plant	15	8.1	53
Joost			
Vegetable	16	8.2	46
Plant	4	8.25	24
Nut	3	8.3	20

Figure 1 shows the overview of nematode community composition at different locations and substrate types based on the number of times a species was characterized. As stated in Table 2, a lot of different unique species have been found. To increase readability, the 6 most abundant species are shown while the rest is gathered in an 'other' group. As 'Heelsum – Soil' only had one community sample included, and 11 unique species were found in total, no scale in abundance could be made and all 11 species are included in the figure. Notable about this graph is that *Caenorhabditis elegans* (CaenEle) is one of the top 6 most present species in all but one (Joost – Nut) types and locations. The genus *Panagrolaimus* is also abundantly present in all types and locations, although divided into 2 known (PGLaSub and PGLaRig, respectively, *P. subelongatus and P. rigidus)* and 2 unknown species.

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Figure 1: Overview of nematode community composition at different locations and substrate types based on the number of times a species was characterized. The data is shown on a scale from 0% to 100%. For complete names see Appendix 2. When a species name could not be assigned due to a lack of information in the database, the genus name + sp(nr). is noted

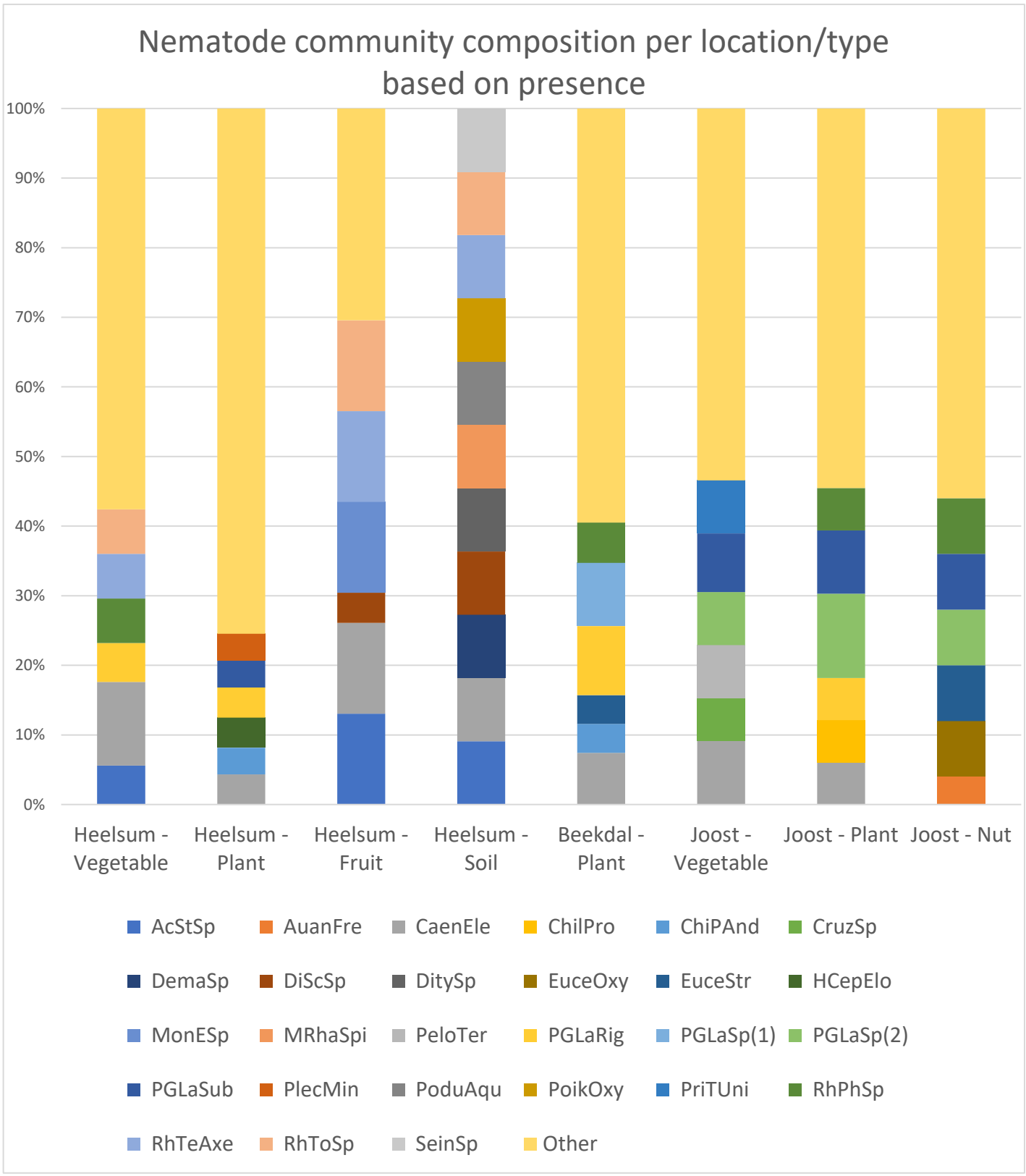
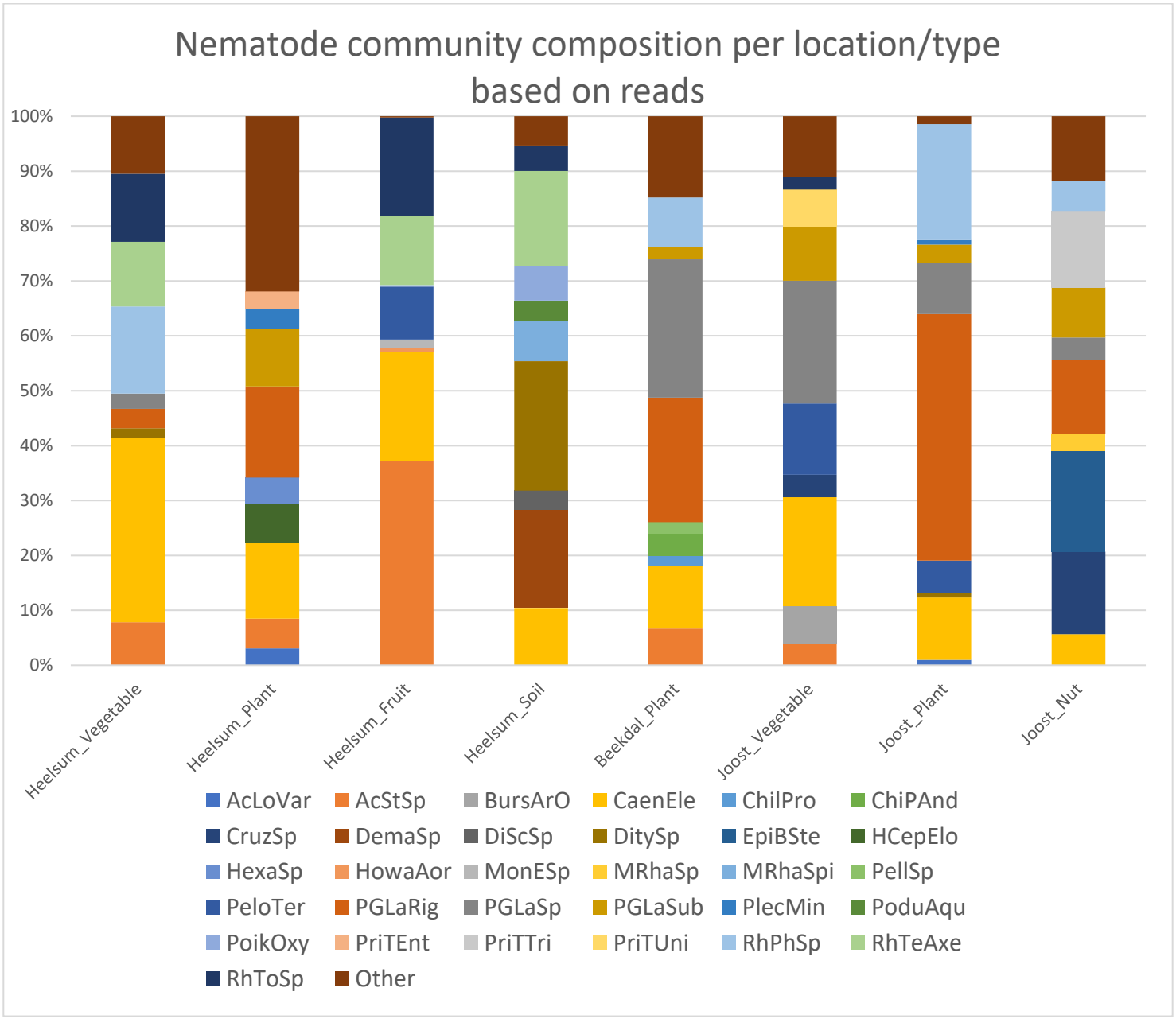


Figure 2 shows the overview of nematode community composition at different locations and substrate types based on the number of reads generated during sequencing. To be able to compare the communities of different locations and types of substrate, the number of reads is shown on a scale from 0% to 100%. Again, the diversity of species characterised is quite high. The 9 most relatively abundant species are shown, and as in figure 1, *Caenorhabditis elegans* (CaenEle) is present in all locations and types of substrate. A difference is that species of the genus *Panagrolaimus* (PGLaRig, PGLaSp,PGLaSub), are now only present in the 9 most abundant species in vegetable and plant material.

Figure 2: Overview of nematode community composition at different locations and substrate types based on the fraction of the number of reads produced by sequencing. For complete names see Appendix 2. When a species name could not be assigned due to a lack of information in the database, the genus name + sp. is noted. The number of reads is presented as fractions of the total amount, hence the 0% to 100% scale on the y-axis.



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RDA analysis

According to the RDA analysis, the amount of variance in nematode community composition that is explained by the abiotic factors is 25%. A permutation test shows temperature, location, substrate type, and pH, as significant contributors to this variation (temperature P=0.02, location P=0.01, substrate type P=0.01, pH P=0.03). Table 4 shows the percentage of variation explained purely by these three individual factors after accounting for the other factors. The substrate type explains most of the variation in nematode community composition, compared to the other factors.

Table 4: Percentage of variation explained by individual environmental factors after accounting for the other factors (constrained variance)

Environmental factor	
Substrate type	8,7%
Location	6,5%
Temperature	1,8%
pH	1,5%

Figure 3 shows the RDA triplot of the relationship between nematode community composition and environmental factors. Three distinct groups of species are found that appear outside the main congregation in the centre of the graph (Table 5). The occurrence of two *Panagrolaimus sp*. In different groups can be explained by them being two different species, but the database lacked the specific species name. The grouping of these species suggests that they occur together. In Figure 3, group A is associated the most with the factors 'Temp', 'TypeFruit', and 'TypeSoil', and the least associated with factor 'pH'. This suggests that these species are associated with a higher temperature, and a lower pH compared to the other species. Group B is mostly associated to 'LocationJoost_Garden' and 'Type_Vegetable'. Group C is associated to the factors 'TypePlant' and 'LocationBeekdal'.

Table 5: Composition of species groups which occur with similar environmental factors. When a species name could not be assigned due to a lack of information in the database, the genus name + sp. is noted.

Group A	Group B	Group C
Caenorhabditis elegans (CaenEle)	*Panagrolaimus sp.* (PGLaSp)	*Panagrolaimus rigidus* (PGLaRig)
Acrostichus sp. (AcStSp)	*Pelodera teres* (PeloTer)	*Panagrolaimus sp.* (PGLaSp)
Mononchoides sp. (MonESp)	*Panagrolaimus subelongatus* (PGLaSub)	
Rhabditella axei (RhTeAxe)	*Pristionchus uniformis* (PriTUni)	
Rhabditoides sp. (RhToSp)	*Cruznema sp.* (CruzSp)	

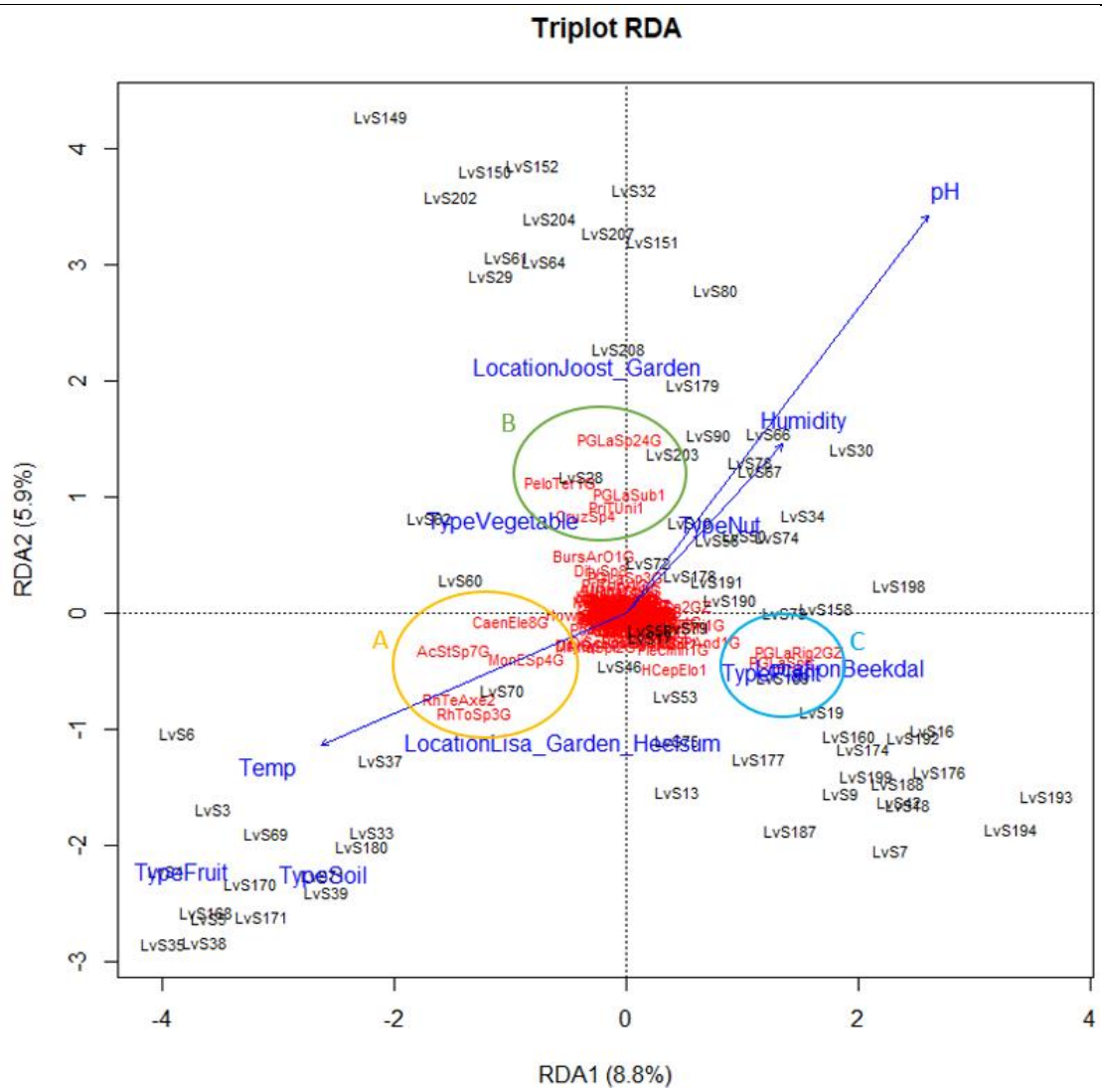

Figure 3: Redundancy analysis (RDA) distance triplot of the relationship between nematode community composition and environmental factors. Species codes are indicated in red, environmental factors in blue, and whole community samples in black.

Discussion and conclusion

Prior work has documented the importance of nematode soil communities to plant health and agricultural production(5,20). Adding to this, the study of Gao et al.(4) shows that studying the nematode community composition is a powerful tool as an indicator of soil health. The traditional ways of characterizing nematode communities using a wide range of taxon-specific qPCR assays, Illumina sequencing, or light microscopy have the inherent weakness that the taxonomic resolution is hindered by the limited number of available qPCR assays or by the limited length of the barcoding region. In this study, the method to use Oxford Nanopore MinION sequencing to characterize nematode communities is evaluated. As the study of Nygaard et al. (21) shows on bacterial communities, this method has a much greater resolution at the genus level and more specifically the species level.

This study was able to generate reads of 1.7kb using the Oxford Nanopore MinION sequencing method with an accuracy of over 97%. With some uncertainties discussed later, 94 unique nematodes were characterised up to species level. The primers and techniques laid out in this article can probably be a promising way to characterize complex nematode community samples. The nematode communities found in the environmental samples turned out to be very diverse, as there was even a difference in species present in samples with the same conditions. However, some nematode species were present in almost all samples. *Caenorhabditis elegans* is the most abundant of these species. This is expected, as the original sampling was done to collect *C. elegans* associated samples. Nematodes from the genus *Panagrolaimus* are abundantly present in all samples when purely counting presence (*Figure 1*). However, this genus was only abundantly present in vegetable and plant material according to the collected reads (Figure 2). While this genus is not part of the 9 most abundant species, it is present in all location/types in the “other” category, excluding “Heelsum_Soil”.

This difference also extends into the observation that all communities are quite diverse, but with only a couple of nematode species resulting in up to 70% or more of the produced reads (Figure 2). The substrate type, location, temperature, and pH of the samples are the most influential factors for nematode community composition. This is as expected, as studies show changes in pH and temperature greatly impact the composition of nematode communities(22–24). In this current study however, the assumption must be made that the sampling temperature was representative as an average temperature for that sample as only one temperature measurement was done. Factors like substrate type, location, temperature, and pH however, are only a small portion of variables that affect nematode communities. Other interacting microbes for example, which this study didn't take into account but have been shown to greatly impact nematode community composition (25).

Some species also occur relatively more often with the same other species compared to all other species (Table 5). There are three recognizable groups which all seem to occur together under certain environmental conditions, like a higher temperature, lower pH, or certain substrate types. The found association of *C. elegans* with substrate type fruit is confirmed by the study of Schulenburg *et al*. (26), as they found *C. elegans* particularly abundant in microbe-rich environments and especially in decomposing fruit matter. Literature is still unclear about if these species of groups are often found together. However, the species found in these groups are all classed as enrichment opportunists (*Appendix 2*) (27), which often occur together under food-rich conditions. Rotting material is often rich in microbes and thusly it can be expected that all these bacterial feeding nematodes are present together.

The method shown in this report for characterizing nematode communities is novel in current academic literature, as the use of Oxford Nanopore MinION sequencing has never before been applied in combination with metabarcoding of nematode communities. The results of this report provide a compelling basis for future research regarding nematodes, as current studies still have problems when characterizing nematode communities, even up to genus level (6).

There are however, also ways to improve the use of Oxford Nanopore sequencing to characterize nematode communities. For example, lowering the number of DNA amplification cycles could decrease the amplification bias. More amplification cycles lead directly to a greater difference in the amount of DNA present after amplification of a species which was already abundantly present and started with a lot of DNA, compared to an obscure species which started the amplification process which much less DNA(28). This study chose 30 cycles to make sure that enough DNA would be produced for sequencing. To decrease sequencing run time, it is recommended for future researchers to pre-emptively decide how complete their communities need to be characterized as over 70% of the nematode community samples consist of only 10 species.

Improvements in the bio-informatics can also be made. The accuracy of characterization can improve by altering the BLAST output of Decona. Currently, it assigns the name with the best identity score to a sequence. However, the script does not handle different names with the same identity scores well. The first name is picked and no convenient way exists to check if another viable name was available, which might result in wrongly characterized nematodes. Also, future researchers are recommended make use of duplex basecalling (29), which can further increase accuracy and reliability.

This report presents the use of Oxford Nanopore sequencing when characterizing complex nematode communities, and finds this technique very promising. This report evaluated the use and showed a direct practical application in the field of ecology. There are however still some pitfalls and recommendations which future research needs to address to further increase the accuracy and reliability of the results.

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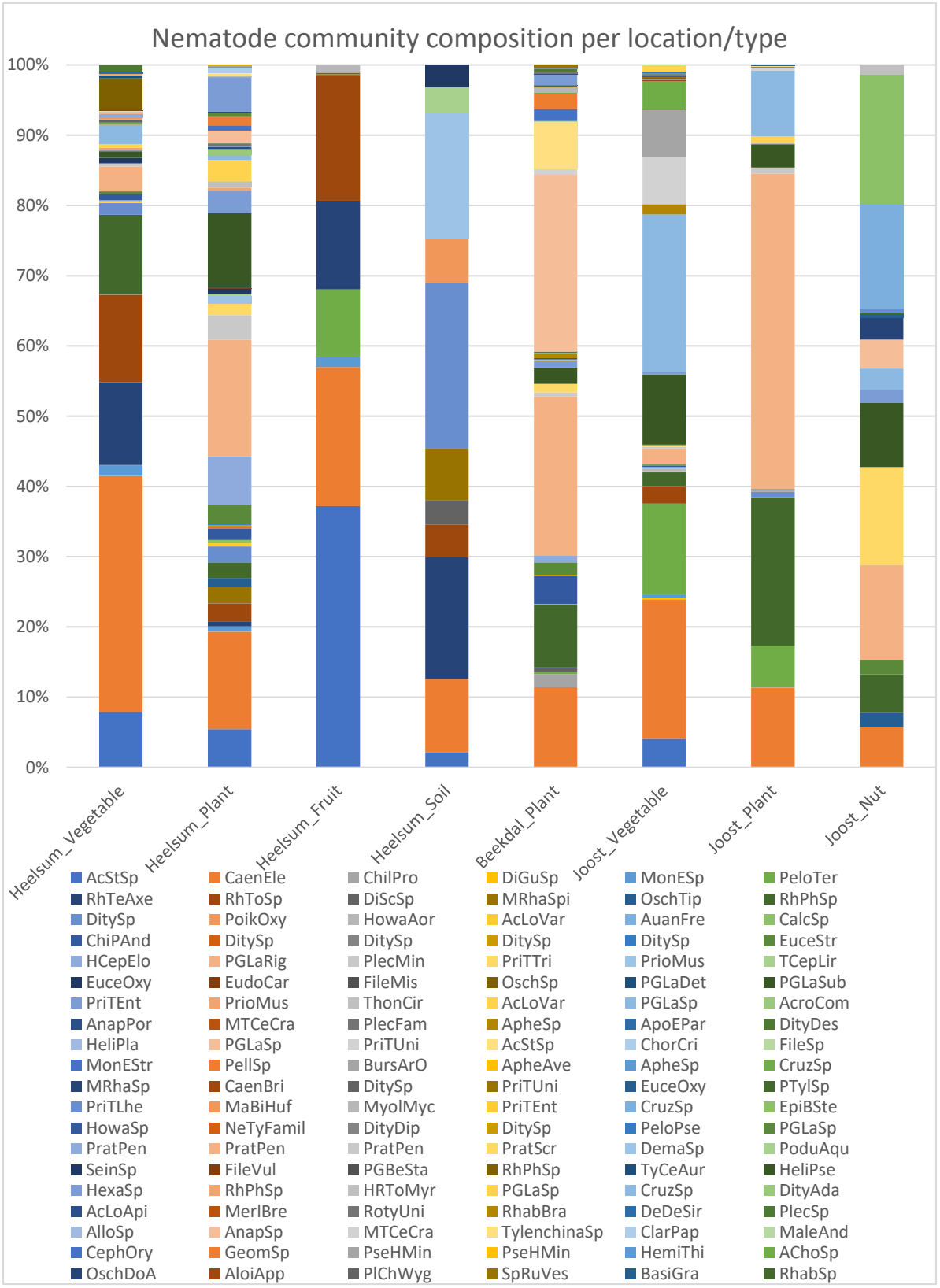
Appendix:

Appendix 1: full nematode community composition per location/type based on the fractions of the amount of reads generated during sequencing. For full genus and species names see Appendix 2



393 *Appendix 2: Overview of species code, full genus and species name, cp type, and feeding type*.

Code	**Genus**	**species**	**cp type**(27)	**feeding**(30)
AChoSp	Aphelenchoides	sp	basal fauna	fungal hyphal feeding
AcLoApi	Acrobeloides	apiculatus	basal fauna	bacterial feeding
AcLoVar	Acrobeloides	varius	basal fauna	bacterial feeding
AcroCom	Acrobeles	complexus	basal fauna	bacterial feeding
AcStSp	Acrostichus	sp.	enrichment opportunist	bacterial feeding
AlloSp	Allodorylaimus	sp.	intermediate succession and disturbance sensitivity	Omnivory
AloiApp	Alloionema	appendiculatum	enrichment opportunist	bacterial feeding
AnapPor	Anaplectus	porosus	basal fauna	bacterial feeding
AnapSp	Anaplectus	sp.	basal fauna	bacterial feeding
ApheAve	Aphelenchus	avenae	basal fauna	fungal hyphal feeding
ApheSp	Aphelenchus	sp.	basal fauna	fungal hyphal feeding
ApoEPar	Aporcelaimellus	Paraobtusicaudatus	long-lived intolerant species	Predation
AuanFre	Auanema	freiburgensis		dispersal stages or animal parasites
BasiGra	Basiria	gracilis	basal fauna	plant feeding
BursArO	Bursaphelenchus	arthuroides	basal fauna	fungal hyphal feeding
CaenBri	Caenorhabditis	brenneri	enrichment opportunist	bacterial feeding
CaenEle	Caenorhabditis	elegans	enrichment opportunist	bacterial feeding
CalcSp	Calcaridorylaimus	sp.	intermediate succession and disturbance sensitivity	Omnivory
CephOry	Cephalobus	oryzae	basal fauna	bacterial feeding
ChilPro	Chiloplacus	propinquus	basal fauna	bacterial feeding
ChiPAnd	Chiloplectus	andrassyi	basal fauna	bacterial feeding
ChorCri	Choriorhabditis (Rhabditis)	cristata	enrichment opportunist	bacterial feeding
ClarPap	Clarkus	Papillatus	intermediate succession and disturbance sensitivity	Predation
CruzSp	Cruznema	sp.	enrichment opportunist	bacterial feeding
DeDeSir	Deladenus	siricidicola	basal fauna	fungal hyphal feeding
DemaSp	Demaniella	sp.	enrichment opportunist	bacterial feeding
DiGuSp	Diplogastrellus	sp.	enrichment opportunist	bacterial feeding
DiScSp	Diploscapter	sp.	enrichment opportunist	bacterial feeding
DityAda	Ditylenchus	adasi	basal fauna	fungal hyphal feeding
DityDes	Ditylenchus	destructor	basal fauna	fungal hyphal feeding
DityDip	Ditylenchus	dipsaci	basal fauna	fungal hyphal feeding
DitySp	Ditylenchus	sp.	basal fauna	fungal hyphal feeding
EpiBSte	Epitobrilus	Stefanskii	early successional opportunists	Predation
EuceOxy	Eucephalobus	oxyuroides	basal fauna	bacterial feeding
EuceStr	Eucephalobus	striatus	basal fauna	bacterial feeding
EudoCar	Eudorylaimus	carteri	intermediate succession and disturbance sensitivity	predation

FileMis	Filenchus	misellus	basal fauna	fungal hyphal feeding
FileSp	Filenchus	sp.	basal fauna	fungal hyphal feeding
FileVul	Filenchus	vulgaris	basal fauna	fungal hyphal feeding
GeomSp	Geomonhystera	sp.	basal fauna	bacterial feeding
HCepElo	Heterocephalobus	elongatus	basal fauna	bacterial feeding
HeliPla	Helicotylenchus	Platyurus	early successional opportunists	plant feeding
HeliPse	Helicotylenchus	Pseudorobustus	early successional opportunists	plant feeding
HemiThi	Hemicycliophora	Thienemanni	early successional opportunists	plant feeding
HexaSp	Hexatylus	sp.	basal fauna	fungal hyphal feeding
HowaAor	Howardula	aoronymphium		dispersal stages or animal parasites
HowaSp	Howardula	sp.		dispersal stages or animal parasites
HRToMyr	Heterorhabditidoides (heterorhabditis)	Myriophilus		dispersal stages or animal parasites
MaBiHuf	Macrobiotus	hufelandi		Outgroup (Tardigrada)
MaleAnd	Malenchus	andrassyi	basal fauna	plant feeding
MerlBre	Merlinius	Brevidens	early successional opportunists	plant feeding
MonESp	Mononchoides	sp.	enrichment opportunist	predation
MonEStr	Mononchoides	striatus	enrichment opportunist	predation
MRhaSp	Mesorhabditis	sp.	enrichment opportunist	bacterial feeding
MRhaSpi	Mesorhabditis	spiculigera	enrichment opportunist	bacterial feeding
MTCeCra	Metateratocephalus	Crassidens	early successional opportunists	bacterial feeding
MyolMyc	Myolaimus	mycophilus	enrichment opportunist	bacterial feeding
NeTyFamil	Neotylenchidae (Family)			
OschDoA	Oscheius	dolichura	enrichment opportunist	bacterial feeding
OschSp	Oscheius	sp.	enrichment opportunist	bacterial feeding
OschTip	Oscheius	tipulae	enrichment opportunist	bacterial feeding
PellSp	Pellioditis	sp.	enrichment opportunist	bacterial feeding
PeloPse	Pelodera	pseudoteres	enrichment opportunist	bacterial feeding
PeloTer	Pelodera	teres	enrichment opportunist	bacterial feeding
PGBeSta	Panagrobelus	stammeri	enrichment opportunist	bacterial feeding
PGLaDet	Panagrolaimus	detritophagus	enrichment opportunist	bacterial feeding
PGLaRig	Panagrolaimus	rigidus	enrichment opportunist	bacterial feeding
PGLaSp	Panagrolaimus	sp.	enrichment opportunist	bacterial feeding
PGLaSub	Panagrolaimus	subelongatus	enrichment opportunist	bacterial feeding
PlChWyg	Plectonchus	wyganti	enrichment opportunist	bacterial feeding
PlecFam	Plectus (Family)			
PlecMin	Plectus	minimus	basal fauna	bacterial feeding
PlecSp	Plectus	sp.	basal fauna	bacterial feeding
PoduAqu	Podura	aquatica		Outgroup (water springtail)
PoikOxy	Poikilolaimus	oxycercus	enrichment opportunist	bacterial feeding
PratPen	Pratylenchus	penetrans	early successional opportunists	plant feeding
PratScr	Pratylenchus	scribneri	early successional opportunists	plant feeding

PrioMus	Prionchulus	Muscorum	intermediate succession and disturbance sensitivity	Predation
PriTEnt	Pristionchus	entomophagus	enrichment opportunist	predation
PriTLhe	Pristionchus	lheritieri	enrichment opportunist	predation
PriTTri	Pristionchus	triformis	enrichment opportunist	predation
PriTUni	Pristionchus	uniformis	enrichment opportunist	predation
PseHMin	Pseudhalenchus	minitus	basal fauna	fungal hyphal feeding
PTylSp	Paratylenchus	sp.	basal fauna	plant feeding
RhabBra	Rhabditis	brassicae	enrichment opportunist	bacterial feeding
RhabSp	Rhabditis	sp.	enrichment opportunist	bacterial feeding
RhPhSp	Rhabditophanes	sp.	enrichment opportunist	bacterial feeding
RhTeAxe	Rhabditella	axei	enrichment opportunist	bacterial feeding
RhToSp	Rhabditoides	sp.	enrichment opportunist	bacterial feeding
RotyUni	Rotylenchus	Uniformis	early successional opportunists	plant feeding
SeinSp	Seinura	sp.	basal fauna	predation
SpRuVes	Sphaerularia	vespae		dispersal stages or animal parasites
TCepLir	Teratocephalus	Lirellus	early successional opportunists	bacterial feeding
ThonCir	Thonus	Circulifer	intermediate succession and disturbance sensitivity	Predation
TyCeAur	Tylocephalus	auriculatus	basal fauna	bacterial feeding
TylenchinaSp	Tylenchina	sp.		very diverse

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Appendix 3: comparison between the number of species found by manual identification, and after sequencing.

Sample	Manual species count	Sequencing species count	Are the two counts the same?
LvS3	7	8	No
LvS4	5	10	No
LvS5	5	8	No
LvS6	7	8	No
LvS7	16	18	No
LvS9	9	18	No
LvS10	11	9	No
LvS13	14	14	Yes
LvS16	9	16	No
LvS17	11	1	No
LvS18	6	10	No
LvS19	4	2	No
LvS28	3	3	Yes
LvS29	4	11	No
LvS30	5	9	No
LvS32	6	12	No
LvS33	6	7	No
LvS34	6	14	No
LvS35	2	7	No
LvS37	1	4	No
LvS38	3	8	No
LvS39	3	6	No
LvS42	6	12	No
LvS46	3	2	No
LvS50	2	7	No
LvS53	3	2	No
LvS56	2	7	No
LvS58	4	2	No
LvS60	3	6	No
LvS61	1	7	No
LvS62	2	5	No
LvS64	1	4	No
LvS66	2	13	No
LvS67	1	8	No
LvS69	2	12	No
LvS70	1	6	No
LvS71	3	11	No
LvS72	4	1	No
LvS73	4	7	No
LvS74	2	9	No
LvS75	6	11	No

LvS78	2	21	No
LvS79	2	6	No
LvS80	3	14	No
LvS90	2	9	No
LvS149	4	11	No
LvS150	4	8	No
LvS151	4	11	No
LvS152	7	8	No
LvS158	3	11	No
LvS160	2	6	No
LvS168	2	5	No
LvS170	5	7	No
LvS171	4	10	No
LvS174	3	20	No
LvS176	3	21	No
LvS177	5	7	No
LvS178	2	6	No
LvS179	3	13	No
LvS180	3	4	No
LvS187	3	14	No
LvS188	3	18	No
LvS189	3	5	No
LvS190	3	6	No
LvS191	2	7	No
LvS192	2	7	No
LvS193	2	10	No
LvS194	4	8	No
LvS198	3	14	No
LvS199	3	6	No
LvS202	5	8	No
LvS203	3	10	No
LvS204	4	6	No
LvS207	1	7	No
LvS208	4	10	No

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