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Scalable isolation of soil genomic DNA from microbial eukaryotes to multicellular micro- and mesofauna

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ABSTRACT

Global initiatives emphasise the need for harmonised soil biodiversity assessments. Efficient DNA extraction methods that accommodate larger soil volumes are essential for capturing organisms at higher trophic levels than bacteria and fungi and for supporting extensive sampling campaigns. We developed and evaluated a scalable, cost-efficient, and automation-ready soil DNA isolation protocol and compared its performance with two widely used commercial extraction methods: Qiagen DNeasy PowerSoil Pro (0.25 g soil input) and Qiagen DNeasy PowerMax Soil (2.5 g soil input). Four contrasting soil types representing agricultural and forest ecosystems (mull, sandy till, peat, and coarse mineral soil) were analysed. The new protocol combines bead-beating in sodium thiocyanate lysis buffer with either silica-membrane or carboxyl-coated magnetic bead purification and was tested using soil inputs of 2.5 g and 5 g for one soil type. DNA extracts were sequenced on an Illumina MiSeq platform using universal eukaryotic 18S rRNA gene primers to characterise soil metazoans and microbial eukaryotes. The developed protocol, combining a tenfold increase in soil input with commercial kit purification, produced the highest DNA yields in three of four soil types, increasing DNA recovery by up to 2.3-fold compared with the commercial maxiprep method. It also consistently detected the greatest metazoan richness, recovering up to 103% more taxa than the conventional miniprep method and up to 82% more taxa than the commercial maxiprep method. The carboxyl-coated magnetic bead workflow also performed well, recovering up to 83% more metazoan taxa than the miniprep method and up to 55% more taxa than the maxiprep method, particularly in forest soils. For microbial eukaryotes and protists, the commercial maxiprep and combination of developed high lysis volume and commercial kit silica purification protocols generally yielded the highest richness in agricultural soils, whereas the developed protocol performed best in coarse xeric forest soils. These results indicate that at least a tenfold increase in soil input relative to conventional 0.25 g extractions is required for reliable characterisation of mesofauna diversity, while further increases may provide additional gains. The developed DNA isolation protocol offers a solution for large-scale soil biodiversity assessments, supporting the integration of higher trophic levels into eDNA-based monitoring frameworks and future soil health monitoring programmes.

1. Introduction

Soil biodiversity underpins essential ecosystem functions, yet its comprehensive assessment remains challenging. In soil science literature, studies on "soil microbiota" have traditionally focused on bacterial communities. Even when a broader perspective is considered, research typically targets either soil microbes such as bacteria and fungi or soil fauna including animal-like protists, rarely both. This separation

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reflects the need for taxonomically specialised expertise and methodological differences between microbial and faunal studies. Furthermore, detecting organisms across trophic levels often requires separate sampling procedures, as larger-bodied organisms demand substantially greater soil volumes (Taberlet et al., 2012; Dopheide et al., 2019). Even single-celled eukaryotes, such as protists, are also largely absent from standard soil microbiological surveys.

Recent advances in environmental DNA (eDNA) technologies, reference databases, and bioinformatics now enable more comprehensive assessments of soil biological communities. However, earlier studies have been constrained by several methodological limitations, including inconsistent assembly of amplicon sequence variants (ASVs), selective targeting of specific organism groups, and variation in DNA extraction methods, all of which hinder comparability and completeness of biodiversity estimates. In addition, differences in soil type and the typically insufficient and non-standardised volumes of soil samples used for DNA sequencing have further limited the representativeness of detected communities. Reliable biodiversity monitoring requires not only adequate laboratory sample sizes but also spatially representative field sampling (Pennanen et al., 1999; Rossi and Nuutinen, 2004). Soil eDNA analysis has become a key tool for monitoring community composition and, in some cases, soil health. Yet, most DNA extraction protocols remain optimised for single-cell organisms.

Soil biota play a critical role in nutrient cycling, organic matter decomposition, and soil structure. These include unicellular protists as well as soil fauna, such as multicellular micro-invertebrates such as Tardigrada, Rotifera, and small nematode species ($\leq$ 0.1 mm), and multicellular mesofauna (>0.2–2 mm), comprising groups such as Collembola, other Arthropoda, Enchytraeid, and larger nematode species. These heterotrophs link microbial decomposers to higher-level consumers and enhance soil porosity through movement. As the European Soil Monitoring Law (Directorate-General for Environment, 2023) moves toward harmonised soil monitoring, there is an urgent need for standardised, cost-efficient, and scalable high-throughput DNA extraction methods. These approaches will support large-scale implementation of eDNA-based monitoring and provide robust data to maintain and restore healthy soils, in line with EU targets for 2050. Recent work by Sánchez-Cueto et al. (2025) highlights the lack of a standardised framework for molecular soil fauna assessment and identifies key methodological gaps, including optimised DNA extraction protocols and well-designed primers supported by comprehensive reference databases to ensure accurate identification. Addressing these limitations is critical for advancing holistic soil biodiversity assessments that integrate trophic complexity.

Current protocols for metazoan and protozoan eDNA community analysis rely heavily on commercial kits, often using small sample sizes (0.25–0.5 g) that limit detection of larger-bodied organisms. Since 2023, studies focusing on metazoan and protist community analysis mainly use commercial kits. Sediment and benthic studies often rely on the DNeasy PowerMax Soil Kit (Qiagen) with 3–10 g of material (Angulo-Preckler et al., 2023; Hale et al., 2024; Han et al., 2022; Jensen et al., 2024; Maciute et al., 2025; Mazurkiewicz et al., 2024; Pawlowski et al., 2024). Some recent studies use smaller sample sizes (0.25–0.5 g) with kits such as FastDNA® SPIN kit for soil (MP Biomedicals, Santa Ana, CA, USA) (Lin et al., 2025), E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) (Ma et al., 2024), or PowerSoil Pro (Qiagen) kit (Xu et al., 2023). Soil metazoan studies typically use DNeasy PowerSoil Pro Qiagen kits with 0.25 g samples (Dendooven et al., 2025; He et al., 2024; Jeanbille et al., 2024; Sánchez-Cueto et al., 2025).

To address the limitations of small sample sizes in commercial kits, representative subsampling requires rigorous homogenisation of the soil material prior to DNA extraction. Some studies additionally employ multiple technical replicates (e.g., Dendooven et al., 2025) or pre-treatment steps such as lyophilisation and homogenisation. For example, Sapkota et al. (2025) lyophilised 40 g of soil and applied three consecutive bead homogenisation steps to ensure complete pulverisation and mixing before subsampling 0.25 g for DNA extraction. Previous comparisons indicate that 1.5–15 g of soil may be required for accurate metazoan diversity assessment (Dopheide et al., 2019), whereas current high-throughput surveys, such as the Land Use/Cover Area Frame Survey (LUCAS), use only 0.2 g of soil, targeting microbial diversity, including bacteria and fungi (Labouyrie et al., 2023), as well as broader eukaryotic diversity (Orgiazzi et al. 2022). Such small sample volumes are unlikely to adequately represent larger soil fauna, emphasising the need for improved, scalable protocols that accommodate larger sample sizes and enable additional study areas to be addressed using the same samples.

Efficient DNA extraction methods that retain the diversity of soil organisms are fundamental to advancing soil biodiversity research and contributing to European as well as global soil health initiatives. To address this gap, we aimed to develop a scalable, cost-efficient, and automation-ready high-throughput soil DNA extraction protocol supporting extensive sampling efforts and capable of processing 10–20-fold sample volumes in comparison to the miniprep kits. Considering the increasing interest in soil biodiversity monitoring, eDNA analytics that take higher trophic levels into account would be highly interesting.

2. Material and methods

2.1. Soil material

We used soil from two agricultural fields and two types of boreal forests. The first was high mull (high organic matter >30%) agricultural soil, pH 5.7 (hereafter Amull), and the second was fine sandy till, pH 6.5 (Asandytill). The third was carex peat forest soil (Fpeat) from mesic upland forest site type (Vaccinium vitis-idaea and myrtillus type), pH 4.2. The fourth was coarse xeric Scots pine forest soil (CT – Calluna type) pH 4.5 (Fcoarse). Agricultural soils were sampled in 2021 from carrot fields located in the Southwest Finland area (mull soil) and the South Savo area (fine sandy till, organic management). At both sites, a composite soil sample was collected by taking six replicate cores (25 mm diameter, 0–10 cm depth) at approximately 1 m intervals from the middle of the bed, which were then pooled and thoroughly homogenised by manual mixing in a bag. Forest soils were sampled in 2022, with the peat soil originating from a mesic site in Northern Ostrobothnia and the coarse mineral soil from a xeric Scots pine stand in Southern Lapland. At both forest sites, 12 soil cores (humus layer, 6 cm diameter, depth 0–10 cm) were collected systematically across a circular plot (20 m diameter), approximately 4 m apart, combined into composite samples, and sieved (6.7 mm) to remove roots, stones and large debris. All soil samples were placed in clean zip-lock bags and stored frozen at −20 °C.

2.2. DNA extraction methods

In short, we tested three different starting amounts of soil: 0.25 g, 2.5 g, and 5 g, processed with PowerMax Soil (Qq1) or the miniprep reference isolation technique DNeasy PowerSoil Pro (Qq3) (Qiagen) kits, as recommended by the manufacturer, or with the developed novel method hereafter called the developed *isolation technique*, or a combination of these (Table 1). Although the DNeasy PowerMax Soil kit can accommodate up to 8–10 g of soil, 2.5 g and 5 g input amounts were selected to facilitate effective homogenisation and standardised sample processing in 15–50 ml tubes while minimising the risk of clogging during downstream purification. Larger soil inputs are possible but can compromise the consistency of lysis and extraction efficiency across samples. To increase the total amount of processed soil, multiple technical replicates may therefore be preferable to substantially increasing lysis volumes. Lysis buffer volumes varied from 800 μl to 15 ml in 2–50 ml test tubes (Table 1), reflecting commonly used extraction formats that are compatible with standard laboratory equipment and semi-automated workflows. During purification, DNA was bound to a solid-phase carrier using either carboxyl-coated magnetic beads (with NaCl

Table 1
The 12 eDNA isolation schemes tested in this study and their abbreviations. F indicates the large-volume pilot test conducted with coarse forest soil (Fcoarse). D = developed method; S = Cytiva carboxylated SpeedBeads; C = in-house carboxylated beads; Q = Qiagen lysis; q = Qiagen downstream purification. Buffers C1-C6, CD1-CD3, and EA were supplied by Qiagen.

	Developed Novel Method								***DNeasy PowerMax Soil***			***DNeasy Power Soil Pro kit***
Isolation technique	Ds1	Ds2	Dc1	Dc2	Dq1	Ds3^{F}	Dc3^{F}	Dq2^{F}	QDq	Qq1	Qq2^{F}	Qq3
Soil type	all					Fcoarse			all		Fcoarse	all
Bead beating 3 x 30 s x 6 m/s	TeenPrep 12 x 15 ml					BigPrep 2 × 50 ml						Quickprep 24 x 2 ml
Sample weight mg	2500					5000			2500		5000	250
Tube Volume	15 ml					50 ml						2 ml
Lysis Matrix	In-house: Silica grains, Ceramic spheres, Garnet sand 1:1:1									0.7 mm garnet beads		Mixed ceramic zirconium bead, 0.1 and 0.5 mm
Lysis Matrix Vol	6 ml					12 ml				5 ml		250 µl
Nr steel ball	1					3				NA		
Lysis buffer	10 ml					15 ml				15 ml PowerBead Solution and 1.2 ml C1		800 µl CD1
Supernatant	6 ml					6 ml			8 ml			700 µl
Inhibitor removal solution	2.5 ml					2.5 ml			5 ml C2 + 4 ml C3			200 µl CD2
Purification volume	600 µl	5 ml	600 µl	5 ml	600 µl				15 ml			600 µl
Binding buffer	in-house binding buffer mixed in 1:1 ratio with supernatant				600 µl CD3	in-house binding buffer mixed in 1:1 ratio with supernatant		600 µl CD3	30 ml C4			600 µl CD3
Purification	0.75 mg, Cytiva Carboxylated SpeedBeads (S)	6.25 mg, S	0.75 mg, Carboxylated (C)	6.25 mg, C	Silica (Qiagen spin column)	0.75 mg, S	0.75 mg, C	Silica (Qiagen spin column)				
Wash	2 x purification volume, 80% EtOH				500 µl EA 500 µl C5	2 x purification volume, 80% EtOH		500 µl EA 500 µl C5	10 ml C5			500 µl EA 500 µl C5
Elution, C6 buffer, 10 mM Tris-HCl (pH8.5)	150 µl	1250 µl	150 µl	1250 µl	100 µl	150 µl		100 µl	5 ml			100 µl

and PEG) or a silica-membrane (with chaotropic salts). The carboxyl-coated particles used were either in-house produced paramagnetic particles, as described in Oberacker et al. (2019), or commercially available particles from Cytiva (U.S.A), sold under trade name Sera-Mag SpeedBeads™ Carboxylate (hydrophilic) (Table 1). The Carboxylate Sera-Mag SpeedBeads™ (hydrophilic) were washed twice with TE-buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) to remove sodium azide, which can interfere with downstream steps. The beads to be used were resuspended in TE buffer to a final concentration of 50 mg ml^{-1}. The bound DNA was washed with 80% ethanol and eluted in buffer C6 (Qiagen), consisting of 10 mM Tris-HCl (pH 8.5). All extractions were made in triplicates except for the Qq2 scheme which was done in duplicate (Table 1).

2.3. DNA extraction ingredients and steps

The in-house sample lysis matrix of the novel isolation technique contained equal parts (1:1:1 vol) of blasting glass spheres (Ø 0.09–0.15 mm), Zirblast® B20 ceramic spheres (Ø 0.6–0.85 mm, Saint-Gobain, France), and garnet sand (Ø 0.50–1.00 mm, JH Mining, China). In addition, one or three Gamo BB steel balls (Ø 4.5 mm, Gamo Outdoors S.L.U., Spain) were added to the lysing matrix (Table 1 and Supplementary Table S3) and assembled into Ultra-high Performance 15- or 50-ml polypropylene conical test tubes (VWR International, U.S.A.). All matrix components were washed with ultrapure water and rinsed once with 70% EtOH before autoclaving at 121 °C for 20 min and oven dried at + 70 °C o/n prior to tube assembly.

Sample lysis in the novel isolation technique is based on mechanical disruption of cells in the presence of a lysis buffer (Sodium Thiocyanate NaSCN 1.25 M 98% extra pure, Thermo Scientific Chemicals, U.S.A.; Disodium Phosphate (Na_2HPO_4) 0.2 M, ≥ 99%, GPR RECTAPUR®, VWR Chemicals, U.S.A.), with a pH of 8.4. Volumes for different isolation techniques are reported in Table 1.

Sample homogenisation in 15- and 50-ml conical test tubes was done in the FastPrep 24 machine, equipped with TeenPrep and BigPrep adapters (MP Biomedicals, U.S.A.) (Table 1). These adapters were used to ensure effective homogenisation: 3 times for 30 s at speed 6 m s^{-1}. After homogenisation, tubes were centrifuged for 2 min at 16 000 x g. The samples for DNeasy PowerSoil Pro (Qiagen) method were lysed according to manufacturer's instructions for 20 min in 2-ml test tubes using Vortex adapters (Vortex-Genie 2, Scientific Industries, U.S.A).

Inhibitor removal in the developed novel isolation techniques was based on the addition of an in-house inhibitor removal solution containing aluminium chloride ($AlCl_3$, 0.095 M) and ammonium acetate

($NH_4CH_3CO_2$, 3.75 M, ≥97%, GPR RECTAPUR®, VWR Chemicals, U.S.A., pH of 6.3) to the centrifuged supernatant from the homogenisation step. Inhibitor removal was based on chemical coagulation of humic acids, proteins, and debris. To summarise, a 6 ml aliquot of the supernatant from homogenised and centrifuged lysate was pipetted into new 15 ml conical test tubes and mixed using a Vortex-Genie 2 for 5 s with 2.5 ml of in-house inhibitor removal solution (Table 1). Tubes were centrifuged for 3 min at 17,000 x g, and supernatant was moved into a new clean tube.

Following this, the DNA from the supernatant was bound to a carrier, either carboxyl-coated particles or a silica-membrane, thus facilitating its separation. In the developed novel method, DNA was bound to the carboxyl-coated particles (1.25 mg ml^{-1} of supernatant) with equal amounts of binding buffer (PEG8000 12.5%, NaCl 1.2 M, Tween20 0.05%) to the supernatant from the inhibitor removal step. The binding solution was shaken at room temperature at 1300 rpm for 5 min. A neodymium magnet was applied to settle all the beads to the side of the tube, and the cleared solution was removed. Beads were then washed twice with an equal volume of 80% ethanol prepared from absolute ethanol (99.9%; Altia) and ultrapure water and shaken as described above. After each washing step, a neodymium magnet was applied to settle all the beads and ethanol was meticulously removed. Particles were incubated at room temperature for 5 min to dry before elution (Table 1). Elution buffer C6 (10 mM Tris-HCl buffer, pH 8.5) was added to the particles, and the mixture was vortexed, and then incubated at room temperature for 5 min. The magnet was applied to the mixture, and the cleared supernatant (containing DNA) was collected to a fresh tube.

DNeasy PowerMax Soil (Qq1, maxiprep) and DNeasy PowerSoil Pro (Qq3, here the miniprep reference isolation technique) kits were used as described by the manufacturer, using dedicated vortex adapters in bead beating. In addition, two additional DNeasy PowerMax Soil DNA isolation schemes were tested; One with 5 g of soil (Qq2) and the other with 2.5 g of soil (QDq) where the kit's own lysis matrix was replaced with in-house lysis matrix with 3 steel balls (Table 1).

The quantity and quality of isolated DNA were measured using a Qubit 4 fluorometer (Thermo Fisher Scientific, U.S.A.) and a NanoDrop One Spectrophotometer (Thermo Fisher Scientific, U.S.A.), respectively. DNA was stored at −20 °C until used in downstream processes. Instructions for using the developed novel method with carboxyl-coated particles on a KingFisher™ Apex semi-automatic purification system (Thermo Fisher Scientific) can be found in the supplementary material (Supplementary Tables S3 and S4). This method is adapted for 3 g soil samples in 24 deep well plates (Saartama et al., 2026).

2.4. Inhibition tests

To assess DNA quality, we ran qPCR inhibition tests for a subset of samples with HOT FIREPol® EvaGreen qPCR Supermix (Solis Biodyne, Estonia) which is not marketed as an inhibition resistant qPCR kit according to the manufacturer. The quality of extracted DNA as a PCR template was tested for a subsample of soils with 0.167, 0.67, and 2.67 ng/μl (low, medium, and high DNA concentration) of extracted DNA mixed with ~10^5 copies of target DNA (self-ligated empty cloning vector) in 6 μl reaction volume. Amplification target DNA was compared to a control reaction without soil DNA containing only target DNA (Supplementary Table S2).

2.5. Sequencing and bioinformatics

All samples were sequenced on an Illumina MiSeq platform using universal eukaryotic primers targeting the 18S rRNA SSU region: Euk575Fngs (ASCYGYGGTAAYWCCAGC) and Euk895Rngs (TCHNHGNATTTCACCNCT), producing an amplicon of approximately 320 bp. These primers are widely used especially for detecting non-fungal eukaryotes, particularly soil-dwelling animals (European Commission, 2018; Guerra et al., 2021; Vasar et al., 2022). However, it is important to note that these primers also amplify fungal sequences (see e.g. Köninger et al. (2023). Samples were sequenced at the DNA Sequencing and Genomics Laboratory (BIDGEN), Helsinki.

Bioinformatic and statistical analyses of eDNA-based communities were performed in R (R Core Team, 2024). Raw 18S rRNA reads were trimmed with Cutadapt (Martin, 2011) and processed using a DADA2-based pipeline (Callahan et al., 2016) on the IT Center for Science (CSC), Finland's national high-performance computing center, Puhti supercomputer. Steps included quality filtering, error learning, merging, chimera removal, and ASV inference. Taxonomy was assigned using the PR2 database v5.0.0 (Guillou et al., 2013). It is important to note that PR2 has limited coverage and resolution for metazoans and some protozoan groups due to incomplete representation, marine bias, and inconsistent taxonomy (Guillou et al., 2013). A cladogram that illustrates sample relationships can be found in Supplementary Figure S1. Phyloseq (McMurdie and Holmes, 2013), Microbiome (Lahti and Shetty, 2019), and vegan (Oksanen et al., 2020) packages were used for data analyses, and ggplot2 (Wickham, 2016) and ggpubr (Kassambara, 2025) for visualisation of the data. All eukaryotes were assessed by filtering the data by the domain "Eukaryota". The metazoans were further assessed by selecting only "Metazoa" Subdivision from the Eukaryotes. Furthermore, to restrict the dataset to unicellular protist microbial eukaryotes, we excluded Fungi, Metazoa, Rhodophyta, Streptophyta and Chlorophyta, as well as taxa with complex or multicellular life stages (Eumycetozoa and Evosea). For a deeper look into the amplicon sequence abundance of metazoa classes please see Supplementary Figure S2. To account for varying sequence depth, we rarefied the data before calculating the alpha diversity. For calculating observed richness, we used both ASV data and data agglomerated to *species level* (like OTUS) by merging ASVs that have the same taxonomy. Aggregating ASVs to the species level merges ASVs with the same species-level rank, resulting in much smaller datasets. For example, the number of ASVs for metazoa decreased from 1778 to 163, and for microbial eukaryotes from 12132 to 437. In assessing beta diversity for ordinations and PERMANOVA (adonis2; vegan) and pairwise comparisons pairwiseAdonis (Martinez Arbizu, 2020) we used P/A matrix instead of relative abundances and Jaccard dissimilarity. Statistically significant differences in species richness between miniprep reference isolation technique Qq3 and all other isolation techniques were tested with aov and glht with Dunnett's post hoc comparison, multcomp (Hothorn et al., 2008).

3. Results

3.1. Isolation technique, DNA quality, yield and purity

DNA yield varied according to the soil type and the isolation technique (Supplementary Table S1). In agricultural soils, the gain was low, from 3 to 14 μg/g in mull, and 14–38 μg/g in sandy till soil. In forest soils, the yield was higher, varying from 21 to 53 μg/g in peat, and from 15 to 38 μg/g in coarse soil. The lowest yields were obtained with Ds2 and Dc2 isolation techniques using mull soil. The highest yield in mull soil resulted in the miniprep reference isolation technique, Qq3. The highest DNA yields from forest soils were achieved with Dq1 (combined developed lysis with silica purification) and Ds1, and the lowest with maxiprep Qq1 and QDq isolation techniques. DNA yield (μg/g soil) and DNA purity (A260/A280, A260/A230, and A340) are reported in detail in Supplementary Table S1, including the mean and standard deviation of the replicates. A340 values, which indicate a high concentration of particles and/or humic acids (Devi et al., 2015), were found to be between five and 150 times higher for eDNA isolated using magnetic beads in comparison to the reference isolation technique (Qq3), depending on the isolation technique and soil type.

The inhibition based on the qPCR test ranged from non-existent to complete, where no amplification of the target DNA occurred. This indicates variable DNA quality. Inhibition was not detected using the Qq3,

Qq1 and Dq1 isolation techniques (see Supplementary Table S2). The highest level of inhibition occurred in schemes Dc1, Dc2 and Ds2, and amplification was either not detected or severely inhibited in reactions spiked with a high DNA concentration. Furthermore, dilution to lower concentration did not improve the situation for Dc1 and Dc2 in agricultural mull soil, while for Ds2, inhibition could only be detected at the highest DNA concentration (see Supplementary Table S2). The inhibition of forest soil DNA was much lower. Overall, silica-based purification produced higher-quality DNA than carboxyl-coated magnetic particles. However, the PCR inhibition decreased when DNA was diluted.

Based on the results above, we concluded the Carboxylate Sera-Mag SpeedBeads™ Cytiva (U.S.A) to be more suitable for further method development than the in-house carboxyl-coated particles. A slightly modified method based on Ds1 and Ds2 isolation techniques was further developed and applied also to semi-automatic scale (the program steps are presented in Supplementary Tables S3 and S4).

3.2. Relative abundances of ASVs

The universal Eukaryote primers Euk575Fngs and Euk895Rngs resulted in amplicons that represented mainly fungi (~55%), metazoa (~15%), cercozoa (~10%) and streptophyta (~10%) (Fig. 1a). The major metazoan taxonomic groups were Annelida, Arachnida and Nematoda inc. class Chromadorea (Fig. 1b), and their relative abundances varied according to soil type and isolation technique. All eukaryote, metazoan and microbial eukaryote (protists) communities varied according to the sample soil, and agricultural and forest soils had markedly different community profiles. Accordingly, also the microbial eukaryote community varied according to soil type and isolation technique (Fig. 1c).

3.3. Species level richness of all eukaryotes, metazoans and microbial eukaryotes

Species richness varied markedly among isolation techniques, with the largest deviations generally observed for the Qq3, Qq1, and Dc1, Dc2 methods compared to others (Fig. 2). ASV richness followed a mostly similar pattern (Supplementary Figure S3).

In agricultural soils, the whole eukaryote and microbial eukaryote species richness was significantly lower in the reference isolation technique Qq3 compared to the other commercial Qq1 isolation technique ($p = 0.020$, $p = 0.006$). In sandy till soil, the Dc1 and Dc2 isolation techniques showed even lower species richness than Qq3 ($p = 0.040$) (Figs. 2a and 2c). However, in forest peat soil, there were no significant differences in all eukaryote and microbial eukaryotes species richness between Qq3 and Qq1 and all the other isolation techniques. For coarse forest soil, the developed Ds3 technique exhibited significantly higher species richness than Qq3 ($p = 0.020$), and Qq1 isolation technique also showed higher richness ($p = 0.009$) (Figs. 2a and 2c).

Regarding metazoan species richness, the reference isolation technique Qq3 did not differ significantly from the other techniques in agricultural soils due to high variability of replicate isolations (Fig. 2b). In contrast, in forest peat soil, Qq3 showed significantly lower metazoan richness compared to the developed Dq1 ($p < 0.001$), and to Dc1, Ds1, and Ds2 ($p = 0.009$) techniques. In coarse forest soil, metazoan richness was significantly lower in Qq3 in comparison to Dq1, Dq2, and Ds3, $Dc3^F$ ($p < 0.001$), as well as Ds1, Ds2, Dc1, and Dc2 ($p = 0.019$) (Fig. 2b).

Extraction volume had a significant effect on metazoan observed richness in forest soil samples: in peat soil midi volume samples had higher richness than maxi and mini volumes ($p < 0.001$), and in coarse forest soil midi volume samples had higher richness than mini ($p < 0.05$). Significant differences in species richness were observed for all eukaryotes and microbial eukaryotes in agricultural soil samples: mini ($p = 0.005$, $p = 0.01$) and midi ($p < 0.001$, $p < 0.001$) had lower richness than maxi in mull, and midi lower than maxi ($p < 0.001$, $p = 0.01$) in sandy till for all eukaryotes and microbial eukaryotes respectively.

The annotated metazoan species showed clearly that some mesofauna species were detected less frequently with the miniprep reference isolation technique Qq3 in comparison to the other techniques that started out with larger soil volumes (Fig. 3). Moreover, all the developed in-house isolation techniques performed well, finding on average 30% more species than the Qq3. Only Qq1 had the same or smaller number of metazoan species found than Qq3. For agricultural mull soil the differences between isolation techniques were minor. Agricultural mull soil had the lowest total richness in QDq (28 metazoan species) and highest in Dq1 (44) (Fig. 3a). The miniprep reference isolation technique Qq3 found 38 taxa. Sandy till soil had the lowest total number of species with the reference isolation technique Qq3 (17) and the highest with Dq1 (23) and Ds2 (24) (Fig. 3b). For forest soil the difference was even more clear and the best isolation technique revealed almost twice the number of species in comparison to the reference isolation technique Qq3. Forest peat soil had the highest number of species with Dq1 (59) and Ds1 (54) isolation techniques, and the lowest with Qq3 (29, Fig. 3c). Coarse forest soil had the highest richness in Dq2 (61) and Dq1 (60), and the lowest in Qq1 and Qq2 (33), and the miniprep reference isolation technique Qq3 (36) (Fig. 3d).

Several important enchytraeid species such as *Cognettia sphagnetorum* (forest soil:Fcoarse, Fpeat), and random occurrence also in agricultural soil) and *Enchytraeus buchholzi* (Amull), were found in samples regardless of the used isolation technique. This indicates that the developed method achieves taxonomic recovery comparable to established commercial kits for key soil fauna taxa. In contrast, in coarse forest soil some larger metazoan genera such as *Tetrodontophora sp.* were only found with isolation techniques Ds1, Dc1, and in peat soil *Carabodes sp.* with Ds2, Dc2, Dq1 and Qq1.

3.4. Metazoan and microbial eukaryotes community composition

The NMDS ordination visualises the dissimilarity of metazoan and microbial eukaryotes species level communities in soil samples by isolation techniques (in-house isolation techniques Dq1, Ds1, Ds2 and commercial kits Qq3, Qq1, QDq). The miniprep reference isolation technique (Qq3) with low starting soil volume, 0.25 g, and the isolation techniques using in-house produced carboxyl beads (Dc) show in general larger deviation than other isolation techniques for metazoans, but the difference disappears when studying the microbial eukaryotes (Fig. 4, Supplementary Figure S5). A similar pattern can be seen when plotting the ASV level (Supplementary Figure S4). For both forest soil types and agricultural mull soil the coefficient of variation (CV) for observed species richness of metazoa shows that miniprep reference isolation technique Qq3 result in relatively high CV means in comparison to the developed methods, thus indicating high relative fluctuation between miniprep samples and thus inconsistent richness (Supplementary Figure S5). For sandy till soil only the developed Ds1 technique showed repeatedly consistent results (Fig. 4, Supplementary Figure S5).

The composition of soil metazoan communities was significantly influenced by extraction volume across soil types, as revealed by PERMANOVA analyses. For the agricultural mull samples (Fig. 4a), extraction volume explained a modest but significant portion of the variation in community composition (R^2=0.146, $p = 0.017$), with pairwise comparisons indicating small to moderate differences between mini and midi volumes (R^2=0.101, $p = 0.040$) and between maxi and midi volumes (R^2=0.10, $p = 0.030$). In contrast, no significant effects of extraction volume or isolation technique were observed for the sandy till samples with the global, pairwise tests suggested a difference between mini and midi samples (R^2=0.112, adjusted $p = 0.027$) (Fig. 4b); this should be interpreted with caution given the non-significant overall test. More substantial effects were detected with the forest peat samples (Fig. 4c), where extraction volume accounted for a considerable fraction of community variation (R^2=0.27, $p < 0.001$). Significant pairwise

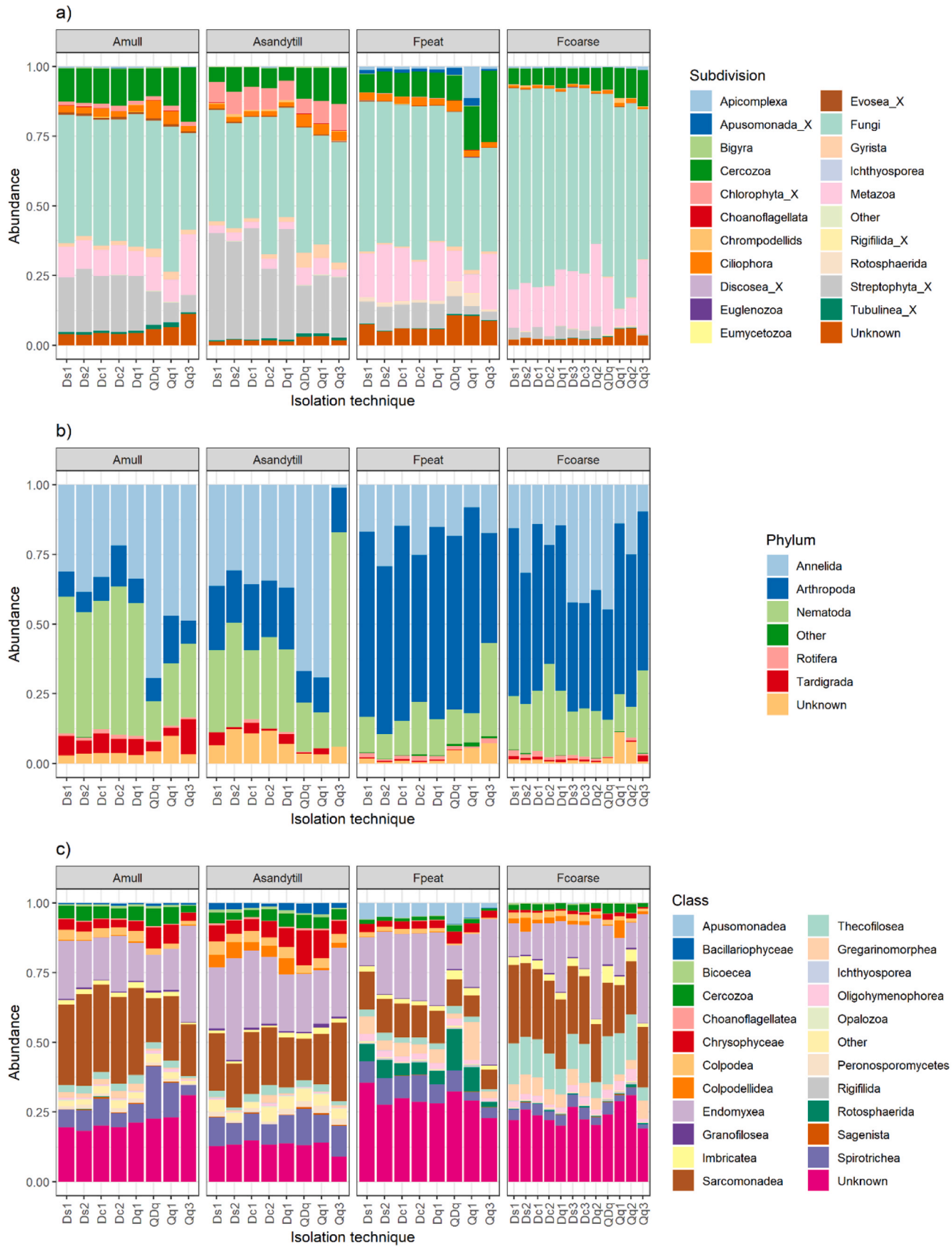

Fig. 1. Relative abundance of a) all eukaryotes (PR2 database subdivision), b) metazoans (Phylum) and c) microbial eukaryotes (Class). The PR2 database uses a taxonomy that may differ from conventional classifications (Guillou et al., 2013). The category **other** refers to small taxa that are agglomerated together to improve readability. DNA isolation technique on the x-axis. Soils include agricultural high mull (Amull) and fine sandy till (Asandytill), and forest carex peat (Fpeat) and coarse xeric soil (Fcoarse).

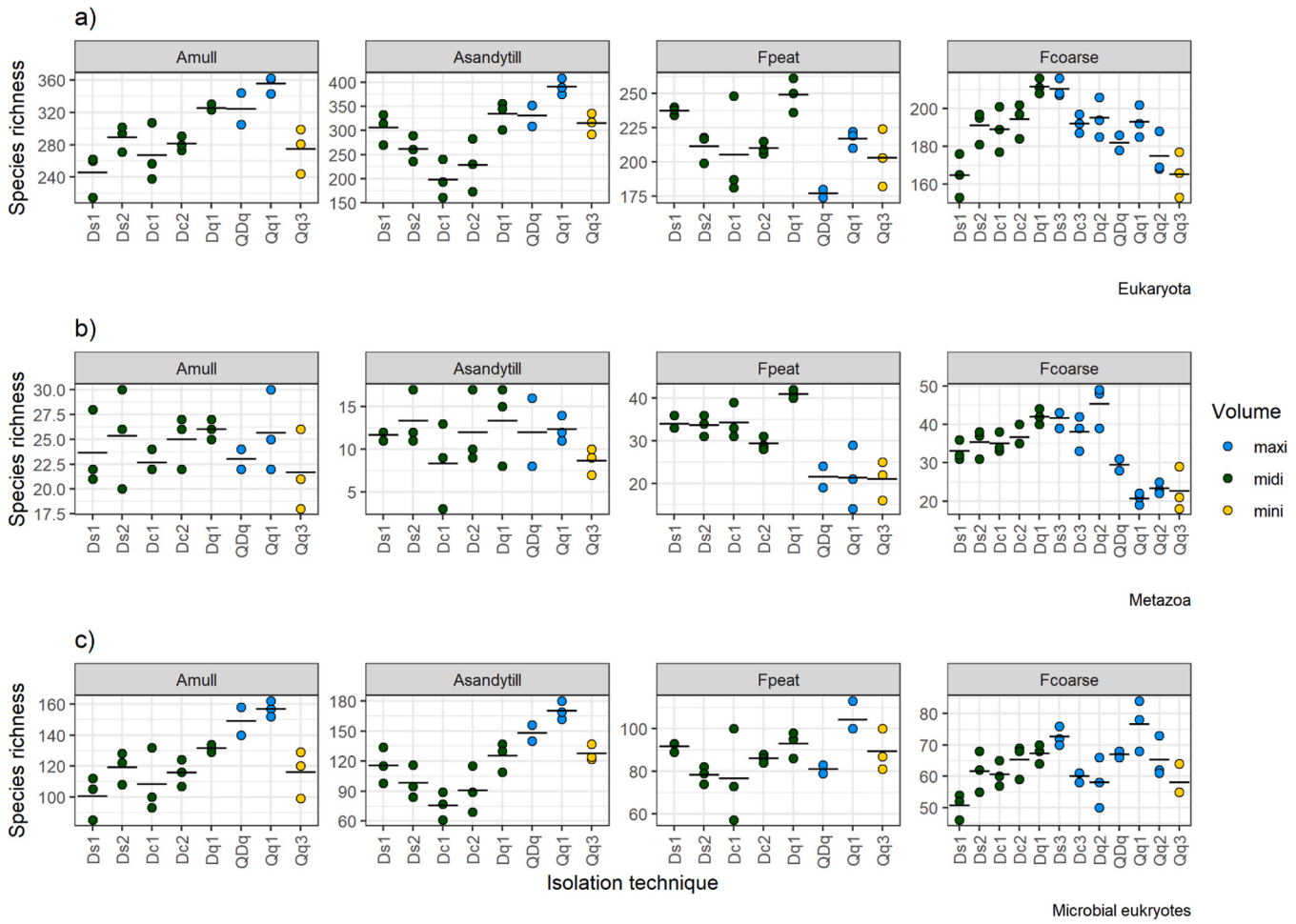

Fig. 2. Observed species level richness of a) all eukaryotes, b) metazoans and c) microbial eukaryotes. DNA isolation technique on the x-axis. Mean value presented as a black horizontal line. Volume refers to tube volume (2–15-50 ml, Table 1). Soils include agricultural high mull (Amull) and fine sandy till (Asandytill), and forest carex peat (Fpeat) and coarse xeric soil (Fcoarse).

differences were found between mini and maxi (R^2=0.254, p = 0.035), mini and midi (R^2=0.205, p = 0.002), and maxi and midi (R^2=0.183, p = 0.003) volumes. For the coarse forest soil samples, both extraction volume (R^2=0.113, p < 0.001) and isolation technique (R^2=0.354, p < 0.001) contributed to explaining the community composition, with pairwise differences between mini and maxi (R^2=0.114, p = 0.036) and mini and midi (R^2=0.163, p = 0.004) extraction volumes reflecting modest effects (Fig. 4d). However, despite the overall isolation technique effect, pairwise method comparisons were not significant after adjustment for multiple testing.

The composition of soil microbial eukaryote communities was also significantly influenced by extraction volume across different soil types, as demonstrated by the PERMANOVA analyses. In the mull samples (Fig. 4e), extraction volume explained a moderate portion of the variation in community composition (R^2=0.161, p < 0.001), with pairwise comparisons revealing a strong difference between mini and maxi volumes (R^2=0.242, p = 0.038) and a moderate difference between maxi and midi volumes (R^2=0.130, p = 0.006). Similarly, for the sandy till samples (Fig. 4f), both extraction volume (R^2=0.156, p = 0.011) and isolation technique (R^2=0.255, p = 0.029) explained modest but significant variation in microbial eukaryote community composition. Pairwise comparisons indicated small differences between midi and maxi volumes (R^2=0.132, p = 0.005), although, as with metazoans, pairwise isolation technique comparisons were not significant after correction for multiple testing. In the peat samples, extraction volume accounted for a moderate portion of community variation (R^2=0.19, p < 0.001). Significant pairwise differences were found between mini and maxi (R^2=0.274, p = 0.019), mini and midi (R^2=0.099, p = 0.003), and maxi and midi (R^2=0.139, p = 0.007) volumes. In the coarse samples (Fig. 4h), both extraction volume (R^2=0.08, p = 0.006) and isolation technique (R^2=0.323, p < 0.001) contributed to explaining microbial eukaryote community composition, though the effect of extraction volume was limited. Notably, significant pairwise differences were detected between midi and maxi extraction volumes (R^2=0.053, p = 0.025). However, consistent with other sites, pairwise isolation technique comparisons did not remain significant after multiple testing adjustments.

4. Discussion

A harmonised DNA extraction methodology is essential for accurate soil biodiversity assessments. Yet many existing methods lack scalability or compromise community representation. We present a cost-efficient, scalable and automation-ready protocol designed for high-throughput processing of gram-scale soil samples (typically 2–5 g). This approach improves recovery of diverse microbial taxa and supports eDNA-based monitoring of micro- and mesofauna as well as unicellular eukaryotes, enabling comprehensive and reproducible biodiversity surveys that advance global soil health initiatives.

We found that the eDNA yields based on in-house developed lysis matrix, lysis buffer, and inhibitor removal solution had variable and soil-specific performances compared to the miniprep reference isolation

Fig. 3. Heatmap of annotated metazoan species for each isolation technique and soil type: a) agricultural high mull and b) fine sandy till, and c) forest carex peat and d) coarse xeric soil. Please note, that the order of the x-axis DNA isolation technique varies according to the ordination distance matrix.

technique (Qq3, PowerSoil Pro, Qiagen), and maxiprep (Qq1, PowerMax Soil, Qiagen) DNA isolation kits. Among all methods tested, Dq1, which combined the developed lysis protocol with commercial silica purification, achieved the best overall balance between DNA quality and biodiversity recovery, producing high DNA yields, no detectable inhibition, and consistently high metazoan richness across soil types (Fig. 5).

The carboxyl-coated paramagnetic bead isolation techniques provided a scalable and automatable alternative for the commercial silica-membrane filter-based kits. The main disadvantage of these methods is the lower quality of the obtained DNA, which leads to PCR inhibition at higher DNA concentrations due to impurities and/or humic acids (Devi et al., 2015). Humic acids are usually considered as the main PCR inhibiting substances in soil which can be removed by chemical flocculation (Braid et al., 2003). Based on the safety datasheets and patents of some commercial DNA extraction kits, $AlCl_3$ is often used for inhibitor removal. The similar size and charge characteristics of double-stranded DNA and humic acids often lead to undesired co-precipitation of DNA (Wnuk et al., 2020) along with humic acids. Here we used 2.4:1 ratio between supernatant and inhibitor removal solution. However, this ratio should be optimised for every soil type especially when using carboxyl-coated paramagnetic beads. In the follow-up work by Saartama et al. (2026), 3:1 ratio was found to be more suitable for agricultural soil. Unlike many commercial kits, our developed protocol uses sodium thiocyanate in the lysis buffer which further diminishes unwanted co-extraction of humic acids into lysate / supernatant after initial homogenisation. It also has lower toxicity than guanidine thiocyanate.

All tested isolation schemes produced qPCR-amplifiable DNA when diluted within the limits recommended by the manufacturer (0.01 ng/μl to 4 ng/μl), as supported by our inhibition tests. Furthermore, high DNA quality and yield was observed with the Dq1 isolation technique, which combined the developed larger volume lysis with miniprep PowerSoil Pro purification steps, where DNA was bound into a silica-membrane in presence of chaotropic salt. However, concerning agricultural mull soil, the yield was slightly lower than for the commercial kits. Nevertheless, the developed carboxyl-coated paramagnetic bead purification techniques outcompeted the reference isolation technique, Qq3, in DNA yield with peat and sandy till soil.

DNA isolation technique accounted for a significant proportion of the variation in community composition and influenced species richness. However, pairwise comparisons between individual techniques were not significant after multiple-testing correction, likely due to limited replication. Effects on richness varied among soil types and organism groups. For all eukaryotes and microbial eukaryotes, the miniprep reference isolation technique (Qq3) yielded lower richness than the commercial maxiprep (Qq1) in agricultural mull and sandy till soils, with some developed isolation techniques performing even worse in sandy till. In forest peat soil, isolation techniques did not differ, whereas several developed isolation techniques yielded higher richness than miniprep (Qq3) in coarse forest soil, indicating higher richness with the developed approaches. For metazoans, miniprep reference isolation technique (Qq3) consistently yielded lower richness in forest peat and coarse soils compared to developed isolation techniques. Extraction volume also mattered. Midi volumes (2.5 g soil in 15 ml lysis) increased metazoan richness in forest soils, while maxi volumes (2.5–5 g soil in 50 ml lysis) gave the highest all eukaryote and microbial eukaryotes richness in agricultural soils. However, in the developed methods, extraction volume and isolation protocol varied together, making it difficult to separate their individual contributions to the observed differences. Larger sample volumes are known to better capture soil heterogeneity (Dopheide et al., 2019; Sapkota et al., 2025; Taberlet et al., 2012). Consistent with Sapkota et al. (2025), no single method captured full diversity; isolation techniques complemented each other for metazoan detection. Our developed method lyses soil samples directly, avoiding sieving and centrifugation that may exclude macrofauna fragments or free DNA (Dopheide et al., 2019). The sample size of DNA extraction seems to be critical for metazoan biodiversity estimates in forest soils, though effects are non-linear. Surprisingly, some developed isolation techniques yielded higher richness than the commercial maxiprep (Qq1)

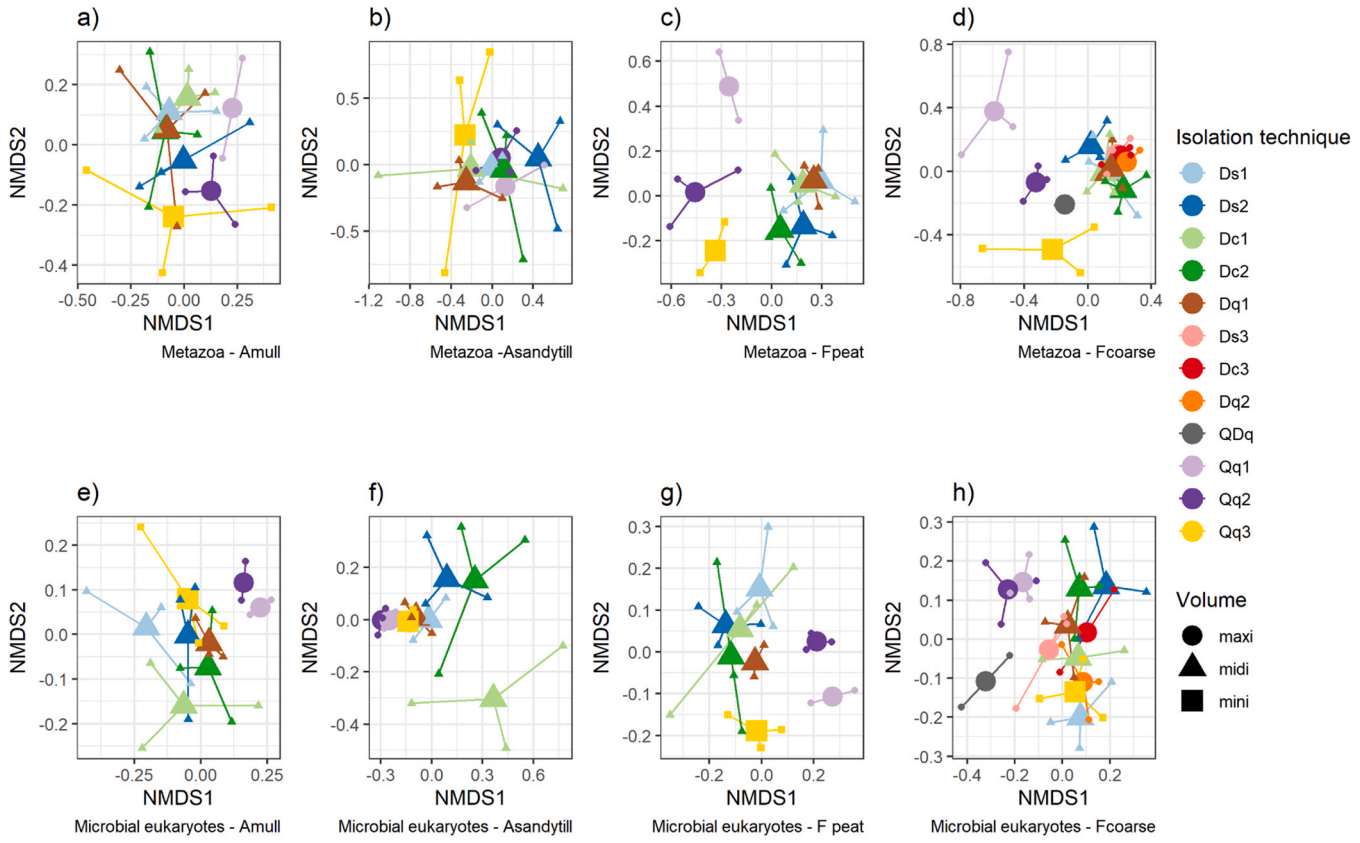

Fig. 4. A species level 2D NMDS ordination of metazoan communities in a) agricultural high mull (Amull) and b) fine sandy till (Asandytill), and c) forest carex peat (Fpeat) and d) coarse xeric soil (Fcoarse), and microbial eukaryote communities e) agricultural high mull, f) fine sandy till, g) forest carex peat, h) coarse xeric soil, based on Jaccard distances calculated from presence–absence matrices. DNA isolation techniques are distinguished by colour, and extraction volumes are indicated by point shape.

kit, although this difference may reflect combined effects of extraction volume and protocol. Unlike Dopheide et al. (2019), we also observed increased microbial eukaryote diversity with larger extraction volumes. Microbial eukaryote communities were even more sensitive to sample size, especially in forest peat and agricultural mull soils, where larger volumes improved representation despite their small size likely reflecting high diversity and spatial heterogeneity. Better detection of protist predators may provide insights into trophic relationships, as microbial richness contributes to soil function through complex microbiome associations (Wagg et al., 2019).

Large variation in the body-size of soil biota from micro- to macrofauna requires attention to sampling strategy and sample size. The available commercial miniprep extraction kits are primarily designed for small-sized microorganisms, whereas the maxiprep method showed inconsistent performance across sample types and is substantially more expensive than the other approaches tested. In traditional morphological identification it is well established that high sample volumes are needed to cover an adequate number of individuals (Smith et al., 2008). However, in eDNA-based analytics, where quantitative interpretation is generally limited, the question of the portion of the truly captured diversity is easily neglected with these bigger and more sparsely occurring organisms (Köninger et al., 2023). Our study did not address sampling size considerations for macrofauna; rather, it focused on scaling soil sample volumes within the range appropriate for high-throughput DNA extraction targeting micro- and mesofaunal communities, alongside developing cost-effective purification protocols compatible with standard laboratory infrastructure. Nevertheless, in contrast to the recent soil eukaryotic diversity monitoring using smaller DNA-sample size and variable soil layers (Köninger et al., 2023), our test set of the most common soil types in Fennoscandia indicated a higher richness of mesofauna in forest soils than in the two agricultural soils, representing higher intensity land use. We therefore propose including micro-and mesofauna in soil microbial analyses as it encompasses keystone species in the decomposer system, such as the omnivorous enchytraeid *Cognettia sphagnetorum* (Huhta et al., 1998).

From a practical implementation perspective, both reagent costs and labour requirements are relevant when evaluating the feasibility of large-scale soil biodiversity monitoring. Based on our laboratory-scale reagent cost estimates, the developed maxiprep-scale method was more than five times cheaper per extraction than the commercial maxiprep kit, while the bead-based workflow adapted to smaller volumes and semi-automated processing was estimated to be moare than twenty times cheaper than the commercial maxiprep and over four times cheaper than the commercial miniprep. The combined method using the developed high-volume lysis and commercial miniprep silica-column purification required similar labour to the commercial miniprep but had 14% higher reagent costs, primarily because it used commercial miniprep silica columns. However, its cost could potentially be reduced in large-scale applications if a comparable high-quality silica-column were available at a lower price. These comparisons should be interpreted as indicative, as exact costs depend on supplier prices, purchasing agreements, batch size, included consumables, and the level of automation. Nevertheless, the estimates indicate substantial potential for reducing per-sample costs in high-throughput applications, and the maxiprep-scale methods did not differ markedly in labour requirements, at least not to the disadvantage of the developed method adapted to semi-automated processing.

Overall, these patterns highlight that scaling extraction volume can significantly alter biodiversity profiles. The combined procedure (developed lysis with silica purification), which took advantage of lysing

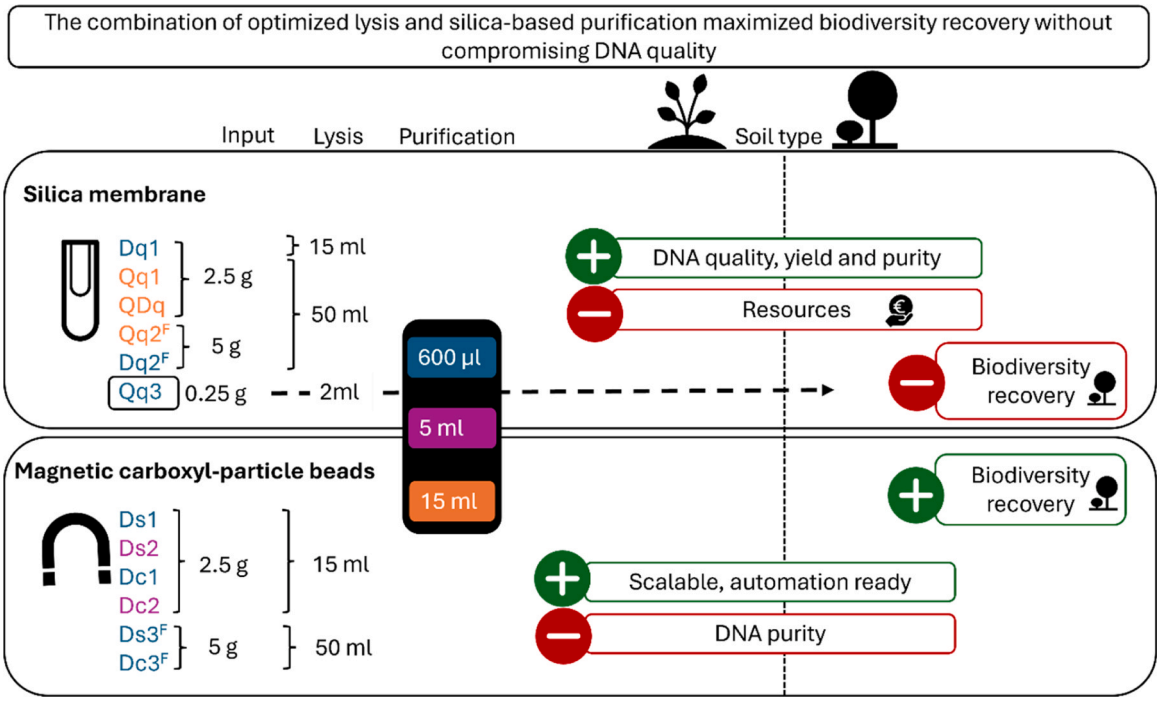

Fig. 5. Summary of DNA isolation techniques based on either silica-membrane spin columns (Qiagen) or Carboxylate Sera-Mag SpeedBeads™ (Cytiva) or the in-house carboxyl-coated particles. The miniprep method Qq3 produced high-quality DNA with negligible PCR inhibition but generally recovered fewer metazoan species and showed greater variability among replicates, particularly in forest soils. The maxiprep method Qq1 also yielded high-quality DNA and improved recovery of total eukaryotic diversity relative to Qq3, although gains in metazoan richness were limited. Among all methods tested, Dq1, which combined the developed lysis protocol with commercial silica purification, achieved the best overall balance between DNA quality and biodiversity recovery, producing high DNA yields, no detectable inhibition, and consistently high metazoan richness across soil types. The Carboxylate Sera-Mag SpeedBeads™ (Cytiva)-based Ds methods (Ds1, Ds2 and Ds3F) also performed well, particularly in forest soils, where they recovered high metazoan richness and a broader range of mesofaunal taxa than Qq3, while remaining compatible with scalable and semi-automated workflows. D for developed method, S Cytiva Carboxylated SpeedBeads, C in-house Carboxylated beads, Q Qiagen lysis, q Qiagen downstream protocol.

10-fold sample mass but purified only a small aliquot of the lysate with a commercial silica-based membrane, seemed to be very good in terms of DNA quality, yield and obtained richness in the tested soil types. However, for a high-throughput method, the isolation techniques using carboxyl-coated paramagnetic beads offer superior scalability at a reasonable cost. Together, these findings underscore that extraction volume is a key factor shaping detection of both soil metazoan and microbial eukaryote community composition, with effects varying by soil type and organism group. In line with our expectations, the effect of analysed soil volume was bigger for mesofaunal than microbial eukaryote communities. While isolation technique effects were detected in some cases, their influence appears more subtle and requires cautious interpretation. Nevertheless, our study highlights the importance of carefully selecting extraction protocols tailored to specific soil environments and target communities to accurately capture soil biodiversity patterns. DNA extraction methods can substantially affect richness estimates, particularly in coarse and organic-rich soils, and that reliance on a single small-scale method may underestimate soil biodiversity. Improved coverage, improved representativeness and cost-efficiency of eDNA-based assessments will be increasingly important for soil monitoring (FAO, 2024).

CRediT authorship contribution statement

Satu Latvala: Writing – review & editing, Investigation, Conceptualization. **Taina Pennanen:** Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Tero Tuomivirta:** Writing – original draft, Methodology, Investigation, Conceptualization. **Juha-Matti Pitkänen:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sannakajsa Velmala:** Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.microb.2026.100786.

Data availability

Data will be made available on request.

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