

Feeding preference as a main determinant of microscale patchiness among terrestrial nematodes

Molecular Ecology Resources

Quist, Casper W.; Gort, Gerrit; Mulder, Christian; Wilbers, Ruud H.P.; Termorshuizen, Aad J. et al

<https://doi.org/10.1111/1755-0998.12672>

This publication is made publicly available in the institutional repository of Wageningen University and Research, under the terms of article 25fa of the Dutch Copyright Act, also known as the Amendment Taverne.

Article 25fa states that the author of a short scientific work funded either wholly or partially by Dutch public funds is entitled to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

This publication is distributed using the principles as determined in the Association of Universities in the Netherlands (VSNU) 'Article 25fa implementation' project. According to these principles research outputs of researchers employed by Dutch Universities that comply with the legal requirements of Article 25fa of the Dutch Copyright Act are distributed online and free of cost or other barriers in institutional repositories. Research outputs are distributed six months after their first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and / or copyright owner(s) of this work. Any use of the publication or parts of it other than authorised under article 25fa of the Dutch Copyright act is prohibited. Wageningen University & Research and the author(s) of this publication shall not be held responsible or liable for any damages resulting from your (re)use of this publication.

For questions regarding the public availability of this publication please contact openaccess.library@wur.nl

Feeding preference as a main determinant of microscale patchiness among terrestrial nematodes

Casper W. Quist¹  | Gerrit Gort² | Christian Mulder³ | Ruud H. P. Wilbers¹ | Aad J. Termorshuizen⁴ | Jaap Bakker¹ | Johannes Helder¹

¹Laboratory of Nematology, Wageningen University and Research Centre (WUR), Wageningen, The Netherlands

²Biometris, Wageningen University and Research Centre (WUR), Wageningen, The Netherlands

³National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

⁴SoilCares Research, Wageningen, The Netherlands

Correspondence

Casper W. Quist, Laboratory of Nematology, Wageningen University and Research Centre (WUR), Wageningen, The Netherlands.
Email: Casper.Quist@wur.nl

Funding information

BE Basic Foundation, Grant/Award Number: FS8.002.002

Abstract

Soil biota are responsible for essential ecosystem services such as carbon storage, nutrient cycling and water retention. However, assessment of the condition of soil biota is hampered by an overwhelming level of diversity. With representatives in all trophic levels of the food web, nematode communities can be used as bioindicators. Accurate assessment of nematode assemblages requires insight into the distribution of specimens with distinct food preferences. With the availability of taxon-specific quantitative PCR assays, distribution patterns of multiple nematode groups can be investigated simultaneously. Here, microscale patchiness of 45 nematode taxa was studied on 12 sampling sites (each with four adjacent microplots) located on arable fields or semi-natural grasslands ('system'), and on marine, river clay or sandy soils ('soil type'). From each microplot, five composite samples were collected. Contrary to our expectations, an increase in the number of cores per composite sample did not result in more accurate measurements, and apparently the levels of microscale patchiness of the taxa are low compared to what has been reported for oligophagous plant-parasites. System and soil type did not affect microscale distribution. To investigate the level of patchiness in more detail, detection probability (DP) and variability of abundances were calculated. Common and widespread bacterivorous and fungivorous taxa had $DP \geq 90\%$, confirming low level of microscale patchiness. With DPs of 40%–70%, predators and most omnivores showed degrees of local clustering. An overview of mean variabilities of abundances is presented that offers insight into how feeding preferences impact the microscale distribution both between and within trophic groups.

KEYWORDS

bioindicators, nematode community, quantitative PCR, spatial distribution, trophic group

1 | INTRODUCTION

Soil biota are responsible for ecosystems services such as nutrient cycling, carbon fixation, water retention, detoxification of a variety of wastes, and specific and general disease suppressiveness (Janzen, 2006; Six & Paustian, 2014; Wagg, Bender, Widmer, & Van Der Heijden, 2014). The societal relevance of soil contrasts with our fragmentary understanding of the functioning of soil biota (Fitter, 2005).

Although technically demanding, the generation of a full inventory of soil life is feasible, but the resulting data are ecologically barely interpretable. Various proxies for soil quality have been identified, such as earthworms (Pansu et al., 2015), mycorrhizas (Jansa, Erb, Oberholzer, Smilauer, & Egli, 2014), collembolans (Nelson, Boiteau, Lynch, Peters, & Fillmore, 2011) and nematodes (Bongers & Ferris, 1999; Neher, 2001). Several motives plea in favour of nematode communities as bioindicators, such as their representation in all major trophic

levels of the soil food web (Holtkamp et al., 2008), their species diversity within each trophic level (Yeates, 2003), their abundance and diversity in virtually any soil, and their extractability from soil samples. In a recent overview article on cost-effective and policy-relevant indicators for the soil biological condition by 39 European soil ecologists, nematodes received the highest score among a selection of 30 types of potential indicators (Griffiths et al., 2016).

The main operational reason that hampers the use of nematodes as bioindicators seems to be the lack of informative morphological characters. Therefore, microscopic characterization of nematode communities is laborious, requires ample expertise, and typically relatively small subsamples are analysed (Wiesel, Daniell, King, & Neilson, 2015). These practical hurdles prompted the development of quantitative PCR (qPCR)-based methods, which have increased the capacity to characterize nematode communities (Floyd, Abebe, Papert, & Blaxter, 2002; Holterman et al., 2008; Quist et al., 2016; Rybarczyk-Mydlowska et al., 2012; Vervoort et al., 2012). Griffiths et al. (2016) identified that the molecular analysis of nematode assemblages was the preferred method for the assessment of the soil biological condition.

Insights into the spatial distribution of nematodes will greatly contribute to our understanding of the ecological functioning of individual taxa. At the same time, this knowledge can be applied to design soil sampling strategies with predictable accuracies. Horizontal spatial distribution of soil biota has been described at two (Ferris, Mullens, & Foord, 1990), three (Ettema & Wardle, 2002) or even four nested scales (Berg, 2012). At each spatial scale, patterning is defined by abiotic and biotic soil characteristics and the dimensions of the chosen scales predominantly depend on the body size of the organismal group of interest (Ettema & Wardle, 2002; Martiny et al., 2006). Nematodes belong to the soil microfauna (animals with body width of $<100\ \mu\text{m}$) as 99% of all soil nematodes have body widths between ~ 10 and $55\ \mu\text{m}$ and body lengths between ~ 150 and $1500\ \mu\text{m}$ (Mulder & Vonk, 2011). Microfaunal organisms are mainly passive dispersers. Typical active migration velocities range from 0 to 3 cm per day; exposure to external stimuli, however, can result in a 10-fold higher migration speed (Bal, Taylor, & Grewal, 2014; Moore, Lawrence, Arriaga, Burmester, & Van Santen, 2010; Wallace, 1958, 1960).

Here, we propose three nested scales of horizontal nematode distribution: microscale, mesoscale and metascale. *Microscale* is the spatial level at which individual nematodes search for food, mate and multiply, are exposed to predators, and cope with abiotic stressors such as locally unfavourable temperature and moisture conditions (microplot-scale dimensions: 0.05–1 m). Major drivers of heterogeneity of nematode microscale distribution are the size and nature of soil aggregates, and the local spread of food and predators. At *mesoscale*, nematode communities are exposed to comparable abiotic conditions and land use (plot or field-scale dimensions: 1–1,000 m). Horizontal patterning at mesoscale is mainly driven by plant inputs and soil texture modifiers such as tunnelling soil fauna and tillage practices. At the *metascale*, multiple connected mesoscale habitats are involved (landscape-scale dimensions: $>1,000$ m). At this

scale, distribution patterns are mainly driven by dissimilarities in land use and soil properties.

So far, most research on spatial distribution of nematodes concentrated on mesoscale distribution; in arable fields focusing on individual plant-parasites (Been & Schomaker, 2006; Duncan & Phillips, 2009; McSorley & Parrado, 1982; Seinhorst, 1982); and in natural areas on trophic group level (Robertson & Freckman, 1995; Simmons, Niles, & Wall, 2008). Information about the microscale distribution of nematode taxa is scarce, and almost fully restricted to obligate plant-parasites (Been & Schomaker, 2006; Rossi, Delaville, & Quénehervé, 1996) in agricultural systems. Microscale distribution of nonplant parasitic nematodes at family level and lower, received even less attention. Viketoft (2013) used a geostatistical approach to determine practical ranges (=limits of spatial dependence) of plant-parasites, bacterivores, fungivores and omnivores/predators in a 6.6×4.2 m plot, located in a semi-natural grassland (smallest distance between sampling points was 0.1 m). The estimated patch sizes were around 1 m, and these were independent of the trophic preference. Microscale patterns of four physiological stages entomopathogenic nematodes, *Steinernema feltiae* and *S. affine*, were studied in great detail (5×5 cm samples from 0.25-m^2 plots) by Spiridonov, Moens, and Wilson (2007). They revealed that levels of aggregation were negatively related with time after emergence from insect hosts.

Keeping in mind that trophic groups consist of evolutionary independent lineages with comparable food preferences as a common denominator (e.g., Quist, Smant, & Helder, 2015), distinct degrees of patchiness can be expected when nematode communities are analysed at lower taxonomic levels (Neher, Wu, Barbercheck, & Anas, 2005; Porazinska, Duncan, McSorley, & Graham, 1999; Quist et al., 2014). The aim of the present study was to investigate the optimal methodology to assess and define micropatchiness of plant parasitic, bacterivorous, fungivorous, omnivorous and predatory nematodes, with representatives of all colonizer–persister (cp) groups. According to their ecological characteristics, nematode families are assigned to one of the five cp groups (Bongers, 1990). Moreover, highly standardized qPCR-based detection approaches facilitate the quantitative detection of individual nematode taxa. Variations in microscale patchiness were subsequently used to design soil sampling strategies with a known level of accuracy. To this end, 12 sampling sites were selected, and in each sampling site four adjacent microplots (0.25 and $1.0\ \text{m}^2$) were defined. Five composite samples (a thorough mixture of multiple soil cores) were collected from each microplot. The number of cores (diameter: 1.5 cm, depth: 0–20 cm) per composite sample was different in each of the four microplots: 3, 6, 12 or 24. Samples were collected from arable fields with crop rotation, and in semi-natural grasslands with high plant diversity, as well as from three soil types—marine clay, river clay and sand. We expect higher degrees of patchiness in natural fields with high plant diversity as compared to the arable fields, reflecting differences in plant diversity and management practices. Further, based on studies on the distributions of oligophagous parasites of higher plants (Been & Schomaker, 2006; McSorley & Parrado, 1982), we hypothesized that

more cores per composite sample would result into a reduction of the degree of variation between replicate composite samples. We examined how the variation in microscale patchiness was related to the trophic ecology of the taxa under investigation. At least at higher taxonomic levels nematodes show limited biogeography (e.g., Finlay, 2002), implying that most families and genera investigated here can be found on all continents. Therefore, the insights presented here may translate to other geographical regions, providing broader relevance for our understanding of the spatial distribution, sampling and ecological relevance of the Nematoda in soils.

2 | MATERIALS AND METHODS

2.1 | Sampling sites and sample collection

Composite samples were collected during March 2012 and March–April 2013, just before the growing season. Eight fields were sampled across the Netherlands in five arable systems and three grasslands with high plant diversity on three soil types; marine clay, river clay and sand (see Table 1, Appendix S1: Table S1 and Fig. S1). Abiotic soil characteristics were determined by Blgg AgroXpertus (Wageningen), a NEN-EN-ISO 17025 certified service laboratory, using standardized procedures. Visually homogeneous sampling sites were localized approximately in the middle of the selected fields. At each of the sampling sites, four adjacent microplots were defined (Figure 1). In the first microplot, composite samples consisted of 3 cores (\varnothing 1.5 cm, depth: 0–20 cm), in the second of 6 cores, in the third of 12 cores and in the fourth of 24 cores. Five composite samples were collected from each microplot. In 2012 composite samples were taken from three arable fields with two sampling sites in each field approximately 10 m apart: microplots at the first sampling site were 1 m² and in the second 0.25 m². In 2013, composite samples were collected from three arable fields and three semi-natural grasslands with high plant diversity, where one sampling site was chosen per field with 0.25-m² microplots only. This selection of sampling sites includes a variety of abiotic conditions, management and plant diversity, thereby allowing us to investigate the effects of sampling in relation to the micropatchiness of an ecologically wide range of nematode taxa. In total 240 composite samples were stored at 4°C immediately after sampling.

2.2 | DNA extraction and qPCR-based analysis

Within 1 week after sampling, composite samples were homogenized thoroughly and nematodes were extracted from a 100 g subsample using an elutriator–cotton wool filter method (Oostenbrink, 1960). All nematode suspensions were concentrated and the DNA was extracted by a lysis buffer including mammalian DNA as an external standard (to monitor losses due to sampling handling and DNA purification) as described by Vervoort et al. (2012). Thereafter, DNA extracts were purified using a glass fibre column-based procedure (Ivanova, Dewaard, & Hebert, 2006). All purified DNA extracts were stored at –20°C.

To assess nematode biodiversity per sampling site, 1- μ l aliquots of all purified DNA extracts from a given site were combined, and mixtures were analysed. Initial analysis with 59 nematode taxon-specific primer combinations generated insight into the nematode biodiversity per sampling site (Appendix S1: Table S2). Depending on the known biodiversity, between 24 and 33 primer sets were selected for quantification of specific taxa in each sample of a given sampling site. A separate qPCR assay was used to assess total nematode density. To generate factors that compensate for DNA losses during sampling handling, DNA levels of the external control were quantified after purification. Quantitative PCRs were executed and C_t values were converted to qPCR counts: nematode densities were approximated by making use of the known linear relationships between C_t values and ¹⁰log of the number of target nematodes. The maxima of the negative first mathematic derivative of the melting curves were checked to confirm the correct nature of the amplicons (Quist et al., 2016; Vervoort et al., 2012).

2.3 | Data analysis

Detection probability (DP) is defined here as the chance that a given taxon is present in a given composite sample, provided this taxon is known to be present at that site. Hence, DP is a qualitative measure, a fraction that can be calculated per microplot by the number of times a taxon is present among the five replicates. To determine the effects of system, soil type, surface and number of cores per composite sample on DP, relatively common or widespread taxa were analysed. Taxa present in <25% of all sampling sites ('rare taxa') were excluded because of the low number of data points. The fraction of composite samples showing presence of a taxon (out of the five composite samples per microplot) was analysed using generalized linear mixed models (Littell, Milliken, Stroup, Wolfinger, & Schabenberger, 2006) with a binomial distribution and a logit link function. As the DP is expected to be related to the number of cores, we hypothesized that an increase in the number of cores per composite sample would result in higher DP. DP could also be related to factor surface (0.25–1 m²; larger surface was expected to be related to higher detection probability) and field traits (system: arable field—semi-natural grassland; soil type: marine clay, river clay, sand). Random effects of fields were introduced into the model to allow for correlations among multiple observations on the same field. For some taxa the DP could not be related to field traits as they were undetectable in some soil types or systems (Appendix S1: Table S3).

The second quantity of interest was the variability of nematode abundance among the five composite samples per microplot. This variability was quantified as the IQR (interquartile range) of transformed qPCR counts (using the natural logarithm (ln) of (y + 0.1), y being the primary qPCR count). We choose the IQR instead of the variance, because the IQR is less sensitive to outliers. To relate this measure of variability to the four experimental factors (number of cores: 3, 6, 12 and 24; system: arable and natural; soil type: marine clay, river clay and sand; surface: 0.25 and 1 m²), we fitted mixed models (Littell et al., 2006), using transformed IQR (ln(IQR + 0.1)) as

TABLE 1 Localization of fields under investigation

Field	GPS coordinates	Soil pH (pH-CaCl ₂)	Total nitrogen (mg N/kg dry soil)	Total phosphorus (mg P ₂ O ₅ /kg dry soil)	Organic matter (%)	Clay (%)	Crops rotation scheme/ dominant plant species
Schoondijke (Zeeland; m)	51°19' N 3°31' E	7.5	980	1,540	2.6	17	Potato, onion, sugar beet, wheat
Lelystad (Flevoland; m)	52°32' N 5°33' E	7.2	1,410	1,410	2.8	17	Potato, onion, sugar beet, wheat
Lauwersmeer (Friesland; m) ^a	53°20' N 6°09' E	7.2	2,360	1,440	5.0	17	<i>Holcus lanatus</i> , <i>Agrostis stolonifera</i> , <i>Ranunculus repens</i> , <i>Trifolium pratense</i>
Houten (Utrecht; r)	52°02' N 5°09' E	6.7	1,760	1,910	3.5	25	Corn
Millingerwaard (Gelderland; r) ^a	51°52' N 6°00' E	7.0	2,050	1,930	3.8	19	<i>Brassica nigra</i> , <i>Solidago gigantea</i> , <i>Calamagrostis epigejos</i> , <i>Erigeron annuus</i>
Wageningen (Gelderland; s)	51°59' N 5°39' E	5.7	720	1,310	2.8	2	Potato, barley, sugar beet, wheat, corn, grass (2 consecutive years)
Sint Kruis (Zeeland; s)	51°16' N 3°30' E	5.3	1,490	1,440	3.8	2	Potato, <i>Lolium perenne</i> (2 consecutive years), barley, wheat, beans
Mossel (Gelderland; s) ^a	52°03' N 5°45' E	5.6	950	1,200	3.2	2	<i>Agrostis capillaris</i> , <i>Jacobaea vulgaris</i> , <i>Achillea millefolium</i> , <i>Holcus lanatus</i> , <i>Plantago lanceolata</i>

^aSoil samples were collected from arable fields (just before the onset of the growing season), and from semi-natural grasslands with high plant diversity. Soil types are indicated by 'm' (marine clay), 'r' (river clay) or 's' (sand) (see also Appendix S1: Table S1 and Fig. S1). For each of the fields, five major abiotic soil characteristics were determined: pH, total nitrogen, total phosphorus, organic matter % and clay content (% soil particles <2 μm).

response, which we named tIQR. The transformation of IQR was needed to obtain more normally distributed responses, as required for mixed models. The mixed models contained random effects for fields and sampling sites per field, as multiple observations from the same field and sampling sites were modelled. Fixed effects were introduced for the four above-mentioned experimental factors. Mixed models were fitted per nematode taxon. For each nematode taxon, microplots with only qPCR counts of zero were excluded from the analysis. To facilitate the interpretation of the results, the mean tIQR per taxon—obtained from mixed models by averaging over all factors—was back-transformed to a relative scale factor (RSF). RSF is the multiplication factor required to go from the first (Q1) to the third quartile (Q3) of qPCR counts within a set of five composite samples per plot (by definition RSF is >1).

Mean DP and RSF with 95% confidence intervals (CI) per nematode taxon were estimated and plotted for comparison among taxa (Figures 2 and 3). The mean DP was estimated using a generalized linear model with binomial distribution and logit link, ignoring all experimental factors (motivated by results from analysis described above). The mean RSF with 95% CI was estimated by back-transformation of results of mixed model analyses for tIQR, averaging over levels of factors surface and cores only.

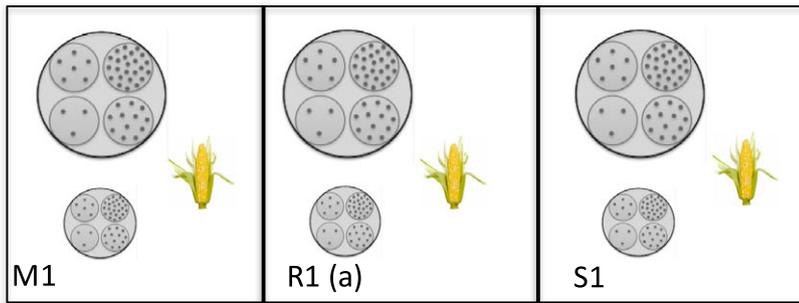
Finally, the relationship between number of replicate composite samples and accuracy of nematode quantification per nematode taxon was studied. To this end, we pooled variability estimates from all separate microplots, ignoring differences in number of cores

(motivated by results, showing no effect of number of cores; see our Section 3), using mixed models for log-transformed qPCR counts ($\ln(y + 0.1)$) for reasons of simplicity. Assuming a lognormal distribution of the qPCR count, the coefficient of variation (CV) for the back-transformed mean qPCR count equals $\sqrt{e^{\sigma^2/n} - 1}$ with σ^2/n the variance of the mean log-transformed qPCR count. Parameter σ^2/n is the within-microplot-variance component, estimated from the mixed models. In this way, the number of replicate composite samples n can be calculated to obtain a given CV for back-transformed mean log qPCR count. Alternatively, the CV can be calculated given the number of replicate composite samples.

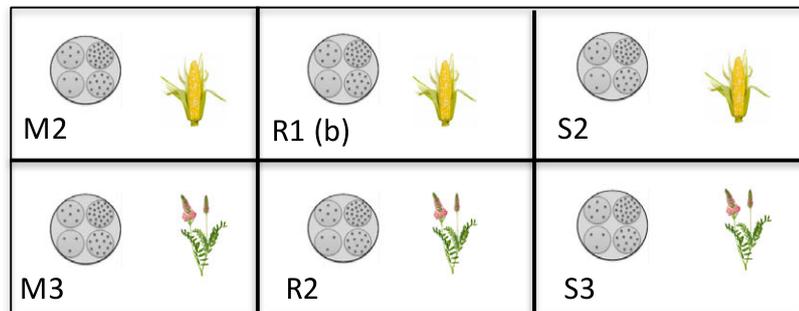
3 | RESULTS

To investigate the micropatchiness among terrestrial nematodes, microplots were sampled in nine fields that varied in land management and soil type (Figure 1, Table 1). We investigated 45 nematode taxa, with representatives from all major trophic groups (bacterivores, fungivores, omnivores, predators, plant-parasites and entomopathogens; Table 2). It is noted that most taxa are determined either at family or at genus level. Genus level is used if the corresponding family is not monophyletic, or in cases a given family harbours only a single genus. The first option is exemplified by the fungivorous Diphtherophoridae; this family can only be detected by measuring the two constituting genera, *Diphtherophora* and

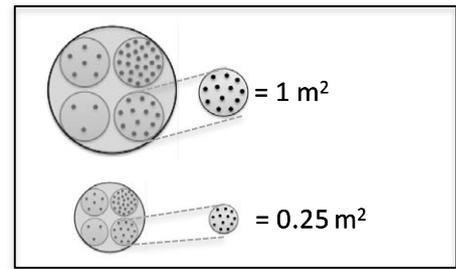
March 2012



March - April 2013



Two sampling surfaces



Sampling site with four adjacent microplots

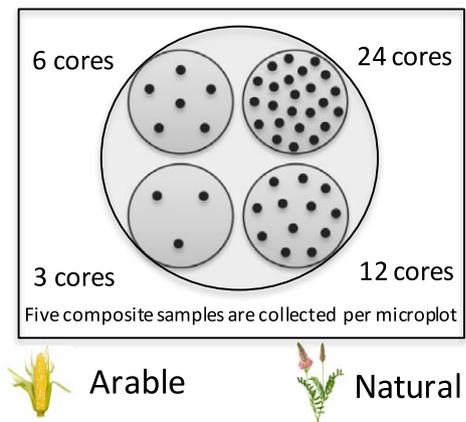


FIGURE 1 Experimental design to investigate the effect of sampling surface and number of cores, in different soil types and systems, on detection probability and quantification accuracy. Fields on marine clay (M1, 2, 3), river clay (R1 (a and b), 2) and sandy soil (S1, 2, 3) in arable and natural systems (with corresponding icons), were sampled in March 2012 and March–April 2013. Per sampling site ($n = 12$, two per field in M1, R1(a) and S1), one in each of the other fields), four adjacent microplots were sampled, indicated in the scheme as grey circles with circular microplots inside. In March 2012 microplots of two surfaces (0.25 and 1 m^2) were sampled at two sampling sites per field. In March–April 2013, four adjacent microplots (0.25 m^2) were sampled at one site per field. Per microplot, five composite samples containing 3, 6, 12 or 24 cores were collected

Tyolaimophorus, individually. Entomopathogenic nematodes are an example of the second option; Heterorhabditidae and Steinernematidae each harbour a single genus, namely *Heterorhabditis* and *Steinernema*. Some plant-parasites such as *Pratylenchus* are measured at species level as the genus in nonmonophyletic (e.g., Rybarczyk-Mydłowska et al., 2014). The selected taxa belong to 27 nematode families residing in 10 of the 12 major nematode clades (Holterman et al., 2006). On the basis of their ecological characteristics, nematode families have been grouped on a colonizer–persister scale (cp 1–5; Bongers, 1990). In the current selection of nematode taxa, all cp categories are represented (Table 2).

3.1 | Effect of the number of cores per composite sample on qualitative and quantitative detection

No significant effects of the number of cores per composite sample on detection probability (DP) were found for the ‘common’ or ‘widespread’ taxa under investigation (Table 3 and Appendix S1: Table S3). Also for the other variables, plot size, soil type and system, rarely an impact on the DP was observed. Plot size was

shown to have significant effects in two instances only: the necromenic bacterivore/predator *Pristionchus* had higher DP in 1-m^2 plots, whereas the omnivorous group Dorylaimida D3 had higher DP in 0.25-m^2 plots ($p < .001$ and $p < .01$, respectively; see Appendix S1: Table S3). Also, no effects of soil type and system on DP of any of the taxa under investigation were found. It is noted that not all variables could be tested for all nematode groups. A few taxa received the label ‘rare’ as they were exclusively detected in either sand (e.g., *Pratylenchus crenatus*, *Cruzinema*) or clay soils (e.g., *Pratylenchus thornei*, Dorylaimida D9B). Probably these taxa would not have received this label in case a single soil type was considered.

Quantitative PCR counts were used to obtain interquartile ranges (IQR) for quantitative analysis. Again, variability of densities of individual nematode taxa was not significantly influenced by system, by sample surface or by the number of cores per composite sample (Appendix S1: Table S4). Soil type affected variabilities of densities of herbivores (including all small Tylenchidae) at trophic group level, which were less variable in sand than in clay ($p < .05$). Abundances of *Basiria* (Tylenchidae) were lower and less variable in sand than in

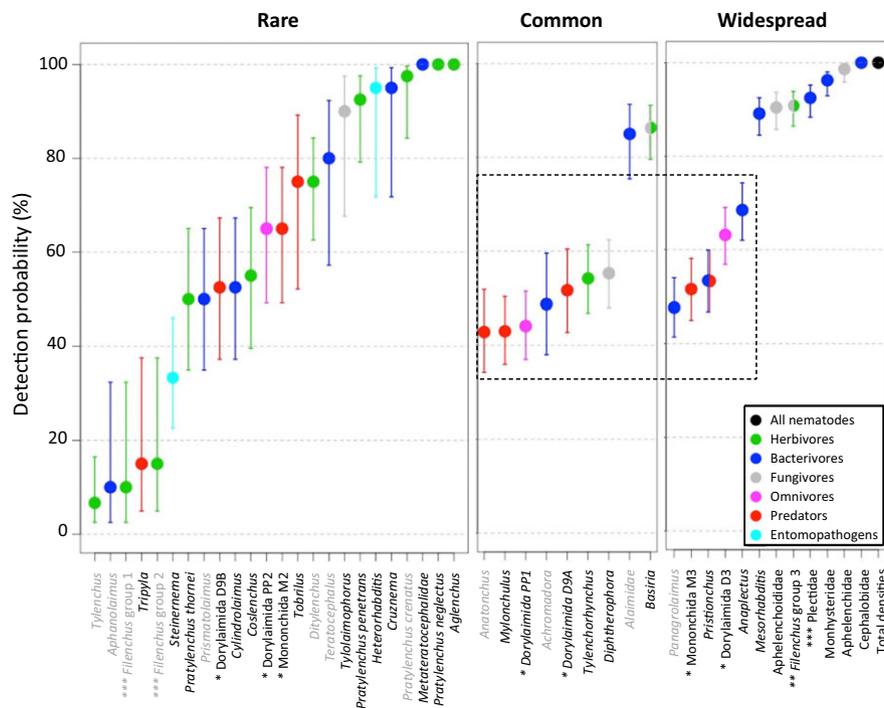


FIGURE 2 Detection probability (DP) with error bars (95% CI) for total nematode densities and individual nematode taxa that were 'Rare' (present in <25% of the sampling sites), 'Common' (present in >25%–75% of the sampling sites) or 'Widespread' (present in >75% of the sampling sites). DP was calculated as n records among five replicates times 100% (sampling sites were excluded when taxa were absent). Trophic ecologies are indicated by colours. The dotted-line demarcated box includes 'Common' or 'Widespread' taxa with a DP between 40% and 70%. Grey printed nematode taxa were present in densities close to the detection limit, and results are less robust than the ones shown for taxa indicated in black. *See Holterman et al. (2008). **See J. Helder et al., manuscript in preparation. ***Except for the genus *Anaplectus*

clay. Abundances of the herbivore *Tylenchorhynchus* were higher and less variable in sand than in clay (respectively Appendix S1: Tables S2 and S4).

As no significant effects of the numbers of cores per composite sample were detected for the taxa under investigation (Table 3; Appendix S1: Tables S3 and S4), the mean DP and relative scale factor (RSF) with 95% confidence interval (CI) were calculated over all microplots (hence regardless of the number of cores) for comparison among individual taxa and to study how the variation in microscale patchiness was related to trophic group, cp value and phylogenetic position.

3.2 | Nematode detection probabilities

Within nematode communities individual taxa were shown to differ in both distribution across the fields of study (e.g., rare, common or widespread) as well as in mean DP with 95% CI (given they were present) (Figure 2). To illustrate this, three examples are briefly discussed. The fungivorous nematode family Aphelenchidae was shown to be 'widespread' (present in >75% of the sites, right panel), and the mean DP—the expected chance of detecting Aphelenchidae in a composite sample—was close to 100%. The predatory Dorylaimida D9A (including the genera *Aquatides*, *Clavicaudoides* and *Nygolaimus* (Holterman et al., 2008)) was 'common' as it occurred in 25%–75% of the sites (middle panel), its mean DP was close to 50% with a

wide 95% CI. The left panel of Figure 2 shows the 'rare' taxa (here defined as present in <25% of the sampling sites). The entomopathogenic genus *Steinernema* was present at three sampling sites only, and at these sites its mean DP was around 35%.

Among the 'widespread' taxa, bacterivores were overrepresented. Aphelenchidae and Aphelenchoididae, two of the four main fungivorous lineages resided in the 'widespread' category as well. Also *Filenchus* group 3 (Tylenchidae) and omnivore Dorylaimida D3 were shown to be widespread. Among the 'common' taxa another set of bacterivores was found (*Anaplectus*, *Alaimidae* and *Achromadora*), together with the fungivore *Diphtherophora*, the supposedly root-hair feeder *Basiria*, and an omnivore group referred to as Dorylaimida PP1 (see Holterman et al., 2008). Further, predatory nematodes (Mononchida M3, Dorylaimida D9A and *Myonchulus*) and an obligate plant-parasite (*Tylenchorhynchus*) were common in our sites. Almost half of all 'rare' taxa were herbivores, whereas bacterivores were underrepresented in this category. Mononchida M2, Dorylaimida D9B (dominant genus: *Paravulvulus*) and *Tripyla* were among the more rarely detected predators. The acidophilic fungivorous genus *Tylo-laimophorus* was detected only once, in an arable field on sand. Representatives of the entomopathogenic genera *Steinernema* and *Heterorhabditis* were 'rare' and they were almost exclusively found in semi-natural grasslands.

The dotted-line demarcated box in Figure 2 was drawn to emphasize 'Common' or 'Widespread' taxa with a DP between 40%

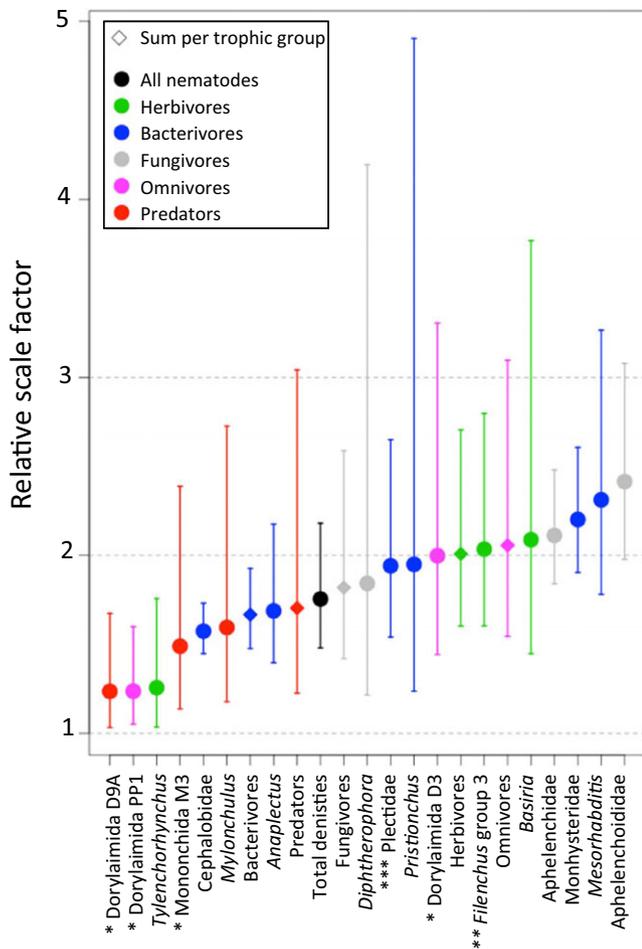


FIGURE 3 Variability of nematode densities for common and widespread taxa and trophic groups over three soil types (marine clay, river clay and sand) in six arable and three natural sites, with plots of 0.25 and 1 m² and by taking five replicate composite samples per plot with 3, 6, 12 or 24 cores per composite sample. This variability of densities is expressed as a relative scale factor (RSF). The interquartile range (IQR) for nematode densities was calculated per microplot and transformed (tiQR) (see Section 2 for details about statistical analysis). To facilitate the interpretation of the results, the mean tiQR per taxon – obtained from mixed models by averaging over all factors – was back-transformed to obtain the RSF. This is the multiplication factor to go from the first (Q1) to the third quartile (Q3) of qPCR counts within the set of five composite samples per plot. Error bars (95% CI) show the range of plausible values of the relative scale factor. The colour of the dots indicates trophic group. Diamonds show the sum per trophic group. *See Holterman et al. (2008). **See J. Helder et al., manuscript in preparation. ***Except for the genus *Anaplectus*

and 70%. Omnivores and predatory nematodes are overrepresented in this box. Also the two nematode taxa that predominantly live in phoretic association with insects, *Pristionchus* and *Panagrolaimidae*, are in the demarcated box. Six of the twelve taxa in the demarcated box belong to the stress-sensitive nematode orders Dorylaimida and Mononchida (Holterman et al., 2008), taxa belonging to cp groups 1, 2 or 3 are present but underrepresented in this box.

3.3 | Variability of nematode densities

Widespread and common taxa displayed a wide range of variabilities of abundances, here quantified by the mean RSF with 95% CI (Figure 3). Differences in mean RSF can be attributed to a combination of spatial variability (within and between microplots and sampling sites), and measurement errors (sum of variation caused by the protocol, from nematode extraction till qPCRs). Within the bacterivores, different degrees of variability of abundances were seen: Cephalobidae and *Mesorhabditis* respectively had the lowest and the highest mean RSFs. Based on the width of the 95% CI, bacterivorous taxa can be approximately divided into two categories: the first category includes taxa that have a narrow 95% CI, representatives of this category are, for example, Cephalobidae, *Anaplectus* and Monhysteridae. The second type includes taxa that have wide 95% CI, such as *Pristionchus*, *Mesorhabditis* and Plectidae (excluding *Anaplectus*). Predators usually had a relatively low mean RSF with wide 95% CI. Comparison of variability of taxa within all trophic groups resulted in significantly different levels of RSF, except for the fungivores (Appendix S1: Table S4). For the majority of widespread and common nematode taxa the RSF was around two (so Q3 is about twice the value of Q1). Cephalobidae showed the narrowest 95% CI, so we are most certain about the mean RSF of this family; other taxa such as *Pristionchus* and *Diphtherophora* had relatively wide 95% CI, indicating a high level of uncertainty about the mean RSF.

3.4 | Optimal sampling to reach increased detection probabilities and more accurate estimates of nematode densities

Contrary to our expectations increased numbers of cores per composite sample above three did not result in increased DP or more accurate assessments of nematode densities of any taxon under investigation. In addition, neither system nor soil type influenced the DP and IQR. Analysis of increased numbers of replicate composite samples, however, results in higher DP as well as in more accurate quantification. A prediction of the expected DP when different numbers of replicate composite samples (*n*) are analysed is obtained by using DP_{mean} from Figure 2: $DP_{\text{expected}} = (1 - (DP_{\text{mean}})^n) \times 100\%$. Table 4 shows the number of composite samples that should be collected from a microplot to obtain a desired CV for common and widespread taxa with well-detectable densities (substantially above the qPCR detection limit). For this reason, four of the ‘common’ and ‘widespread’ taxa in grey font in Figure 2, *Anatonchus*, *Achromadora*, *Alaimidae* and *Panagrolaimus*, are not represented in Table 4. In this table, nematode taxa are arranged by predicted quantification accuracies, and this arrangement mirrors the results presented in Figure 3.

4 | DISCUSSION

Contrary to our expectations, increasing the number of cores per composite sample did not affect the detection probability nor the

TABLE 2 Overview of main characteristics of nematode taxa detected by qPCR assays

Trophic group	Rare, Common, Widespread	cp value	Clade	Family	Taxon targeted by qPCR
B	W	1	10	Panagrolaimidae	<i>Panagrolaimus</i>
B	R	1	9	Rhabditidae	<i>Cruzema</i>
B	W	1	9	Rhabditidae	<i>Mesorhabditis</i>
B	W	2	11	Cephalobidae	Cephalobidae
B	W	2	6	Plectidae	<i>Anaplectus</i>
B	W	2	6	Plectidae	Plectidae ^a
B	W	2	5	Monhysteridae	Monhysteridae
B	R	3	7	Teratocephalidae	<i>Teratocephalus</i>
B	R	3	6	Aphanolaimidae	<i>Aphanolaimus</i>
B	R	3	6	Metateratocephalidae	Metateratocephalidae
B	R	3	5	Diplopeltidae	<i>Cylindrolaimus</i>
B	R	3	1	Prismatolaimidae	<i>Prismatolaimus</i>
B	C	4	1	Alaimidae	<i>Alaimidae</i>
B/P	W	1	9	Neodiplogastridae	<i>Pristionchus</i>
E	R	1	10	Steinernematidae	<i>Steinernema</i>
E	R	1	9	Heterorhabditidae	<i>Heterorhabditis</i>
F	W	2	12	Aphelenchidae	Aphelenchidae
F	W	2	10	Aphelenchoididae	Aphelenchoididae
F	C	3	1	Diphtherophoridae	<i>Diphtherophora</i>
F	R	3	1	Diphtherophoridae	<i>Tyololaimophorus</i>
F/H	C	2	12	Tylenchidae	<i>Basiria</i>
F/H	R	2	12	Tylenchidae	<i>Ditylenchus</i>
F/H	W	2	12	Tylenchidae	<i>Filenchus</i> group 3 ^b
H	R	2	12	Tylenchidae	<i>Aglenchus</i>
H	R	2	12	Tylenchidae	<i>Coslenchus</i>
H	R	2	12	Tylenchidae	<i>Filenchus</i> group 1 ^b
H	R	2	12	Tylenchidae	<i>Filenchus</i> group 2 ^b
H	R	2	12	Tylenchidae	<i>Tylenchus</i>
H	C	3	12	Belonolaimidae	<i>Tylenchorhynchus</i>
H	R	3	12	Pratylenchidae	<i>Pratylenchus crenatus</i>
H	R	3	12	Pratylenchidae	<i>Pratylenchus neglectus</i>
H	R	3	12	Pratylenchidae	<i>Pratylenchus penetrans</i>
H	R	3	12	Pratylenchidae	<i>Pratylenchus thornei</i>
O	R	4	2	Qudsianematidae	<i>Dorylaimida</i> PP2 ^c
O	C	4	2	Qudsianematidae/Nordiidae	<i>Dorylaimida</i> PP1 ^c
O	W	4	2	Qudsianematidae/Nordiidae/ Dorylaimidae	<i>Dorylaimida</i> D3 ^c
P	R	3	1	Tobrilidae	<i>Tobrilus</i>
P	R	3	1	Tripylidae	<i>Tripyla</i>
P	C	4	2	Anatonchidae	<i>Anatonchus</i>
P	R	4	2	Mononchidae	Mononchida M2 ^c
P	W	4	2	Mononchidae	Mononchida M3 ^c
P	C	4	2	Mylonchulidae	<i>Mylonchulus</i>
P	C	5	2	Nygolaimidae	<i>Dorylaimida</i> D9A ^c
P	R	5	2	Nygolaimidae	<i>Dorylaimida</i> D9B ^c
U	C	3	3	Achromadoridae	<i>Achromadora</i>

Trophic group is given by single capitals: B, bacterivores; E, entomopathogens; H, herbivores; O, omnivores; P, predators; and U, unicellular eukaryote feeders. For feeding preferences, we adhered to Yeates, Bongers, de Goede, Freckman, and Georgieva (1993). Distribution of individual nematode taxa over sampling sites: R, rare: present in <25% of the sampling sites; C, common: present in >25%–75% of the sampling sites; or W, widespread: present in >75% of the sampling sites. Attributed cp value, a 1–5 colonizer–persister scales defined at family level by Bongers (1990) and phylogenetic position (clades 1–12; Holterman et al., 2006) are provided.

^aExcept for the genus *Anaplectus*.

^bSee J. Helder et al., manuscript in preparation.

^cSee Holterman et al. (2008).

variability of nematode densities, pointing at relatively low degrees of microscale patchiness. Comparison of the distribution of nematode taxa across our study sites show that being rare, common or widespread was strongly related to feeding type. Most plant-feeding and entomopathogenic nematodes reside in the rare category, reflecting their dependence on the availability of specific hosts and/or preferences for specific site characteristics. Most bacteria feeders and two of four fungivores reside in the widespread category and seem to either be less picky or consume a very commonly available food source. Predators and omnivores were mainly found in the common category. Detection probabilities were related to feeding behaviour as well: common and widespread bacterivorous and fungivorous taxa had $DP \geq 90\%$, confirming the low levels of microscale patchiness. Predators and most omnivores, however, showed degrees of local clustering based on their lower DPs (between 40% and 70%). Variation in densities, however, was highly taxon-dependent and not related to trophic group, cp group or phylogenetic position. For common and widespread nematode taxa of this study, we made predictions about the number of composite samples that should be collected from microplots to obtain desired quantification accuracies.

4.1 | An increase in the number of cores per composite sample does not affect the detection probability nor the variability of nematode densities

Except for the *Dorylaimida* PP1, an increase in the number of cores per composite sample above three did not result in a higher DP nor in less variability of abundances among replicated samples (IQR). Studies on microscale patchiness of nematodes are rare and vary in scope. In some studies, the microscale distribution of nematodes in general is investigated (Klironomos, Rillig, & Allen, 1999); others concentrate on major trophic groups (Viketoft, 2013), whereas a third category focuses on individual genera or species (e.g., Been & Schomaker, 2006; Rossi et al., 1996). In a semi-natural grassland in south-central Sweden, Viketoft (2013) observed small and similar ranges for four major trophic groups, viz., plant, fungal and bacterial feeding nematodes and omnivores/predators (≈ 1 m). In this study, density data from 7 (fungivores) up to 27 genera (bacterivores) were lumped and subsequently analysed. Reports on microscale distributions of individual genera or species almost exclusively focus on oligophagous plant-parasites such as *Globodera pallida*, *Heterodera* spp. and four tropical plant-parasites in agro-ecosystems (Been & Schomaker, 2006; Fenwick, 1961; Rossi et al., 1996). For these plant-parasites with narrow host ranges, the observed microscale patchiness was mainly attributed to host plant distribution, root architecture and developmental stage of host plant (Rossi et al., 1996). Seinhorst (1988) detected low degrees of patchiness for two polyphagous plant parasitic species; the beet cyst nematode *Heterodera schachtii* (host range includes over 200 plant species including 80% of the Chenopodiaceae and Brassicaceae; Steele, 1965) and the stem nematode *Ditylenchus dipsaci* (approximately 500 host plant

species; Janssen, 1994). Although the number of well-documented examples is limited, polyphagy among plant parasitic nematodes seems to be associated with low levels of microscale patchiness. To the best of our knowledge, no well-documented examples have been published on the microscale distribution of free-living nematode at genus or species level in bulk soil. Detailed information about the distribution of the bacterial and fungal communities at this spatial scale might be indicative for the distribution of bacterivorous and fungivorous nematodes. Members of these trophic groups show food preferences (e.g., Moens, Verbeeck, De Maeyer, Swings, & Vincx, 1999; Quist et al., 2014; Shtonda & Avery, 2006), but too little is known about the nature of these preferences under field conditions to make any statement about the anticipated distribution of individual nematode taxa. Hence, the observed low degree of patchiness of polyphagous plant-parasites matches with previous findings. With regard to the microscale distribution of free-living nematodes, the scarcity of information hampers a more detailed reflection on our finding.

4.2 | Technical and biological factors affecting the accuracy of nematode density assessments

Ideally, we would have analysed total DNA extracts of the 100 g soil subsamples directly. However, there is currently no routine protocol available that allows the handling of such large quantities of soil. The Oostenbrink elutriator-cotton wool filter method for the extraction of nematodes from the soil matrix is relatively efficient (Nijs & Van Den Berg, 2013), but variations have been reported with regard to the yield for individual nematode genera. This might, for example, have resulted in an underestimation of the Plectidae and the Alaimidae concentrations (Verschoor & de Goede, 2000).

A biological factor that introduced some bias in our results is soil type-dependent density differences. This can be illustrated by the widespread and common bacterivorous family Cephalobidae. In marine clay soils, densities of 40–100 individuals per 100 g soil were found, whereas typically 300–750 individuals were observed in sandy sites. The reverse was observed for the root-hair feeder *Basiria*. This genus was systematically present in higher densities in marine and river clay as compared to sandy soils (see Appendix S1: Table S2). In both cases no significant effect of soil type on the microscale distribution was observed (and for further analysis results were lumped), but we might have found effects of numbers of cores on taxon detectability and quantification if more replicate samples were studied per soil type.

A third factor that could have affected our results is the increase in the noise-to-signal ratio for quantitative PCR data from nematode taxa present at very low densities. Low numbers of target individuals (typically 1–10) per 100 g soil result in high C_t values with a relatively high technical noise (Karlen, McNair, Perseguers, Mazza, & Mermod, 2007). It is noted that taxa present in low densities can be present in almost all samples ('widespread'). Nematode taxa that generally occur in low densities are presented in grey font in Figure 2, and these results should be appreciated accordingly.

TABLE 3 Effect of the number of soil cores per composite sample (3, 6, 12 or 24) on the detection probabilities of 'common' (C) or 'widespread' (W) nematode taxa (see Table 2)

Taxon	Effect cores			
	df (n)	df (d)	F-value	p-value
<i>Panagrolaimus</i>	3	36	1.50	.23
<i>Mesorhabditis</i>	3	36	0.38	.76
Cephalobidae	3	36	Present in all samples	
<i>Anaplectus</i>	3	33	0.91	.44
Plectidae (except <i>Anaplectus</i>)	3	36	Present in nearly all samples	
Monhysteridae	3	36	Present in nearly all samples	
Alaimidae	3	36	Present in nearly all samples	
<i>Pristionchus</i>	3	33	1.28	.30
Aphelenchidae	3	36	Present in nearly all samples	
Aphelenchoididae	3	33	0.77	.52
<i>Diphtherophora</i>	3	26	0.14	.93
<i>Filenchus</i> group 3	3	36	0.1	.96
<i>Basiria</i>	3	20	0.54	.66
<i>Tylenchorhynchus</i>	3	26	1.73	.19
Dorylaimida PP1 ^a	3	26	3.06	.05
Dorylaimida D3 ^a	3	36	2.12	.12
<i>Anatonchus</i>	3	17	1.09	.38
Mononchida M3 ^a	3	33	0.16	.92
Dorylaimida D9A ^a	3	15	0.41	.75
<i>Mylonchulus</i>	3	26	0.95	.43
<i>Achromadora</i>	3	9	1.69	.24

df (n): degrees of freedom, numerator; and df (d), denominator; F values; and p values are given. $p \leq .05$ is considered to indicate significant differences and are given in **bold**. For taxa present in (nearly) all samples no test result could be generated by generalized linear mixed models. For details about statistical results, see Appendix S1: Table S3, and for details about statistical analysis of data, see Section 2.

^aSee Holterman et al. (2008).

4.3 | Explanations for the contrasting DP between trophic groups of nematodes

To explain the differences in DP of predatory, omnivorous and insect-associated nematode taxa on the one hand, and the higher mean DP of bacterivorous, fungivorous and putative root-hair feeders on the other hand, we will concentrate on taxa in the categories 'common' and 'widespread' (taxa in black font in Figure 2). For these taxa, we assume that the mean DP is mainly related to spatial variability at microscale: higher DP would correspond to more evenly distributed taxa, while lower DP would point at a degree of patchiness for a taxon. Four of the twelve 'common' and 'widespread' taxa with a relatively low DP were predators (below 60%). This set of predators resides at the top of the food chain of the soil food web, and, as a consequence, they are present at low densities. Hence, these predators could—by matter of chance—be absent in some of the composite samples. If this were correct, it would have resulted in lower DP.

Six of the twelve taxa in the demarcated box in Figure 2 belong to the stress-sensitive nematode orders Dorylaimida and Mononchida (Holterman et al., 2008). Representatives of these groups have a relatively thin and permeable cuticle, and as such, they are more affected by local chemical and/or physical stressors than most other categories of terrestrial nematodes. This is further illustrated by the Maturity Index, an ecological index that categorizes terrestrial nematode families into five so-called cp groups (c, colonizer; p, persister) (Bongers, 1990). All Dorylaimida and Mononchida taxa considered here belong to the cp groups 4 and 5, the most stress-sensitive categories. Hence, chemical and/or physical microheterogeneity might explain the observed patchiness as well.

The only two nematode taxa that predominantly live in phoretic association with insects, *Pristionchus* and Panagrolaimidae, are situated in the demarcated box in Figure 2. *Pristionchus* varied enormously between sampling sites: at some sites *Pristionchus* was present in high densities in each of the five replicates, whereas on other sites this genus was present in low densities and was detected in a fraction of the replicates only. Recently, *Pristionchus pacificus* populations were shown to harbour bacterivorous as well as predatory individuals (Seroby, Ragsdale, & Sommer, 2014). The at least occasional predatory lifestyle of *Pristionchus* (indicated in Figure 2 by a partially red and blue sphere) fits in the overrepresentation of predatory nematodes in this specific category. Another noteworthy explanation for the observed microscale patchiness relates to the remarkable behaviour of *Pristionchus* that results in the formation 'Dauer towers', a strategy to efficiently reach beetle hosts. These towers consist of up to a thousand individuals (Penkov et al., 2014) and would result in extreme micropatchiness. *Pristionchus* has a necromenic association with scarab beetles. Numerous members of the family Panagrolaimidae live in association with bark beetles (Massey, 1974). Results on Panagrolaimidae, however, should be viewed with caution as densities of representatives of this family were close to the detection limit. It could be envisaged that close association with insects results in some degree of patchiness at microplot scale for both taxa.

4.4 | Distribution of plant-parasites

Mainly because of their dependence of a specific host plant, most herbivorous taxa were detected in only a few fields. However, the polyphagous root and root-hair feeders *Tylenchorhynchus*, *Basiria* and *Filenchus* group 3 were common or widespread. *Tylenchorhynchus* constitutes a polyphagous genus feeding on cytoplasm of root-hairs and epidermal cells (Brinkman, Duyts, & van der Putten, 2008). Tylenchidae, such as *Basiria* and *Filenchus*, are usually labelled as epidermal cell and root-hair feeders that might use lower plants or algae as an alternative food source. The fact that *Filenchus* group 3 and *Basiria* are commonly present in combination with high DP (~90%) was shown to be different from other Tylenchidae (such as *Aglenchus*, *Coslenchus* and *Filenchus* groups 1 and 2), and similar to common and abundant bacterivores and fungivores. This observation suggests that *Filenchus* group 3 and *Basiria* feed on omnipresent resources such as fungi and/or algae.

TABLE 4 Taxon-specific estimation of the number of times a composite soil sample should be collected from a microplot to obtain given coefficients of variation (CV [%]), as well as the estimated CVs for a given number of composite samples collected from a microplot

Taxon	Estimated number of replicates per microplot to reach a given CV (%)				Estimated CV with <i>n</i> replicates (%)				
	100%	50%	25%	10%	1	2	3	5	10
Dorylaimida PP1 ^a	1	1	1	3	16	11	9	7	5
Dorylaimida D9A ^a	1	1	1	3	17	12	10	7	5
<i>Tylenchorhynchus</i>	1	1	1	6	24	17	13	10	7
<i>Mylonchulus</i>	1	1	2	7	26	18	15	11	8
Cephalobidae	1	1	2	7	26	18	15	11	8
Total nematode densities	1	1	2	8	27	19	15	12	8
<i>Basiria</i>	1	1	2	11	33	23	19	14	10
Plectidae ^c	1	1	2	11	33	23	19	15	10
<i>Anaplectus</i>	1	1	2	12	36	25	20	16	11
<i>Diphtherophora</i>	1	1	2	12	36	25	20	16	11
Mononchida M3 ^a	1	1	3	13	36	25	20	16	11
Monhysteridae	1	1	3	13	37	26	21	16	11
Aphelenchidae	1	1	3	14	38	27	22	17	12
<i>Mesorhabditis</i>	1	1	3	16	41	28	23	18	12
<i>Filenchus</i> group 3 ^b	1	1	3	18	44	30	25	19	13
Aphelenchoideidae	1	1	4	22	49	34	27	21	15
Dorylaimida D3 ^a	1	2	6	32	61	41	33	26	18
<i>Pristionchus</i>	1	3	8	48	78	52	42	32	22

^aSee Holterman et al. (2008).

^bSee J. Helder et al., manuscript in preparation.

^cExcept for the genus *Anaplectus*.

Mean CV were calculated per taxon, aiming to estimate sampling accuracies when different numbers of replicates are analysed (see Section 2 for details about statistical analysis).

4.5 | Distribution of bacterivores and fungivores

Individual bacterivorous and fungivorous taxa showed considerable variation in terms of commonness and DP. Taxa showing relatively high DP, such as the bacterivorous Cephalobidae, Monhysteridae, Plectidae (except *Anaplectus*), *Mesorhabditis*, Metateratocephalidae and *Cruznama*, and fungivorous Aphelenchidae, Aphelenchoideidae and Tyloilaimophorus, probably feed on ubiquitous food resources, whereas other microbivores *Anaplectus*, *Pristionchus*, *Cylindrolaimus* and *Diphtherophora* might be more selective with regard to their food preferences.

4.6 | Differences in quantification accuracies of 'common' and 'widespread' nematode taxa and their use as bioindicators

Nematodes at taxon as well as trophic group level showed relative scale factors (RSFs) ranging from 1.2 (Dorylaimida D9A, a predator) to 2.4 (the fungivorous family Aphelenchoideidae). Considering common and widespread taxa only, we observe a positive correlation between RSF and the variation in nematode densities. Further, a narrow 95% CI points at relatively small differences in variability of densities between sampling sites, and/or larger sample size, and vice versa for wide 95% CI.

Mesorhabditis, Aphelenchoideidae and *Basiria* showed relative high RSF, suggesting that their densities—as compared to other taxa—show a degree of patchiness, which might be related to aggregation near certain food sources that are not ubiquitous at microscale. The lowest RSF was found for Dorylaimida groups D9A and PP1 (see Holterman et al., 2008), and *Tylenchorhynchus*. This suggests very low spatial variability for these taxa. However, densities of these taxa tended to be low in our microplots, in which these taxa were usually detected in only one or two of five replicates, thereby pushing the RSF towards 1. For the remaining common and widespread taxa, the RSF and 95% CI show their degree of microscale patchiness and (dis)similarity of this patchiness between fields, respectively. *Pristionchus* and *Diphtherophora* had the widest 95% CI, indicating relative large differences in variability between sampling sites, and apparently *Pristionchus* and *Diphtherophora* are sensitive to site-specific characteristics. Recently, Ito et al. (2015) demonstrated that *Pristionchus* was one of the most responsive taxon to soil management practices under humid subtropical conditions in Japan. The effects of soil management on *Diphtherophora* have been studied in a multivariate meta-analysis of data sets collected across the world; *Diphtherophora* appeared to be one of the genera that was consistently reduced by cultivation (Zhao & Neher, 2013). However, we did not observe a significant effect of 'system' (arable fields versus

semi-natural grasslands) for these two genera (Appendix S1: Table S3).

Cephalobidae, probably the most abundant family in terrestrial habitats in temperate climate zones, showed a remarkably narrow 95% CI. This family of bacterivores harbours highly common genera, such as *Eucephalobus*, *Acrobeloides*, *Cervidellus* and *Cephalobus*, which, when considered individually, might show more variability.

5 | CONCLUSIONS AND OUTLOOK

Studies on microscale distribution of nonplant parasitic nematode taxa are scarce, and it is largely unknown how nematode distribution patterns are related to nematode traits such as feeding type, cp value and phylogenetic position. For all nematode taxa included in this study, detection accuracies were not affected by an increase in the number of cores per composite sample above three. Notwithstanding this observation, a certain level of spatial variability within and between trophic groups was demonstrated upon analysis of detection probabilities and variability of abundances. The detection probability of several predatory and omnivorous taxa deviated from 100%, and points at a degree of local clustering. The variation of detection probabilities and variability of abundances expressed by individual taxa can be explained by field effects, whereas no significant effect of soil type and land-use system was found. It is noted that this conclusion will probably not hold for oligotrophic plant-parasites, a category of nematodes that was not included in this study.

Hence, to obtain insight into the presence or absence of a given nematode group in a given area, a relatively simple soil sampling strategy can be used for taxa within nematode communities at microplot level provided it is homogeneous in terms of land use and/or vegetation type. Accurate quantitative analysis may require the analysis of multiple composite samples, and the number was shown to be taxon-dependent, and—evidently—dependent on the level of accuracy required. Recently, we demonstrated distinct food preferences for individual lineages within a trophic group (Quist et al., 2014), and more detailed insights into trophic relationship between nematodes and other organismal groups in the soil food web will contribute to a better ecological understanding of shifts in nematode communities in bulk soil and the rhizosphere.

In this research, we used a quantitative, real-time PCR-based approach as we aimed to generate both qualitative and quantitative information about the spatial distribution of terrestrial nematode taxa. DNA metabarcoding approaches have been developed to assess the biodiversity of soil Metazoa (e.g., Capra et al., 2016), but it should be noted that high throughput sequencing approaches are not yet suitable for quantitative analyses of nematode assemblages.

Recently, nematodes received the highest score among a selection of 30 potential indicators (Griffiths et al., 2016). The detailed insights into the spatial distribution of nematodes at microplot scale presented here teach us about the impact of trophic preferences on the spatial distribution of individual nematode taxa and also will allow for the design of statistically sound soil sampling strategies.

This will bring the robust mapping of effects of anthropogenic and nonanthropogenic physical, chemical or biological disturbances on soil life within reach.

ACKNOWLEDGEMENTS

We are grateful to farmers Jos van Waes, Marijn Uitdewilligen, Unifarm and PPO Lelystad and nature conservation organizations Staatsbosbeheer and Natuurmonumenten for allowing us sampling on their properties. We gratefully thank Sven van den Elsen and Paul Mooijman for their technical support. We thank Erik Slootweg for his help selecting appropriate semi-natural locations. This research was supported by BE Basic Foundation, grant FS8.002.002.

AUTHOR CONTRIBUTION

C.Q., C.M., R.W., A.T. and J.H. designed the study; C.Q. generated the qPCR data, which were analysed by G.G. and C.Q.; and C.Q. and J.H. wrote the manuscript aided by all authors.

DATA ACCESSIBILITY

Nematode taxon specific densities determined by quantitative PCR assays were uploaded to Dryad (doi: 10.5061/dryad.1 g439).

REFERENCES

- Bal, H. K., Taylor, R. A. J., & Grewal, P. S. (2014). Ambush foraging entomopathogenic nematodes employ 'sprinters' for long-distance dispersal in the absence of hosts. *Journal of Parasitology*, *100*, 422–432.
- Been, T. H., & Schomaker, C. H. (2006). Distribution patterns and sampling. In R. N. Perry, & M. Moens (Eds.), *Plant nematology* (pp. 302–326). Wallingford: CABI.
- Berg, M. P. (2012). Patterns of biodiversity at fine and small spatial scales. In D. H. Wall, R. D. Bardgett, V. Behan-Pelletier, J. E. Herrick, T. H. Jones, K. Ritz, ... W. H. van der Putten (Eds.), *Soil ecology and ecosystem services* (pp. 136–152). Oxford, UK: Oxford University Press.
- Bongers, T. (1990). The maturity index – an ecological measure of environmental disturbance based on nematode species composition. *Oecologia*, *83*, 14–19.
- Bongers, T., & Ferris, H. (1999). Nematode community structure as a bioindicator in environmental monitoring. *Trends in Ecology and Evolution*, *14*, 224–228.
- Brinkman, E. P., Duyts, H., & van der Putten, W. H. (2008). Interactions between root-feeding nematodes depend on plant species identity. *Soil Biology and Biochemistry*, *40*, 2186–2193.
- Capra, E., Giannico, R., Montagna, M., & Pizzi, F. (2016). A new primer set for DNA metabarcoding of soil Metazoa. *European Journal of Soil Biology*, *77*, 53–59.
- Duncan, L. W., & Phillips, M. S. (2009). Sampling root-knot nematodes. In R. N. Perry, M. Moens & J. L. Starr (Eds.), *Root-knot nematodes* (pp. 275–300). Wallingford, UK: CABI Publishing.
- Ettema, C. H., & Wardle, D. A. (2002). Spatial soil ecology. *Trends in Ecology and Evolution*, *17*, 177–183.
- Fenwick, D. W. (1961). Estimation of field populations of cyst forming nematodes of the genus *Heterodera*. *Journal of Helminthology*, *35* (Suppl S1), 63–76.

- Ferris, H., Mullens, T. A., & Foord, K. E. (1990). Stability and characteristics of spatial description parameters for nematode populations. *Journal of Nematology*, 22, 427–439.
- Finlay, B. J. (2002). Global dispersal of free-living microbial eukaryote species. *Science*, 296, 1061–1063.
- Fitter, A. H. (2005). Darkness visible: Reflections on underground ecology. *Journal of Ecology*, 93, 231–243.
- Floyd, R., Abebe, E., Papert, A., & Blaxter, M. (2002). Molecular barcodes for soil nematode identification. *Molecular Ecology*, 11, 839–850.
- Griffiths, B. S., Römbke, J., Schmelz, R. M., Scheffczyk, A., Faber, J. H., Bloem, J., ... Stone, D. (2016). Selecting cost effective and policy-relevant biological indicators for European monitoring of soil biodiversity and ecosystem function. *Ecological Indicators*, 69, 213–223.
- Holterman, M., Rybarczyk, K., Van den Elsen, S., Van Megen, H., Mooijman, P., Peña Santiago, R., ... Helder, J. (2008). A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. *Molecular Ecology Resources*, 8, 23–34.
- Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., ... Helder, J. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution*, 23, 1792–1800.
- Holtkamp, R., Kardol, P., van der Wal, A., Dekker, S. C., van der Putten, W. H., & de Ruiter, P. C. (2008). Soil food web structure during ecosystem development after land abandonment. *Applied Soil Ecology*, 39, 23–34.
- Ito, T., Araki, M., Higashi, T., Komatsuzaki, M., Kaneko, N., & Ohta, H. (2015). Responses of soil nematode community structure to soil carbon changes due to different tillage and cover crop management practices over a nine-year period in Kanto, Japan. *Applied Soil Ecology*, 89, 50–58.
- Ivanova, N. V., Dewaard, J. R., & Hebert, P. D. N. (2006). An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes*, 6, 998–1002.
- Jansa, J., Erb, A., Oberholzer, H. R., Smilauer, P., & Egli, S. (2014). Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. *Molecular Ecology*, 23, 2118–2135.
- Janssen, G. J. W. (1994). The relevance of races in *Ditylenchus dipsaci* (Kuhn) Filipjev, the stem nematode. *Fundamental and Applied Nematology*, 17, 469–473.
- Janzen, H. H. (2006). The soil carbon dilemma: Shall we hoard it or use it? *Soil Biology and Biochemistry*, 38, 419–424.
- Karlen, Y., McNair, A., Perseguers, S., Mazza, C., & Mermod, N. (2007). Statistical significance of quantitative PCR. *BMC Bioinformatics*, 8, 131.
- Klironomos, J. N., Rillig, M. C., & Allen, M. F. (1999). Designing below-ground field experiments with the help of semi-variance and power analyses. *Applied Soil Ecology*, 12, 227–238.
- Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R. D., & Schabenberger, O. (2006). *SAS system for mixed models*, 2nd ed.. Cary, North Carolina: SAS Institute Inc..
- Martiny, J. B. H., Bohannan, B. J. M., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L., ... Staley, J. T. (2006). Microbial biogeography: Putting microorganisms on the map. *Nature Reviews Microbiology*, 4, 102–112.
- Massey, C. L. (1974). *Biology and taxonomy of nematode parasites and associates of bark beetles in the United States*. In Agricultural Handbook no. 446. USDA Forest Service. Washington, DC: U.S. Government Printing Offices.
- McSorley, R., & Parrado, J. L. (1982). Estimation relative error in nematode numbers from single soil samples composed of multiple cores. *Journal of Nematology*, 14, 522–529.
- Moens, T., Verbeeck, L., De Maeyer, A., Swings, J., & Vincx, M. (1999). Selective attraction of marine bacterivorous nematodes to their bacterial food. *Marine Ecology Progress Series*, 176, 165–178.
- Moore, S. R., Lawrence, K. S., Arriaga, F. J., Burmester, C. H., & Van Santen, E. (2010). Natural migration of *Rotylenchulus reniformis* in a No-Till cotton system. *Journal of Nematology*, 42, 307–312.
- Mulder, C., & Vonk, J. A. (2011). Nematode traits and environmental constraints in 200 soil systems: Scaling within the 60–6,000 μm body size range. *Ecology*, 92, 2004.
- Neher, D. A. (2001). Role of nematodes in soil health and their use as indicators. *Journal of Nematology*, 33, 161–168.
- Neher, D. A., Wu, J., Barbercheck, M. E., & Anas, O. (2005). Ecosystem type affects interpretation of soil nematode community measures. *Applied Soil Ecology*, 30, 47–64.
- Nelson, K. L., Boiteau, G., Lynch, D. H., Peters, R. D., & Fillmore, S. (2011). Influence of agricultural soils on the growth and reproduction of the bio-indicator *Folsomia candida*. *Pedobiologia*, 54, 79–86.
- Nijs, L. D., & Van Den Berg, W. (2013). The added value of proficiency tests: Choosing the proper method for extracting Meloidogyne second-stage juveniles from soil. *Nematology*, 15, 143–151.
- Oostenbrink, M. (1960). Estimating nematode populations by some selected methods. *Nematology*, 6, 85–102.
- Pansu, J., De Danieli, S., Puissant, J., Gonzalez, J., Gielly, L., Cordonnier, T., ... Cécillon, L. (2015). Landscape-scale distribution patterns of earthworms inferred from soil DNA. *Soil Biology and Biochemistry*, 83, 100–105.
- Penkov, S., Ogawa, A., Schmidt, U., Tate, D., Zagoriy, V., Boland, S., ... Kurzchalia, T. V. (2014). A wax ester promotes collective host finding in the nematode *Pristionchus pacificus*. *Nature Chemical Biology*, 10, 281–285.
- Porazinska, D. L., Duncan, L. W., McSorley, R., & Graham, J. H. (1999). Nematode communities as indicators of status and processes of a soil ecosystem influenced by agricultural management practices. *Applied Soil Ecology*, 13, 69–86.
- Quist, C. W., Schrama, M., De Haan, J. J., Smant, G., Bakker, J., Van der Putten, W. H., & Helder, J. (2016). Organic farming practices result in compositional shifts in nematode communities that exceed crop-related changes. *Applied Soil Ecology*, 98, 254–260.
- Quist, C. W., Smant, G., & Helder, J. (2015). Evolution of plant parasitism in the phylum nematoda. *Annual Review of Phytopathology*, 53, 289–310.
- Quist, C. W., Vervoort, M. T. W., Van Megen, H., Gort, G., Bakker, J., Van der Putten, W. H., & Helder, J. (2014). Selective alteration of soil food web components by invasive Giant goldenrod (*Solidago gigantea*) in two distinct habitat types. *Oikos*, 123, 837–845.
- Robertson, G. P., & Freckman, D. W. (1995). The spatial distribution of nematode trophic groups across a cultivated ecosystem. *Ecology*, 76, 1425–1432.
- Rossi, J.-P., Delaville, L., & Quénehervé, P. (1996). Microspatial structure of a plant-parasitic nematode community in a sugarcane field in Martinique. *Applied Soil Ecology*, 3, 17–26.
- Rybarczyk-Mydlowska, K., Mooijman, P., Van Megen, H., Van den Elsen, S., Vervoort, M.T.W., Veenhuizen, P., ... Helder, J. (2012). Small sub-unit ribosomal DNA-based phylogenetic analysis of foliar nematodes (*Aphelenchoides* spp.) and their quantitative detection in complex DNA backgrounds. *Phytopathology*, 102, 1153–1160.
- Rybarczyk-Mydlowska, K., Van Megen, H., Van den Elsen, S., Mooijman, P., Karssen, G., Bakker, J., & Helder, J. (2014). Both SSU rDNA and RNA polymerase II data recognise that root-knot nematodes arose from migratory Pratylenchidae, but probably not from one of the economically high-impact lesion nematodes. *Nematology*, 16, 125–136.
- Seinhorst, J. W. (1982). The distribution of cysts of *Globodera rostochiensis* in small plots and the resulting sampling errors. *Nematologica*, 28, 285–297.

- Seinhorst, J. W. (1988). *The estimation of densities of nematode populations in soil and plants*. Växtskyddrapporter. Jordbruk 51. Uppsala, Sweden: Research information Center of the Swedish University of Agricultural Sciences.
- Serobyán, V., Ragsdale, E. J., & Sommer, R. J. (2014). Adaptive value of a predatory mouth-form in a dimorphic nematode. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20141334.
- Shtonda, B. B., & Avery, L. (2006). Dietary choice behavior in *Caenorhabditis elegans*. *Journal of Experimental Biology*, 209, 89–102.
- Simmons, B. L., Niles, R. K., & Wall, D. H. (2008). Distribution and abundance of alfalfa-field nematodes at various spatial scales. *Applied Soil Ecology*, 38, 211–222.
- Six, J., & Paustian, K. (2014). Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. *Soil Biology and Biochemistry*, 68, A4–A9.
- Spiridonov, S. E., Moens, M., & Wilson, M. J. (2007). Fine scale spatial distributions of two entomopathogenic nematodes in a grassland soil. *Applied Soil Ecology*, 37, 192–201.
- Steele, A. E. (1965). The host range of the sugar beet nematode, *Heterodera schachtii* Schmidt. *Journal of Sugarbeet Research*, 13(7), 573–603.
- Verschoor, B. C., & de Goede, R. G. M. (2000). The nematode extraction efficiency of the Oostenbrink elutriator-cottonwool filter method with special reference to nematode body size and life strategy. *Nematology*, 2, 325–342.
- Vervoort, M. T. W., Vonk, J. A., Mooijman, P. J. W., Van den Elsen, S., Van Megen, H., Veenhuizen, P., ... Helder, J. (2012). SSU ribosomal DNA-based monitoring of assemblages reveals distinct seasonal fluctuations within evolutionary heterogeneous feeding guilds. *PLoS ONE*, 7, e47555.
- Viketoft, M. (2013). Determinants of small-scale spatial patterns: Importance of space, plants and abiotics for soil nematodes. *Soil Biology and Biochemistry*, 62, 92–98.
- Wagg, C., Bender, S. F., Widmer, F., & Van Der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 5266–5270.
- Wallace, H. R. (1958). Movement of eelworms. II. A comparative study of the movement in soil of *Heterodera schachtii* Schmidt and *Ditylenchus dipsaci* Kuhn) Filipjev. *Annals of Applied Biology*, 46, 86–94.
- Wallace, H. R. (1960). Movement of eelworms. VI. The influence of soil type, moisture gradients and host plant roots on the migration of the potato-root eelworm *Heterodera rostochiensis* Wollenweber. *Annals of Applied Biology*, 48, 107–120.
- Wiesel, L., Daniell, T. J., King, D., & Neilson, R. (2015). Determination of the optimal soil sample size to accurately characterise nematode communities in soil. *Soil Biology and Biochemistry*, 80, 89–91.
- Yeates, G. W. (2003). Nematodes as soil indicators: Functional and biodiversity aspects. *Biology and Fertility of Soils*, 37, 199–210.
- Yeates, G. W., Bongers, T., de Goede, R. G. M., Freckman, D. W., & Georgieva, S. S. (1993). Feeding habits in soil nematode families and genera - an outline for soil ecologists. *Journal of Nematology*, 25(3), 315–331.
- Zhao, J., & Neher, D. A. (2013). Soil nematode genera that predict specific types of disturbance. *Applied Soil Ecology*, 64, 135–141.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Quist CW, Gort G, Mulder C, et al. Feeding preference as a main determinant of microscale patchiness among terrestrial nematodes. *Mol Ecol Resour.* 2017;00:1–14. <https://doi.org/10.1111/1755-0998.12672>