Extracting DNA from soil or directly from isolated nematodes indicate dissimilar community structure for Europe-wide forest soils

Jonathan Donhauser [a,b], Maria J.I. Briones [c], Juha Mikola [d,e,f], Davey L. Jones [g,h],
Reinhard Eder [i], Juliane Filser [j], Aline Frossard [a], Paul Henning Krogh [k], José Paulo Sousa [l],
Jérome Cortet [m], Ellen Desie [n], Xavier Domene [o,p], Simoneda Djuric [q], Davorka Hackenberger [r],
Juan J. Jimenez [s], Maria Iamandei [t], Cornelia Rissmann [u], Olaf Schmidt [v], Merrit Shanskiy [w],
Tarja Silfver [e,x], Karen Vancampenhout [n], Martina Vasutova [y], Emiliya Velizarova [z], Beat Frey [a,*]

[a] Rhizosphere Processes Group, Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland
[b] Department of Biology, University of Copenhagen, Copenhagen, Denmark
[c] Departamento de Ecología y Biología Animal, Universidad de Vigo, Vigo, Spain
[d] Natural Resources Institute Finland (Luke), Helsinki, Finland
[e] Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, University of Helsinki, Lahti, Finland
[f] Kevo Subarctic Research Institute, Biodiversity Unit of the University of Turku, Utsjoki, Finland
[g] Environment Centre Wales, Bangor University, Bangor, Gwynedd, UK
[h] SoilsWest, Centre for Sustainable Farming Systems, Food Futures Institute, Murdoch University, Murdoch, Australia
[i] Group of Fodder Crop Breeding, Agroscope, Zurich, Switzerland
[j] University of Bremen, FB 02 - Ecology, Center for Environmental Research and Sustainable Technology (UFT), Bremen, Germany
[k] Department of Ecoscience, Aarhus University, Denmark
[l] Centre for Functional Ecology, Associated Laboratory TERRA, Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[m] CEFE, Univ. Montpellier, CNRS, EPHE, IRD, Univ. Paul-Valéry Montpellier 3, Montpellier, France
[n] Division Forest, Nature and Landscape, KU Leuven Campus Geel, Geel, Belgium
[o] CREAF, Bellaterra, Cerdanyola del Vallès, Catalonia, 08193, Spain
[p] Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), Catalonia, 08193, Spain
[q] University of Novi Sad, Novi Sad, Serbia
[r] Department of Biology, University of Osijek, Osijek, Croatia
[s] Pyrenean Institute of Ecology (IPE-CSIC), Jaca, Huesca, Spain
[t] Research Development Institute for Plant Protection, Bucharest, Romania
[u] Institute for Biology, Humboldt University, Berlin, Germany
[v] UCD School of Agriculture and Food Science, and UCD Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland
[w] Chair of Soil Science, Estonian University of Life Sciences, Tartu, Estonia
[x] Natural Resources Institute Finland (Luke), Maaninka, Finland
[y] Department of Carbon Storage in the Landscape, Global Change Research Institute, Czech Academy of Sciences, Ceske Budejovice, Czech Republic
[z] Ministry of Environment and Water, Sofia, Bulgaria

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ABSTRACT

Nematodes are numerous in soils and play a crucial role in soil food-webs. DNA metabarcoding offers a time-effective alternative to morphology-based assessments of nematode diversity. However, it is unclear how different DNA extraction methods prior to metabarcoding could affect community analysis. We used soils with woody vegetation from a European latitudinal gradient (29 sites, 39 to 79°N, ~4500 km, covering six biomes) to systematically evaluate the effect of two sources of nematode DNA either directly extracted from soils vs. extracted from nematodes previously isolated from soils hypothesizing that the DNA source material may produce different diversities, community structures and abundances of feeding types. Nematode-sample DNA exhibited a higher richness, while no difference in Shannon diversity was found between the approaches. The DNA sources also created significantly different community structures, with greater differences observed across soil-extracted DNA than nematode-sample DNA. The most overrepresented species in nematode-sample DNA were *Heterocephalobus elongatus*, *Eucephalobus striatus* and *Hexatylus* sp., whereas *Phasmarhabditis* sp. and

* Corresponding author. Swiss Federal Research Institute WSL, Zürcherstrasse 111, CH-8903, Birmensdorf, Switzerland.
E-mail address: beat.frey@wsl.ch (B. Frey).

Eumonhystera filiformis were overrepresented in soil-extracted DNA. Read abundances of feeding types significantly differed between the DNA sources and across sites, with a significant effect of biome on both ecto- and endoparasitic herbivores in soil-extracted DNA and for ectoparasitic herbivores only in nematode-sample DNA. Collectively, our data suggest that choice of the DNA source material may lead to different patterns of nematode community composition across space and environmental conditions. Improving the sensitivity of the soil-extracted DNA method by developing protocols using larger amounts of soil and designing nematode-specific primers will make this approach an efficient screening tool to analyse nematode diversity and community structure complementing the labour-intensive isolation of intact nematodes from soils (nematode-sample DNA).

1. Introduction

Among soil biota, nematodes are important components of soil food webs (Bardgett and van der Putten, 2014; Bar-On et al., 2018; van den Hoogen et al., 2019) and play a critical role in the below-ground mineralization of plant-derived organic C and N by feeding on various food sources and releasing nutrients via their excrements (Wardle, 2006; Osler and Sommerkorn, 2007; Briones, 2014; Andriuzzi et al., 2016). Nematodes cover many of the main trophic groups or feeding types of soil fauna, i.e., bacterivores, fungivores, omnivores, predators and root herbivores (Yeates et al., 1993; Neher, 2001; Yeates, 2003) and are valuable indicators of the complexity of soil food web structure and soil quality (Bongers and Ferris, 1999; Ferris et al., 2001). In recent years, they have received increasing attention, particularly in agricultural soils (Stone et al., 2016b; Treonis et al., 2018; Bongiorno et al., 2019). Nematodes respond quickly and in a taxon-specific manner to environmental changes, and are therefore widely used as bioindicators to reflect changes in the soil environment (Bongers and Ferris 1999; Neher, 2001; Yeates, 2003; Eisenhauer et al., 2012).

In order to develop nematode-based guidelines for policy makers and sustainable land use at the international scale, it is pivotal to take into account the biogeography of nematode diversity and community composition to locally calibrate the nematode indicators (Stone et al., 2016a, 2016b). Survey-based sampling along latitudinal or altitudinal gradients allows exploring potential shifts in nematode community diversity and composition in response to environmental gradients, as has been shown for other soil organisms, such as bacteria, fungi and protists (Bates et al., 2013; Tedersoo et al., 2014; Bahram et al., 2018; Delgado-Baquerizo et al., 2018). To date, however, few assessments of nematode communities at large geographical scales are available and most of them are of relatively limited sample size. Existing biogeographical studies indicate that climate, vegetation and soil abiotic conditions were the main drivers shaping soil nematode communities (Nielsen et al., 2014; Sylvain et al., 2014; Chen et al., 2015; Song et al., 2017; van den Hoogen et al., 2019; Wilschut et al., 2019; Li et al., 2020). In a comprehensive global study, including 6759 samples across all terrestrial biomes and continents, van den Hoogen et al. (2019) found a peak of nematode abundances at high latitudes, associated with large soil carbon stocks. The abundance of feeding types also followed the spatial patterns in total abundances, although some distinct drivers for single feeding types were revealed: herbivore-dominated communities were most influenced by vegetation, whereas bacterivores were most influenced by soil edaphic factors (i.e. sand content and pH).

Traditionally, intact nematodes are isolated from soil and then the bulk nematode samples are classified based on morphological traits. However, it is known that methods to isolate intact nematodes from soil create a bias, with small nematodes as well as large nematodes with a long body being discriminated by these methods (Viglierchio and Schmitt, 1983; Verschoor and de Goede, 2000; EPPO, 2013; Cesarz et al., 2019). Recently, DNA metabarcoding has increasingly been used to assess the diversity of nematodes previously isolated from soils, representing a less laborious alternative to morphological quantification (Powers et al., 2011; Darby et al., 2013; Treonis et al., 2018; Bell et al., 2021). Currently, the 18S rRNA gene is the most widely used molecular marker for nematode identification (Porazinska et al., 2009; Sapkota

and Nicolaisen, 2015; Avó et al., 2017; Treonis et al., 2018; Ahmed et al., 2019), although other markers, such as the cytochrome *c* oxidase sub-unit I (COI), have also successfully been used (Derycke et al., 2010; Macheriotou et al., 2019). Studying nematode communities using DNA directly extracted from soils (Griffiths et al., 2018), instead of extracting DNA from animals that were first isolated from soil, is less investigated, despite this method being a very promising tool that has successfully been implemented in several ongoing soil biodiversity monitoring programmes that target soil bacteria, fungi, protists and multicellular animals using metabarcoding (Zinger et al., 2016; George et al., 2019; Gschwend et al., 2021a, 2021b).

To date, the effect of the source material for DNA extraction (i.e., direct extraction of DNA from soil versus extraction of DNA from previously isolated nematode sample) on the assessment of nematode diversity and community composition has only been investigated in one study of limited sample size (Griffiths et al., 2018). In this study, DNA extracted from a nematode sample (nematode-sample DNA) was shown to contain a higher fraction of nematode sequences, and therefore yielded a higher diversity of nematode taxa (Griffiths et al., 2018). However, as a disadvantage, this method is laborious and may introduce biases, such as a size-based discrimination of some taxa, which can be circumvented by directly extracting DNA from soils (Griffiths et al., 2018). It therefore remains unclear how differences between these sources of DNA could affect the quantification of nematode diversity and community structure at larger spatial scales. This is relevant because global patterns revealed using nematode samples isolated from soil samples, as in van den Hoogen et al. (2019), might not match those inferred from DNA directly extracted from soil. To our knowledge, no studies have investigated how differences in the source material of DNA extraction could affect the characterization of nematode communities across biomes. Calibrating nematode information derived from the two approaches across large spatial scales is however essential to reliably use soil nematodes as bioindicators for soil food web structure and soil quality (Geisen et al., 2018; Griffiths et al., 2018).

In the present study, we used DNA metabarcoding on a comprehensive set of soil samples collected along a European latitudinal gradient (from 37°N to 79°N, ~4500 km) including 18 countries, covering six biomes and a wide range of climatic and edaphic conditions. We characterized nematode communities based on DNA extracted from bulk nematode samples previously isolated from soil ("nematode-sample DNA") or DNA directly extracted from soil ("soil-extracted DNA"). Our study has three main goals: (1) to compare two sources of nematode DNA obtained by two different extraction approaches prior to metabarcoding of 18S rRNA genes at biome scale, (2) to explore if nematode richness and community composition show geographical patterns in European forests, and (3) to evaluate whether the richness and composition of communities can be predicted using environmental data such as biome, climate and soil type. Specifically, we want to reveal whether the different approaches in DNA extraction could result in significant differences in the estimates of nematode diversity and community structure, and thus affect the ecological conclusions of large-scale field studies. Finally, besides looking at the diversity and composition of communities, we also examined the abundances of nematode feeding types to test whether they were affected by the source material of the DNA extraction.

2. Material and methods

2.1. Sampling sites

We conducted a continent-scale field study of soils containing woody vegetation (forests) analysing soil nematode communities in six biomes (alpine, Arctic, Atlantic, boreal, continental and Mediterranean) across Europe (Fig. 1). We define forest here as terrain containing woody vegetation. For the Arctic sites in Svalbard we selected spots covered by *Salix arctica*. "Alpine" refers to mountain regions below the treeline. At least three sites were included in each biome (Table 1), yielding a total of 29 forest sites. The sites cover a wide range of environments across a latitudinal gradient from 39°N to 79°N, a distance of ~4500 km. Most of these forest sites belong to existing long-term national monitoring programmes (e.g. LTER, ICP Forests, ICP IM), and detailed information is available on their soil characteristics (pH, texture and organic matter content), vegetation (species diversity, standing biomass), local climate, and forest management (Cools et al., 2014; De Vos et al., 2015; Martins da Silva et al., 2016; Stone et al., 2016a). The soils from the sites have been classified according to well-accepted classification schemes (Zanella et al., 2011). For each site, we extracted the mean values of mean annual temperature (°C) and total annual precipitation (mm) from the nearest weather station. The mean annual temperatures range from −5.2 to 16.5 °C across the sites, the mean annual precipitation from 375 to 1357 mm, and the elevation from 20 to 1900 m above sea level (Table 1).

2.2. Field sampling

Soil sampling was carried out in 2017 according to a standardized protocol (Stone et al., 2016a). At each site, four plots (1 m² each) at a distance of 10 m from each other were selected in areas with no understorey vegetation and similar soil type to minimize the heterogeneity. In each plot, five soil cores (5 cm Ø × 10 cm deep) were taken at short distances from each other – two cores for the isolation of nematodes and three cores pooled and homogenized for direct soil DNA extraction and soil physico-chemical analyses. This resulted in 8 cores for nematode isolation and 12 cores for soil DNA extraction and soil physico-chemical analyses per site. It is a common approach to use separate soil cores for different analyses that require different processing in nation-wide soil monitoring programmes (George et al., 2017, 2019). Litter was also collected from the four plots at each site. Continental and Mediterranean sites, but also the Atlantic sites that experience drought, were sampled in late spring (May), while the sites regularly exposed to
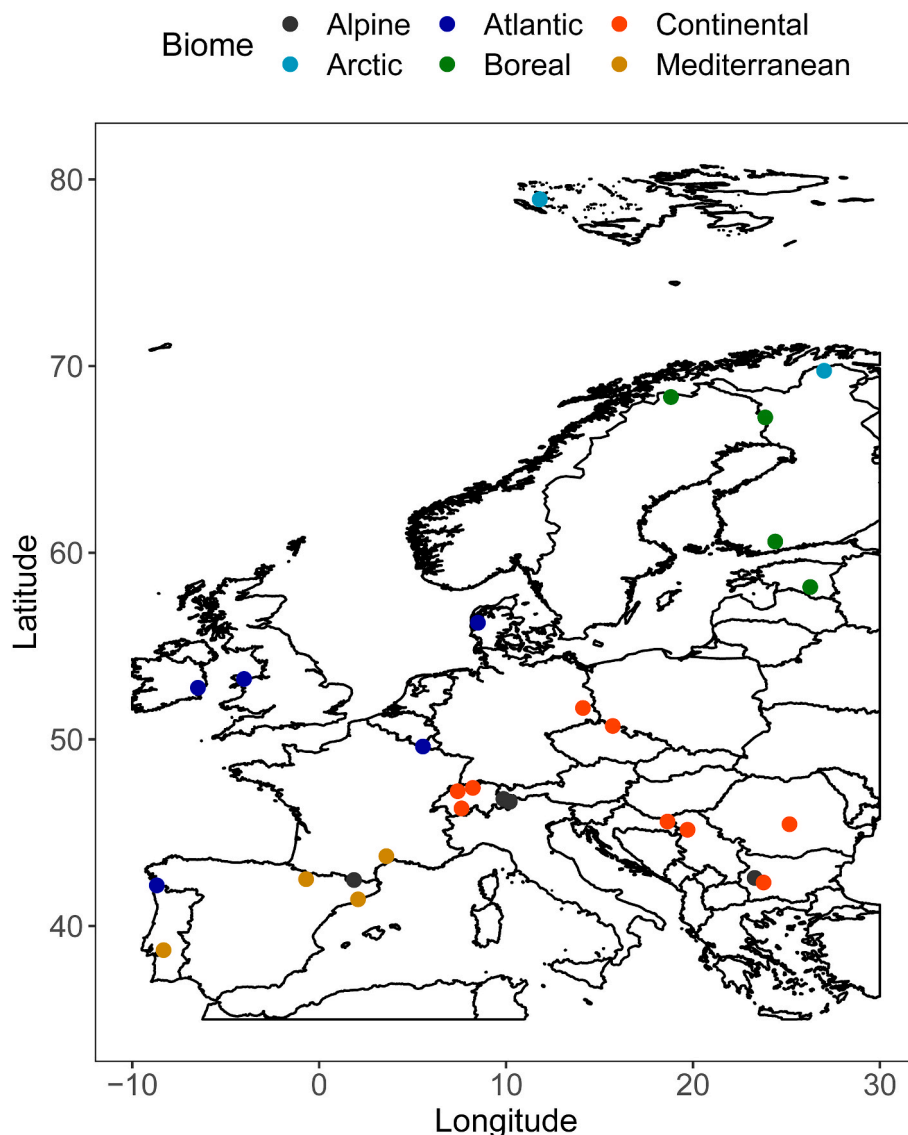

Fig. 1. The location of the twenty-nine Europe-wide sites involved in the study.

Table 1

Geographical, climatic and soil physico-chemical properties of the forest sites. MAT = mean annual temperature, MAP = mean annual precipitation, BD = bulk density, OM = organic matter, SM = soil moisture. NA = not analysed; n = 4 per site.

Site	Country	Biome	Latitude	Longitude	Altitude (m a.s.l.)	MAT (°C)	MAP (mm)	Forest type	Bedrock	pH	C soil (%)	C litter (%)	N soil (%)	N litter (%)	BD (g cm^{-3})	OM (%)	SM (%)	Sand (%)	Silt (%)	Clay (%)
Alp1	Switzerland	Alpine	46.81	9.86	1650	3.9	1000	Evergreen	Paragneiss	3.2 ± 0.2	33 ± 17	45 ± 2	1.1 ± 0.5	1.3 ± 0.1	0.4 ± 0.2	64 ± 32	50 ± 18	64	22	14
Alp2	Switzerland	Alpine	46.66	10.20	1900	4.7	847	Evergreen	Dolomite	6.7 ± 0.3	18 ± 4	33 ± 6	0.5 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	21 ± 3	41 ± 3	47	33	21
Alp3	Spain	Alpine	42.46	1.86	1660	9.1	940	Evergreen	Till	3.9 ± 0.7	10 ± 2	47 ± 0	0.3 ± 0.0	1.3 ± 0.1	0.5 ± 0.0	12 ± 1	26 ± 10	71	22	7
Alp4	Bulgaria	Alpine	42.58	23.30	1823	11.6	604	Shrub	Paragneiss	4.4 ± 0.3	14 ± 1	45 ± 2	0.9 ± 0.0	1.6 ± 0.1	0.5 ± 0.1	26 ± 6	38 ± 4	70	11	19
Arc1	Finland	Arctic	69.75	27.01	110	−0.5	404	Deciduous	Paragneiss	3.2 ± 0.1	15 ± 12	49 ± 1	0.3 ± 0.3	1.4 ± 0.1	0.4 ± 0.2	6 ± 3	27 ± 7	91	6	3
Arc2	Svalbard	Arctic	78.93	11.80	42	−5.2	375	Arctic tree line	Glacial moraine	6.7 ± 0.3	3 ± 1	NA ± NA	0.2 ± 0.0	NA ± NA	1.1 ± 0.0	5 ± 2	17 ± 1	64	29	7
Arc3	Svalbard	Arctic	78.94	11.81	40	−5.2	375	Arctic tree line	Glacial moraine	7.4 ± 0.1	2.5 ± 0	NA ± NA	0.1 ± 0.0	NA ± NA	1.3 ± 0.0	3 ± 0	15 ± 3	77	17	6
Atl1	Denmark	Atlantic	56.25	8.49	30	7.5	762	Deciduous	Sandstone	3.8 ± 0.1	14 ± 17	43 ± 6	0.5 ± 0.5	1.3 ± 0.1	0.4 ± 0.1	20 ± 3	38 ± 11	96	2	2
Atl2	UK	Atlantic	53.24	−4.02	20	9.5	927	Deciduous	Glacial till	4.6 ± 0.0	3 ± 0	44 ± 2	0.2 ± 0.0	1.6 ± 0.3	0.9 ± 0.0	7 ± 0	22 ± 2	58	30	13
Atl3	Ireland	Atlantic	52.76	−6.49	161	9.5	968	Semi-deciduous	Mudstone	3.3 ± 0.1	13 ± 4	43 ± 4	0.7 ± 0.1	2.0 ± 0.1	0.8 ± 0.0	33 ± 14	55 ± 10	60	26	14
Atl4	Belgium	Atlantic	49.62	5.55	377	8.7	994	Semi-deciduous	Loess	3.9 ± 0.7	3 ± 1	29 ± 12	0.1 ± 0.0	1.3 ± 0.5	0.9 ± 0.1	6 ± 2	11 ± 4	79	10	11
Atl5	Spain	Atlantic	42.18	8.69	450	14.9	1303	Evergreen	Granite	4.4 ± 0.3	13 ± 1	47 ± 1	0.8 ± 0.0	1.7 ± 0.1	0.6 ± 0.0	29 ± 5	27 ± 4	71	13	16
Bor1	Finland	Boreal	60.6	24.40	140	4.5	650	Semi-deciduous	Gabbro	3.8 ± 0.3	3 ± 1	41 ± 3	0.1 ± 0.0	1.1 ± 0.0	0.8 ± 0.0	5 ± 1	19 ± 3	69	27	5
Bor2	Finland	Boreal	67.25	23.87	184	−1.1	477	Evergreen	Biotite paragneiss	3.5 ± 0.1	4.0 ± 1	47 ± 1	0.0 ± 0.0	1.1 ± 0.1	0.7 ± 0.0	4 ± 1	19 ± 1	76	20	4
Bor3	Sweden	Boreal	68.34	18.82	400	0.2	387	Deciduous	Quartzite	4.0 ± 0.7	34 ± 12	47 ± 1	1.1 ± 0.4	1.4 ± 0.2	0.4 ± 0.0	64 ± 35	65 ± 21	57	34	9
Bor4	Estonia	Boreal	58.16	26.27	100	5	612	Evergreen	Glacial till	4.6 ± 0.4	3 ± 0	15 ± 6	0.1 ± 0.0	0.5 ± 0.2	0.9 ± 0.0	6 ± 1	17 ± 3	68	27	5
Cont1	Germany	Continental	51.68	14.11	76	9.2	563	Semi-deciduous	Loess	3.9 ± 0.4	4 ± 2	30 ± 5	0.2 ± 0.0	1.3 ± 0.2	1.0 ± 0.1	5 ± 4	5 ± 2	89	8	4
Cont2	Czech Republic	Continental	50.72	15.71	1214	4.6	799	Evergreen	Gneiss	3.2 ± 0.2	32 ± 6	47 ± 0	1.5 ± 0.3	1.8 ± 0.0	0.5 ± 0.0	55 ± 22	63 ± 12	46	11	43
Cont3	Croatia	Continental	45.59	18.64	93	11	686	Deciduous	Limestone	6.8 ± 0.3	9 ± 2	33 ± 5	0.6 ± 0.1	1.9 ± 0.3	0.8 ± 0.1	16 ± 3	36 ± 5	36	40	25
Cont4	Romania	Continental	45.45	25.16	830	6.6	756	Deciduous	Gneiss	3.8 ± 0.1	4 ± 1	42 ± 3	0.3 ± 0.0	1.7 ± 0.1	0.8 ± 0.1	8 ± 3	22 ± 4	65	22	14
Cont5	Bulgaria	Continental	42.33	23.77	746	8.3	599	Deciduous	Gneiss	4.3 ± 0.1	2 ± 0	33 ± 7	1.0 ± 0.0	1.3 ± 0.2	1.0 ± 0.0	3 ± 1	9 ± 2	84	11	5
Cont6	Serbia	Continental	45.16	19.71	NA	11.3	631	Deciduous	Serpentinite/marls	5.3 ± 0.3	4 ± 1	37 ± 3	0.2 ± 0.0	1.5 ± 0.1	1.0 ± 0.0	7 ± 0	20 ± 1	14	62	24
Cont7	Switzerland	Continental	47.40	8.22	500	9.2	1040	Deciduous	Moraine	4.1 ± 0.2	4 ± 2	35 ± 3	0.2 ± 0.0	1.3 ± 0.1	0.7 ± 0.1	9 ± 6	24 ± 3	48	39	13
Cont8	Switzerland	Continental	47.22	7.41	1150	8.9	925	Deciduous	Limestone	6.6 ± 0.3	10 ± 2	35 ± 5	0.3 ± 0.1	0.9 ± 0.0	0.5 ± 0.0	18 ± 6	24 ± 2	50	39	11
Cont9	Switzerland	Continental	46.18	7.37	615	7.4	657	Evergreen	colluvial deposit	5.5 ± 0.7	15 ± 3	39 ± 8	0.5 ± 0.1	1.2 ± 0.3	0.5 ± 0.0	23 ± 4	21 ± 1	49	38	13
Med1	France	Mediterranean	43.44	3.35	270	13,4	722	Evergreen	Limestone	7.0 ± 0.1	9 ± 3	22 ± 11	0.5 ± 0.1	0.9 ± 0.3	0.6 ± 0.0	18 ± 5	27 ± 4	78	8	15
Med2	Spain	Mediterranean	41.43	2.08	252	16,5	612	Grassland	Colluvia of shales	7.1 ± 0.1	3 ± 0	25 ± 0	0.3 ± 0.0	1.1 ± 0.2	1.0 ± 0.1	7 ± 1	17 ± 1	49	31	20
Med3	Spain	Mediterranean	42.51	−0.70	1415	9,5	779	Evergreen	Sandstone	4.8 ± 0.3	6 ± 1	33 ± 12	0.2 ± 0.0	1.2 ± 0.3	0.9 ± 0.1	8 ± 2	23 ± 5	63	22	14
Med4	Portugal	Mediterranean	38.70	−8.32	172	16,1	662	Deciduous	Sandstone	5.0 ± 0.2	2 ± 1	20 ± 15	0.1 ± 0.0	0.9 ± 0.4	1.0 ± 0.1	4 ± 1	6 ± 2	65	22	13

winter frost (Arctic, Atlantic, alpine and boreal) were sampled in early summer (June and July). In each country, samples were transported to a local laboratory in cooler boxes containing ice packs and then immediately shipped overnight to the laboratory at WSL.

2.3. Abiotic soil variables

Immediately upon arrival in the laboratory, the three cores for soil DNA extraction and soil physico-chemical analyses were homogenized by sieving them using stainless steel sieves (mesh size 4 mm). Sieves were cleaned between samples by rinsing them under tap water and spraying them with ethanol. An aliquot (>25 g) of the sieved soils was frozen at $-20\,°C$ for direct soil DNA extraction. The basic soil properties such as texture, pH and organic matter content, as well as total C and N concentrations in both the soil and litter, were determined after drying the samples for 48 h at $60\,°C$. Soil particles were fractionated into sand, silt and clay using the pipette method (Gee and Bauder, 1986). Total C and N concentrations were analysed using fine-ground samples by dry combustion using a CN analyser NC 2500 (CE Instruments, Wigan, United Kingdom). Inorganic carbon in the samples with a pH > 6.5 was removed with acid vapour prior to the analysis of organic carbon (Frey et al., 2021). Bulk density was calculated by dividing the weight of the dried fine fraction (<2 mm) by the volume of the full soil sample. The pH of dry soil samples was measured potentiometrically in 0.01 M $CaCl_2$ (soil:solution ratio = 1:2; 30 min equilibration time).

2.4. Nematode isolation from soil cores

The soil cores for nematode isolation and DNA extractions were processed immediately upon arrival. Nematodes were isolated from the soil cores (8 cores per site = 4 replicated plots x 2 cores per plot) using Oostenbrink dish method (Oostenbrink, 1960; Verschoor and de Goede, 2000). The Oostenbrink dish method is a standard method proposed from EPPO (European and Mediterranean Plant Protection Organization) to isolate nematodes from soils prior to DNA metabarcoding for community analysis (EPPO, 2013). The soil of one core was mixed and split in 2 parts (approximately of 200 g of soil each) before these were placed on a double cotton milk filter (FT25 Sana Vliesstoff-Filter, Zeltner Systemtechnik AG, Fulenbach, Switzerland) on a sieve in a dish. Afterwards the dish was gently filled with tap water from the side until the bottom of the sieve just reached the water. The nematodes were allowed to migrate through the filter into the water at 23 °C. After 72 h the nematodes of both aliquots were collected from the dishes on a 20-μm sieve and transferred to centrifugation tubes. This resulted in relatively clean suspensions for nematode counting. All nematodes in 3 x 1-ml subsample of the 10 ml suspension were counted at $40 \times$ magnification under a microscope. After counting, the subsamples were put back complementing the 10 ml suspension which was used for DNA extraction. Counts of all nematode taxa were extrapolated to the entire sample and expressed per 100 g dry soil for further analyses.

2.5. DNA extraction and amplicon sequencing

"Nematode-sample DNA" was extracted from Oostenbrink-extracted nematodes using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), as previously described (Resch et al., 2022). "Soil-extracted DNA" was extracted directly from 10 g of soil using the PowerMax Soil DNA extraction kit (Qiagen), as previously described (Frey et al., 2016; Resch et al., 2021). DNA was quantified using high-sensitivity Qubit assay (Thermo Fisher Scientific, Reinach, Switzerland) and diluted to 2.69 ng uL^{-1} for PCRs. Subsequently, we amplified a 360-bp fragment of the 18S rRNA gene (Primers NF-1 and 18Sr2b; V6–V8 region; Porazinska et al., 2009). These primers pairs give good coverage of soil nematodes and have been widely used, but are not nematode specific and also amplify other eukaryotes. The nuclear small subunit ribosomal RNA gene (18S) was chosen as the most commonly used marker in nematode

barcoding due to the availability of extensive database information (Macheriotou et al., 2019; Ahmed et al., 2019). The 5′ ends of the primers were tagged with the CS1 (forward) and CS2 (reverse) adapters required for multiplexing samples using the Fluidigm Access Array System (Fluidigm, South San Francisco, CA, USA). The PCR conditions to amplify the 18S RNA gene fragments consisted of an initial denaturation at 95 °C for 2 min, 35 cycles of denaturation at 95 °C for 40 s, annealing at 58 °C for 40 s, and elongation at 72 °C for 1 min, followed by a final elongation at 72 °C for 10 min (Resch et al., 2022). Each sample was amplified in triplicates and pooled prior to purification with Agencourt AMPure XP beads (Beckman Coulter, Berea, CA, USA) and quantification with the Qubit 2.0 fluorometric system (Life Technologies, Paisley, UK). Amplicon pools were sent to the Génome Québec Innovation Centre at McGill University (Montréal, Canada) for library preparation, and paired-end sequencing (2×250 bp) was performed on the Illumina MiSeq v3 platform (Illumina Inc., San Diego, CA, USA).

2.6. Bioinformatics

Bioinformatics analyses were carried out using the Quantitative Insights Into Microbial Ecology 2 program (QIIME 2, ver. 2021.4, Bolyen et al., 2019). The raw paired-end FASTQ files were imported into the QIIME2 program and demultiplexed using a native plugin. Thereafter, the Cutadapt plugin was processed primer trimmed. The Divisive Amplicon Denoising Algorithm 2 (DADA2) plugin in QIIME2 was used to determine amplicon sequence variants (ASVs; Callahan et al., 2016; Callahan et al., 2017). To remove low quality bases and reads, we truncated forward reads at 240 bp and reverse reads at 215 bp and discarded reads not fulfilling quality criteria (maxN = 0, maxEE = 2). Taxonomic assignment was accomplished using the Naive Bayes q2-feature-classifier (Bokulich et al., 2018) in QIIME2 against the PR^2 database v4.14.0 (Guillou et al., 2013) with 99% as the species delineation clustering threshold. Raw sequences were deposited in the NCBI Sequence Read Archive under the BioProject accession identifier PRJNA562119 and PRJNA793632.

Nematode taxa were assigned to feeding types (herbivores, fungivores, bacterivores, omnivores and predators) and C-p/P-p (colonizerpersister) classes using NINJA (Nematode INdicator Joint Analysis; https://shiny.wur.nl/ninja/, Sieriebriennikov et al., 2014) based on genus level taxonomy (from ASV table). Genera that could not be assigned were designated as unclassified. C-p/P-p classes divide nematodes in five groups based on their growth rate and therefore ability to colonize disturbed habitats for free living (C-p) and plant associated (P-p) nematodes, respectively (Bongers, 1990). These range from r-strategists (C-p/P-p 1) to K-strategists (C-p/P-p 5).

2.7. Statistical analyses

All statistical analyses were conducted in R version 4.1.2 (R Core Team, 2021). All figures were created using the *ggplot2* package (Wickham, 2009). The R script is provided in the Supplementary material. *P*-values <0.05 were considered significant. The effects of site, biome and latitude on the abundance of nematodes were calculated using a generalized linear (mixed) model (GLM or GLMM) with a negative binomial distribution. The best fitting distribution was chosen using the 'fitdist' function in the *fitdistrplus* package (Delignette-Muller and Dutang, 2015). For testing the effects of biome and latitude, the site was included as a random factor and *P*-values were calculated using a likelihood ratio test that compared the models against a null-model with the random factor only.

Using datasets that included nematode sequences only, we compared the alpha-diversity indices (species richness and Shannon index at the ASV level) of the two DNA extraction methods (DNA sources) across the biomes. For the calculation of α-diversity (ASV) indices, samples were rarefied to an even number of sequences using the *phyloseq* package (McMurdie and Holmes, 2013). Rarefying of data was only performed

for the analysis of alpha-diversity indices. The effect of site on the observed ASV richness and Shannon diversity was estimated using a one-way analysis of variance (ANOVA). A Bartlett test and a Shapiro test were used to check the homoscedasticity and normality assumptions of the ANOVA. The effects of biome and DNA extraction method (DNA source material) were tested using a linear mixed model with site as a random factor. For these models, homoscedasticity was tested by plotting residuals against means and normality using qqnorm plot, respectively. Rarefaction curves were calculated using the *vegan* package (Oksanen et al., 2019).

Community structure was analysed based on Bray-Curtis distances using relative read abundances. Principal coordinate analyses (PCoAs) were performed using the 'ordinate' function in the *phyloseq* package (McMurdie and Holmes, 2013). A permutational multivariate analysis of variance (PERMANOVA) was conducted to assess differences in community structure using the 'adonis2' function in the *vegan* package. The effects of site and biome were calculated for data subsets corresponding to each DNA extraction method (DNA source). For site, unrestricted permutations were used, whereas for biome, samples from each site were kept together to account for the hierarchical structure of the data (site nested within biome). The effect of the DNA extraction method (DNA source) was calculated together with the effect of site for the full dataset. To determine the *P*-value for the effect of DNA extraction, samples were only allowed to permute within sites without permuting the sites. Environmental parameters were regressed against the axes of the PCoA using the 'envfit' function in the *vegan* package. The 'procrustes' function in *vegan* was used to compare differences in community structure across the dataset between the two DNA sources.

To reveal if particular taxa were over- or underrepresented for either of the extraction methods (DNA sources), we calculated $\log_2$-fold changes in the abundance of the species in soil-extracted DNA over the abundance of species in the nematode-sample DNA. For the calculation of $\log_2$-fold changes between the two extraction methods (DNA sources) we used the *DESeq2* package (Love et al., 2014; Donhauser et al., 2021). Normalization (i.e. calculation of size factors) was done at the ASV level. We calculated $\log_2$-fold changes between extraction methods on counts aggregated at the species level, controlling for the effect of site.

As the relative abundances of the nematode feeding types derived from the ASV taxonomic assignment were strongly heteroscedastic and not normally distributed, GLMs/GLMMs were used to test their responses to site, biome and DNA extraction method (DNA source). The effect of site was tested using separate data subsets for each extraction method (DNA source), the effect of biome using site as a random factor in data subsets of each extraction method (DNA source), and the effect of extraction method (DNA source) using site as a random factor over the full dataset. To this end, count tables were normalized using the normalization from the *DESeq2* package at the ASV level rounded to integers. The function 'fitdist' from the *fitdistrplus* package (Delignette-Muller and Dutang, 2015) was then used to decide the best fitting distribution, which was the negative binomial distribution for all feeding types. The relationship between environmental variables and feeding type abundances was tested with a separate GLMM model for each variable with site as random factor in a subset for each DNA extraction method (DNA source). *P*-values were calculated using a likelihood ratio test that compared each of the models against a null-model with the random factor only.

3. Results

3.1. Soil properties and total nematode abundance

The sampled forest soils showed a wide range of properties (Table 1), with pH ranging from 3.2 to 7.4, total C from 2% to 33.7%, total N from 0.07% to 1.5%, sand content from 13.9% to 95.7%, silt content from 2.4% to 62.3%, and clay content from 1.9% to 42.7%. Soil pH was highest in Mediterranean samples and lowest in Atlantic and boreal

samples. Total C and N were both highest in alpine samples and C was lowest in Mediterranean samples, whereas N was lowest in the Arctic samples. Sand content was lowest in the continental and highest in the Arctic soils, whereas silt and clay contents showed the inverse trends. Accordingly, the total abundance of nematodes ranged from 33 to 1834 individuals per 100 g of dry soil (Fig. 2) and varied significantly across sites ($P < 0.001$; GLM). Differences among biomes were not significant ($P = 0.30$; GLMM), but were lowest in the Atlantic sites and highest in the alpine sites. Abundances increased slightly with latitude (Fig. 2), but this effect was neither significant ($P = 0.13$, GLMM).

3.2. Description of the sequencing data

When extracting DNA directly from the soil ("soil-extracted DNA"), we obtained 2,270,778 high-quality sequences ($20,833 \pm 2725$ per sample), which formed 7894 ASVs (163 ± 46 per sample; Tables S1 and S2). Of these sequences, 117,462 (5.2%) were classified as nematodes (1078 ± 944 per sample), resulting in 713 nematode ASVs (19 ± 7 per sample). Non-nematode sequences mostly belonged to Fungi (66%), other Metazoa (17%) and Streptophyta (3.4%). Of the nematode ASVs, 422 could be classified at the species level and additional 114 ASVs at the genus level.

When extracting DNA from nematode samples ("nematode-sample DNA"), we obtained 2,762,057 high-quality sequences ($25,340 \pm 4911$ per sample), which formed 3016 ASVs (75 ± 20 per sample; Tables S3 and S4). Of these sequences, 2,375,818 (86%) were identified as nematodes ($21,797 \pm 5037$ per sample), resulting in 2020 nematode ASVs (54 ± 16 per sample). Non-nematode sequences mostly belonged to other Metazoa (7.4%), Ciliophora (2.8%) and Ochrophyta (1.8%). Of the nematode ASVs, 1066 could be classified at the species level and additional 322 ASVs at the genus level.

3.3. Effect of the DNA source on α-diversity

When the datasets of the two methods were rarefied together, ASV richness of the nematode-sample DNA (ranging from 16 to 34) was higher than the ASV richness of the soil-extracted DNA (ranging from 8 to 25) (Fig. 3a, Table 2). Shannon diversity of the nematode-sample DNA (ranging from 1.6 to 3.1) did not significantly differ from the Shannon diversity of the soil-extracted DNA (ranging from 1.4 to 2.8) (Fig. 3b, Table 2). For the nematode-sample DNA, richness and Shannon diversity differed across sites, but not across biomes, whereas for the soil-extracted DNA, richness and Shannon diversity differed across both sites and biomes, although site had a stronger effect (Table 2). We found similar patterns for diversity indices at the species level (Figs. S1a and b). Species richness ranged from 4 to 14 for soil-extracted DNA and from 7 to 17 for nematode-sample DNA and was not significantly different between the two extraction methods opposed to ASV level. Shannon diversity ranged from 0.85 to 2.3 for soil-extracted DNA and from 0.97 to 2.3 for nematode-sample DNA.

Although much smaller numbers of nematode sequences were retained in the soil-extracted DNA than in the nematode-sample DNA, in most samples for both methods the number of sequences was sufficient to recover the full diversity of nematodes in each sample (Fig. 3c). However, the rarefaction curves reached a plateau at much lower ASV numbers (Richness) for the soil-extracted DNA than for the nematode-sample DNA, indicating a much lower richness in these samples (no additional ASVs would be recovered with a higher sequencing depth) in these samples. The same pattern was found calculating rarefaction curves at the species level (Fig. S1c).

3.4. Effect of the DNA source and soil properties on community structure

Community structure differed across sites in both DNA extraction methods (Fig. 4a and b, Table 3), with a slightly stronger effect for soil-extracted DNA (Table 3). Variation in community structure across sites

(average distance from the median) was greater in the soil-extracted DNA (Fig. 4c, Table 3) compared to the nematode-sample DNA (0.63 compared to 0.61, $F_{1,217}$ = 5.4, P = 0.02) In the soil-extracted DNA, biome also had a significant effect on the community structure, whereas this was not the case in the nematode-sample DNA (Table 3). Of the soil properties, only total C concentration showed a significant relationship with community structure in the nematode-sample DNA (Fig. 4a), whereas in the soil-extracted DNA all nine soil properties had a significant relationship with community structure along the first two PCoA axes (Fig. 4b). Among these variables, pH, total soil C concentration, total soil N concentration, organic matter content and soil moisture showed the strongest relationships. We also assessed the correlations among environmental variables and found strong positive relationships among total C, total N, OM and soil moisture, all of which showed a strong negative relationship with bulk density. Moreover, we found strong negative relationships between sand and silt as well as sand and clay (Fig. S2).

3.5. Effect of extraction method (DNA source) on species abundance

We found 72 species whose abundance differed ($P_{adjusted}$ < 0.05) between the two DNA extraction methods (DNA source): of these, five were overrepresented and 67 were underrepresented in the soil-extracted DNA. Among the 50 most abundant species showing a significant $\log_2$-fold change, *Phasmarhabditis* sp., *Eumonhystera filiformis*, *Eumonhystera* sp. and *Prodesmodora circulata* in the phylum Chromadorea were more abundant in the soil-extracted DNA, while *Heterocephalobus elongatus*, *Eucephalobus striatus* and *Hexatylus* sp. (synonym *Neotylenchus*) in the phylum Chromadorea and *Prionchulus muscorum* in the phylum Enoplea were the most overrepresented species/genera in the nematode-sample DNA (Fig. 5a). As nematode extraction from soil may exclude taxa of certain body size, we tested the relationship between $\log_2$-fold change and size (weight derived from the NINJA tool but not length was used as a proxy for size), but found no evidence of body size affecting the $\log_2$-fold change (Fig. 5b). Moreover, we did not find a relationship between persister-colonizer groups (C-p/P-p) and differential abundance between the extraction methods. Thus, underrepresented species for soil-extracted DNA or overrepresented species for nematode-sample DNA comprised all persister-colonizer groups, such as *Rhabditis brassicae* (C-p 1), *Heterocephalobus elongatus* (C-p 2), *Prodesmodora* (C-p 3), *Prionchulus muscorum* (C-p 4) and *Xiphinema diversicaudatum* (P-p 5).

We also tested if there was a relationship between the rank abundance of nematode species and relative representation between the two extraction methods, which could arise as a result of different sampling depths between the methods. We found that species with a lower rank abundance were more strongly underrepresented in the soil-extracted DNA compared to the nematode-sample DNA (Fig. S3). This effect was strongest for rank abundances in the soil-extracted dataset (R^2 = 0.11, $F_{1,70}$ = 8.9, P = 0.004), but was also significant for rank abundances in the nematode-sample dataset (R^2 = 0.67, $F_{1,70}$ = 139.8, P < 0.0001) and in both datasets together (R^2 = 0.16, $F_{1,70}$ = 13.24, P = 0.0005).

When we compared the read abundance of the seven most abundant species in the two DNA extraction methods (DNA sources) (Fig. S4), we found that the relative abundance of *Eumonhystera* sp. was consistently lower in soil-extracted DNA across all sites and that the relative abundance of *Rhabditis terricola* was higher in these samples in almost all sites (Fig. S4). For the other five most abundant species, the over- or underrepresentation of either extraction method was less clear across the sites. Relative read abundances of species varied among sites within biomes, and few differences were found between biomes in either extraction method. Only *Tylolaimophorus typicus* was clearly more abundant in the Atlantic and continental sites than in the alpine, Arctic and Mediterranean sites for both extraction methods (DNA sources). *Aphelenchoides saprophilus* showed the highest relative abundances in the Atlantic and continental sites in the nematode-sample DNA, but the highest values in

alpine sites in the soil-extracted DNA, thus highlighting the potentially significant role of the DNA source material when examining species read abundances across habitats.

3.6. Effects of the DNA source and soil properties on the abundance of feeding types

In the nematode-sample DNA and soil-extracted DNA, 80% and 83% of the sequences, respectively, could be classified into feeding types using the NINJA tool. Bacterivores constituted the majority of the sequences in both the nematode-sample DNA and soil-extracted DNA (45% in both), followed by fungivores (19% and 15%, respectively). The extraction method had a highly significant effect on the abundance of all feeding types (Table 4). Site had a significant effect on the abundance of all feeding types for both methods (Table 4). Biome had a significant effect on the abundance of ectoparasitic and endoparasitic herbivores in the soil-extracted DNA, but an effect on the abundance of ectoparasitic nematodes only in the nematode-sample DNA (Table 4). The abundance of bacterivores increased with latitude in the DNA of both extraction methods, but this trend was significant for soil-extracted DNA only (Fig. 6, Table 5). In contrast, omnivores decreased significantly with latitude in the nematode-sample DNA. Overall, the variation in feeding type abundances among replicates within a site was large (Fig. 6).

When assessing the role of soil properties, we found 13 significant associations between feeding types and environmental variables in the nematode-sample DNA and 16 significant associations in the soil-extracted DNA (Table 5). For nematode-sample DNA, most of the significant relationships appeared between the abundance of ectoparasitic herbivores and all variables except latitude, and between the abundance of omnivores and latitude. For soil-extracted DNA most of the associations were observed for bacterivores (with C soil concentration, C litter concentration, soil moisture and latitude) and for fungivores (with N litter concentration). Furthermore, significant relationships were found for ectoparasitic herbivores (with C soil concentration, C litter concentration, N soil concentration, N litter concentration and soil moisture) and for omnivores (with pH, C litter concentration, N soil concentration, N litter concentration, bulk density and soil moisture) (Table 5).

4. Discussion

4.1. Total nematode abundance

We observed large variation in nematode abundances across sites, but no significant difference among biomes. Overall, our abundances were of a similar order of magnitude as observed in earlier studies (Song et al., 2017; van den Hoogen et al., 2019). These studies reported a peak in nematode abundances either at approx. 50°N (Song et al., 2017) or in Arctic regions (van den Hoogen et al., 2019), whereas we did not find a clear latitudinal trend. One possibility for the lack of a latitudinal trend is that we did not include sites south of 30°N. Also, while in our study the highest nematode abundance was found in the Subarctic site (70°N), the two high-Arctic sites (78°N) showed the lowest abundances in the whole dataset. This contrast of abundances in the three northernmost sites suggests that latitude may not be the main driver of nematode abundances. Nematode communities are also known to differ among land-cover types (Neher et al., 2005; Li et al., 2020), and one reason for our results differing from the trends reported earlier might be that we entirely focused on forest soils.

4.2. Effect of DNA source on α-diversity

Using universal 18S rRNA primers, the proportion of nematode sequences in the DNA extracted directly from soil (soil-extracted DNA) was 5–10% while the proportion was 85% in the DNA extracted from nematode samples that were previously isolated using the Oostenbrink dish method (nematode-sample DNA). These results confirm those
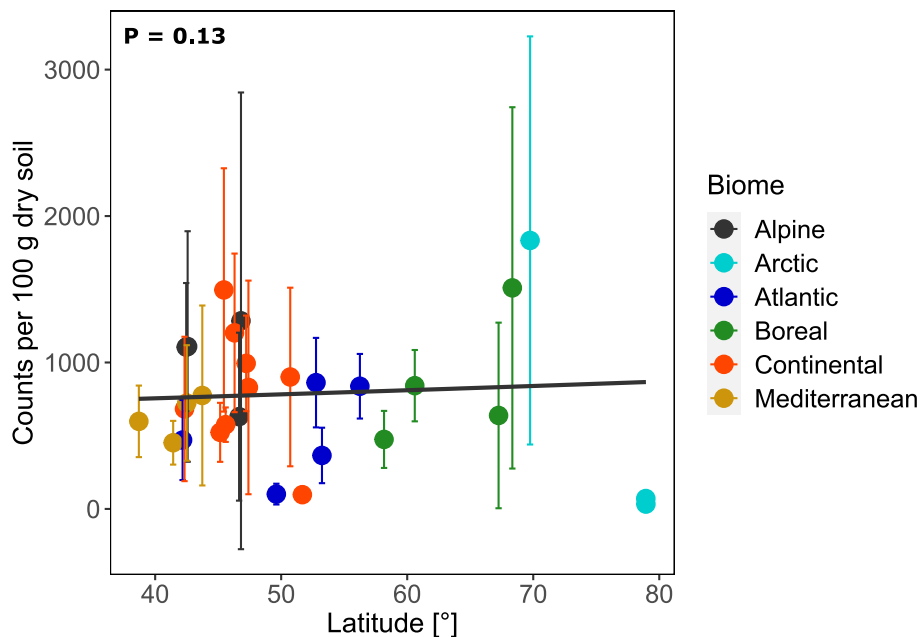
Fig. 2. Nematode abundance counted in nematode samples isolated from soil in relation to latitude (39°N to 79°N, ~4500 km, from six biomes) of the different sites. Mean ± SD per biome. N_{sites} = 29 (4 alpine, 3 arctic, 5 atlantic, 4 boreal, 9 continental, 4 mediterranean), n = 4 per site.

reported by Griffiths et al. (2018) who used the same primers. With direct soil DNA extraction a large amount of sequences were of non-nematode origin, which highlights the need for the development of nematode-specific primers (Sapkota and Nicolaisen, 2015; Peham et al., 2017; Sikder et al., 2020; Kawanobe et al., 2021).

After rarefying to an even sequencing depth, we observed a slightly higher richness of nematode ASVs in the nematode-sample DNA than in the soil-extracted DNA, which may result from the much larger amount of soil in the former method (see discussion below). The richness of ASVs was comparable to values reported by Griffiths et al. (2018) for mid-latitude agricultural soils based on operational taxonomic units (OTUs). We found a significant effect of biome on nematode species richness in the soil-extracted DNA, with higher values recorded in Arctic sites. This finding is in contrast to the distribution of richness, with a minimum at mid latitudes, observed by Song et al. (2017), and to the much lower richness in Antarctic than temperate sites found by Nielsen et al. (2014). Unlike species richness, Shannon diversity did not differ between the extraction methods (DNA source), indicating that a higher evenness in the soil-extracted DNA compensated for the lower richness detected. These results suggest that the extraction method (DNA source) modifies the rank abundance distributions of nematode ASVs, which could be due to the Oostenbrink nematode extraction selectively enriching the sample with particular ASVs, for instance by discriminating against taxa with long bodies or relatively immotile nematodes, as reported previously (Verschoor and de Goede, 2000; EPPO, 2013). We did not find a relationship between weight as a proxy for size and overrepresentation of taxa (see below), suggesting that activity and life status may be more important. Alternatively, lower amounts of soil used for the soil-extracted DNA may lead to less rare species in the sample resulting in more even rank abundance distribution among more frequent species.

When samples were not rarefied to an even sequencing depth, the nematode-sample DNA had a much higher richness than the soil-extracted DNA although the rarefaction analysis confirmed the sequencing depth after rarefying being sufficient to cover the diversity in each sample for both methods. Our results thus suggest that the true richness was higher in the nematode-sample DNA, which likely reflects the much larger amount of soil used for isolating nematodes from soil (up to 200 g) than for extracting the DNA from soil (10 g). Accordingly,

Wiesel et al. (2015) also reported different community compositions depending on the amount of soil used for extraction. For methods extracting intact nematodes, >100 g soil is typically used (Wiesel et al., 2015), whereas the amount of soil used for DNA extraction directly from soil is typically 0.25–10 g (Peham et al., 2017; George et al., 2019). Our results suggest that 10 g soil is not enough to cover the local diversity such that more taxa are recovered using larger amounts. This highlights the need to develop direct soil DNA extraction methods that permit using larger amounts of soil. For instance, existing protocols for soil amounts as large as 100 g (Yeates et al., 1998; Taberlet et al., 2012; Gorny et al., 2018) could be combined with a commercial purification kit to remove PCR-inhibiting soil substances that can hamper amplification of soil DNA (Peham et al., 2017). In addition, the relationship between the recovery of additional species and the amount of soils should be studied more systematically, to determine the minimal amount sufficient to represent nematode diversity and to adapt DNA extraction protocols accordingly.

4.3. Effect of DNA source on community structure and ecological relationships

We found significant differences in nematode community structure among sites with both extraction methods (soil-extracted DNA vs. nematode-sample DNA), while the effect of biome was significant in the soil-extracted DNA only. Interestingly, differences in community structure across the whole dataset were much smaller for the nematode-sample DNA than for the soil-extracted DNA. One explanation is that nematode isolation from soil leads to converging community structures in dissimilar samples. This could be a result of size and/or immotile nematode exclusion effect, as stated above (Verschoor and de Goede, 2000; EPPO, 2013), and indicates that across samples similar taxa are favoured by the extraction. Alternatively, lower amounts of soil (10 g of soil) for soil-extracted DNA may have resulted in an incomplete representation of the nematode community in each sample compared to the classical isolation of intact nematodes (from around 200 g of soil) and thus led to differences between samples.

With both extraction methods (DNA sources), total soil C concentration appeared as an important driver of nematode community structure, which is in agreement with findings in previous studies (Stone
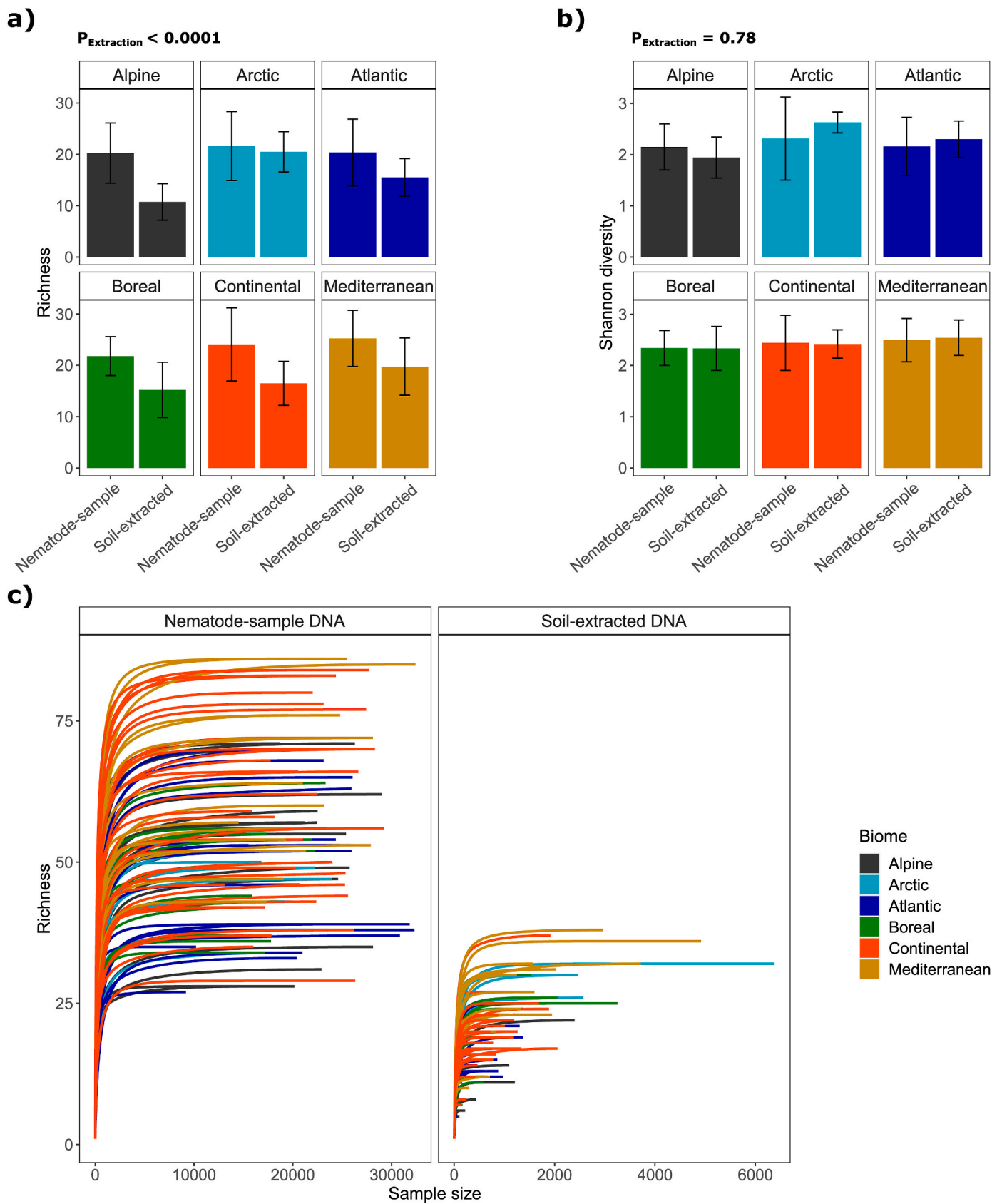
Fig. 3. Alpha-diversity. (a) Observed ASV richness and (b) Shannon diversity for nematode-sample DNA and soil-extracted DNA samples rarefied together. If a sample had <100 sequences for one of the extraction methods, it was removed for both methods. Mean ± SD per biome, N_{sites} = 29 (4 alpine, 3 arctic, 5 atlantic, 4 boreal, 9 continental, 4 mediterranean), n = 4 per site. (c) Rarefaction curves for nematode-sample DNA (left) and for soil-extracted DNA (right).

Table 2

Effects of site, biome and extraction method on nematode ASV richness and Shannon diversity index. The effects of site and biome were assessed in separate subsets for the two DNA extraction approaches. To test the site effect, a one-way ANOVA was used, and to test the effects of biome and the DNA extraction method, linear mixed models with site as a random factor were used. Samples with <100 sequences were removed: when the analyses were conducted for data subsets of the two DNA extraction methods, samples with <100 sequences were removed for the respective method only, and when both DNA extraction methods were included, samples with <100 sequences were removed for both methods. For ANOVA results, subscripted indices indicate numerator and total degrees of freedom; for results of the linear mixed models, subscripted indices indicate numerator and denominator degrees of freedom. Significant values are in bold letters. N_{sites} = 29 (4 alpine, 3 arctic, 5 atlantic, 4 boreal, 9 continental, 4 mediterranean), n = 4.

	Factor	Richness		Shannon index	
		F	*P*	*F*	*P*
	Site				
Nematode-sample		$4.8_{(27, 106)}$	**<0.0001**	$2.6_{(27, 106)}$	**<0.0001**
Soil-extracted		$5.9_{(27, 105)}$	**<0.0001**	$5.0_{(27, 105)}$	**<0.0001**
	Biome				
Nematode-sample		$1.8_{(5, 22)}$	0.15	$0.89_{(5, 22)}$	0.5
Soil-extracted		$3.3_{(5, 22)}$	**0.021**	$3.2_{(5, 22)}$	**0.026**
	Extraction method				
		$80_{(1, 181)}$	**<0.0001**	$0.075_{(1, 182)}$	0.78

et al., 2016b; Bongiorno et al., 2019; van den Hoogen et al., 2019). Using direct extraction of DNA from the soil, in accordance with previous studies (Nielsen et al., 2014; van den Hoogen et al., 2019), we further found soil texture (sand, silt and clay percentages) and bulk density to associate with nematode community structure. As nematodes are too small to move soil particles, their range of motion depends on pore spaces and aggregate structure, which in turn depend on the soil texture (Sechi et al., 2018). Soil structure likely selects nematode groups based on their size and feeding preferences, as the structure of soil influences access to food sources (Mikola and Sulkava, 2001; Neher, 2010). In addition, we found soil moisture to be strongly associated to community structure. Nematodes are aquatic organisms living in water films surrounding soil particles, and moisture also influences the availability of food sources, such as bacteria, fungi and plant roots (Neher, 2010). Our results thus confirm previous findings that soil moisture and precipitation can be important drivers for nematode communities (Nielsen et al., 2014; Sylvain et al., 2014; Chen et al., 2015; Wilschut et al., 2019) and that response of soil moisture to temperature might explain the temperature effects across latitudinal gradients (Song et al., 2017).

4.4. Selection of nematode species by the DNA source

In our comprehensive dataset, most of those nematodes that differed in read abundance between the two DNA extraction methods (DNA sources) were more abundant in the nematode-sample DNA. In the class Chromadorea, those species that differed between the extraction methods were predominantly small, with a weight <1 μg. Exceptions with a greater body weight were *Rhabditis brassicae*, three species belonging to the genus *Anaplectus* and *Pristionchus lheritieri*, all of which were underrepresented in the soil-extracted DNA, and *Phasmarhabditis* sp., which was overrepresented in the soil-extracted DNA. In the class Enoplea, no overrepresented taxa were found in soil-extracted DNA. In contrast, this class had many underrepresented taxa of larger body weight, the heaviest being *Anatonchus tridentatus*. Overall, we did not find an unequivocal relationship between the size of species (using weight as a proxy) and difference in abundance between the two extraction methods, i.e., species of similar size were both over- and underrepresented. If the isolation of intact nematodes prior to DNA extraction did select against taxa of certain sizes, one possible reason for not finding a clear difference in their relative abundances between the two extraction methods might be that both very large and very small nematodes can be excluded during the different steps of the Oostenbrink method (Verschoor and de Goede, 2000; EPPO, 2013). Another possible

explanation is that the bias between the two extraction methods is related to the motility of nematodes rather than their size: in the final step of the Oostenbrink dish method, nematodes migrate actively through a cotton-wool filter, such that eggs, nematodes in non-motile resting stages, and relatively immotile or otherwise inactive nematodes are excluded (Verschoor and de Goede, 2000; EPPO, 2013; Cesarz et al., 2019). Conversely, soil-extracted DNA targets any nematode DNA, independent of life stage and activity status.

We found a strong relationship between the rank abundance and the differential abundance of taxa between the two methods, i.e. rare taxa were more underrepresented in the soil-extracted DNA. This could be a result of lower amounts of soils and a large fraction of sequences of non-nematode origin in the soil-extracted DNA approach leading to a lower sensitivity to detect rare taxa. Consequently, rare taxa seem to be underrepresented compared to the nematode-sample DNA. These findings additionally emphasize the need to develop methods to extract DNA from larger amounts of soil and to design nematode-specific primers.

In agreement with findings from Griffiths et al. (2018), we found a lower abundance of taxa affiliated with the families Aporcelaimidae, Cephalobidae, Paratylenchidae, Plectidae and Tylenchidae and a higher abundance of taxa affiliated with the family Monhysteridae in the soil-extracted DNA. However, opposite patterns were observed for taxa within the families Aphelenchoididae, Diphtherophoridae and Microlaimidae. One reason for the contrasting results could be the taxonomic resolution, i.e., family level in Griffiths et al. (2018) and species level in our study. For instance, within the family Rhabditidae, we found both over- and underrepresented species in soil-extracted DNA. Differences could also be attributed to different soil types (Verschoor and de Goede, 2000; Cesarz et al., 2015), the sequencing depth and platform used, the bioinformatic processing of the sequences, and the database used for taxonomic classification.

4.5. Effects of the DNA source on nematode feeding types

Nematode feeding types are important indicators of soil food-web dynamics and have been widely studied across different soil habitats (Nielsen et al., 2014; Stone et al., 2016b; Song et al., 2017; Treonis et al., 2018; van den Hoogen et al., 2019; Vazquez et al., 2019; Wilschut et al., 2019; Li et al., 2020). In this study, we found a highly significant difference in the abundance of all feeding types between the two extraction methods (DNA sources). In agreement with findings from previous studies, bacterivores were the most abundant group with both extraction methods, followed by fungivores, whereas omnivores and predators
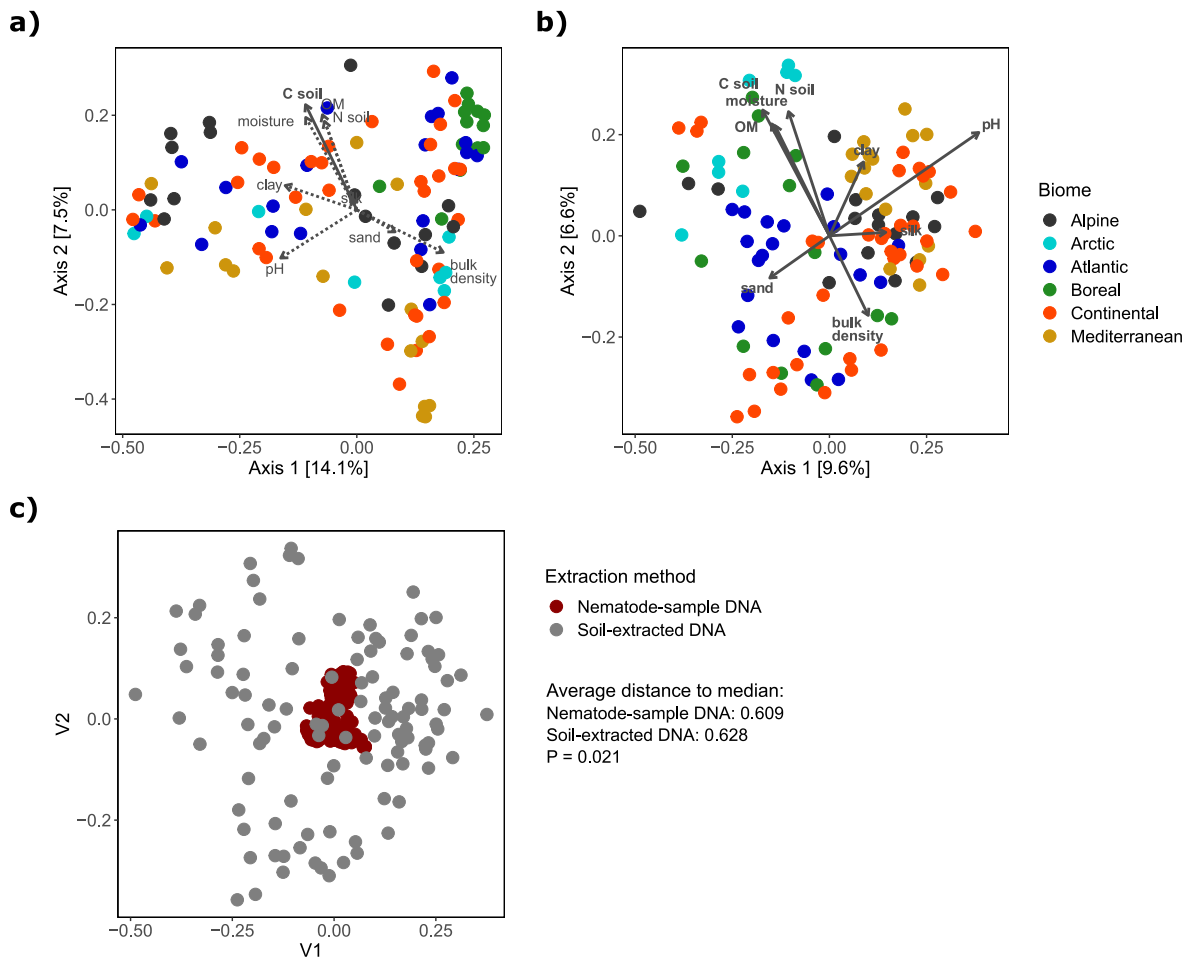
Fig. 4. Relationships between nematode community structure and environmental variables. (a) Nematode-sample DNA, (b) Soil-extracted DNA. PCoAs were conducted based on Bray-Curtis distances of relative read abundances. Vectors represent a regression of environmental parameters against the PCoA axes. Significant parameters are represented by solid lines. (c) Comparison of community structure across biomes for nematode-sample DNA and soil-extracted DNA. n = 4 per site.

were the least abundant (Bongiorno et al., 2019).

The abundance of feeding types was highly variable among sites within the same biome, and also across biomes. In the soil-extracted DNA, herbivores (ecto- and endoparasites) differed significantly among biomes, whereas in the nematode-sample DNA, this was the case for ectoparasitic herbivores only. Van den Hoogen et al. (2019) reported that the absolute abundance of all feeding types followed the total abundance of nematodes with a peak at high latitudes, indicating that the relative abundances of different feeding types did not exhibit a latitudinal trend in their study. Conversely, we found an increase in the proportion of bacterivores with increasing latitude in the soil-extracted DNA. In accordance with Song et al. (2017), we found soil moisture to be significantly associated with the abundance of bacterivores. In addition, we also found a significant association between soil and litter C concentration and bacterivore abundance as well as between litter N concentration and fungivores. These soil properties have previously been identified as important drivers of bacterial and fungal communities (Delgado-Baquerizo et al., 2016; George et al., 2019; Smith et al., 2021), thus having potential to affect the abundance of bacterivores and fungivores. The effect of soil properties on herbivores, which was revealed with both extraction methods, is likely to be mediated by the plants these nematodes feed on as plant characteristics have earlier been identified as primary drivers of herbivorous nematodes (van den Hoogen et al., 2019). Collectively, our findings suggest that feeding type abundances and their associations with environmental variables are influenced by the extraction method (DNA source), highlighting the need to take into account the potential methodological biases when making

ecological interpretations of DNA metabarcoding data.

4.6. General implications

Across all analyses, we observed differences between the DNA extraction methods across the large spatial extent of our study. A previous methodological comparison that aimed at validating the use of molecular approaches for the ecological assessment of nematodes demonstrated a good agreement between morphological, qPCR-based and high throughput sequencing-based analyses of nematode communities (Geisen et al., 2018). As a result, the authors deemed molecular characterization of nematode communities a valid approach and highlighted the increased diversity and taxonomic resolution compared with the morphological approach. Notably, all the approaches compared were based on the Oostenbrink extraction. Overall, the molecular characterization of nematode communities is increasingly used, but the vast majority of studies have first isolated the nematodes from soil and only very few studies have included direct DNA extraction from the soil (environmental DNA). Griffiths et al. (2018) found differences in nematode communities depending on the extraction method applied at a small scale. Here, we were able to demonstrate that the choice of the DNA source material (nematode-sample DNA vs. soil-extracted DNA) can lead to significantly different ecological interpretations at a large spatial scale including sites with a wide range of edaphic and climatic conditions. This suggests that previous studies using isolation of intact nematodes from soil might have led to different conclusions if conducted using direct DNA extraction from the soil. One possible source of

Table 3
Effects of site, biome and extraction method on nematode community structure were tested using permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations. The effect of site and biome were analysed in separate subsets for each extraction method, while the effect of extraction method was analysed using the full dataset. In the subsets, when testing the effect of biome, samples were not permuted across sites to account for increased similarity among replicates within sites. In the full dataset, the effect of extraction method was tested together with the effect of site, but only the effect of extraction is shown. Data was permuted in pairs of samples for nematode-sample DNA and soil-extracted DNA to account for the dependence between DNA extracted with the two different methods in the same plot. The function permutest with 9999 permutations was used to assess homogeneity of variance. Significant values are in bold letters. $N_{sites} = 29$ (4 alpine, 3 arctic, 5 atlantic, 4 boreal, 9 continental, 4 mediterranean), n = 4.

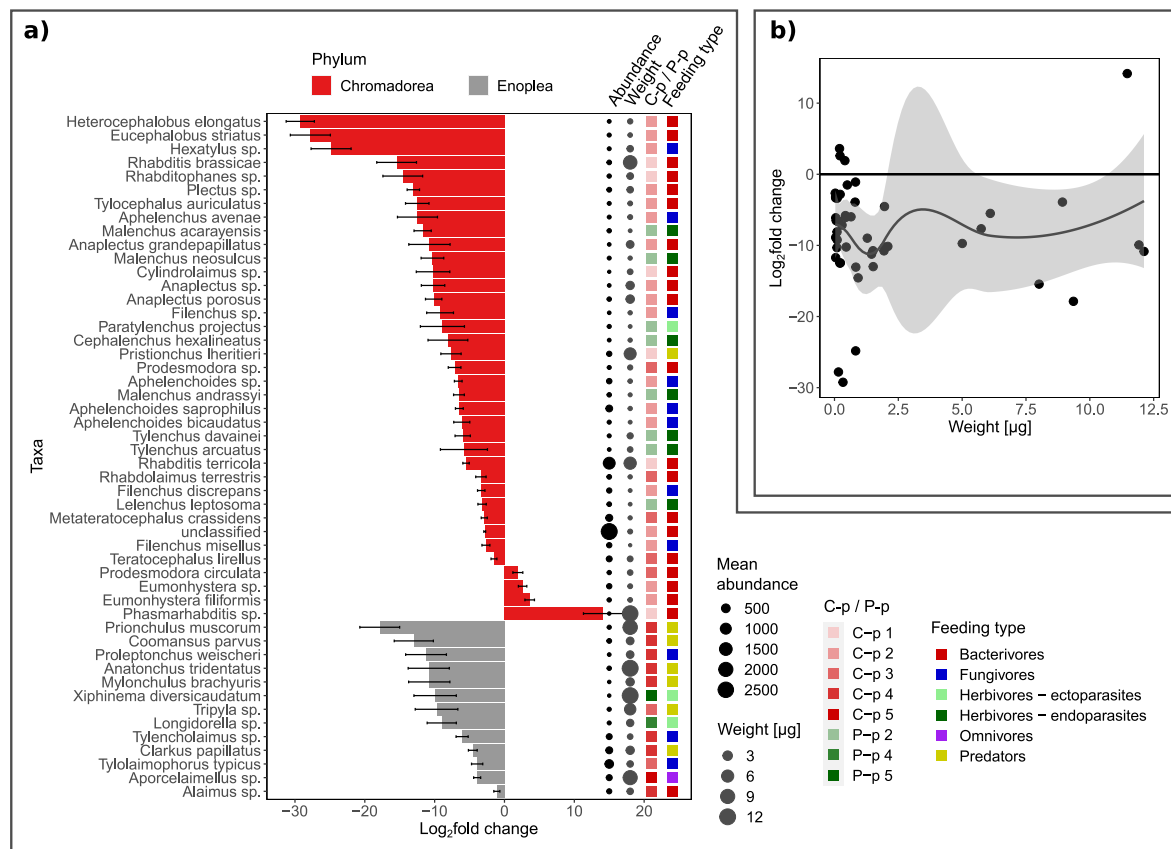|	Factor	Nematode-sample DNA		Soil-extracted DNA	
		$F_{(28,108)}$	P	$F_{(28,108)}$	P
PERMANOVA	**Site**	2.9	**0.0001**	3.4	**0.0001**
Betadispersion		1.1	0.41	1.7	**0.034**
	Biome	Nematode-sample DNA		Soil-extracted DNA	
		$F_{(5,95)}$	P	$F_{(5,95)}$	P
PERMANOVA		2.6	0.051	2.9	**0.018**
Betadispersion		7.0	**0.0021**	11	**0.0002**
		Extraction method (accounting for site)			
		$F_{(1,217)}$		P	
PERMANOVA		10		**0.0001**	
Betadispersion		–		–	

Fig. 5. (a) Log$_2$-fold changes in species abundances between extraction methods (soil-extracted DNA vs. nematode-sample DNA), controlling for the effect of site. Significant log$_2$-fold changes ($P_{adjusted} < 0.05$) are shown. Normalization in *DESeq2* was conducted at the ASV level. Error bars represent the estimated standard error for the log$_2$-fold changes from the model (*DESeq2*). Positive log$_2$-fold changes indicate that taxa were overrepresented in soil extracted DNA, while negative log$_2$-fold changes indicate that taxa were overrepresented in nematode-sample DNA. The size of the black points represents the mean normalized read abundance of the respective species. The size of the grey points represents the weight of the species. C-p/P-p represents colonizer-persister classes for free-living and plant-associated nematodes. C-p 1/P-p 1 = highest growth rate; C-p 5/P-p 5 = lowest growth rate. (b) Relationship between log$_2$-fold changes and weight of the species. The trendline was obtained by loess smoothing. $N_{sites} = 29$ (4 alpine, 3 arctic, 5 atlantic, 4 boreal, 9 continental, 4 mediterranean), n = 4 per site.

differences between approaches compared here, is the preferential extraction of some taxa in the Oostenbrink method depending on size and activity. (Verschoor and de Goede, 2000; EPPO, 2013). Such a bias could be significantly reduced using, a combination of Oostenbrink extraction method followed by a sugar centrifugal flotation including active, inactive and dead nematodes (McSorley and Frederick, 2004;

Table 4

Effects of site and biome on the abundance of nematode feeding types for nematode-sample DNA and soil-extracted DNA, as well as the effect of the extraction method. The site effect was estimated using a generalized linear model with a negative binomial distribution, based on read abundances normalized at the amplicon sequence variant (ASV) level using DESeq2. The effects of biome and extraction method were estimated using a generalized linear mixed model with site as a random factor. Subscripted indices indicate numerator and total degrees of freedom. N_{sites} = 29 (4 alpine, 3 arctic, 5 atlantic, 4 boreal, 9 continental, 4 mediterranean), n = 4.

|	Site $_{(28,108)}$		Biome		Extraction method
	Nematode-sample DNA	Soil-extracted DNA	Nematode-sample DNA	Soil-extracted DNA	
Bacterivores	**<0.0001**	**<0.0001**	0.64	0.35	**<0.0001**
Fungivores	**<0.0001**	**<0.0001**	0.10	0.37	**<0.0001**
Herbivores (ectoparasites)	**<0.0001**	**<0.0001**	**<0.0001**	**0.0043**	**0.025**
Herbivores (endoparasites)	**<0.0001**	**<0.0001**	0.31	**0.012**	**<0.0001**
Omnivores	**<0.0001**	**<0.0001**	0.11	0.20	**<0.0001**
Predators	**<0.0001**	**<0.0001**	0.12	0.68	**<0.0001**
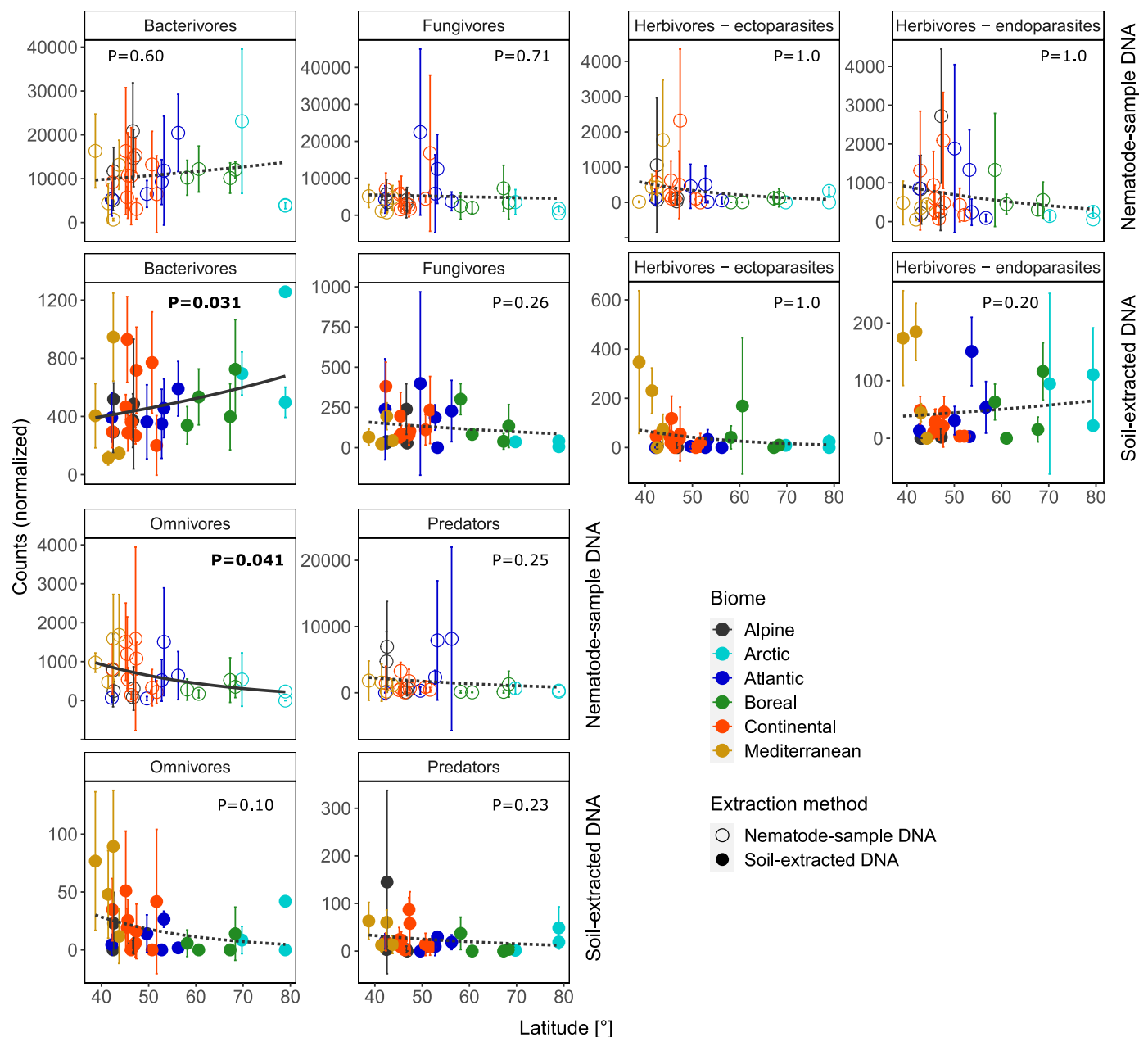
Fig. 6. Abundances of nematode feeding types as a function of latitude for the two extraction approaches (nematode-sample DNA vs. soil-extracted DNA). Amplicon sequence variants (ASVs) were classified into feeding types based on their taxonomic affiliation at the genus level. Abundances were normalized at the ASV level using *DESeq2*. Lines represent regressions using a generalized linear mixed model with significant regressions in solid. Mean ± SD (n = 4) for each site is shown.

Table 5

Relationship between the abundance of nematode feeding types and environmental parameters for nematode-sample DNA (top) and for soil-extracted DNA (bottom). Amplicon sequence variants (ASVs) were classified into feeding types based on their taxonomic affiliation at the genus level and abundances were normalized at the ASV level using DESeq2. Feeding type abundances were modelled separately for each parameter using a generalized linear mixed model with a negative binomial distribution and site as random factor. P-values were obtained from a likelihood ratio test comparing each model against a null model with the random factor only. Significant values are in bold letters. N_{sites} = 29 (4 alpine, 3 arctic, 5 atlantic, 4 boreal, 9 continental, 4 mediterranean), NA = not analysed; n = 4.

	pH	C soil	C litter	N soil	N litter	Bulk density	OM	Soil moisture	Sand	Silt	Clay	Latitude
Bacterivores	0.83	0.18	0.20	0.18	0.18	0.06	0.13	0.17	1.0	NA	0.64	0.60
Fungivores	**0.026**	0.74	0.17	0.59	0.13	0.83	0.40	0.13	0.23	0.22	0.60	0.71
Herbivores (ectoparasites)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	1.0
Herbivores (endoparasites)	0.074	0.67	0.49	0.57	0.99	0.97	0.47	1.0	0.78	0.94	0.49	1.0
Omnivores	0.44	0.43	0.76	0.59	0.45	0.79	0.30	0.62	NA	0.56	0.29	**0.041**
Predators	0.17	0.63	0.45	0.43	0.56	0.59	0.44	0.08	0.36	0.17	0.86	0.25
Bacterivores	0.09	**0.047**	**0.011**	0.16	0.06	0.74	0.06	**0.0031**	1.0	0.86	0.80	**0.031**
Fungivores	0.34	0.47	0.64	0.51	**0.046**	0.29	0.53	0.81	1.0	0.39	0.82	0.26
Herbivores (ectoparasites)	1.0	**0.0062**	**<0.0001**	**0.041**	**<0.0001**	1.0	0.09	**0.0077**	0.19	0.26	0.51	1.0
Herbivores (endoparasites)	0.90	0.84	1.0	0.59	0.92	0.56	0.26	0.81	1.0	0.36	0.55	0.20
Omnivores	**<0.0001**	0.13	**0.0017**	**0.00014**	**<0.0001**	**0.00039**	0.20	**<0.0001**	0.86	0.92	0.83	0.10
Predators	0.15	0.28	0.15	0.73	0.77	0.51	0.41	0.68	0.51	0.84	0.09	0.23

Cesarz et al., 2019; Gattoni et al., 2022). Soil-extracted DNA appeared to be biased by a lack of sensitivity to detect rare taxa resulting from low amounts of soil used for extraction and a large fraction of non-nematode sequences. Thus, this approach would significantly benefit from extraction protocols for large amounts of soils (>100 g) and from the need of designing nematode-specific primers.

Geisen et al. (2018) pointed out that sequencing methods are not capable of retrieving full quantitative information on the abundance of taxa or functional groups, and for this reason, van den Hoogen et al. (2019) used morphological data in their global modelling study. Using taxon-specific qPCR primers, as used in some recent studies (Vervoort et al., 2012; Quist et al., 2016; Geisen et al., 2018) may contribute more quantitative information on particular groups of interest. Also, it is important to remember that the Oostenbrink extraction approach relies on active migration of individuals and thus targets only living nematodes, whereas direct DNA extraction from the soil includes both active and inactive/dead nematodes. Research questions targeting both life stages could be tackled by targeting ribosomal RNA that is rapidly degraded in dead cells, an approach that has been applied in numerous amplicon and metagenome-based microbial studies (Carini et al., 2020; Donhauser et al., 2020).

In conclusion, nematode communities assessed with two different molecular approaches (soil-extracted DNA and nematode-sample DNA) were comparable although with some differences in nematode community composition at a large spatial scale. Direct extraction of DNA from the soil can be a high-throughput screening approach to target nematode diversity and community structure, which can complement laborious classical methods. In particular, for monitoring programmes where DNA is directly extracted from soils to study bacterial and fungal communities using soil-extracted DNA (Gschwend et al. 2021a, 2021b) will allow to analyse nematode community structure at a similar throughput and to assess cross-kingdom trophic structures. A key problem with direct soil extraction is that a large amount of sequences are of non-nematode origin, pointing to a need for developing nematode-specific primers (Kawanobe et al., 2021) and that low amounts of soil used for extraction reduced sensitivity to detect rare taxa. If this obstacle can be overcome, metabarcoding of nematode communities directly extracted from soil can be a powerful, efficient approach that could be implemented in large-scale monitoring programs using nematode-based indicators of soil health.

Data accessibility

Raw sequences were deposited in the NCBI Sequence Read Archive under the BioProject accession identifier PRJNA562119 and PRJNA793632. R script is available in the Supplementary Material.

Author contributions

AF and BF designed the study, all authors contributed to the soil sampling and collection of data, JD, and BF analysed the data, JD and BF drafted the manuscript, to which all authors critically contributed.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2023.109154.

References

Ahmed, M., Black, M.A., Prior, T., Karssen, G., Lawson, R., Adams, I., Sapp, M., 2019. Metabarcoding of soil nematodes: the importance of taxonomic coverage and availability of reference sequences in choosing suitable marker(s). Metabarcoding Metagenomics 3, e36408.

Andriuzzi, W.S., Ngo, P.T., Geisen, S., Keith, A.M., Dumack, K., Bolger, T., Bonkowski, M., Brussaard, L., Faber, J.H., Chabbi, A., Rumpel, C., Schmidt, O., 2016. Organic matter composition and the protist and nematode communities around anecic earthworm burrows. Biology and Fertility of Soils 52, 91–100.

Avó, A.P., Daniell, T.J., Neilson, R., Oliveira, S., Branco, J., Adão, H., 2017. DNA Barcoding and morphological identification of benthic nematodes assemblages of estuarine intertidal sediments: advances in molecular tools for biodiversity assessment. Frontiers in Marine Science 4, 66.

Bahram, M., Hildebrand, F., Forslund, S.K., Anderson, J.L., Soudzilovskaia, N.A., Bodegom, P.M., et al., 2018. Structure and function of the global topsoil microbiome. Nature 560, 233–237.

Bar-On, Y.M., Phillips, R., Milo, R., 2018. The biomass distribution on Earth. Proceedings of the National Academy of Sciences of the United States of America 115, 6506–6511.

Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505–511.

Bates, S.T., Clemente, J.C., Flores, G.E., Walters, W.A., Parfrey, L.W., Knight, R., Fierer, N., 2013. Global biogeography of highly diverse protistan communities in soil. The ISME Journal 7 (3), 652–659.

Bell, C.A., Namaganda, J., Urwin, P.E., Atkinson, H.J., 2021. Next-generation sequencing of the soil nematode community enables the sustainability of banana plantations to be monitored. Applied Soil Ecology 166, 103999.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., et al., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6, 1–17.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37, 852–857.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83 (1), 14–19.

Bongers, T., Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology & Evolution 14, 224–228.

Bongiorno, G., Bodenhausen, N., Bünemann, E.K., Brussaard, L., Geisen, S., Mäder, P., Quist, C.W., Walser, J.C., de Goede, R.G.M., 2019. Reduced tillage, but not organic matter input, increased nematode diversity and food web stability in European long-term field experiments. Molecular Ecology 28 (22), 4987–5005.

Briones, M.J.I., 2014. Soil fauna and soil functions: a jigsaw puzzle. Frontiers of Environmental Science 2, 7.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J., Holmes, S.P., 2016. DADA2: high resolution sample inference from Illumina amplicon data. Nature Methods 13, 581–583.

Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME Journal 11, 2639–2643.

Carini, P., Delgado-Baquerizo, M., Hinckley, E.-L.S., Holland-Moritz, H., Brewer, T.E., Rue, G., Vanderburgh, C., McKnight, D., Fierer, N., 2020. Effects of spatial variability and relic DNA removal on the detection of temporal dynamics in soil microbial communities. mBio 11 (1), e02776–e12719.

Cesarz, S., Reich, P.B., Scheu, S., Ruess, L., Schaefer, M., Eisenhauer, N., 2015. Nematode functional guilds, not trophic groups, reflect shifts in soil food webs and processes in response to interacting global change factors. Pedobiologia 58 (1), 23–32.

Cesarz, S., Schulz, A.E., Beugnon, R., Eisenhauer, N., 2019. Testing soil nematode extraction efficiency using different variations of the Baermann-funnel method. Soil Organisms 91, 61–72.

Chen, D., Cheng, J., Chu, P., Hu, S., Xie, Y., Tuvshintogtokh, I., Bai, Y., 2015. Regional-scale patterns of soil microbes and nematodes across grasslands on the Mongolian plateau: relationships with climate, soil, and plants. Ecography 38, 622–631.

Cools, N., Vesterdal, L., De Vos, B., Vanguelova, E.I., Hansen, K., 2014. Tree species is the major factor explaining C:N ratios in European forest soils. Forest Ecology and Management 311, 3–16.

Darby, B., Todd, T.C., Herman, M.A., 2013. High-throughput amplicon sequencing of rRNA genes requires a copy number correction to accurately reflect the effects of management practices on soil nematode community structure. Molecular Ecology 22, 5456–5471.

De Vos, B., Cools, N., Ilvesniemi, H., Vesterdal, L., Vanguelova, E., Carnicelli, S., 2015. Benchmark values for forest soil carbon stocks in Europe: results from a large scale forest soil survey. Geoderma 251–252, 33–46.

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Trivedi, P., Osanai, Y., Liu, Y., Hamonts, K., Jeffries, T.C., Singh, B.K., 2016. Carbon content and climate variability drive global soil bacterial diversity patterns. Ecological Monographs 86 (3), 373–390.

Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge, D. J., Bardgett, R.D., Maestre, F.T., Singh, B.K., Fierer, N., 2018. A global atlas of the dominant bacteria found in soil. Science 359, 320–325.

Delignette-Muller, M.L., Dutang, C., 2015. Fitdistrplus: an R package for fitting distributions. Journal of Statistical Software 64, 1–34.

Derycke, S., Vanaverbeke, J., Rigaux, A., Backeljau, T., Moens, T., 2010. Exploring the use of Cytochrome Oxidase c subunit 1 (COI) for DNA barcoding of free-living marine nematodes. PLoS One 5, e13716.

Donhauser, J., Niklaus, P.A., Rousk, J., Larose, C., Frey, B., 2020. Temperatures beyond the community optimum promote the dominance of heat-adapted, fast growing and stress resistant bacteria in alpine soils. Soil Biology and Biochemistry 148, 107873.

Donhauser, J., Qi, W., Bergk-Pinto, B., Frey, B., 2021. High temperatures enhance the microbial genetic potential to recycle C and N from necromass in high-mountain soils. Global Change Biology 27, 1365–1386.

Eisenhauer, N., Cesarz, S., Koller, R., Worm, K., Reich, P.B., 2012. Global change belowground: impacts of elevated CO_2, nitrogen, and summer drought on soil food webs and biodiversity. Global Change Biology 18, 435–447.

EPPO, 2013. PM 7/119 (1) Nematode extraction. EPPO Bulletin 43, 471–495.

Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Applied Soil Ecology 18, 13–29.

Frey, B., Rime, T., Phillips, M., Stierli, B., Hajdas, I., Widmer, F., Hartmann, M., 2016. Microbial diversity in European alpine permafrost and active layers. FEMS Microbiology Ecology 92, 1–17.

Frey, B., Walthert, L., Perez-Mon, C., Stierli, B., Koechli, R., Dharmarajah, A., Brunner, I., 2021. Deep soil layers of drought-exposed forests harbor poorly known bacterial and fungal communities. Frontiers in Microbiology 12, 674160.

Gattoni, K., Gendron, E.M.S., Borgmeier, A., McQueen, J.P., Mullin, P.G., Powers, K., et al., 2022. Context-dependent role of abiotic and biotic factors structuring nematode communities along two environmental gradients. Molecular Ecology 31, 3903–3916.

Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In: Klute, A., et al. (Eds.), Methods of Soil Analysis: Part 1, Physical and Mineralogical Methods, second ed. American Soc Agro Madison WI, pp. 383–411.

Geisen, S., Snoek, L.B., Hooven, F.C. ten, Duyts, H., Kostenko, O., Bloem, J., Martens, H., Quist, C.W., Helder, J.A., van der Putten, W.H., 2018. Integrating quantitative morphological and qualitative molecular methods to analyse soil nematode community responses to plant range expansion. Methods in Ecology and Evolution 9, 1366–1378.

George, P.B., Keith, A.M., Creer, S., Barrett, G.L., Lebron, I., Emmett, B.A., Robinson, D. A., Jones, D.L., 2017. Evaluation of mesofauna communities as soil quality indicators in a national-level monitoring programme. Soil Biology and Biochemistry 115, 537–546.

George, P.B.L., Lallias, D., Creer, S., Seaton, F.M., Kenny, J.G., Eccles, R.M., Griffiths, R. I., Lebron, I., Emmett, B.A., Robinson, D.A., Jones, D.L., 2019. Divergent national-scale trends of microbial and animal biodiversity revealed across diverse temperate soil ecosystems. Nature Communications 10, 1–11.

Gorny, A.M., Hay, F.S., Wang, X., Pethybridge, S.J., 2018. Isolation of nematode DNA from 100 g of soil using Fe_3O_4 super paramagnetic nanoparticles. Nematology 20 (3), 271–283.

Griffiths, B.S., de Groot, G.A., Laros, I., Stone, D., Geisen, S., 2018. The need for standardisation: exemplified by a description of the diversity, community structure and ecological indices of soil nematodes. Ecological Indicators 87, 43–46.

Gschwend, F., Hartmann, M., Hug, A., Enkerli, J., Gubler, A., Frey, B., Meuli, R.G., Widmer, F., 2021a. Long-term stability of soil bacterial and fungal community structures revealed in their abundant and rare fractions. Molecular Ecology 30, 4305–4320.

Gschwend, F., Hartmann, M., Mayerhofer, J., Hug, A.S., Enkerli, J., Gubler, A., et al., 2021b. Site and land-use associations of soil bacteria and fungi define core and indicative taxa. FEMS Microbiology Ecology 97 (12) fiab165.

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., Del Campo, J., Dolan, J.R., Dunthorn, M., Christen, R., 2013. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Research 41 (D1), D597–D604.

Kawanobe, M., Toyota, K., Ritz, K., 2021. Development and application of a DNA metabarcoding method for comprehensive analysis of soil nematode communities. Applied Soil Ecology 166, 103974.

Li, X., Zhu, H., Geisen, S., Bellard, C., Hu, F., Li, H., Chen, X., Liu, M., 2020. Agriculture erases climate constraints on soil nematode communities across large spatial scales. Global Change Biology 26, 919–930.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15, 550.

Macheriotou, L., Guilini, K., Bezerra, T.N., Tytgat, B., Nguyen, D.T., Phuong Nguyen, T. X., Noppe, F., Armenteros, M., Boufahja, F., Rigaux, A., Vanreusel, A., Derycke, S., 2019. Metabarcoding free-living marine nematodes using curated 18S and CO1 reference sequence databases for species-level taxonomic assignments. Ecology and Evolution 9, 1211–1226.

Martins da Silva, P., Carvalho, F., Dirilgen, T., Stone, D., Creamer, R.E., Bolger, T., Sousa, J.P., 2016. Traits of collembolan life-form indicate land use types and soil properties across an European transect. Applied Soil Ecology 97, 69–77.

McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8 (4), e61217.

McSorley, R., Frederick, J.J., 2004. Effect of extraction method on perceived composition of the soil nematode community. Applied Soil Ecology 27, 55–63.

Mikola, J., Sulkava, P., 2001. Responses of microbial-feeding nematodes to organic matter distribution and predation in experimental soil habitat. Soil Biology and Biochemistry 33, 811–817.

Neher, D.A., 2001. Role of nematodes in soil health and their use as indicators. Journal of Nematology 33, 161–168.

Neher, D.A., 2010. Ecology of plant and free-living nematodes in natural and agricultural soil. Annual Review of Phytopathology 48, 371–394.

Neher, D.A., Wu, J., Barbercheck, M.E., Anas, O., 2005. Ecosystem type affects interpretation of soil nematode community measures. Applied Soil Ecology 30 (1), 47–64.

Nielsen, U.N., Ayres, E., Wall, D.H., Li, G., Bardgett, R.D., Wu, T., Garey, J.R., 2014. Global-scale patterns of assemblage structure of soil nematodes in relation to climate and ecosystem properties. Global Ecology and Biogeography 23, 968–978.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. vegan: community ecology package. R package version 2.5-4. https://cran.r-project.org/package. vegan.

Oostenbrink, M., 1960. Estimating nematode populations by some selected methods. In: N, S.J., R, J.W. (Eds.), Nematology. Chapel Hill: The University of North Carolina Press, pp. 85–102.

Osler, G.H.R., Sommerkorn, M., 2007. Toward a complete soil C and N cycle: incorporation the soil fauna. Ecology 88 (7), 1611–1621.

Peham, T., Steiner, F.M., Schlick-Steiner, B.C., Arthofer, W., 2017. Are we ready to detect nematode diversity by next generation sequencing? Ecology and Evolution 7 (12), 4147–4151.

Porazinska, D.L., Giblin-Davis, R.M., Faller, L., Farmerie, W., Kanzaki, N., Morris, K., Powers, T.O., Tucker, A.E., Sung, W., Thomas, W.K., 2009. Evaluating high-throughput sequencing as a method for metagenomic analysis of nematode diversity. Molecular Ecology Resources 9, 1439–1450.

Powers, T., Harris, T., Higgins, R., Mullin, P., Sutton, L., Powers, K., 2011. MOTUs, Morphology, and Biodiversity Estimation: a case study using nematodes of the suborder *Criconematina* and a conserved 18S DNA barcode. Journal of Nematology 43, 35–48.

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.

R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Resch, C., Schütz, M., Buchmann, N., Frey, B., Graf, U., van der Putten, W., Zimmermann, S., Risch, A., 2021. Evaluating long-term success in grassland restoration: an ecosystem multifunctionality approach. Ecological Applications 31, e02271.

Resch, C., Schütz, M., Ochoa-Hueso, R., Buchmann, N., Frey, B., Graf, U., van der Putten, W., Zimmermann, S., Risch, A., 2022. Long-term recovery of above-and belowground interactions in restored grasslands after topsoil removal and seed addition. Journal of Applied Ecology 59 (9), 2299–2308.

Sapkota, R., Nicolaisen, M., 2015. High-throughput sequencing of nematode communities from total soil DNA extractions. BMC Ecology 15, 1–8.

Sechi, V., De Goede, R.G.M., Rutgers, M., Brussard, L., Mulder, C., 2018. Functional diversity in nematode communities across terrestrial ecosystems. Basic and Applied Ecology 30, 76–86.

Sieriebriennikov, B., Ferris, H., de Goede, R.G.M., 2014. NINJA: an automated calculation system for nematode-based biological monitoring. European Journal of Soil Biology 61, 90–93.

Sikder, M.M., Vestergård, M., Sapkota, R., Kyndt, T., Nicolaisen, M., 2020. Evaluation of metabarcoding primers for analysis of soil nematode communities. Diversity 12 (10), 388.

Smith, L.C., Orgiazzi, A., Eisenhauer, N., Cesarz, S., Lochner, A., Jones, A., Bastida, F., Patoine, G., Reitz, T., Buscot, F., Rillig, M.C., Heintz-Buschart, A., Lehmann, A., Guerra, C.A., 2021. Large-scale drivers of relationships between soil microbial properties and organic carbon across Europe. Global Ecology and Biogeography 30 (10), 2070–2083.

Song, D., Pan, K., Tariq, A., Sun, F., Li, Z., Sun, X., Zhang, L., Olusanya, O.A., Wu, X., 2017. Large-scale patterns of distribution and diversity of terrestrial nematodes. Applied Soil Ecology 114, 161–169.

Stone, D., Blomkvist, P., Hendriksen, N.B., Bonkowski, M., Jørgensen, H.B., Carvalho, F., Dunbar, M.B., Gardi, C., et al., 2016a. A method of establishing a transect for biodiversity and ecosystem function monitoring across Europe. Applied Soil Ecology 97, 3–11.

Stone, D., Costa, D., Daniell, T.J., Mitchell, S.M., Topp, C.F.E., Griffiths, B.S., 2016b. Using nematode communities to test a European scale soil biological monitoring programme for policy development. Applied Soil Ecology 97, 78–85.

Sylvain, Z.A., Wall, D.H., Cherwin, K.L., Peters, D.P.C., Reichmann, L.G., Sala, O.E., 2014. Soil animal responses to moisture availability are largely scale, not ecosystem dependent: insight from a cross-site study. Global Change Biology 20, 2631–2643.

Taberlet, P., Prud'Homme, S.M., Campione, E., Roy, J., Miquel, C., Shehzad, W., Gielly, L., Rioux, D., Choler, P., Clément, J.-C., Melodelima, C., Pompanon, F., Coissac, E., 2012. Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. Molecular Ecology 21, 1816–1820.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., et al., 2014. Global diversity and geography of soil fungi. Science 346, 1256688.

Treonis, A.M., Unangst, S.K., Kepler, R.M., Buyer, J.S., Cavigelli, M.A., Mirsky, S.B., Maul, J.E., 2018. Characterization of soil nematode communities in three cropping systems through morphological and DNA metabarcoding approaches. Scientific Reports 8, 2004.

van den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D.A., de Goede, Ron, G.M., Adams, B.J., Ahmad, W., Andriuzzi, W.S., Bardgett, R.D., Bonkowski, M., 2019. Soil nematode abundance and functional group composition at a global scale. Nature 572, 194–198.

Vazquez, C., de Goede, R.G.M., Korthals, G.W., Rutgers, M., Schouten, A.J., Creamer, R., 2019. The effects of increasing land use intensity on soil nematodes: a turn towards specialism. Functional Ecology 33 (10), 2003–2016.

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.J.J., van Megen, H.H. B., Veenhuizen, P., et al., 2012. SSU Ribosomal DNA-based monitoring of nematode assemblages reveals distinct seasonal fluctuations within evolutionary heterogeneous feeding guilds. PLoS One 7 (10), e47555.

Viglierchio, D.R., Schmitt, R.V., 1983. On the methodology of nematode extraction from field samples: comparison of methods for soil extraction. Journal of Nematology 15, 450–454.

Wardle, D.A., 2006. The influence of biotic interactions on soil biodiversity. Ecology Letters 9, 870–886.

Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis. New York: Springer Publishing Company, Incorporated.

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.

Wilschut, R.A., Geisen, S., Martens, H., Kostenko, O., Hollander, M. de, Hooven, F.C. ten, Weser, C., Snoek, L.B., Bloem, J., Caković, D., Čelik, T., Koorem, K., Krigas, N., Manrubia, M., Ramirez, K.S., Tsiafouli, M.A., Vreš, B., van der Putten, W.H., 2019. Latitudinal variation in soil nematode communities under climate warming-related range-expanding and native plants. Global Change Biology 25, 2714–2726.

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, 315–331.

Yeates, C., Gillings, M.R., Davison, A.D., Altavilla, N., Veal, D.A., 1998. Methods for microbial DNA extraction from soil for PCR amplification. Biological Procedures Online 1 (1), 40.

Zanella, A., Jabiol, B., Ponge, J.F., Sartori, G., De Waal, R., Van Delft, B., Graefe, U., Cools, N., Katzensteiner, K., Hager, H., Englisch, M., 2011. A European morphofunctional classification of humus forms. Geoderma 164, 138–145.

Zinger, L., Chave, J., Coissac, E., Iribar, A., Louisanna, E., Manzi, S., et al., 2016. Extracellular DNA extraction is a fast, cheap and reliable alternative for multi-taxa surveys based on soil DNA. Soil Biology and Biochemistry 96, 16–19.