European Journal of Soil Biology 117 (2023) 103512

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: www.elsevier.com/locate/ejsobi

Original article

Communities of nematodes, bacteria and fungi differ among soils of different wild cabbage populations

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ARTICLE INFO

Handling editor: Zoë Lindo

Keywords:
Belowground community ecology
Brassica oleracea
Glucosinolates
Rhizosphere
Soil biota
Soil-root interphase

ABSTRACT

Plants exhibit significant variation in morphological and chemical traits of shoots and roots in response to an array of biotic and abiotic selection pressures, and this variation in turn affects their interactions with the biotic and abiotic environment. Thus far, most studies examining these interactions have focused on the aboveground domain, which is easier to study than the belowground domain. However, soil organisms significantly affect plant fitness directly through mutualisms e.g. growth promotion, or antagonisms e.g. herbivory and disease. Natural populations of wild *Brassica oleracea* L. growing along the south coastline of Great Britain exhibit significant differences in growth form and secondary chemistry. Studies in the field have shown that these differences affect aboveground plant-insect interactions, whereas soil communities have not been explored. We sampled belowground communities of nematodes, bacteria and fungi associated with roots, rhizosphere and bulk soil in five coastal wild cabbage populations in Dorset, England, and found significant differences among these communities. Site-related differences in nematode community composition were primarily found for nematodes in bulk soil and were consistent over two years of sampling. Nematode communities in roots of wild cabbage did not significantly differ across the cabbage populations but did differ between the two years. Results for communities in rhizosphere soil were spatially and temporally variable. The composition of nematode communities in cabbage roots differed strongly from those in the rhizosphere and bulk soil, showing that plants attract a subset of nematodes from the bulk soil community. For microbes, we analysed only rhizosphere samples, and found that fungal communities differed more strongly among plant populations than bacterial communities. Thus, while there is spatio-temporal variation in belowground communities, soil and/or plant properties differentially affect the assembly of nematodes, fungi and bacteria.

1. Introduction

The study of interactions between plants and other organisms is often focused on the aboveground domain. Less is known about how interactions between plant roots and soil organisms shape phenomena such as plant defense, intra- and interspecific competition, dispersal and colonization and belowground community structure. The soil is teeming with both micro- and macro invertebrates as well as microbes. Often, specific communities of soil organisms are adapted to certain plant species and genotypes as well as local soil conditions [1–3]. Some microbes in the soil or those that directly interact with the plant roots affect plant fitness either negatively or positively. For example, rhizobacteria and arbuscular mycorrhizal fungi can promote plant growth, whereas pathogenic and parasitic microbes can be detrimental or impair plant health [4–7]. Interactions between soil organisms and plants can also indirectly feedback to affect plants, e.g. through changes in soil fertility [8,9] or by acting as biocontrol agents [5]. Lastly, organisms in the rhizosphere can affect the growth and quality of plant tissues

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https://doi.org/10.1016/j.ejsobi.2023.103512
Received 11 October 2022; Received in revised form 6 May 2023; Accepted 11 May 2023
Available online 9 June 2023

aboveground and *vice-versa*, with corresponding effects on the behavior and development of consumers up the food chain [10–15]. Consequently, knowledge of factors affecting multitrophic interactions occurring aboveground is enhanced when processes in the soil are considered [13].

Most soil bacteria and fungi feed on living or dead plant tissues, whereas nematodes occupy a variety of trophic levels. Bacterial and fungal interactions with plants range from neutral to symbiotic to pathogenic [4,7]. Nematodes exhibit diverse lifestyles, from free-living bacterivores, fungivores, carnivores and omnivores to a variety of feeding types on plant roots [16–18]. Advancements in screening techniques, such as next generation sequencing, have promoted the study of geographical patterns and community structure of soil microbiota and the underlying factors that determine their distribution, but the field is still in its infancy and it remains underappreciated [2,19]. The long-standing assumption that bacteria generally have a global distribution in their specific habitats is being increasingly debated among microbial ecologists as endemic soil bacterial genotypes are increasingly being reported [19–21]. Bacterial and fungal biogeography is thought to be controlled primarily by chemical and physiological soil variables, such as pH [22,23]. Nematode communities are also known to exhibit strong variation in their spatial and temporal dynamics [3,24,25].

Microscale heterogeneity in soils caused by the arrangement of aggregates, referred to as soil structure, together with soil texture, is of particular importance in regulating biotic activity because of its influence on water content and aeration, and it plays a major role in the microscale distribution of soil biota [26]. However, the distribution of symbiotic, pathogenic or commensal biota is largely determined by that of their host plant [21]. The rhizosphere, in which a considerable amount of the net fixed carbon can be excreted, is a 'hot spot' for microbial activity where root exudates and other rhizo-deposits play a major role in determining the composition of the microbial community in the root-soil interface [7,27]. These biota regulate soil processes, such as nutrient recycling, and disease control, which are critically important for plant growth and health [27,28]. Although soil biota are an important driving force determining plant community structure and composition, plants in turn regulate the composition of soil microbiota and their functioning by providing nutrients and excreting defensive compounds [29–31].

The composition of soil microbiota among different populations of the same plant species growing in their natural environment has been rarely studied [32]. Wild cabbage, *Brassica oleracea*, is a perennial plant species that is native to coastlines of western and southern Europe, including the United Kingdom [33]. In the county of Dorset (UK), wild cabbage grows in small distinct populations primarily on maritime calcareous cliffs. Despite growing close (<10 km) to one another, the Dorset populations of *B. oleracea* have been spatially stable for at least 70 years with high genetic differentiation [34,35]. Wild cabbage has proven to be a model plant for exploring how local selection pressures drive the expression of chemical defenses in aboveground shoots. Several studies have reported that there is considerable variation in the types and concentrations of foliar secondary metabolites (i.e. glucosinolates) among the Dorset populations, which in turn affects development of aboveground insects and their natural enemies [36–39]. *Brassica oleracea*, like other species in the Brassicales, produces glucosinolates in all tissues, including the roots, which convey resistance to plant antagonists, in particular poorly-adapted species that are limited in their ability to metabolize and detoxify these secondary metabolites [40,41]. Glucosinolate concentrations also vary in root tissues of wild cabbage populations originating from different locations in the UK and these affect interactions with insect root herbivores [42,43]. Plant roots release large amounts of chemicals in the soil and these root exudates play an important role in the establishment of plant-microbe interactions [44,45]. Variation in root chemistry in the Dorset cabbage populations provide a model system to investigate to what extent belowground communities of microorganisms associated with plants differ across populations of the same species growing in their natural environment.

The main aim of this study is to compare the diversity and abundance of nematodes, bacteria and fungi in the soil and fine roots among populations of wild cabbage from Dorset. Based on earlier studies, which have reported differences in plant quality among the different cabbage populations affecting performance of shoot insect herbivores, we hypothesize that communities of these microorganisms will also vary belowground. The establishment of these communities in the soil is determined by complex interactions between soil type and plant species [46] and differs for bulk soil, the rhizosphere and the roots [32]. We compared community composition of microbes and nematodes in bulk soil, rhizosphere soil and the roots of cabbage plants originating from five different cabbage populations. With this approach, we will be able to disentangle, at least to some extent, whether differences in nematode and microbial composition are determined more by differences related to local soil conditions or by differences between the cabbage populations themselves. To determine robustness of the patterns, we sampled and analysed nematode communities in two consecutive years.

2. Materials and methods

2.1. Site description

In August of 2011 and 2012 we sampled the roots, rhizosphere soil and bulk soil of plants from five wild cabbage populations along a 25–30 km coastline in Dorset (UK)(Fig. S1). The vegetation type of these sites has been classified as maritime grassland [34]. The minimum distance between sites is ca. 2 km and the maximum is ca. 10 km. At the Durdle Door site (coordinates 50.621, −2.277), which is a partially secluded beach surrounded by hills, we sampled plants growing on the hill and at the back of the beach. The Kimmeridge site (50.604, −2.126) is located on top of a cliff and is exposed to the prevailing south-westerly wind. Plants were sampled along a trail that follows the coastline. At St. Aldhelm's Head (50.580, −2.058) we sampled plants at different elevations on a steep hillside which is partially secluded from wind exposure. Winspit valley (50.584, −2.033) is a sheltered and rocky location where we sampled plants from small patches among the rocks. The Old Harry population (50.641, −1.924) is located on top of a cliff facing the east and is therefore less exposed to the wind than Kimmeridge. From here onwards, we will refer to the populations as DD (Durdle Door), KIM (Kimmeridge), SAH (St Aldhelms Head), WIN (Winspit), and OH (old Harry).

2.2. Sample collection

At each population, six plants were randomly selected for sampling, maintaining a distance between sampled plants of at least 5 m. Root tissues, rhizosphere soil and bulk soil were collected for nematode community analysis in 2011 and 2012. For bacterial and fungal DNA analysis, which was only conducted in 2011, soil brushed from the roots (1–3 g) was collected in a plastic 50-ml vial. For the root tissues, part of the plant's root system was exposed by removing soil and a representative sample of both thick and thin roots was collected in a plastic 50-ml vial. For the rhizosphere soil, we collected ca. 2 g soil surrounding the roots of a single plant. For the bulk soil, we collected approximately 0.5 kg soil (top 25 cm) 1 m from the sample plant and placed it into plastic bags. All samples were stored after sampling and during transportation in a cool box. In the laboratory, soil samples for bacterial and fungal analysis were stored at −80 °C, while samples for nematode analysis were stored at 4 °C, until further processing. The root and soil samples were analysed for bacteria, fungi and nematode communities at the Netherlands Institute of Ecology in Wageningen.

2.3. Nematode extraction

Roots – Nematode samples were processed within a week after sampling. Nematodes were extracted from the root tissues at room temperature by incubating cut pieces of the root samples submerged in tap water using a funnel in a humidifier chamber. After 24 h, the funnels lined with filter paper holding the root samples were drained and the filtrate was allowed to settle for an additional 24 h. Most of the water was removed through suction until 10 ml remained which was followed by another round of sedimentation and removal of excess water until 2 ml of nematode suspension remained. The nematodes where then fixed in 10 ml 4% formalin and stored at 4 °C.

Soil - Nematodes from both the rhizosphere and bulk soil were extracted from approximately 100 g fresh bulk soil and the complete rhizosphere sample, respectively, by rinsing in Oostenbrink elutriators [47]. The water fraction containing the nematodes was sieved four times over sieves decreasing in mesh size (75-45 μm). The nematodes were separated from the remaining fluid by allowing the nematodes to migrate through a double cotton filter for 24 h at 20 °C. The nematodes were concentrated and fixated as described for root nematodes. After extraction, the soil samples were dried in an oven at 70 °C for 48 h and weighed. Nematode densities were expressed as the number per 100 g soil dry weight.

2.4. Nematode abundance and identification

For the root tissues, all the nematodes in 5 ml of the original 10 ml nematode suspension were counted and identified morphologically. Nematodes were identified to family or genus level using a light microscope (Olympus, type BX40). The identified nematodes were divided into feeding groups according to Yeates et al. [18] and Bongers & Bongers [17] as herbivores, fungivores, bacterivores, carnivores or omnivores. If there were more than 150 nematodes in 5 ml, all nematode were counted but only 150 were identified, and numbers were multiplied to correct for this. Nematodes that were too deformed to be properly identified were included in the total counts. After extraction, the root samples were dried in an oven at 70 °C for 48 h and weighed. Nematode densities were expressed as the number per 100 g root dry weight.

For the rhizosphere and bulk soil, total nematode abundance was determined as described for the roots samples in only 1 ml from the original 10 ml nematode suspension. To express the nematode density per 100 g dry soil, a subsample of soil was taken, weighed fresh, dried at 105 °C for 72 h and then weighed again so the soil moisture content could be calculated.

2.5. T-RFLP analysis of bacterial and fungal DNA

We used terminal restriction fragment length polymorphism (T-RFLP) analysis to determine the composition of the bacteria and fungi soil communities. This high-throughput fingerprinting method was popular in the first decade of this century until more advanced, cheap, sequencing methods became available, which were only starting to be developed when the samples from this study were analysed. In short, a targeted gene is amplified and labelled with fluorescent dyes using PCR and consequently digested with an restriction enzyme [48]. The resulting mixture of fragments is separated using electrophoresis. Each peak in the sample detected by the fluorescence detector is considered to correspond to one taxonomic unit. Treatments (here population site) can be compared using presence/absence patterns of peaks in each sample.

Total DNA was extracted from 0.25 g frozen soil (−20 °C) with a Power Soil DNA isolation kit (MOBIO laboratories, Inc.) using a bead beating system. DNA concentration was measured using the NanoDrop 2000. For the samples with a DNA concentration below 10 ng/μl a new DNA-extraction was performed.

Fungi - For the analysis of the fungi, the ITS region of the rDNA was amplified by PCR using the primers ITS1F (5′ CTTGTTCATTTAGAGGAA 3′ [49]) and ITS4R (5′ TCCTCCGCTTATTGATATGC 3’ [50]), which were dual-end labelled with 6FAM and NED respectively.

The PCR reaction contained: 13.8 μL Milli-Q water, 2.5 μL 10x Fast Start High Fidelity Reaction Buffer (Roche Diagnostics), 2.5 μL dNTP mix (2 mM of each), 2.5 μL ITS1Ff-6FAM primer (10 μM), 2.5 μL ITS4r-NED primer (0.2 μM), 0.2 μL Fast Start High Fidelity Enzyme Blend (5 U μL^{-1}) (Roche Diagnostics) and 1 μL template DNA. PCR cycle conditions: 5 m at 95 °C, 34 cycles of 30 s at 95 °C, 40 s at 55 °C and 1 m at 72 °C, followed by 10 m at 72 °C before cooling. PCR product presence and quality were verified on 1.5% agarose gels prior to restriction digestion.

Two restriction enzymes, *Hha*I and TaqαI (New England Biolabs, Ipswich, MA, USA), were used to digest the dual end-labelled DNA amplicons in a mixture containing: 3.5 μL ddH_2O, 1 μL buffer, 0.1 μL Bovine Serum Albumin, 5 μL PCR product and 0.4 μL restriction enzyme, incubated at 37 °C (*Hha*I) or at 65 °C (TaqαI) for 3 h, followed by inactivation at 80 °C for 20 min. Restriction products were purified using ethanol precipitation.

Bacteria - Bacterial DNA was amplified by PCR using the fluorescently-labelled forward primer 27f-FAM (5′ AGAGTTT-GATCCTGGCTCAG 3′) and the unlabeled reverse primer 1492r (5′ GR TACCTTGTTACGACTT 3’), which target the bacterial 16S rRNA gene ([51]). The PCR reaction contained: 17.8 μL Milli-Q water, 2.5 μL 10x Fast Start High Fidelity Reaction Buffer (Roche Diagnostics), 2.5 μL dNTP Mix (2 mM of each), 0.5 μL of each primer 27f-FAM and 1492r (10 μM), 0.2 μL Fast Start High Fidelity Enzyme Blend (5 U μL^{-1}, Roche Diagnostics) and 1 μL template DNA. PCR amplification conditions were: 5 min at 95 °C, 34 cycles of 30 s at 95 °C, 40 s at 55 °C and 1 min at 72 °C, followed by 10 min at 72 °C before cooling. PCR product presence and quality were verified on 1.5% agarose gels prior to restriction digestion.

The restriction enzymes *Hha*I and *Msp*I (New England Biolabs, Ipswich, MA, USA) were used to digest the amplified DNA in a mixture containing: 3.5 μL ddH_2O, 1 μL buffer, 0.1 μL Bovine Serum Albumin, 5 μL PCR product and 0.4 μL of each restriction enzyme, held at 37 °C for 3 h, followed by enzyme inactivation at 80 °C for 20 min. Restriction products were purified using ethanol precipitation.

Fragment length polymorphism analysis was performed on an automated 3130 Genetic Analyser sequencer, using GeneScan-500 LIZ (Applied Biosystems, Carlsbad, CA, USA) as a size standard. Samples which were over- (highest peak >80 000 rfu) or underloaded (highest peak <1000 rfu) were re-run with an adjusted concentration. A clustering threshold of 0.5 bp was used to align peaks to TRFs among the samples. Each fragment is considered an OTU (Operational Taxonomic Unit). Only fragments with a size between 50 and 500 base pairs were included in the analysis. Peaks with heights lower than 0.3% of the sum of all heights for that sample were considered ‘noise’ and were removed from that sample.

2.6. Data analysis

All analyses were performed in R 4.0.2 [52]. The total number of nematodes in the roots, rhizosphere soil and bulk soil, respectively, were analysed using General Linear Model (GLM) analysis of variance with cabbage population, year and their interaction terms as fixed explanatory variables. Nematode numbers were standardized as their number per 100 g dried soil/roots. Total numbers were log-transformed to meet assumptions of homoscedasticity and normality. Post-hoc Tukey multiple comparison tests were performed when any of the terms is the model was significant ($P < 0.05$).

Non-metric multidimensional scaling (NMDS) was used to compare the composition of fungal, bacterial and nematode communities in the various samples. NMDS maximises rank-order correlations between distance measures in the ordination space. The distance matrix was based on pairwise Bray-Curtis dissimilarities between samples. The number of dimensions was set at 2 unless the final stress level was high (>0.2), and an additional dimension was included. Stress is a measure of

the disagreement between the ordination configuration and the predicted values from the regression (low stress is better). We first determined whether β-dispersion was similar among the samples of each group of interest, using the betadisper function in the *vegan* package [53]. This is a multivariate ordination variant of testing for homoscedasticity and it determines whether the average distance of group members to the group centroid is similar among groups. Beta dispersions (βdisp) based on dissimilarity indices were analysed using a GLM with sample type, year, or population as a fixed factor depending on the analysis. NMDS analysis was conducted to compare whether nematode species composition differed 1) among sample types (bulk soil, rhizosphere and roots) in each of the two years, 2) between the two years for each soil type, and 3) among populations for each soil type in each year. For fungi and bacteria in rhizosphere soil we only analysed the effect of plant population in 2011. For the nematodes we used the number of nematodes of each species per 100 g dried sample. For the bacteria and fungi, we used absence/presence data of the OTUs in each sample. We used the metaMDS and the adonis2 function of the *vegan* package. The adonis2 function was used to determine the extent in which explanatory variables explained variance based on PERMANOVA tests. The explanatory variables were population, sample type, and year, depending on the model (see results). If any of these terms was significant, pairwise comparisons were conducted using the adonis.pair function of the *EcolUtils* package [54]. To determine which nematode genera characterized grouping of the community, the multipatt function of the *idicspecies* package was used [55]. Alpha diversity computed as the Shannon index was also subjected to GLM analysis with the same explanatory variables as described for NMDS analysis. In addition, Mantel tests were conducted to investigate whether the NMDS dissimilarity distance matrices of the three communities in the rhizosphere correlated (Spearman) pair-wise according to population.

3. Results

3.1. Nematodes

In total 82 nematode taxa were identified in bulk soil, 77 in the rhizosphere, and 36 in the roots (Tables S1–S3). Soil and root samples for nematode analysis were collected in both 2011 and 2012. The total abundance of nematodes was significantly higher in 2012 than in 2011, both in the roots and in the rhizosphere, whereas the converse was found for bulk soil (Table 1, Fig. 1). In the roots, the year difference was mainly caused by higher numbers of bacterivorous nematodes that dominated the root-nematode community in 2012 (Fig. 1f).

Nematode abundance was significantly lower at the KIM site than at the OH and WIN site and were intermediate for the other two sites (Fig. 1a and b). The population effects were more pronounced in 2012, which led to the significant year-population interaction term (Table 1). Results on total abundance of nematodes in the rhizosphere of the five populations were more variable and were not consistent between the two years (Table 1, Fig. 1c vs 1d). Cabbage population had no significant effect on total nematode abundance in the roots in both years (Table 1, Fig. 1e and f).

Each of the three soil types were characterized by specific nematode communities. The difference in nematode composition was largest between the roots on the one hand, and rhizosphere and bulk soil on the other. In addition, the composition of nematodes in bulk soil and rhizosphere also differed significantly (pairwise PERMANOVA tests $P = 0.001$) in both years (Fig. 2a and b). The variation in nematode composition among samples was significantly higher in root samples in 2011 but lower in 2012, compared to the variation among bulk soil and rhizosphere samples (Table 2, beta dispersion; Fig. 2). Moreover, alpha diversity (Shannon index) of nematode taxa was significantly lower in the roots than in bulk and rhizosphere soil in both years (Table 2; Fig. 3a and b). The nematode community in bulk soil and the rhizosphere was dominated by herbivorous and bacterivorous nematodes (ca. 75% and 85% of total, respectively). The remaining nematodes were mostly omnivorous (almost 20% in bulk soil and more than 10% in rhizosphere soil). For a small number of nematode taxa (ca. 7% abundance in both soil types) the feeding guild is not clear, as these nematodes can feed on plant tissues, fungi or both. These patterns of feeding guild distribution were stable over the two years in bulk and rhizosphere soil, whereas the feeding guild composition in the roots was highly variable (Fig. 1e and f). For instance, in 2012, more than 90% of the nematodes were bacterivorous (predominantly two Rhabtidid genera; one unidentified and *Diploscapter*), whereas in 2011 bacterivorous nematodes contributed less than 30% to total nematode abundance. The remaining root nematodes were plant feeders, fungal feeders, or both, whereas there were few omnivorous or carnivorous nematodes in the roots (<0.5%). The roots harbored only a few taxa in higher densities (with a relative abundance of 10% or more), e.g. *Eucephalobus*, *Pratylensis* (both years), *Aphelnchoides*, *Tylenchorchynchus* (both in 2012), *Acrobeloides* and *Aphelenchus* (both in 2011). In rhizosphere soil, *Filenchus*, an unidentified Rhabtidid genus and *Eucephalobus* (only in 2011) were the most abundant genera that were present in most of the samples. More prominent nematodes in bulk soil were in the genera *Malenchus, Thonus*, *Eucephalobus* and an unidentified Tylenchid genus; the latter nematode genus contributed almost 20% to nematode abundance in 2011, whereas the distribution of these four genera was more homogeneous in 2012. For a detailed description of the nematode composition in samples taken from the different soil samples see Tables S1–S3.

Differences in nematode composition were also significant among the cabbage populations but only for bulk and rhizosphere soil (Table 2, Fig. 4). Especially, the nematode community in bulk soil from the KIM site clearly differed from that of the other locations in both years. This distinction could not be attributed to the presence of specific nematode taxa. However, overall nematode (alpha) diversity was lower at KIM than at the other sites (Fig. 5a and b). Nematode composition of SAH and OH bulk soils also differed consistently in both years (Fig. 4a and b). SAH and OH bulk soil each contained nematodes that were significantly more abundant at those than at the other sites. Alpha diversity was highest in OH bulk soil (Fig. 5a and b). Population-related differences in rhizosphere nematode communities were more idiosyncratic, but some consistencies were found. The OH nematode community in the rhizosphere differed from those in WIN and KIM rhizosphere soil, respectively in 2011 and 2012 (Fig. 4c and d). Also community composition differed in DD and SAH rhizosphere soil, in both years. As found for bulk soil, alpha diversity tended to be higher in OH rhizosphere soil, though this effect was not statistically significant (Fig. 5c and d).

Table 1
Statistical results on total abundance of nematodes in bulk soil, rhizosphere and the roots. General linear model analyses of variance conducted with the factor year, population (Pop) and their interaction term (Inter) as explanatory variables and log-transformed total nematode abundances (per 100 g dry material) as the response variable. Nematode data were analysed separately for bulk soil, rhizosphere soil and roots. F(df's): F-statistic with degrees of freedom between brackets.

Sample type	Year F (df's)	P-value	Pop F (df's)	P-value	Inter	P-value
Bulk soil	4.28 (1,49)	0.004	7.41 (4,49)	0.009	4.85 (4,49)	0.002
Rhizosphere	12.9 (1,50)	<0.001	2.45 (4,50)	0.057	4.16 (4,50)	0.005
Root	6.89 (1,49)	0.011	0.75 (4,49)	0.56	0.82 (4,49)	0.51

3.2. Bacteria in the rhizosphere

In total 433 bacterial OTUs were detected in the rhizosphere soil associated with wild cabbage. Of these OTUs 119 were found in only one sample and 25 were found in all or all but one of the samples from each

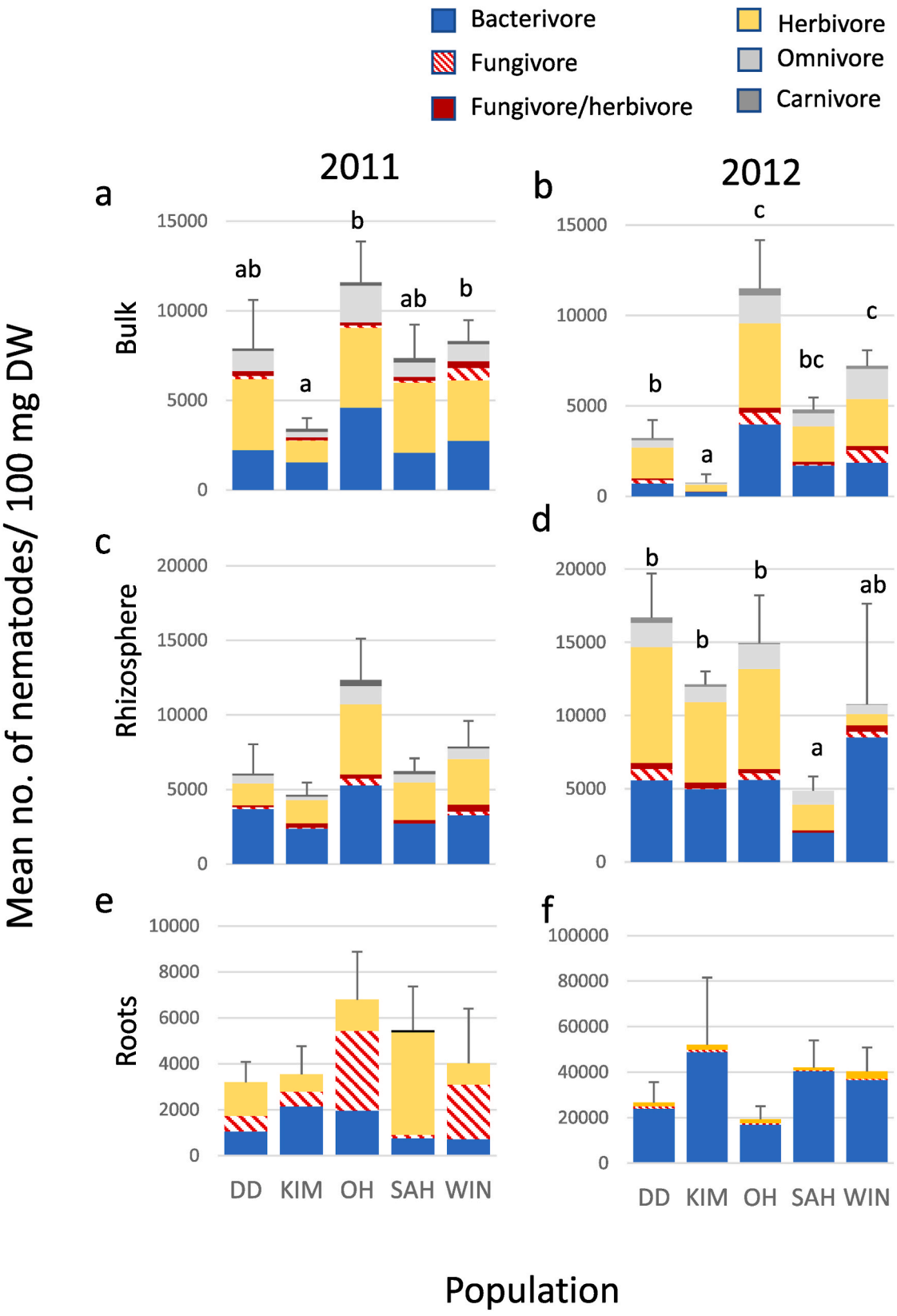

Fig. 1. Total nematode abundance (mean ± SE per 100 g dry material) in bulk soil (a, b), rhizosphere (c, d) and roots (e, f) collected at five sites (DD, KIM, OH, SAH, WIN) along the Dorset coast (UK) where wild cabbage grows naturally. Samples (n = 6 per population) were collected in 2011 (a, c, e) and 2012 (b, d, f). Different letters indicate significant differences between populations (Tukey tests, $P < 0.05$). Nematodes were classified as bacterivorous (blue), fungivorous (hatched in red), herbivorous (yellow), omnivorous (light grey) or carnivorous (dark grey). Some nematode can be feeding on both plants and fungi (red). In roots omnivourous and carnivorous nematodes are combined. Note the 10-fold difference in scale for root nematodes in 2011 and 2012.

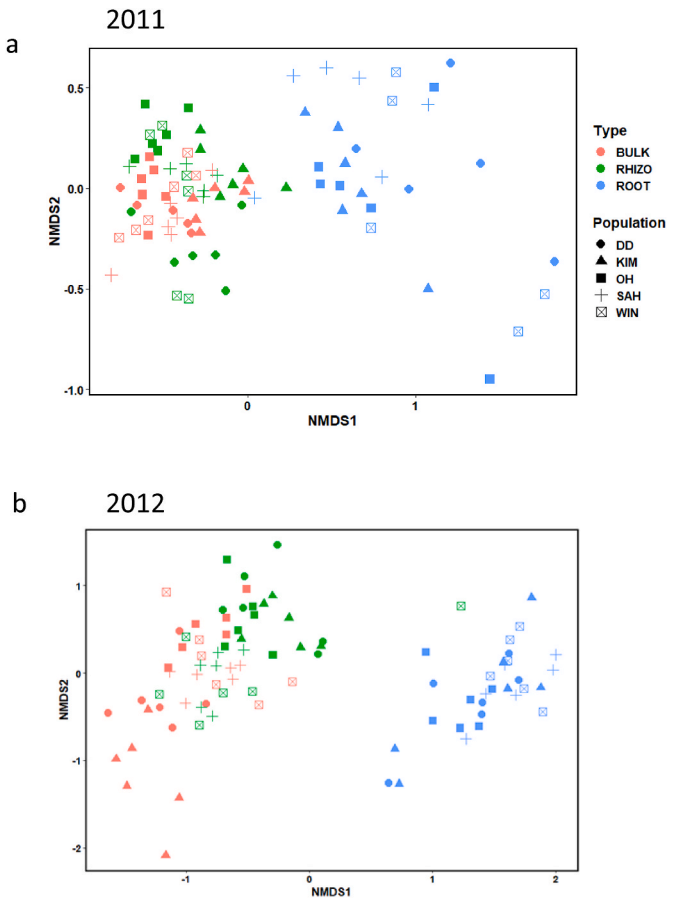

Fig. 2. NMDS ordination of nematode taxa based on their abundance per 100 g dry material in bulk soil (red), rhizosphere (green), and roots (blue) collected at five sites (DD, diamonds; KIM, filled triangles; OH, filled boxes; SAH, plusses; WIN, boxes with x) along the Dorset coast (UK) where wild cabbage grows naturally. Samples were collected in 2011 (a) and 2012 (b). Each point is a sample. Stress levels were 0.151 and 0.119 for (a) and (b), respectively, with models parameters set at two dimensions (see Materials and Methods for further details).

location. Eight and eight OTUs were uniquely associated with one or two populations, respectively. Beta-dispersion of the bacteria was similar among samples from the five locations ($F_{4,24}$ = 1.01, P = 0.42). The composition of the bacterial community differed according to sampling location (F_{pseudo} = 1.79, P = 0.001, R^2 = 0.23, permutations = 999, stress = 0.183, Fig. 6a). The bacterial communities in the rhizosphere of DD and OH, and of SAH and WIN differed significantly (P < 0.05), whereas those in the rhizosphere of KIM and DD plants were not significantly different (Fig. 6a). The KIM bacterial community also overlapped to some degree with that of the other four populations (Fig. 6a). Alpha diversity did not differ across the populations ($F_{4,24}$ =

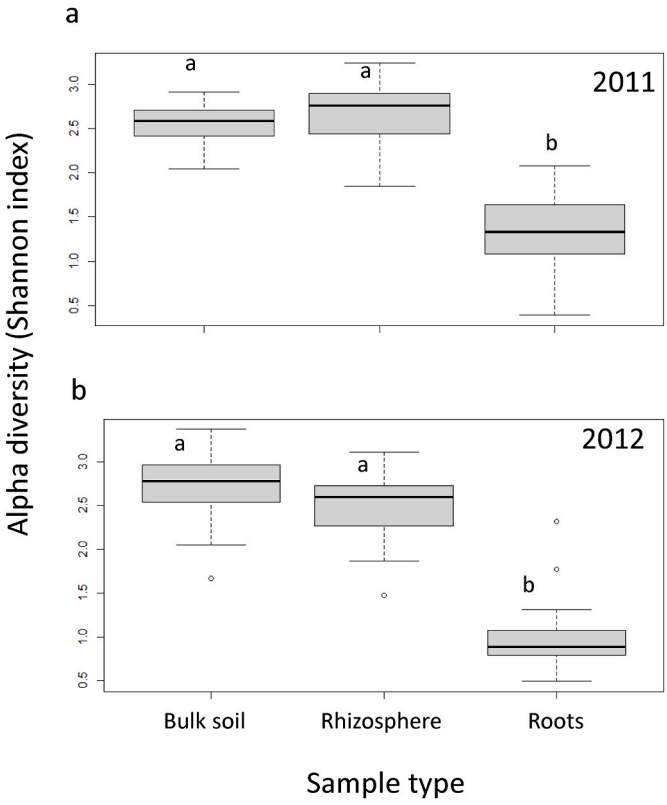

Fig. 3. Boxplots of alpha diversity of the nematode community per 100 g dry material in bulk soil, rhizosphere and roots pooled accross samples from the five sites (DD, KIM, OH, SAH, WIN) along the Dorset coast (UK) where wild cabbage grows naturally. Samples were collected in 2011 (a) and 2012 (b). Different letters indicate significant differences between sample types (Tukey tests, α < 0.05).

Table 2
Statistical results on multivariate analysis (NMDS) of rhizosphere bacterial, fungal, and nematode communities respectively. Nematodes communities were also compared in bulk soil and the roots. Beta dispersion (βdisp) was based on Bray-Curtis dissimilarity indices and analysed using a GLM. Permutational analysis of variance, generating a pseudo f-statistic (F_{sp}), was used to determine whether there were differences in community composition among sample types (bulk soil, rhizospheres or roots) in each year, between years for each sample types, and among the cabbage populations for each sampe type by year. Alpha diversity (α-div) using the Shannon index was analysed using a GLM. P provides the P-value for the statistic with the degrees of freedom (df).

	Factor	βdisp	P	F_{sp}	P	R^2	α-div	P	df
Nematodes									
2011	Sample type	8.91	<0.001	9.74	0.001	0.19	56.7[a]	<0.001	1,84
2012	Sample type	6.61	0.002	24.0	0.001	0.35	198	<0.001	1,87
Bulk	Year	3.86	0.054	5.21	0.001	0.08	0.91	0.34	1,57
Rhizosphere	Year	0.003	0.96	3.33	0.001	0.05	2.73	0.10	1,57
Root	Year	1.9	0.001	23.6	0.001	0.30	20.8	<0.001	1,56
Bulk-2011	Population	0.99	0.42	2.26	0.001	0.27	0.91	0.34	4,24
Bulk-2012	Population	0.55	0.69	3.48	0.001	0.36	10.1	<0.001	4,25
Rhizo-2011	Population	3.33	0.026	2.40	0.001	0.28	3.46	0.002	4,25
Rhizo-2012	Population	1.07	0.39	2.89	0.001	0.32	2.56	0.062	4,25
Root-2011	Population	1.01	0.42	1.29	0.10	0.18	3.32	0.026	4,24
Root-2012	Population	0.73	0.57	1.35	0.20	0.18	0.56	0.69	4,25
Bacteria	Population	1.01	0.42	1.79	0.001	0.23	1.83	0.15	4,24
Fungi	Population	2.47	0.07	1.51	0.001	0.19	0.75	0.57	4.25

[a] Kruskal-Wallis test was used due to violation of equal variance assumption.

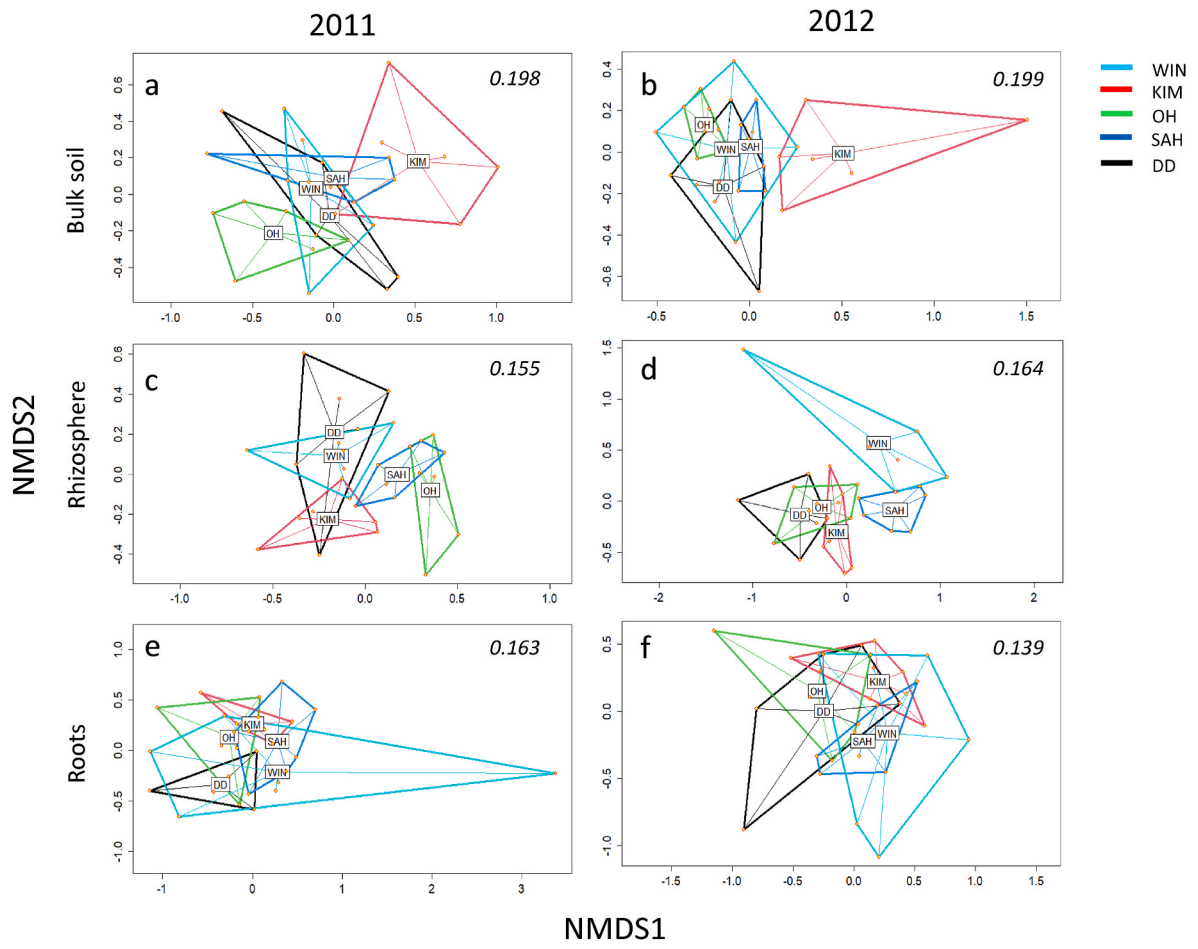

Fig. 4. NMDS ordination of bacterial (a) and fungal OTUs (b) based on their presence/absence 0.25 g frozen rhizosphere soil collected in 2011 at five sites (DD, black; KIM, red; OH, green; SAH, blue; WIN, light blue) along the Dorset coast (UK) where wild cabbage grows naturally. Each point is a sample. Numbers in italics depict stress levels with models parameters set at two (a, b, d, e) or three dimensions (c, f), see Materials and Methods for further details.

1.83, P = 0.15, Shannon index (mean ± SE) 4.68 ± 0.03).

3.3. Fungi in the rhizosphere

In total 1026 fungal OTUs were detected in the cabbage rhizosphere with 314 OTUs found in only one sample and seven found in five or more samples per population. Ten and 11 OTUs were unique for one or two populations, respectively. Beta-dispersion of the fungi was variable but did not differ statistically across populations ($F_{4,25}$ = 2.47, P = 0.07). The variation among the DD samples tended to be larger than the population variation for the other samples and was lowest for KIM plants (Fig. 6b). Fungal composition differed across the populations (F_{pseudo} = 1.51, P = 0.001, R^2 = 0.19, permutations = 999, stress = 0.154 with three dimensions). Almost all pairwise comparisons were significant (P < 0.05), except DD and WIN (P = 0.41) and for OH vs SAH (P = 0.09) (Fig. 6b). As for bacterial communities, alpha diversity did not differ among the populations ($F_{4,25}$ = 0.75, P = 0.57, Shannon index (mean ± SE) 5.16 ± 0.03.

In an additional analysis, we compared distance matrices of each of the three taxonomic communities for the samples taken at each location. None of the pair-wise Spearman correlation coefficients was significant (Table S4). Thus population-specific differences in community composition of nematodes, fungi and bacteria were not consistent.

4. Discussion

We found significant differences in soil communities of nematodes, bacteria and fungi among the five wild populations of Dorset *B. oleracea* plants. Site-related differences in nematode community composition were primarily found for nematodes in bulk soil and were consistent over the two years of sampling, whereas the nematode communities inside the roots of wild cabbage did not significantly differ across the plant populations and only differed between the two years. Population-related differences for rhizosphere soil were more variable and differed between the two years. The composition of the nematode communities in the roots of cabbage differed strongly from those in the rhizosphere and bulk soil. In rhizosphere soil, population differences for bacterial communities were less pronounced than for fungal communities. Population-related differences were also not consistent for the three types of communities. In other words the community structure of nematodes, fungi and bacteria in the rhizosphere of wild cabbage varied independently in each population.

Soil biota are distributed horizontally and vertically within the soil matrix [8,20,56,57]. Vertical gradients have been well documented and, in general, biomass of soil organisms is known to decline with soil depth [20]. Depending on the studied organism and habitat, soil biota also exhibit horizontal patchiness at variable scales and this is often attributed to physicochemical conditions of the soil, human activity (i.e. disturbance) and biotic processes [20,56]. At microscales (mm), soil porosity, and rhizodeposits play an important role in the distribution of nematodes, whereas at coarser spatial scales (<1 m), resources, plant community composition and diversity, ecosystem type and human disturbance become more important [58]. The spatial distribution of the Dorset wild cabbage populations has changed little over at least the past

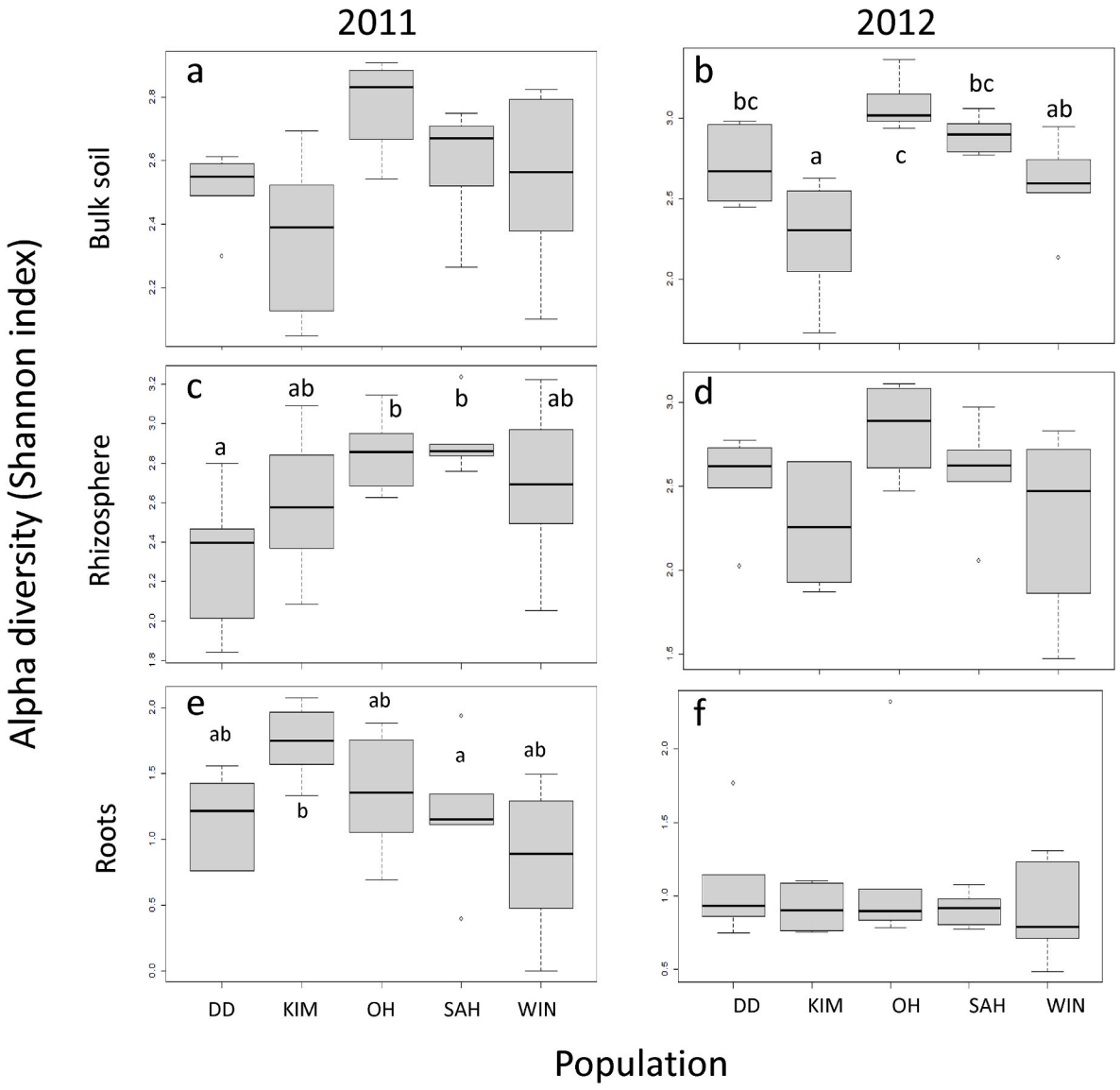

Fig. 5. Boxplots of alpha diversity (Shannon index) of nematode taxa per 100 g dry material in bulk (a, b), rhizosphere (c, d) and roots (e, f) collected at five sites (DD, KIM, OH, SAH, WIN) along the Dorset coast (UK) where wild cabbage grows naturally. Samples (n = 6) were collected in 2011 (a) and 2012 (b). Different letters indicate significant differences between sample types (Tukey tests, $\alpha < 0.05$).

70 years, suggesting that these populations are quite stable within this time frame [34], although they seem to show metapopulation processes of colonization and rescue effects [35]. Our results showed that, over a relative short time period of two years, population differentiation of the nematode communities was consistent for bulk soil, but not for the rhizosphere. Moreover, in both years, no population differentiation was found for nematodes associated with the roots.

At the soil-root interphase and within the roots, the abundance and composition of the nematode community was highly variable. In a study by Ettema et al. [59], nematodes were shown to be spatially structured up to 70 m, depending on the species and most of the large-scale spatial trends were persistent in time. Interestingly, in the same study, the authors found only a weak correlation between the distribution of the nematodes and soil abiotic variables (e.g. water content, nitrate, total N, total C). Moreover, temporal dynamics were more pronounced for nematodes than for soil resources. Quist et al. [60] also reported species-specific spatial variability in nematode abundance. The best predictor explaining spatial variability was organic matter content, which correlated positively with abundance of nematode belonging to colonizer-persister (cp) group 1 and 2 (mainly herbivores and fungivores) and negatively with those belonging to cp 3 and 4 (omnivores and predators) [60]. Cp classification orders nematodes from colonizers (cp1) to persisters (cp5) and can be used to determine a maturity index (MI) of the soil, which can serve as a tool to classify soil as disturbed and nutrient enriched (MI $\leq$ 2) to undisturbed and pristine (MI $\pm$4) [61]. The MI of the bulk soil samples of this study varied between 2.2 and 2.8 (Table S3). In our study, results for nematode composition were most consistent for bulk soil; cabbage population patterns were similar over the two years. The most contrasting populations were OH and KIM in terms of nematode composition, overall diversity of nematode taxa, and total nematode abundance. Alpha diversity and total nematode abundance were highest in OH and lowest in KIM bulk soil compared to bulk soil from the other sites. These differences were not determined by geographical distance; OH and DD are the furthest apart, but had greatly overlapping nematode communities.

Monroy et al. [25] found that community similarity of grassland nematodes and bacteria in the soil decreased with increasing geographic distance (up to 200 km). This effect was not related to changes in plant community composition or soil chemistry. However, they also found some site-specific structuring of the nematode communities. Notably,

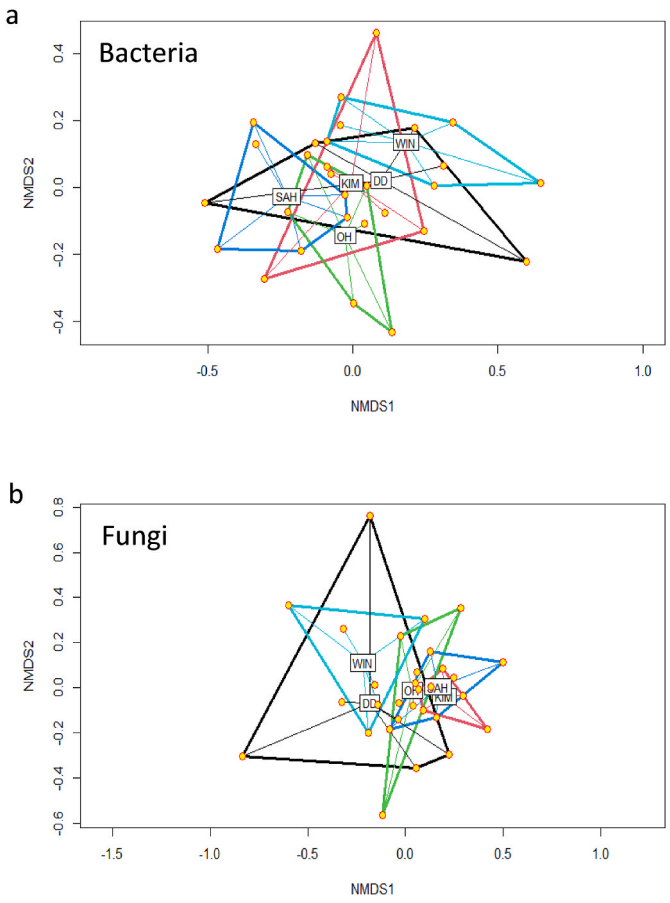

Fig. 6. NMDS ordination of bacterial (a) and fungal OTUs (b) based on presence/absence in 0.25 g rhizosphere samples (n = 6) collected in 2011 at five sites (DD, black; KIM, red; OH, green; SAH, blue; WIN, light blue) along the Dorset coast (UK) where wild cabbage grows naturally. Each point is a sample.

that study was also performed in the south of the UK, albeit further inland, although some of the sites were located less than 30 km away from the sites sampled here. Some degree of differentiation by distance was also found for nematode communities sampled in organic farmlands in different regions of Minnesota (USA) covering a distance of ca. 650 km [62]. We only sampled a limited number of sites located relatively close to one another (2–25 km), which may explain why such a pattern did not emerge.

Nematode community attributes were most variable in the rhizosphere soil. Interestingly, nematode community composition and abundance in the roots did not differ among the plant populations, whereas they did between the two years. The year effect could be attributed to Rhabditidae, which were highly abundant in the cabbage roots sampled in 2012. Species in this family of bacterivores are easily transported through insect phoresy and rapidly respond to resource opportunities [63]. Our results show that site-specific structural differentiation of the nematode community in bulk soil disappears in the roots and converges to a plant specific nematode community. Living in close association with plant roots requires specific adaptations and this explains why root-associated nematode communities are in general more specific than nematodes that live in the soil [64]. Our results also suggest that there are site-specific differences that affect nematodes in bulk soil, but also that a similar subset of these nematodes associate with the roots of these plants across the cabbage populations. Thus, most of the root nematode taxa that associate with *B. oleracea* are present at all sites. Moreover, nematode communities in the rhizosphere and roots clearly show temporal dynamics. The wild cabbage population in the UK are confined to maritime cliffs and grow in well-drained substrates of chalk or limestone with relatively high concentrations of exchangeable phosphorous [33]. Nevertheless, site-specific differences in biotic and abiotic soil characteristics may promote site-specific nematode communities in bulk soil, whereas temporal differences in soil conditions may greatly influence the nematode community in the rhizosphere and in the roots. These results illustrate the dynamic interplay between soil and plant derived properties influencing nematode community compositions and that they operate at different spatial scales within the soil matrix.

The distribution of rhizosphere bacteria was quite homogeneous among the cabbage populations, though some spatial differentiation did occur. By contrast, spatial differentiation was most pronounced for the fungal communities; the rhizosphere of most of the cabbage populations was characterized by site-specific fungal communities. Root exudates and other rhizo-deposits play a major role in determining the composition of the microbial community in the root-soil interphase [7,9,27,45]. The composition of root exudates is highly diverse and some of their constituents have clear antibiotic properties [45]. Trytophan-derived metabolites such as indole glucosinolates have potent antimicrobial activities and are exuded by the roots [45]. In addition, it has been shown that *Brassica* crops can act as soil biofumigation agents to control parasitic nematodes and plant pathogens through volatilization of glucosinolates [65]. The roots of the five populations used in this study exhibit quantitative variation in their glucosinolate profiles, including indole glucosinolates [42,43]. This, in addition to variation in the active transport of exudate phytochemicals (glucosinolates and other metabolites), could explain differences in the assemblage of the fungal community, but not the bacterial community, as cabbage population effects on bacteria were only marginal. Alternatively, local variation in soil properties among the cabbage sites is more deterministic in shaping the fungal communities than variation in (chemical) cabbage traits and this affects fungi stronger than bacteria. Lundberg et al. [32] studied the belowground bacterial community associated with eight different *Arabidopsis thaliana* accessions when grown in two natural soils that differed geochemically. The bacterial communities in bulk soil, rhizosphere soil and roots were strongly influenced by soil type [32]. However, the *Arabidopsis* root microbiomes from both soils were overlapping and characterized by low-complexity communities that were enriched in Actinobacteria and specific families from other phyla. They only reported quantitative differences in the root microbiome of the various genotypes. These results conform with our results for the nematode community; decreasing differentiation in nematode community composition from bulk soil to rhizosphere soil, to the roots. Geochemical measurements at the sites where these populations grow have been conducted before [34], but in order to link these soil properties to soil community attributes these measurements have to be conducted in both rhizosphere and bulk soil. Moreover, more advanced next generation sequencing techniques of fungi and bacteria would improve the resolution and identification of microbial communities in highly diverse soil samples compared to the now considered outdated T-RFLP high-throughput methodology.

The observed differences in belowground communities show that the wild cabbage populations not only differ in their aboveground communities [37,38], which has been attributed to variation in chemical profiles [39,66,67]. In the rhizosphere, plant population effects differed among the three types of soil organisms from little to no effect for bacteria, some differentiation for nematodes and stronger effects for fungi. To what extent these taxonomic differences are driven by variation in responses to chemicals in or released by the roots, in particular glucosinolates, merits further investigation. The soil serves as a reservoir for soil organisms that can potentially interact with the roots of a plant. The establishment of these communities is determined by complex interactions between soil type and plant species [46] and differs for bulk soil, the rhizosphere and the roots. The underlying mechanisms for these interactions clearly differ for bacteria, fungi and nematodes. Our nematode results and those by Kowalchuk et al. [68] further show that once

the relationship with the host plant is more intimate, host plant effects can override soil effects. As many bacteria and fungi feed on plant material, either alive or dead, some are adapted to associate with specific plant species and consequently plant community composition plays a role in their distribution and abundance [20,23]. However, the interaction between plants and organisms in the soil is complicated by the fact that the distribution of plants is also determined by soil biota and plant-soil feedback mechanisms mediated by these organisms [69,70].

Since we have just begun to explore the belowground compartment of these natural wild cabbage populations in the UK, important questions remain unanswered. For example, what are the consequences of these differences in soil biota for the plants in each population? Soil communities of nematodes, bacteria and fungi can affect plant fitness, biodiversity and community structure. It is unclear to what extent these wild cabbage populations have been affected by their soil communities of these micro-organisms and *vice versa* how these populations have affected their associated belowground communities. Moreover, it is also unknown to what extent naturally occurring communities of soil biota of these populations affect the outcomes of aboveground-belowground (multi)trophic interactions in these cabbage populations [13]. The composition of nematodes differed between the two years of sampling. Climate warming and concomitant extreme events, such as drought and flooding, can change the abundance, diversity and composition of soil biota communities [71–73]. To what extent the effects of anthropogenic changes contribute to temporal variation in soil communities in the study area is unknown. Future studies addressing these questions will greatly enhance our understanding of more complex biotic interactions across variable spatial and temporal scales.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank Pedro Beschoren da Costa for help with the statistical analysis. This research was supported by grant no. 821006 from the Earth and Life Sciences Foundation (ALW), which is subsidized by the Netherlands Organization for Scientific Research (NWO). JMB was funded by UKCEH project 06895.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2023.103512.

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