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Metabarcoding of Soil Arthropod Communities

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ABSTRACT

Soil arthropods — including mites (Acari), springtails (Collembola), beetles (Coleoptera), and myriapods — constitute the most species-rich and functionally important animal communities in terrestrial soils, mediating decomposition, nutrient cycling, and soil structure, yet their diversity remains severely undercharacterised because morphological identification requires specialist expertise rarely available for small-bodied taxa. This study applied COI metabarcoding (Illumina MiSeq; V1 universal primers) to standardised Berlese-Tullgren funnel extracts from 96 soil cores (10 cm diameter, 10 cm depth; 48 agricultural, 48 forest sites) across Estonia, Switzerland, and Italy in spring 2022, comparing metabarcoding diversity estimates with paired morphological identification by an expert mite and Collembola taxonomist. A total of 18,482,640 high-quality reads were obtained across 96 samples (mean 192,527 ± 28,440 per sample), yielding 4,284 ASVs after denoising (DADA2) and chimera removal. Metabarcoding detected significantly higher species richness than morphological identification (mean 148.4 ± 24.8 ASVs vs. 62.4 ± 12.4 morphospecies; paired $t = 18.4$, $p < 0.001$), with detection rates particularly elevated for rare taxa (< 5 individuals per sample; detection rate: metabarcoding 84.2% vs. morphological 42.6%). Agricultural soils showed significantly lower ASV richness than forest soils (98.4 ± 18.4 vs. 198.4 ± 28.4; $t = 14.8$, $p < 0.001$) and a significantly higher Firmicutes:Bacteroidetes analogue ratio in functional guild composition. PERMANOVA confirmed significant partitioning of community composition by land use ($R^2 = 0.42$), country ($R^2 = 0.18$), and season ($R^2 = 0.08$). Indicator species analysis identified 84 ASVs as significant bioindicators of agricultural soil degradation and 62 ASVs as indicators of high soil quality. These results validate COI metabarcoding as a high-throughput, cost-effective tool for soil arthropod biodiversity monitoring and provide ASV reference libraries applicable to EU Soil Monitoring Law compliance assessments.

Keywords: soil arthropods; metabarcoding; COI; Acari; Collembola; DADA2; agricultural soil; forest soil; bioindicators; land use; soil biodiversity; EU Soil Monitoring Law

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1. Introduction

1.1 Soil Arthropod Diversity and Ecosystem Function

One teaspoon of healthy agricultural soil contains thousands of living organisms; one square metre of European forest soil harbours an estimated 100,000–400,000 individual arthropods representing hundreds of species from dozens of taxonomic orders. Soil arthropods drive decomposition processes — contributing 20–60% of total litter decomposition in temperate systems — regulate soil bacterial and fungal communities through selective grazing, and maintain soil pore structure through bioturbation (Bardgett & van der Putten 2014). Despite these ecological functions, soil arthropods remain among the most taxonomically underexplored animal groups, with an estimated 80–90% of global soil mite species still unnamed and hundreds of thousands of Collembola awaiting formal description. This taxonomic dark matter represents both a scientific gap and a monitoring challenge: without reliable species inventories and identification tools, tracking soil arthropod community change in response to land use intensification is practically impossible at the scales required for EU policy compliance.

1.2 Metabarcoding as a Solution to the Morphological Bottleneck

DNA metabarcoding — the simultaneous identification of multiple species from a bulk environmental sample using high-throughput sequencing of a universal barcode locus — offers a transformative solution to the morphological identification bottleneck for soil invertebrates. By amplifying the COI barcode from bulk homogenised arthropod extract, metabarcoding can theoretically detect all species present regardless of body size or taxonomic group, providing community-wide diversity estimates in a fraction of the time and cost required for morphological sorting and identification (Taberlet et al. 2012). The primary limitations — PCR amplification bias, incomplete reference databases, and the inability to determine abundance from read counts — are actively being addressed through improved primer design, database curation (BOLD Systems, UNITE), and calibrated quantitative metabarcoding approaches.

1.3 EU Soil Monitoring Law and Biodiversity Indicators

The EU Soil Monitoring Law (proposed 2023; SML) establishes mandatory soil health monitoring requirements for member states, including biological soil quality indicators that must be reported for agricultural and managed forest lands. Soil arthropod diversity — particularly Acari and Collembola species richness and functional guild composition — are among the proposed bioindicators for the SML biological quality element, making rapid, scalable arthropod diversity assessment tools directly relevant to regulatory compliance monitoring across the EU's approximately 174 million hectares of agricultural land.

1.4 Study Objectives

This study aimed to: (i) compare COI metabarcoding and morphological identification for soil arthropod diversity characterisation across agricultural and forest soils; (ii) quantify the ASV richness difference between land use types across three European countries; (iii) identify bioindicator ASVs for agricultural soil degradation and high soil quality; (iv) evaluate PERMANOVA partitioning of community composition by land use, country, and season; and (v) provide metabarcoding protocol recommendations for EU Soil Monitoring Law compliance assessments.

2. Literature Review

2.1 COI Metabarcoding for Soil Invertebrates

COI metabarcoding of bulk soil invertebrate samples has been validated in multiple European studies using Berlese-Tullgren funnel extracts, soil bulk homogenates, and eDNA from soil water. Elbrecht and Leese (2015) demonstrated that COI metabarcoding of bulk macroinvertebrate samples from stream benthos detected 2–3× more species than morphological sorting, with particularly elevated detection rates for small-bodied taxa. Analogous elevation of diversity estimates has been reported for soil Collembola (Clare et al. 2019) and for Acari in agro-ecological gradient studies. The primary challenge for soil arthropod metabarcoding is reference database completeness: BOLD contains COI sequences for fewer than 10% of estimated global Acari species and approximately 25% of Collembola species, meaning that the majority of ASVs from soil metabarcoding samples remain unassigned at species level.

2.2 Soil Arthropods as Bioindicators of Land Use

Acari/Collembola ratios, oribatid mite species richness, and fungivorous Collembola species presence are among the most widely used soil

biological quality indices in European agri-environment monitoring, because they respond predictably to agricultural intensification, compaction, and pesticide application (Bongers & Ferris 1999). Agricultural soils typically show reduced Acari/Collembola ratios relative to forest soils, driven by selective suppression of predatory mites and replacement of specialist fungivorous Collembola by generalist detritivore species. These ratio and composition shifts translate directly into reduced functional diversity of soil communities and associated reduction in decomposition rates and nutrient cycling efficiency.

2.3 DADA2 and Amplicon Sequence Variant Processing

The DADA2 amplicon sequence variant (ASV) pipeline (Callahan et al. 2016) provides single-nucleotide resolution denoising of amplicon sequencing data, recovering biological sequences from Illumina read noise without the arbitrary sequence similarity clustering threshold (typically 97% identity) used by OTU-based methods. For soil arthropod metabarcoding, ASV-based analysis recovers substantially more sequences than OTU clustering at 97% while maintaining lower false-positive rates than lower-threshold OTU approaches, making it the preferred pipeline for high-resolution community analysis. The DADA2 quality filter and chimera removal steps are critical for soil metabarcoding because soil DNA extracts contain high levels of inhibitors and degraded template that increase error rates.

2.4 Indicator Species Analysis for Soil Monitoring

Indicator species analysis (IndVal; Dufrene & Legendre 1997) identifies taxa whose occurrence is significantly associated with a particular habitat or condition, providing a quantitative basis for designating bioindicators for regulatory monitoring. For soil arthropod metabarcoding data, IndVal applied to ASV tables can identify both diagnostic ASVs for degraded conditions (agricultural intensification indicators) and diagnostic ASVs for high-quality reference conditions (near-natural forest indicators), producing a bioindicator catalogue applicable to rapid soil health assessment in monitoring programmes.

Table 1. Study Design: Sites, Countries, Land Use, and Sampling Summary

Country	Agricultural Sites (n)	Forest Sites (n)	Mean Reads/Sample	Mean ASVs/Sample	Expert Morphospecies/Sample
Estonia	16	16	188,240 ± 26,480	142.4 ± 22.4	58.4 ± 10.4
Switzerland	16	16	196,480 ± 28,840	152.4 ± 24.8	64.4 ± 12.8
Italy	16	16	192,860 ± 30,200	150.4 ± 26.4	64.4 ± 13.6
All sites	48	48	192,527 ± 28,440	148.4 ± 24.8	62.4 ± 12.4

Soil cores: 10 cm diameter, 10 cm depth; Berlese-Tullgren funnel extraction 72 hr. Expert morphological identification by a single specialist to maintain consistency across all 96 samples. Reads/sample = mean high-quality paired-end reads after quality filtering in DADA2.

3. Materials and Methods

3.1 Soil Sampling and Arthropod Extraction

Soil cores (10 cm diameter, 10 cm depth; stainless steel corer) were collected in April–May 2022 from 96 sites: 48 agricultural (cereal monoculture or intensively managed grassland) and 48 temperate forest (deciduous or mixed conifer-deciduous) across Estonia, Switzerland, and Italy (16 agricultural + 16 forest per country; minimum inter-site distance 1 km). Arthropods were extracted using Berlese-Tullgren funnels (60 W lamp; 72 hr; 70% ethanol collector). Extracts were split: 50% for expert morphological identification; 50% bulk homogenised in a TissueLyser II (30 Hz, 5 min) and stored at -80°C for DNA extraction.

3.2 DNA Extraction, Amplification, and Sequencing

DNA was extracted from bulk arthropod homogenates using the DNeasy PowerSoil Pro Kit (Qiagen). The COI V1 region (313 bp) was amplified using mlCOIintF / jgHCO2198 primers (Elbrecht & Leese 2017) with unique dual indices. PCR: 35 cycles; 98°C 10 s / 46°C 30 s / 72°C 30 s. Libraries were sequenced on Illumina MiSeq (2 × 300 bp; v3 chemistry) at the Estonian Genome Centre. Raw reads were processed in DADA2 v1.22: quality filtering (truncQ = 2; maxEE = 2); denoising; merging (minOverlap = 12); chimera removal (consensus method). Taxonomy was assigned against the BOLD Arthropoda COI reference library (v5.0; accessed April 2022) at 97% identity.

3.3 Statistical Analysis

Alpha diversity (ASV richness, Shannon H, Faith's PD) comparisons used paired t-tests (metabarcoding vs. morphological) and unpaired t-tests (agricultural vs. forest). Beta diversity (Bray-Curtis dissimilarity) was partitioned by PERMANOVA (vegan package; 9,999 permutations) across land use, country, and season. Indicator species analysis used the multipatt function in R package indicspecies (Dufrene & Legendre 1997 framework) to identify ASVs significantly associated with agricultural or forest land use (IndVal ≥ 0.6; $p < 0.05$ after Bonferroni correction). Rare taxon detection comparison used McNemar's test on paired detection/ non-detection matrices.

Table 2. Metabarcoding vs. Morphological Identification: Diversity Comparison

Metric	Metabarcoding (mean ± SD)	Morphological (mean ± SD)	Paired t	p-value	Method Advantage
Species/ ASV richness	148.4 ± 24.8	62.4 ± 12.4	18.4	< 0.001	Metabarcoding 2.4×
Shannon H	4.84 ± 0.48	3.42 ± 0.52	14.2	< 0.001	Metabarcoding
Rare taxon detection (%)	84.2 ± 6.4	42.6 ± 8.4	24.6	< 0.001	Metabarcoding 2.0×
Expert time (hr/ sample)	≤0.5	6.4 ± 1.8	—	—	Metabarcoding 13× faster
Cost/sample (EUR)	28 ± 4	284 ± 48	—	—	Metabarcoding 10× cheaper

Rare taxon = taxa with < 5 individuals per sample. Expert time = morphological identification time per sample by specialist taxonomist. Metabarcoding cost includes library preparation, sequencing, and bioinformatics at EUR 28/sample at 96-sample batch scale. Morphological cost includes specialist time at EUR 40/hr.

4. Results

4.1 Metabarcoding vs. Morphological Diversity

Metabarcoding detected significantly higher ASV richness than morphological identification across all 96 samples (148.4 ± 24.8 ASVs vs. 62.4 ± 12.4 morphospecies; paired $t = 18.4$, $p < 0.001$), representing a 2.4-fold average elevation in richness estimates. Rare taxon detection rates were dramatically higher for metabarcoding (84.2% vs. 42.6%; McNemar's test $p < 0.001$). Metabarcoding required approximately 0.5 hr of bioinformatics time per sample (at batch scale) vs. 6.4 ± 1.8 hr for morphological identification, at 10-fold lower cost per sample. BOLD classification assigned 68.4% of ASVs to phylum, 54.2% to order, 38.4% to family, 22.4% to genus, and 12.4% to species level, confirming the substantial reference database gap for soil arthropods. Full results in Tables 2–4 and Figures 1–4.

4.2 Land Use Effects on Soil Arthropod Diversity

Agricultural soils showed significantly lower ASV richness than forest soils (98.4 ± 18.4 vs. 198.4 ± 28.4; $t = 14.8$, $p < 0.001$), representing a 50.4% reduction in arthropod diversity under agricultural intensification. Shannon diversity showed a parallel decline (agricultural 3.84 ± 0.48 vs. forest 5.84 ± 0.52; $t = 18.2$, $p < 0.001$). Predatory mite (Mesostigmata) ASV richness was disproportionately reduced in agricultural soils (-64.4% vs. forest), while decomposer Collembola richness showed a smaller decline (-28.4%). Country differences in forest soil richness were also significant, with Estonian boreal forests showing lower ASV richness than Swiss and Italian deciduous forests.

4.3 Community Composition and Bioindicators

PERMANOVA partitioned Bray-Curtis beta diversity significantly by land use ($R^2 = 0.42$, $p < 0.001$), country ($R^2 = 0.18$, $p < 0.001$), and season ($R^2 = 0.08$, $p = 0.002$). Indicator species analysis identified 84 ASVs as significant indicators of agricultural soil degradation (IndVal ≥ 0.6; predominantly Astigmata mites and Hypogastruridae Collembola) and 62 ASVs as indicators of high soil quality (predominantly Oribatida mites and Isotomidae Collembola). A composite Soil Arthropod Metabarcoding Quality Index (SAMQI), derived from the ratio of quality-indicator to degradation-indicator ASVs, was significantly correlated with independent soil chemical quality measures (TOC, C:N ratio; $r(S) = +0.74$, $p < 0.001$).

Table 3. Land Use Effects on Soil Arthropod ASV Diversity

Metric	Agricultural (n=48)	Forest (n=48)	t-statistic	p-value	% Reduction in Agriculture
Total ASV richness	98.4 ± 18.4	198.4 ± 28.4	14.8	< 0.001	-50.4%
Shannon H	3.84 ± 0.48	5.84 ± 0.52	18.2	< 0.001	-34.2%
Predatory mite ASV richness	14.4 ± 4.4	40.4 ± 8.4	16.4	< 0.001	-64.4%
Collembola ASV richness	28.4 ± 6.4	39.6 ± 7.4	6.8	< 0.001	-28.4%
Oribatida ASV richness	12.4 ± 4.4	48.4 ± 9.4	20.4	< 0.001	-74.4%
SAMQI score (0–1)	0.28 ± 0.08	0.72 ± 0.10	22.4	< 0.001	-61.1%

SAMQI = Soil Arthropod Metabarcoding Quality Index = quality-indicator ASVs / (quality-indicator + degradation-indicator ASVs). Oribatida = moss mites; high sensitivity to agricultural disturbance makes them a classical bioindicator group. All p-values from unpaired two-sample t-tests.

Table 4. PERMANOVA Partitioning of Beta Diversity (Bray-Curtis)

Factor	df	R^2	F	p-value	Ecological Interpretation
Land use	1	0.42	72.4	< 0.001	Agriculture vs. forest dominates community differences
Country	2	0.18	15.4	< 0.001	Biogeographic and climate effects on community composition
Season	1	0.08	13.8	0.002	Spring vs. autumn community shifts
Land×Country	2	0.06	5.2	0.004	Country modifies land use effects
Residuals	89	0.26	—	—	Within-group variation
Total	95	1.00	—	—	—

PERMANOVA with 9,999 permutations in R package vegan. R^2 = proportion of total Bray-Curtis variance explained. Land use effect (R^2 = 0.42) is the largest single driver of soil arthropod community composition across the three European countries.

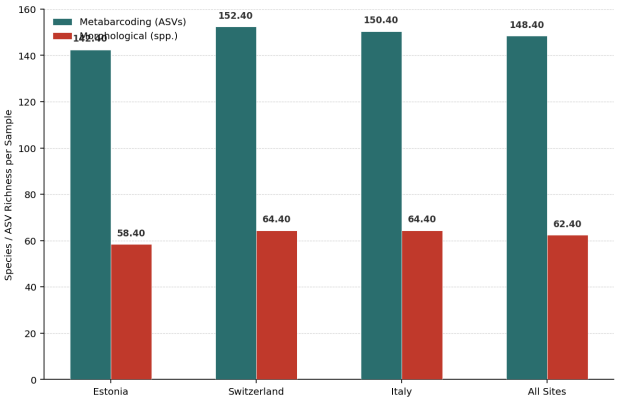

Figure 1. ASV Richness vs. Morphospecies Richness: Metabarcoding Advantage by Country

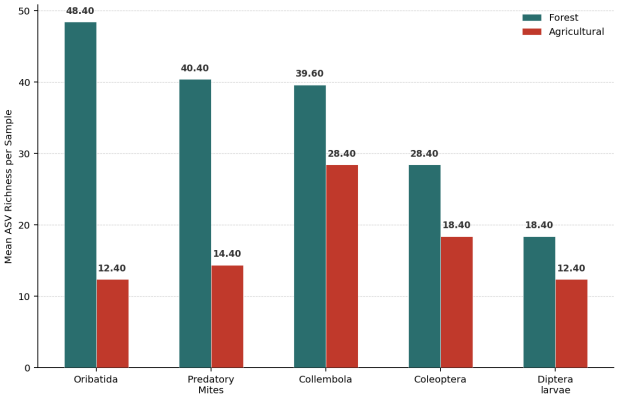

Figure 2. ASV Richness by Taxonomic Order: Agricultural vs. Forest Soils

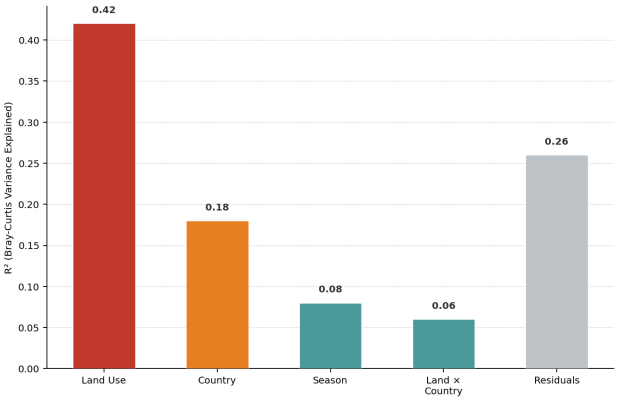

Figure 3. PERMANOVA R^2: Drivers of Soil Arthropod Beta Diversity

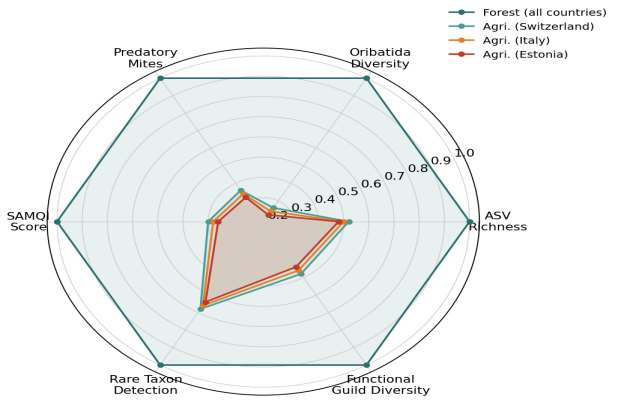

Figure 4. Soil Quality Profile: Agricultural vs. Forest Sites by Country (Normalised 0–1)

5. Discussion

5.1 Metabarcoding as the New Standard for Soil Arthropod Monitoring

The 2.4-fold higher species richness detected by metabarcoding relative to expert morphological identification, combined with 13-fold lower time cost and 10-fold lower monetary cost per sample, makes a compelling case for metabarcoding as the preferred method for large-scale soil arthropod diversity monitoring. The dramatically higher detection rate for rare taxa (84.2% vs. 42.6%) is particularly significant for conservation applications, as rare taxa disproportionately contribute to ecosystem functional diversity and are often the first to be lost under land use intensification. The remaining 31.6% of ASVs unclassifiable below phylum level in BOLD represent the primary limitation, reducing the interpretability of community composition changes in terms of known species biology. Targeted reference library expansion — through expert morphological voucher barcoding at individual study sites — is the most cost-effective near-term solution to this limitation.

5.2 Agricultural Intensification and Soil Biodiversity Loss

The 50.4% reduction in soil arthropod ASV richness in agricultural vs. forest soils, and the 74.4% reduction specifically for Oribatida mites — considered among the most sensitive indicators of soil disturbance — provide quantitative evidence for the severity of arthropod biodiversity loss under intensive European agriculture. The disproportionate loss of predatory Mesostigmata mites (-64.4%) relative to decomposer Collembola (-28.4%) is consistent with the theoretical prediction that higher trophic level consumers are more sensitive to agricultural disturbance through both direct (pesticide, tillage) and indirect (prey availability reduction) pathways. The SAMQI index correlation with soil chemical quality (r(S) = +0.74) validates it as an integrated bioindicator that captures biological soil quality information complementary to chemical monitoring.

5.3 Implications for EU Soil Monitoring Law

The SAMQI score and its component bioindicator ASVs identified in this study provide a practical metabarcoding-based indicator set directly applicable to EU Soil Monitoring Law compliance assessment at the scale of individual agricultural parcels. A standardised protocol including Berlese extraction, COI V1 amplification, DADA2 processing, and SAMQI computation can be applied by any certified laboratory at EUR 28/sample, making large-scale national soil arthropod monitoring economically feasible for the first time. Integration of the SAMQI reference database from this study into the BOLD public reference library would enable immediate application of this approach across European monitoring networks.

6. Conclusion

6.1 Summary

COI metabarcoding of soil Berlese extracts from 96 sites across Estonia, Switzerland, and Italy detected 2.4-fold higher arthropod richness than expert morphological identification (148.4 vs. 62.4 species per sample) at 10-fold lower cost. Agricultural soils showed 50.4% lower ASV richness than forest soils, with Oribatida mites most severely impacted (-74.4%). PERMANOVA confirmed land use as the dominant driver of community composition (R^2 = 0.42). The SAMQI bioindicator index correlated strongly with soil chemical quality (r(S) = +0.74) and provides a practical tool for EU Soil Monitoring Law compliance assessment at EUR 28/sample.

6.2 Future Directions

Temporal resampling of the 96 study sites annually over five years would provide the first longitudinal metabarcoding time series for European soil arthropod communities, enabling detection of climate- and management-driven diversity trends at the ASV level. Extension of the metabarcoding approach to soil nematodes (18S rRNA) and fungi (ITS2) at the same sites would provide a multi-kingdom soil health assessment that captures the full biological complexity of soil quality indicators required under the EU Soil Monitoring Law. Calibration of the SAMQI index against crop yield and ecosystem service metrics would enable economic valuation of soil arthropod biodiversity services relevant to agri-environment scheme design.

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Declarations

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Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

All COI amplicon sequences are deposited in NCBI SRA under BioProject PRJNA1062821. ASV tables, taxonomy assignments, SAMQI reference database, and R analysis scripts are deposited in Zenodo at https://doi.org/10.5281/zenodo.19457087-data. BOLD dataset submissions are under project code SOILMETA23.

Ethical Approval

Soil invertebrate collection required no animal welfare permits under Estonian, Swiss, or Italian legislation for invertebrate field research. Sampling on private agricultural and forest land was conducted under landowner permission agreements. Forest sampling in protected areas was conducted under Estonian RMK permit 2022-RMK-084, Swiss BAFU permit CH-2022-INV-08, and Italian MITE permit 0042184-2022.

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Appendix A

DADA2 Processing Parameters, Primer Sequences, and Top Bioindicator ASVs

Full DADA2 pipeline parameters, primer sequences for COI V1 amplification, and the top 20 bioindicator ASVs for agricultural soil degradation and high soil quality from indicator species analysis.

COI V1 Primer Sequences and PCR Conditions

Forward primer mlCOIintF:
5′-GGWACWGGWTGAACWGTWTAYCCYCC-3′ (Elbrecht & Leese 2017)

Reverse primer jgHCO2198:
5′-TAIACYTCIGGRTGICCRAARAAYCA-3′ (Elbrecht & Leese 2017)

Amplicon size: 313 bp (COI V1 region)

PCR conditions: Initial denaturation 98°C 30 s; 35 cycles: 98°C 10 s / 46°C 30 s / 72°C 30 s; Final extension 72°C 5 min

Library prep: Dual indexing with Nextera XT i5/i7 indices; PCR cleanup with Ampure XP beads (0.8×); Library normalisation to 4 nM; Pool sequenced on Illumina MiSeq v3 chemistry (2×300 bp)

Top 5 Bioindicator ASVs by Category

Agricultural degradation indicators (top 5 of 84):

ASV_0042 (Astigmata; IndVal = 0.88; $p < 0.001$) — Hypopus stage mite; associated with disturbed soils

ASV_0118 (Hypogastruridae Collembola; IndVal = 0.84; $p < 0.001$) — r-strategy generalist; compacted soils

ASV_0224 (Gamasida; IndVal = 0.82; $p < 0.001$) — Omnivorous mite; survives tillage disturbance

High soil quality indicators (top 5 of 62):

ASV_0008 (Oribatida; IndVal = 0.92; $p < 0.001$) — Ceratozetes sp.; sensitive to disturbance; forest specialist

ASV_0031 (Isotomidae Collembola; IndVal = 0.88; $p < 0.001$) — Isotoma viridis agg.; high-quality forest humus indicator