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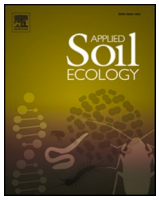

Soil microarthropod biodiversity in agricultural landscapes: Revisiting the QBS index through DNA metabarcoding

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ABSTRACT

The Qualità Biologica del Suolo (QBS-ar) index provides a rapid, low-cost measure of soil biological quality by assigning arthropods in morphotaxonomic groups named biological forms. Although widely used, its low taxonomic resolution and reliance on expert-defined scores limits its sensitivity to subtle management effects. We therefore evaluated whether DNA metabarcoding can complement and refine QBS-based assessments by analysing soil microarthropod communities across seven agricultural treatments differing in tillage intensity and production system. Using COI metabarcoding, we compared α- and β-diversity patterns between molecular and QBS datasets, evaluated different QBS index variants in relation to DNA amplicon sequence variant (ASV) richness, and explored potential for a preliminary DNA-derived index based on QBS-like trait scoring. DNA metabarcoding resolved clear community separation among production systems and treatments that the QBS only partially detected and revealed indicator taxa characteristic of reduced-disturbance and organic management. The QBS indices distinguished major production systems but were less responsive to within-system variation. Correlations between ASV richness and QBS-ar varied among production systems, indicating context-dependent index performance. The experimental DNA-derived QBS index (QBS-DNA) retained a QBS-like trait signal, showing positive treatment-mean correlations with morphology-based QBS-ar and QBS-ar_BF, but it did not significantly distinguish treatments. These results support QBS-DNA as a proof-of-concept framework for translating trait-based soil-quality indicators into molecular biodiversity assessments. As molecular tools and trait databases expand, metabarcoding enables the development of next-generation soil biodiversity indicators based on explicit, species-level functional traits, moving beyond the constraints of classical QBS formulations while retaining their ecological intent.

1. Introduction

Soil biodiversity forms the living foundation of terrestrial ecosystems, as soil organisms drive nutrient cycling and other essential soil processes that support ecosystem functioning and agricultural productivity (Neher and Barbercheck, 2019), thereby sustaining more than 95% of global food production (Delgado-Baquerizo et al., 2025). Microarthropods help fulfil a key ecological role in this ecosystem. Mostly mites and springtails dominate the animal component, accounting for more than 95% of global soil arthropod abundance and ~ 20% of their biomass (Rosenberg et al., 2023). Their roles are diverse, including trophic versatility linking microbial decomposers with higher tiers, accelerating nutrient turnover and redistributing microbial 'hotspots' (Gergócs et al., 2022; Heneghan and Bolger, 1998) among others. Because many complete multiple generations within a single cropping season (Fountain and Hopkin, 2005) and respond within days to shifts in moisture, temperature or chemical inputs (Liu et al., 2017), microarthropods are increasingly promoted as sensitive bioindicators of soil

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health and agricultural sustainability (Meyer et al., 2021).

Traditional microarthropod survey methods rely on morphological identification of individuals extracted from soil cores, often by Berlese-Tullgren funnels or MacFadyan high gradient extractors (Bruckner, 2024; Petersen, 1978). While informative, these identification methods are labor-intensive, require taxonomic expertise, and are limited in their resolution, particularly for cryptic or juvenile life stages (Porco et al., 2012; Sun et al., 2017). Furthermore, inter-operator variability can introduce bias and reduce comparability across studies. Standardization (e.g., ISO 23611-2:2024) exists precisely to mitigate these issues. Additional guidance is being developed, for example by the FAO Global Soil Partnership, whose GLOSOLAN manual will include standardised procedures for QBS assessments (FAO, 2025). Nevertheless, a shortage of taxonomic expertise further limits throughput (Löbl et al., 2023). To address these limitations, the QBS index (Qualità Biologica del Suolo) was developed by Parisi et al. (2005) as a rapid bioindication tool. It is based on the presence or absence of predefined morphotaxonomic groups named biological forms (BF), each assigned a score reflecting presumed adaptation to life in the soil. Its simplicity, low cost and limited need for taxonomic expertise have led to its adoption in some soil studies, particularly in Europe, where studies have demonstrated its utility in detecting broad differences between, for example, land uses, pollution levels, or soil management types (Gallese et al., 2025; Gardini et al., 2025; Reilly et al., 2023). Nevertheless, the QBS approach faces some limitations. Firstly, eco-morphological index (EMI) scores, values assigned to each biological form, reflecting the degree of morphological and functional adaptation to life in the soil (e.g. body pigmentation, eye loss), are expert-derived approximations that may not capture trait variability across biogeographical regions as the majority of studies were historically performed in Italy (Menta et al., 2018). However, it is true that the QBS-ar index has also been applied in some other biogeographical regions in recent years (Galli et al., 2021; Lakshmi et al., 2021). Secondly, the index collapses some entire taxonomic groups into a single value, sacrificing taxonomic and functional resolution (Tóth et al., 2025). Thirdly, its reliance on presence/absence data may make results highly sensitive to sampling effort and rare taxa (Gallese et al., 2025).

In this context, metabarcoding of environmental DNA has emerged as a promising method for soil biodiversity assessment (Basset et al., 2022). By targeting a standardised genetic marker (such as the mitochondrial cytochrome *c* oxidase I, COI), metabarcoding can rapidly yield taxonomic lists from bulk soil samples in a fraction of the time required for traditional morphology-based identifications (Oliverio et al., 2018; Young and Hebert, 2022), thus overcoming many limitations of morphological identification. However, several methodological and interpretive constraints remain: reference libraries remain incomplete, leaving many soil sequences unassigned (Recuero et al., 2024), PCR amplification biases, variable mitochondrial copy numbers and differences in organism size, among other factors, mean that read counts are not reliable proxies for organismal abundance, and intra-individual COI variants can even be misclassified as separate taxa (Deiner et al., 2017; Elbrecht and Leese, 2015). Soil DNA extracts may also contain extracellular and relic DNA, so detection does not always imply the presence of living organisms (Carini et al., 2016; Nagler et al., 2018). Finally, bioinformatic pipelines are not yet standardised, and ecological interpretation still requires linking taxonomic lists to functional traits or indicator frameworks (Pawlowski et al., 2018). Ongoing improvements in reference databases (e.g. initiatives developed under the Biodiversa+ European Biodiversity Partnership), laboratory protocols (e.g. GLOSOLAN, EUSO), and best-practice guidelines (Ramirez et al., 2015) are gradually mitigating these challenges, yet they must be acknowledged whenever metabarcoding is proposed as a monitoring tool. Moreover, once these limitations are accounted for, metabarcoding enables standardised, reproducible assessments that can be readily scaled across studies, ecosystems, and taxa.

Recent studies increasingly employ DNA metabarcoding to explore soil microarthropod diversity across agricultural landscapes (Köninger et al., 2025; Sahdra et al., 2025; Sapkota et al., 2025); however, its broader application within agricultural monitoring represents only a small share of eDNA research globally (Kestel et al., 2022). These studies often reveal higher richness and finer taxonomic resolution than morphotaxonomy-based surveys (Basset et al., 2022) and can detect subtle differences between treatments that would otherwise be missed. Metabarcoding provides an independent validation of morphological observations (Cuartero et al., 2025). Beyond this quality-control function, sequence data may enrich the QBS framework by assigning EMI scores with greater confidence, revealing species-level turnover within each BF, and detecting obligate euedaphic lineages whose inclusion would sharpen the index's sensitivity to disturbance. Normalised read counts could potentially even support a dominance-weighted DNA-QBS-ab analogue. Conversely, classical QBS scores provide a morphological cross-check on DNA outputs, highlighting samples where amplification bias, relic DNA, DNA from aboveground organisms or database gaps may distort community profiles. The newly adopted EU Soil Monitoring Law explicitly encourages multi-indicator integration (European Parliament and Council of the European Union, 2025). Embedding these feedback loops into routine workflows would move soil monitoring from parallel indicators toward a unified, trait-informed system.

Earlier studies demonstrated that arthropod community structure is affected by habitat characteristics (Lazzerini et al., 2007) and agricultural management practices; reduced tillage and organic or less intensive systems generally support higher soil arthropod richness (Bengtsson et al., 2005; Lichtenberg et al., 2017; Tsiafouli et al., 2015). Our objectives were therefore: (1) to compare soil biodiversity metrics derived from BF identification for the QBS index, and COI-DNA metabarcoding; (2) to evaluate the ability of each method to detect differences between production systems and treatments; and (3) to explore whether DNA metabarcoding can be used to complement or improve the calculation and interpretation of the QBS index. We hypothesised that (H1) DNA metabarcoding would capture richer communities, (H2) β-diversity derived from metabarcoding would separate production systems more clearly than the QBS-based approach and (H3) high-throughput sequencing of the COI barcode region would detect treatment-level differences and fine compositional shifts that the coarser, presence-based QBS approach may overlook. We expect QBS indices to discriminate among production systems but to be less sensitive to finer treatment effects. Finally, we suggest that linking metabarcoding data to species-level trait information could help refine EMI scores and may contribute to the development of new, function-oriented indicators that complement the morphology-based QBS framework. Building on a previous QBS-focused baseline survey (Naglič et al., 2025), several methodological approaches were employed to evaluate the response of soil microarthropod communities to different management regimes within agricultural production systems. Those provided contrasts that form the basis for the empirical testing of our objectives and hypotheses.

2. Materials and methods

2.1. Study sites

The study was conducted across two research locations of the Agricultural Institute of Slovenia, including three distinct agricultural production systems: the arable field at the Infrastructure Centre Jablje (46.141204 N, 14.571509 E), and the strawberry system and orchard at the Infrastructure Centre Brdo (46.166927 N, 14.680106 E). These sites share comparable climatic conditions. Both locations are situated in the temperate warm humid climatic zone (Cfbw' according to the Köppen classification) with warm summers and autumnal precipitation peaks (Ogrin et al., 2023). Annual precipitation ranges between 1300 and 1400 mm and average annual temperatures range from 10 to 12 °C (Agencija Republike Slovenije za okolje, 2023). Soil at the study site Jablje is classified as Eutric Cambisol on alluvial pebble and sand with

silty-loam texture, while Dystric Cambisol silty-loam soil type is present at the Brdo site. A schematic representation of the sampling design is presented in the Supplementary Fig. S1.

2.2. Experimental design and soil sampling

Sampling was conducted in spring 2023 in three production systems: (1) three adjacent arable fields (each measuring 490 × 24 m) under three tillage systems (conventional, minimum tillage, and no-till), (2) a strawberry system under organic and integrated production systems, and (3) an apple orchard also managed under organic and integrated production. For clarity in figures and statistical outputs, each treatment was identified by a concise two-block code: the first block denotes the management regime and the second the cropping system. These codes, and their consistent plotting colours, are used throughout the Results section. Thus CT-A, MT-A and NT-A refer to conventional-, minimum- and no-till arable treatments (plotted in blue); INT-O and ORG-O denote the integrated and organic apple orchards (plotted in green); and INT-S and ORG-S mark the integrated and organic strawberry systems (plotted in orange). A trailing numeral distinguishes replicates (e.g., NT-A-1/3). Each field within a treatment group (system-management combination) was represented by ten replicates ($n = 10$) and evenly distributed across the treatments. A total of 70 soil samples were collected. Three cores forming one QBS replicate were taken approximately 5 m apart, after which sampling moved ~10 m along the field to collect the next set of three cores. A third set consisted of a fourth additional core collected following the same spacing. The additional subsample acted as a buffer in case of sample loss or processing failure. In the strawberry system and orchard, where plots were smaller, the same sampling strategy was performed but with shorter distances between samples. In those two systems, samples were taken both from the within-row areas (under the crop canopy, 6 samples) and inter-row areas (4 samples), ensuring coverage of typical microhabitats. All soil samples were taken using a standardised soil corer (11.3 cm inner diameter) to a depth of 10 cm, resulting in approximately 1 l of soil per sample. Samples for QBS analysis were collected following the QBS protocol (Parisi et al., 2005), which requires the collection of an intact soil core without disturbing the structure, allowing the extraction and identification of soil micro-arthropods later on. Ten soil samples for DNA analysis were taken immediately adjacent (within <10 cm of the corresponding core) to the QBS samples, using the same corer and depth, ensuring spatial comparability between the two datasets. While the soil collected for QBS analysis was left undisturbed, the soil for molecular analysis was homogenised by hand on site inside a sealed plastic bag. A subsample of about 40 ml was stored for DNA extraction and subsequent analysis, and the remaining soil was discarded. No technical replicates were taken for DNA analyses to align with the QBS approach, which uses a single intact core. As both methods targeted the same small-scale unit in homogenous fields, only biological replicates (ten per field) were collected to ensure comparability between methods.

2.3. QBS analysis of soil microarthropods

After field sampling, soil samples were transported to the laboratory in plastic bags, which were stored in an insulated box. Samples were not actively cooled during transport but were protected from heating and extracted within 24 h of collection. The assessment of soil mesofauna followed the standardised QBS-ar protocol (Parisi et al., 2005), which was adapted for calculation of two additional indices: QBS-ar_BF (D'Avino et al., 2024) and QBS-ab (Mantoni et al., 2021). Extraction of soil mesofauna was performed using a Kempson extractor (ecoTech, Bonn, Germany) following QBS-ar extraction guidelines (D'Avino et al., 2024), using intact soil cores. Samples were exposed to gradual heating from above using warm air, with the extractor set to 30 °C for approximately 10 days. To promote a vertical temperature and moisture gradient within the soil sample, the extractor doors were kept slightly open during extraction. No active cooling from below was applied. Apart from this configuration, no deviations from standard QBS extraction protocols were applied. Soil samples were placed on a double mesh system consisting of a 5 mm supporting mesh and a 2 mm extraction mesh. Arthropods migrating downward were collected in vials containing a preservative solution of 70% ethanol with added glycerol placed beneath the funnel. After extraction, all arthropods were sorted and counted under a stereomicroscope. Following the original QBS protocol, organisms were assigned to taxonomic groups at the class or order level (e.g., Collembola, Acarina, Diplura, Isopoda), and then further classified according to the ecomorphological index (EMI), which ranges from 1 to 20, with higher values indicating greater adaptation to edaphic environments. EMI classification uses morphological features such as body pigmentation, size, presence of eyes, and limb morphology. These are defined as BFs, an operational unit that blends higher taxonomic classification with morphospecies style traits distinctions to reflect edaphic adaptation. All extracted arthropod individuals were counted as BFs, thus providing a full description of the arthropod community based on the QBS approach. Three indices were calculated for each sample: QBS-ar: summing the highest EMI value per taxonomic group per sample, QBS-ar_BF: summing all EMI values for all identified BFs in each sample (D'Avino et al., 2024) and QBS-ab: applying a log transformation to the abundance before multiplying with the EMI value, thus down-weighting highly abundant groups such as Collembola and Acarina (Mantoni et al., 2021). Definitions and mathematical formulations of all soil biological quality indices used in this study are provided in Table 1. The QBS index is traditionally calculated by merging data from three adjacent subsamples per treatment replicate, taking the maximum EMI value observed for each group, emphasizing highly adapted taxa across the sampled area. In our study, we applied the QBS index in two distinct ways: (1) adhering to the standard protocol by merging three geographically closest subsamples per treatment replicate (Supplementary Table S4), and (2) using permutations to systematically combine different sets of subsamples within each homogeneous field (Results). This permutation approach allowed us to leverage the available data, providing a statistically more robust dataset by capturing the inherent variability and ecological signal across multiple subsample combinations.

2.4. DNA analysis of soil microarthropods

Soil samples for DNA analysis were manually homogenised in a sealed plastic bag, and an aliquot of approximately 200 g was subsampled and stored at −20 °C until further processing. Later, a 40 ml aliquot of that soil sample was freeze-dried for 72 h and homogenised in a bead mill homogeniser (SPEX SamplePrep 1600 MiniG®) using 2.4 mm metal beads (Metal Bead Media, Omni International, USA). DNA

Table 1
List of the soil biological quality indices used in this study together with their mathematical formulations.

Index	Within-group rule	Formula
QBS-ar (Parisi et al., 2005)	Max BF within each group; presence only	$\sum_g \max_{bf \in g}(EMI_{bf} \cdot P_{bf})$
QBS-ar_BF (D'Avino et al., 2024)	No within-group max (all present BFs)	$\sum_{bf} EMI_{bf} \cdot P_{bf}$
QBS-ab (Mantoni et al., 2021)	Abundance-weighted	$\sum_{bf} EMI_{bf} \cdot \log_{10}(n_{bf} + 1)$
QBS-DNA (this paper)	Species level; presence only	$\sum_{s \in S} Score_s$

* g – taxonomic group (e.g. Collembola, Coleoptera); BF – biological form (eco-morphological unit inside a taxonomic group g); s – species; S – set of species detected in a sample; r – subsample (replicate; $r = 1,2,3$).
EMI_{bf} – eco-morphological index score of biological form bf; $X_{bf,r}$ – abundance (count) of biological form bf in replicate r; P_{bf} – presence indicator of biological form bf (Pbf = 1 if $X_{bf,r} > 0$ for any r, otherwise 0); n_{bf} – total abundance of biological form bf; $Score_s$ – (EMI-like) score assigned to species s.

was extracted from 0.25 g of homogenised soil using DNeasy PowerSoil Pro Kit (Qiagen), following the manufacturer's instructions. DNA concentrations were quantified using a Qubit 4.0 Fluorometer (Invitrogen, USA). The subsequent metabarcoding workflow followed the protocol described by Sapkota et al. (2025). The COI region was amplified with mlCOlintF/jgHCO2198 primers (Geller et al., 2013) using a two-step PCR and dual indexing for Illumina MiSeq sequencing. During the first PCR (PCR#1), amplicons were generated using 0.25 µl HiFi polymerase (PCR Biosystems), 5 µl HiFi buffer, 0.5 µl 10 µM of each forward and reverse primer, 0.5 µl BSA, 2 µl template and nuclease-free water to a total volume of 25 µl. The PCR#1 thermocycler program consisted of an initial denaturation temperature of 95 °C for 5 min, followed by ten cycles of 94 °C for 30s, 50 °C for 30 s, 72 °C for 1 min, another 10 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min, followed by 15 cycles of 94 °C for 30 s, 54 °C for 30 s, with the final elongation at 72 °C for 2 min. For dual indexing, the second PCR (PCR#2) included 5 µl of PCR#1 product and 2 µl of primers with Illumina adaptors and indexes (Illumina Inc., 2023) using the PCR reaction described above. The PCR#2 program consisted of 98 °C for 1 min, followed by 13 cycles of 98 °C for 10 s, 55 °C for 20 s, and 68 °C for 40 s, and a final elongation of 68 °C for 5 min. Negative controls without sample template were included in both PCR's. They were verified on agarose gels prior to sequencing. As no amplification products were detected, these controls were not included in the sequencing library. To ensure comparability with the QBS approach, technical PCR/sequencing replicates were not included. Successfully amplified PCR products were cleaned with HighPrep™ magnetic beads (MagBio Genomics Inc.), and their concentration was quantified using a Qubit 4.0 Fluorometer. Samples were then equimolarly pooled and sequenced on an Illumina MiSeq using the 500 cycles V2 reagent kit at the Department of Environmental Science, Aarhus University (Denmark).

2.5. Bioinformatic data processing

Paired-end Illumina reads were processed using QIIME2 (v2023.5), following the official installation and execution guidelines (Bolyen et al., 2019). Details on data processing, filtering and statistics are provided in the Supplementary Table S1. Briefly, reads were imported using a manifest file and quality-checked with qiime demux summarize to guide trimming decisions. Primer sequences were removed, and reads were truncated based on visual quality profiles using DADA2 (Callahan et al., 2016) via *qiime dada2 denoise-paired*. Primer sequences and low-quality leading bases were removed by trimming 20 bp from the 5′ end of both forward and reverse reads (−-p-trim-left-f 20; −-p-trim-left-r 20). Reads were then truncated where the 25th-percentile quality score dropped below Q25. DADA2 performed filtering, error correction, merging of paired reads, chimera removal, and dereplication to produce Amplicon Sequence Variants (ASVs), using the following key parameters: trim-left = 20 bp (both reads), trunc-len = 230 bp (forward) / 180 bp (reverse), max-EE = 2.0, trunc-Q = 2, and min-overlap = 12 bp with consensus chimera removal and independent sample pooling. Representative ASV sequences were first exported from QIIME 2 as a FASTA file. They were then taxonomically assigned with BOLDigger v3 release 1.4.4 (Buchner and Leese, 2020; Buchner and Shah, 2025), querying the Barcode of Life Data Systems (BOLD) v5 API (accessed 28. February 2025) in mode 3, which triggers the "Exhaustive Search" option. Assignments followed BOLD's default identity thresholds of 98%, 95%, 90%, and 85% for species-, genus-, family-, and order-level matches, respectively. All query batches were concatenated and merged with the QIIME 2 ASV table via custom Python scripts. We first removed non-metazoan lineages (e.g., fungi, protists, plants) and then restricted the dataset to ASVs classified within Arthropoda, thereby excluding non-arthropod metazoans such as nematodes and annelids. Further cleaning in R eliminated singletons (ASVs detected in just one sample), very low-abundance ASVs (total reads <10 or relative abundance <0.005%), and samples with fewer than five arthropod ASVs. One sample with fewer than 100 reads was removed during filtering, leaving 69 samples for downstream analyses. Sampling completeness was assessed using coverage-based rarefaction implemented in the R package *iNEXT* v3.0.2 (Hsieh et al., 2016). Details about filtering steps are available in the Supplementary Table S2. Finally, sample columns were relabeled to encode treatment and replicate information for subsequent ecological analyses.

2.6. Statistical analyses

Statistical analyses were performed in R (version 4.3.1) (R Core Team, 2021) using the packages *vegan* (v2.7–1; Oksanen et al., 2022), *phyloseq* (v1.44.0; McMurdie and Holmes, 2013), and *ggplot2* (v3.5.2; Wickham, 2009). Alpha diversity metrics were based on richness of QBS biological forms and on species richness based on the filtered ASV table for DNA data. ASVs were used for community composition and diversity analyses, whereas taxonomic aggregation of ASVs was applied for indicator analyses and calculation of the QBS-DNA index. Sample-based rarefaction curves were generated (*vegan*::*specaccum*, method = "random") to assess sampling completeness. Because singletons/low-abundance ASVs were removed from the DNA table and QBS richness is bounded at 38 biological forms, we did not apply abundance-based richness estimators (e.g., Chao1). Normality of ANOVA residuals was evaluated with the Shapiro–Wilk test, and homogeneity of variances was assessed with Levene's test. When both assumptions were met, α-diversity metrics were compared among production systems and treatments using one-way ANOVA followed by Tukey's HSD. Otherwise, a Kruskal–Wallis test followed by Dunn's post-hoc test with Holm correction was applied. For beta diversity, Bray–Curtis dissimilarities were computed for QBS and DNA datasets. For the DNA dataset, we additionally performed equal-depth subsampling to 581 reads to evaluate the potential influence of uneven sequencing depth. Because the results did not differ significantly from those obtained using the original dataset, we retained the original analysis in the final results. Both were visualized using non-metric multidimensional scaling (NMDS). To assess differences in community composition between treatments and production systems (e.g., tillage, orchard, and strawberry systems), permutational multivariate analysis of variance (PERMANOVA) was conducted using the *adonis2* function with 999 permutations. Relative abundance was visualized with stacked bar plots, and differences in the relative abundance of major taxa among treatments and production systems were assessed using non-parametric tests (Kruskal–Wallis), followed by Dunn's post-hoc tests with Holm correction where applicable. Indicator species analysis was conducted with the package *indicspecies* (v1.8.0; Cáceres and Legendre, 2009) using the *multipatt* function with 999 permutations. Analyses were run at the genus and order levels. For DNA metabarcoding data, indicator analysis was performed on taxonomic groups, whereas for QBS data it was applied to biological forms (BFs). Single groups and combinations of groups were evaluated, and significant indicators were retained. To compare the performance of QBS and DNA assessments, DNA-based ASV richness was correlated with QBS biological form richness using Pearson correlation coefficients. Because a single QBS value is defined from three pooled subsamples, all QBS analyses operate at the level of subsample triads. Two approaches to triad construction were used: 1) Standard QBS values were calculated following the original protocol by merging three spatially adjacent subsamples within each treatment, 2) a permutational QBS approach in which three subsamples out of ten within each treatment were randomly grouped to generate alternative triad combinations. Because QBS-ar is inherently based on three subsamples and any single choice of triplets is to some extent arbitrary, more subsamples than the minimum required were collected, enabling a permutational approach to assess the robustness of QBS indices to subsample selection rather than to increase replication. Distributional properties of QBS indices derived from random and adjacent triplets were evaluated using non-parametric tests of central tendency (Mann–Whitney), dispersion (Levene or Brown–Forsythe), and multivariate dispersion (betadisper).

These results are available in Supplementary Table S3. Permutational QBS was used exclusively for visualization and sensitivity assessment of triad selection and was not treated as generating independent biological replicates. To ensure methodological equivalence when comparing QBS indices with DNA-based richness, the same three-subsample aggregation was applied to the DNA data. For each treatment and production system, all unique combinations of three subsamples were enumerated to generate matched QBS and DNA triplets. These aggregated values were used exclusively for method-comparison analyses and not for testing treatment or production system differences. We constructed a presence-only DNA-derived QBS index from the ASV table using relaxed filtering to maximise taxon retention. Of the 1020 Arthropoda ASVs retained after filtering (505,623 reads), only 32 ASVs (≈3% of ASVs, ≈5% of reads) were assigned to species level and could therefore be used in the calculation of the QBS-DNA index. Exact thresholds and settings are available in Supplementary Material S1. We refer to this index as QBS-DNA. The mathematical formula for the index is provided in Table 1. Each metabarcoding-derived taxon was assigned an EMI score, following the QBS-ar biological-form framework (D'Avino et al., 2024; Parisi et al., 2005), reflecting its degree of adaptation to soil life based on diagnostic morphological traits (e.g. body form, pigmentation and appendage reduction). Where necessary, the original QBS framework was refined to accommodate species-level DNA data and taxa comprising multiple biological forms. The table with assigned scores is available in Supplementary Table S5. Euedaphic taxa received high scores, epiedaphic taxa low scores. Intermediate values were applied where life stage could not be inferred from DNA data; for example, Diptera sequences may originate from adults classified as "flies: 2 wings and 2 halteres" (EMI = 2) or from larval forms described as "fly larva, mostly no legs, small or absent head" (EMI = 10), and were therefore assigned an intermediate score of EMI = 6. Summing these scores across all taxa detected in a sample yielded a single DNA-derived index value (no abundance weighting). We report two visualisations: a sample-level index and a triplet-level index that merges consecutive subsamples using the same triplet aggregation logic as the morphology-based QBS. The dataset was restricted to QBS-relevant groups and species-level identifications. All figures were prepared using *ggplot2*, and statistical significance was considered at $p < 0.05$.

3. Results

3.1. Sequencing output and filtering

Across the 70 QBS samples collected from the three agricultural production systems and five management regimes, we recorded 6999 soil microarthropod individuals, representing 30 biological forms (BFs) observed in the dataset out of the 38 BFs considered in the QBS framework. For the DNA metabarcoding dataset, sequence data were obtained for all samples, but one conventional arable sample failed the minimum read-depth threshold and was removed, yielding 69 DNA samples. After removal of this sample and all filtering steps, the final Arthropoda table comprised 1020 ASVs and 505,623 reads, representing 32 named species, 40 genera, 38 families, and 19 orders across the filtered dataset.

3.2. Richness

Using DNA metabarcoding at the ASV level, overall richness was higher than with BF richness (Fig. 1), but DNA ASV richness did not differ significantly among production systems. In contrast, BF richness was significantly higher in Arable than in Strawberry ($p = 0.006$), while Arable vs Orchard and Orchard vs Strawberry were not significant. Within systems, DNA ASV richness varied in Arable: conventional tillage (CT-A) had lower richness than both minimum tillage (MT-A; $p = 0.012$) and no-till (NT-A; $p = 0.001$), whereas MT-A and NT-A did not differ. In Strawberry, DNA richness was higher in organic (ORG—S) than in integrated (INT—S) management ($p = 0.021$), while in Orchard it did not differ between INT-O and ORG—O. BF richness showed no within-system treatment differences in any system (all $p \geq 0.161$). Sample-

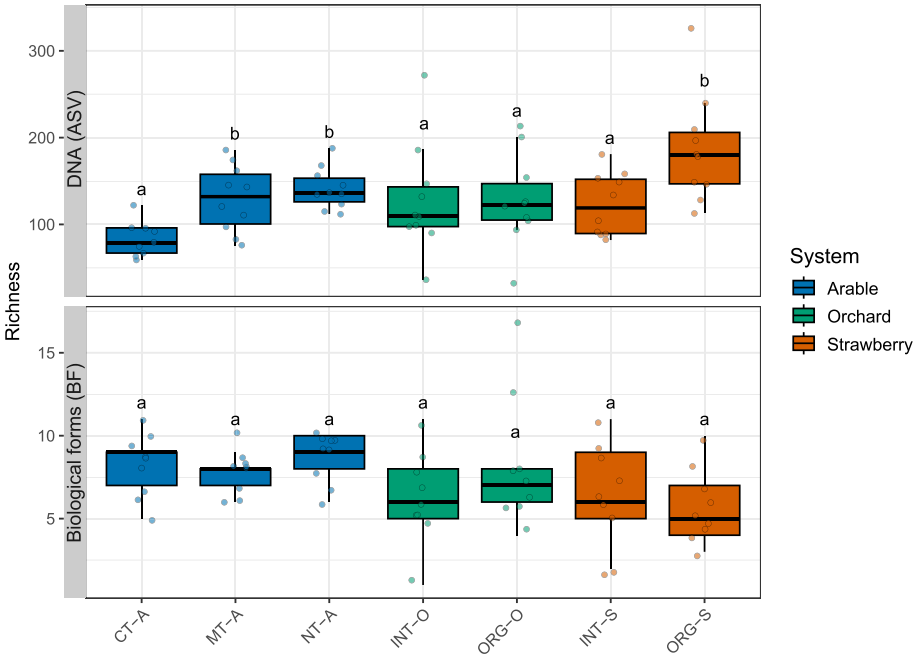

Fig. 1. DNA (ASV-level) and BF richness by production system and treatment. Boxes show sample distributions with jittered points; colours denote systems (Arable = blue, Orchard = green, Strawberry = orange). Management treatments are abbreviated as follows: CT-A = conventional tillage (arable), MT-A = minimum tillage (arable), NT-A = no-tillage (arable), INT-O = integrated management (orchard), ORG-O = organic management (orchard), INT-S = integrated management (strawberry), and ORG-S = organic management (strawberry). Compact letters indicate statistically distinct treatments within each system, computed separately per system (letters are only comparable within the same system). Between-system contrasts are not annotated on the plot and are reported in the text. Note that DNA and BF panels use free y-scales.

based rarefaction curves (Supplementary Fig. S2) showed system-level richness approaching an asymptote in both datasets. Treatment-level curves rise more gradually, suggesting dominant groups were well-sampled, while additional samples would chiefly add rarer taxa and fill remaining within-treatment richness. Correlations between DNA ASV richness and BF richness were not statistically significant (p = 0.96), indicating no clear relationship between richness patterns derived from the two methods.

3.3. Community composition

DNA and BF community composition differed among production systems and within them (Fig. 2). DNA and BF data showed similar production-system effects (DNA: R2 = 0.28, F = 12.97, p = 0.001; BF: R2 = 0.27, F = 12.71, p = 0.001). However, DNA detected clearer within-system treatment differences, separating arable tillage treatments and organic/integrated treatments in both orchard and strawberry systems. In Arable fields, no-till, minimum-till, and conventional plots formed distinct communities based on DNA (R^2 = 0.23, F = 3.79, p = 0.001). DNA also distinguished communities between organic and integrated treatments in Orchard (R^2 = 0.09, F = 1.87, p = 0.018), and in Strawberry system (R^2 = 0.21, F = 4.93, p = 0.001). In contrast, BF showed only partial separation among different management treatment in Arable system (R^2 = 0.14, F = 2.13, p = 0.043) while BF community composition did not differ significantly among treatments in either Orchard (R^2 = 0.066, F = 1.27, p = 0.265) or Strawberry (R^2 = 0.031, F = 0.58, p = 0.807). All NMDS ordinations were well fitted (stress <0.15). Direct comparison of the two ordination spaces showed strong congruence (Procrustes r = 0.879, p = 0.009; co-inertia RV = 0.876, with 81.9% of the co-structure captured by the first axis), indicating that DNA and BF ordinations capture the same dominant beta-diversity gradient (Fig. 2; Supplementary Fig. S3).

DNA-based relative abundance profiles revealed pronounced shifts in order-level community structure among production systems and management regimes (Fig. 3). In arable soils (CT-A, MT-A and NT-A), communities were strongly dominated by Hemiptera and

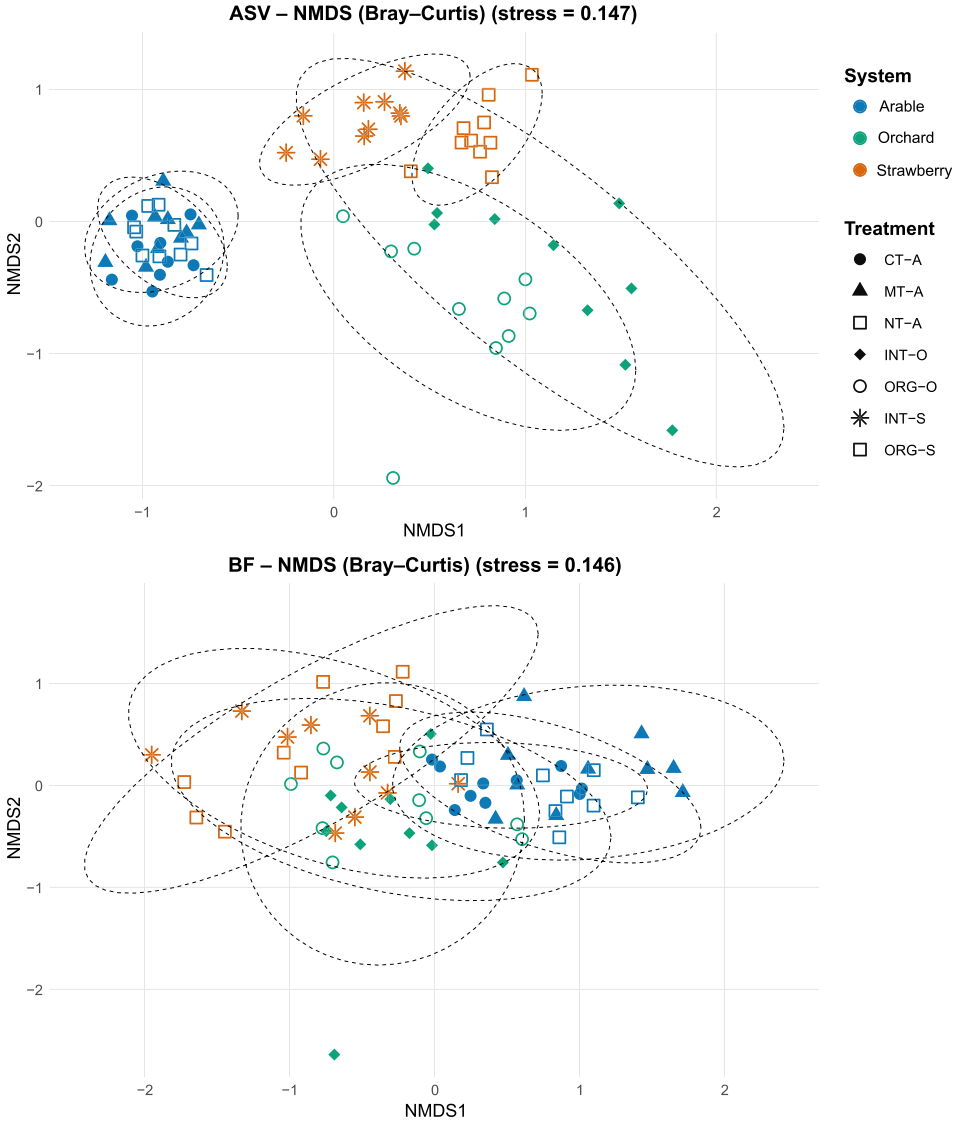

Fig. 2. Non-metric multidimensional scaling (NMDS) ordination of soil mesofauna communities based on DNA metabarcoding (top) and BF data (bottom). Each plot shows the full dataset across all seven treatments. Symbols represent individual treatments, using filled shapes to distinguish management types, and colours indicate production systems. Management treatments are abbreviated as follows: CT-A = conventional tillage (arable), MT-A = minimum tillage (arable), NT-A = no-tillage (arable), INT-O = integrated management (orchard), ORG-O = organic management (orchard), INT-S = integrated management (strawberry), and ORG-S = organic management (strawberry). Dashed ellipses denote 95% confidence envelopes for each treatment. Panel titles include the corresponding stress values. Axes represent NMDS1 and NMDS2.

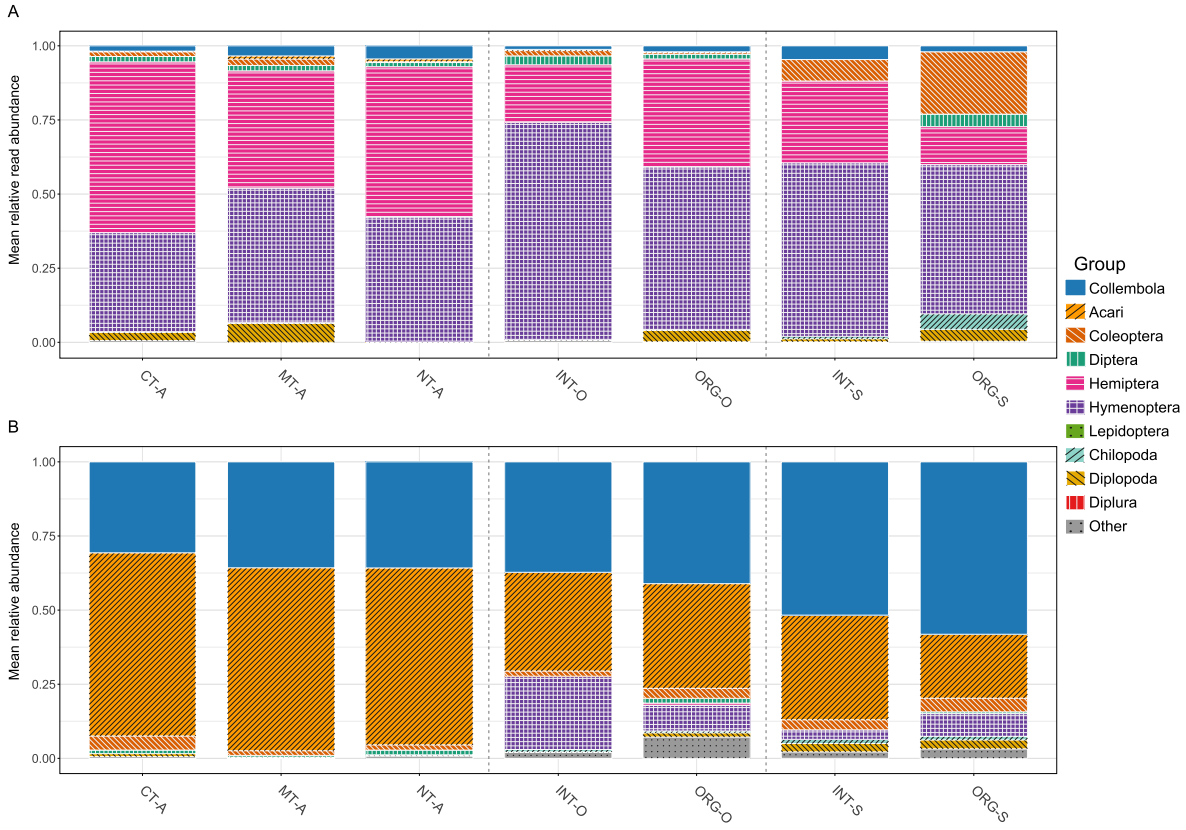

Fig. 3. Relative community composition by treatment based on DNA read abundances (A) and BF abundances (B). Each stacked bar shows the mean relative abundance within a treatment, after normalising each sample to 100% and then averaging across replicates. Colours/patterns denote taxonomic/BF groups and are matched between panels to facilitate comparison; groups not among the display set or not assigned are merged into "Other". Vertical dashed lines separate production systems. Management treatments are abbreviated as follows: CT-A = conventional tillage (arable), MT-A = minimum tillage (arable), NT-A = no-tillage (arable), INT-O = integrated management (orchard), ORG-O = organic management (orchard), INT-S = integrated management (strawberry), and ORG-S = organic management (strawberry). Panel A summarises DNA reads at the Order level; Panel B aggregates BF counts in the same categories used for DNA. Both plots share the same colours/patterns of groups displayed.

Hymenoptera, which together accounted for more than 85% of sequence reads. Both orders differed significantly among arable treatments (Hemiptera: $H = 28.4$, $df = 2$, $p = 1.3 \times 10^{-7}$; Hymenoptera: $H = 25.6$, $df = 2$, $p = 2.0 \times 10^{-6}$), with conventional and no-till arable soils showing a stronger dominance of Hemiptera, whereas minimum tillage soils exhibited a significantly higher relative contribution of Diplopoda ($H = 6.5$, $df = 2$, $p = 0.0107$). Orchard systems were characterized by a marked predominance of Hymenoptera. Integrated orchards were almost exclusively dominated by this order (≈ 75% of reads), while organic orchards retained a high Hymenoptera contribution (≈ 55%) together with minor contributions from Diplopoda. Strawberry systems displayed the most distinct DNA-based profiles. Integrated strawberry soils remained Hymenoptera-dominated but also contained substantial proportions of Coleoptera, which differed significantly among systems ($H = 36.9$, $df = 4$, $p = 4.4 \times 10^{-8}$). In organic strawberry soils, Coleoptera relative read abundances increased markedly, accompanied by a pronounced decline in Hemiptera. Patterns derived from BF assessments emphasized different components of the soil fauna (Fig. 3). Arable treatments were dominated by Acarina (≈ two-thirds of individuals) and Collembola (≈ one-third). Orchard soils exhibited reduced Acarina and increased Hymenoptera, while strawberry soils displayed the greatest BF diversity. Organic strawberry soils were characterized by the highest relative abundance of Collembola (≈ 60%) and the lowest proportion of Acarina (≈ 25%), with the remaining community distributed among Diptera, Coleoptera, Hemiptera, Hymenoptera and Diplopoda. These differences were statistically supported: Acarina were more abundant in arable than orchard or strawberry soils ($H = 21.9$, $df = 2$, $p = 2.7 \times 10^{-5}$; all pairwise $p_{adj} \leq 5.7 \times 10^{-5}$), Collembola were more abundant in strawberry than arable soils ($H = 6.7$, $df = 2$, $p = 0.0096$; $p_{adj} = 0.0074$), and Hymenoptera were more abundant in orchard than arable soils ($H = 26.1$, $df = 2$, $p = 1.7 \times 10^{-6}$; $p_{adj} = 7.7 \times 10^{-7}$).

Indicator species analysis provided finer taxonomic resolution, albeit sometimes at different taxonomic ranks than the order-level groups depicted in Fig. 3. At the system level, the DNA dataset linked orchards to *Diplura* (Rhabdura; r.g = 0.354, $p = 0.018$), whereas the EMI data associated orchards with ants *(formicidae)* (Hymenoptera_5; r.g = 0.529, $p = 0.001$), *Symphyla* (Symphyla_20; r.g = 0.446, $p = 0.002$) and *Hemiptera* (Hemiptera_1; r.g = 0.376, $p = 0.013$). Arable systems were characterized by mite orders (*Sarcoptiformes*, r.g = 0.418, $p = 0.004$; *Trombidiformes*, r.g = 0.409, p = 0.002) and springtail genera (*Ceratophysella*, *Lepidocyrtus*, *Parisotoma*), together with mite genera (*Acrotritia*, *Nenteria*) in DNA; the EMI dataset highlighted epedaphic Collembola_2 (r.g = 0.431, p = 0.001) and winged Diptera_1 (r.g = 0.435, p = 0.004). Strawberry systems showed strong DNA signals for myriapod orders (*Lithobiomorpha*, r.g = 0.451, p = 0.001; *Julida*, r.g = 0.438, p = 0.001) and for beetle and edaphic taxa such as *Polydrusus* and *Thalassaphorura*, while the EMI data recovered high-scoring edaphic groups (Protura_20 and Pauropoda_20) and a high Diplopoda_20 score. At the treatment scale, DNA resolved specific associations, including *Ceratophysella* and *Parisotoma* with MT-A and NT-A, *Acrotritia* with NT-A, *Lithobiomorpha* with INT-S + ORG-S and a strong *Polydrusus* signal in ORG—S. EMI indicators included ants across both orchard treatments, hemiedaphic Collembola_10 across minimum-tillage arable and organic orchard treatments, and winged Diptera_1 across the arable treatments. Overall,

these indicator taxa corroborate the community differences seen in the abundance profiles (Fig. 3) and identify characteristic taxa for each system and management regime.

3.4. QBS indices

QBS indices calculated using the standard approach (merging three adjacent subsamples per replicate) revealed some system- and treatment-level patterns, but no statistically significant differences were observed across treatments within systems or among systems, except for the QBS values that were higher in Strawberry than in Arable. The full table summarizing mean ± standard error of QBS-ar, QBS-ar_BF, and QBS-ab across treatments is provided in Supplementary Table S4. To explore within-treatment variability and ensure comparability to the DNA-based analysis, we calculated QBS values by permutations of the 10 collected samples as described in Methods. The distribution of permutational QBS indices showed clear system-level patterns. Strawberry fields had the highest QBS-ar and QBS-ar_BF values, while Arable fields had the highest QBS-ab values (Fig. 4). At the treatment level, the visual trends, which were not statistically tested, suggest that minimum tillage in Arable fields generally yields lower QBS-ar and QBS-ar_BF values than conventional or no-till treatments. Organic orchards tend to score higher than integrated orchards across indices.

Associations between permutational QBS indices and permutational DNA ASV richness aggregated using identical triplets varied among production systems (Fig. 5). Associations were strongest in Orchard systems, moderate in Arable fields, and weakest in the Strawberry system. In Orchards, all three indices - QBS-ar ($\rho = 0.42$), QBS-ar_BF ($\rho = 0.37$), and QBS-ab ($\rho = 0.35$) - showed moderate positive associations with permutational DNA richness. Arable soils also showed positive relationships for QBS-ab ($\rho = 0.38$), while QBS-ar displayed a weaker association ($\rho = 0.14$). In contrast, Strawberry systems showed only weak relationships, with QBS-ab exhibiting a modest positive trend ($\rho = 0.15$) and no consistent patterns in the other indices. At the treatment level, patterns diverged. In Arable soils, the no-tillage (NT-A) treatment showed a clear split: QBS-ar and QBS-ar_BF were negatively associated with DNA richness (slopes = −0.30 and − 0.36, respectively), whereas QBS-ab revealed a strong positive relationship (slope = +0.59). Other Arable treatments (CT-A and MT-A) did not show consistent relationships for any index. In Orchards, positive associations were particularly evident for QBS-ar and QBS-ar_BF. For instance, INT-O showed strong positive relationships across all indices, including QBS-ar (slope = +0.18) and QBS-ab (slope = +0.26). ORG-O also displayed a positive slope for QBS-ar (slope = +0.21), whereas no clear trend was observed for QBS-ab. In the Strawberry system, only the organic treatment (ORG—S) showed clear and consistent positive relationships, including QBS-ar_BF (slope = +0.35) and QBS-ab (slope = +0.47). The integrated Strawberry system (INT—S), however, did not exhibit consistent trends. Detailed correlation statistics are provided in Supplementary Table S6.

3.5. DNA-derived QBS index (QBS-DNA)

QBS-DNA varied across treatments (Fig. 6), with lowest values under CT-A and generally higher values under reduced-disturbance systems, but treatment differences were not significant at the sample level (Kruskal–Wallis $\chi^2 = 3.46$, df = 6, $p = 0.75$). Triplet-sum aggregation increased index magnitudes and reduced dispersion but still yielded no treatment effect ($\chi^2 = 7.06$, df = 6, $p = 0.315$). At the treatment-mean level, the sample-sum index correlated positively with morphology-based QBS-ar (Spearman $\rho = 0.86$, $p = 0.014$) and QBS-ar_BF ($\rho = 0.79$, $p = 0.036$), whereas the association with DNA ASV richness was weaker and non-significant ($\rho = 0.46$, $p = 0.294$).

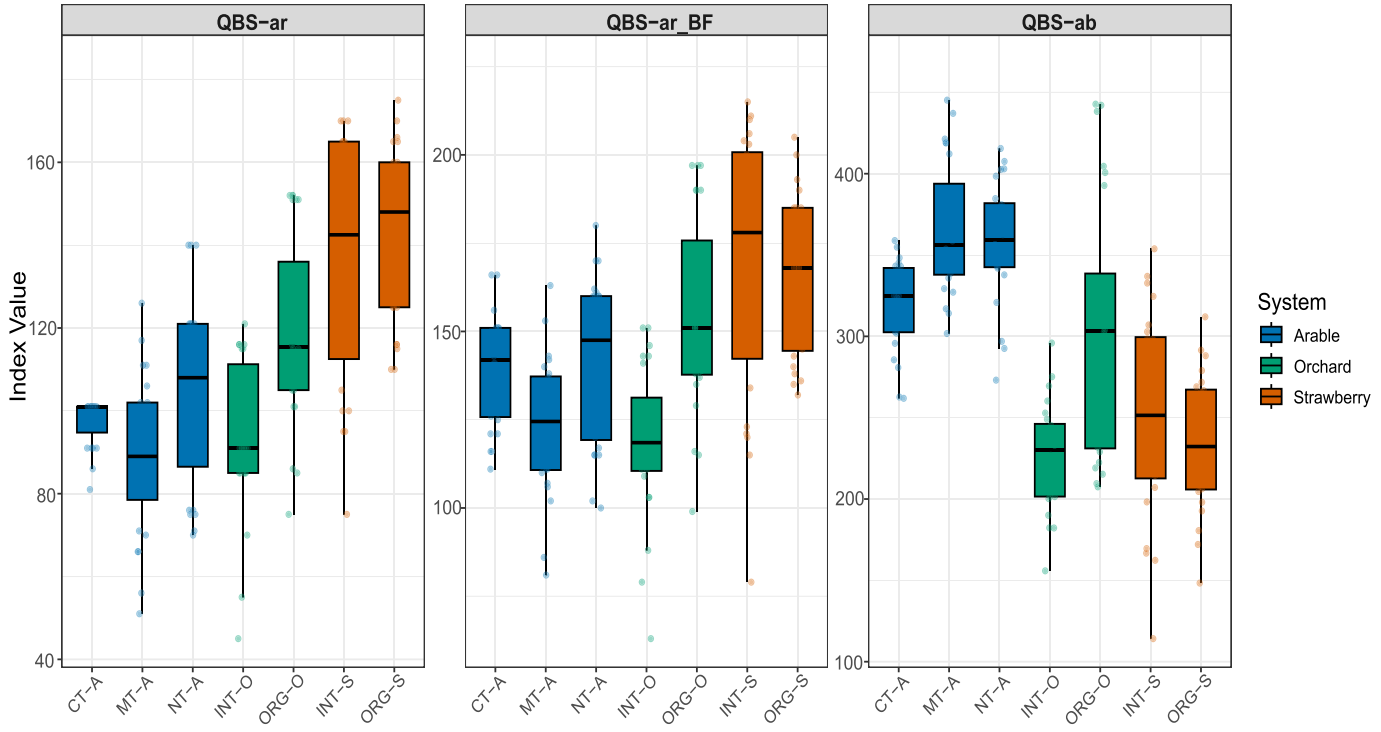

Fig. 4. Comparison of three permutational QBS soil biological quality indices across seven agricultural management treatments. Panels show box-and-jitter plots for (left to right) QBS-ar, QBS-ar_BF, and QBS-ab. Boxes summarize index values calculated from randomly generated three-subsample triplets within each treatment; points represent individual permutational triplets. Fill colours denote production systems. Management treatments are abbreviated as follows: CT-A = conventional tillage (arable), MT-A = minimum tillage (arable), NT-A = no-tillage (arable), INT-O = integrated management (orchard), ORG-O = organic management (orchard), INT-S = integrated management (strawberry), and ORG-S = organic management (strawberry). Panels use free y-scales.

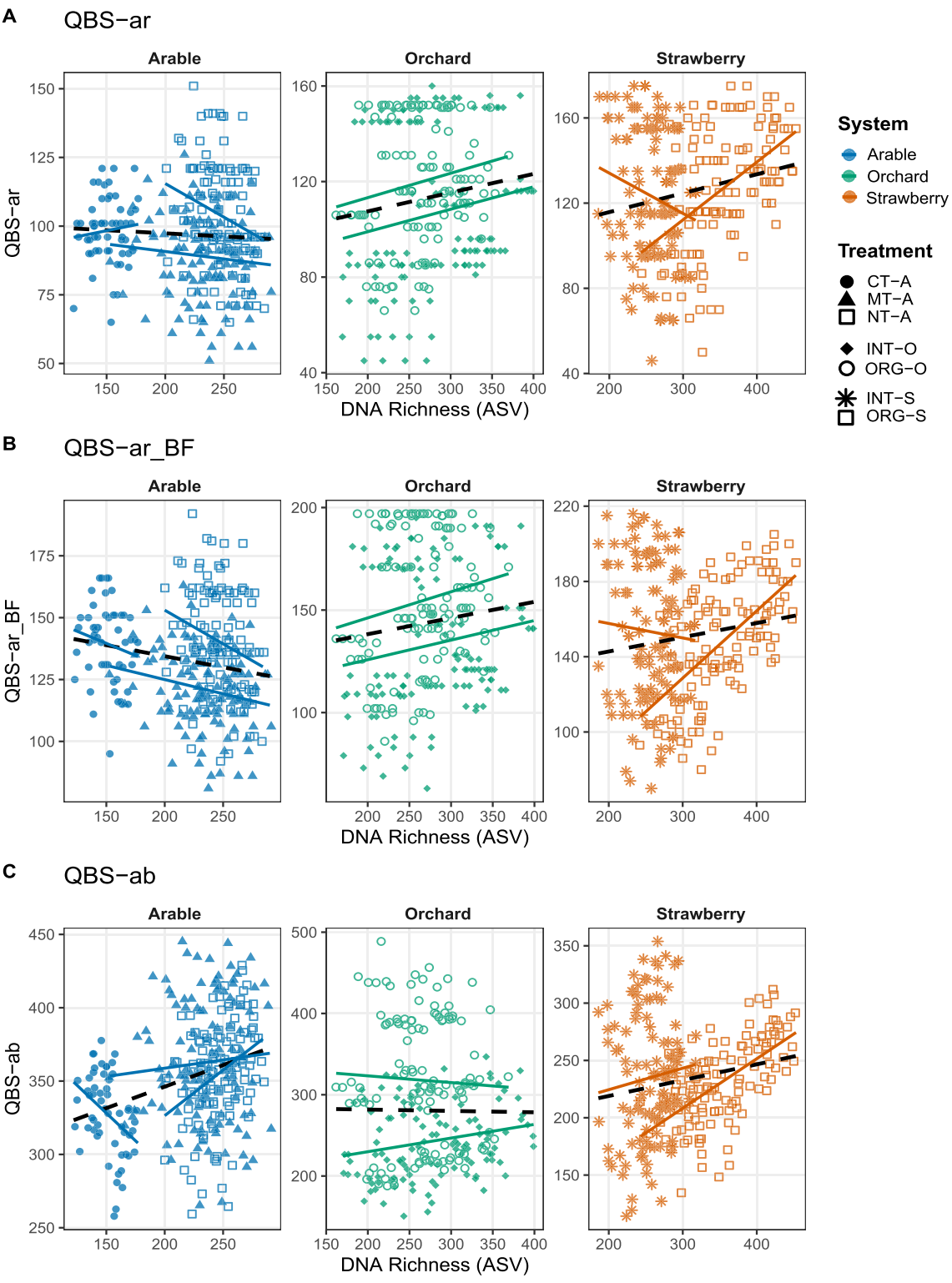

Fig. 5. Associations between permutational QBS soil biological quality indices and permutational DNA ASV richness across agricultural systems. Panels A–C show system-stratified scatterplots for QBS-ar (A), QBS-ar_BF (B), and QBS-ab (C). Each point represents one enumerated triplet aggregation; triplet points are not independent biological replicates and statistics are descriptive. Management treatments are abbreviated as follows: CT-A = conventional tillage (arable), MT-A = minimum tillage (arable), NT-A = no-tillage (arable), INT-O = integrated management (orchard), ORG-O = organic management (orchard), INT-S = integrated management (strawberry), and ORG-S = organic management (strawberry). Colored regression lines indicate treatment-level fits; thick dashed lines indicate system-level trends. Correlation coefficients and slope estimates are provided in Supplementary Table S6.

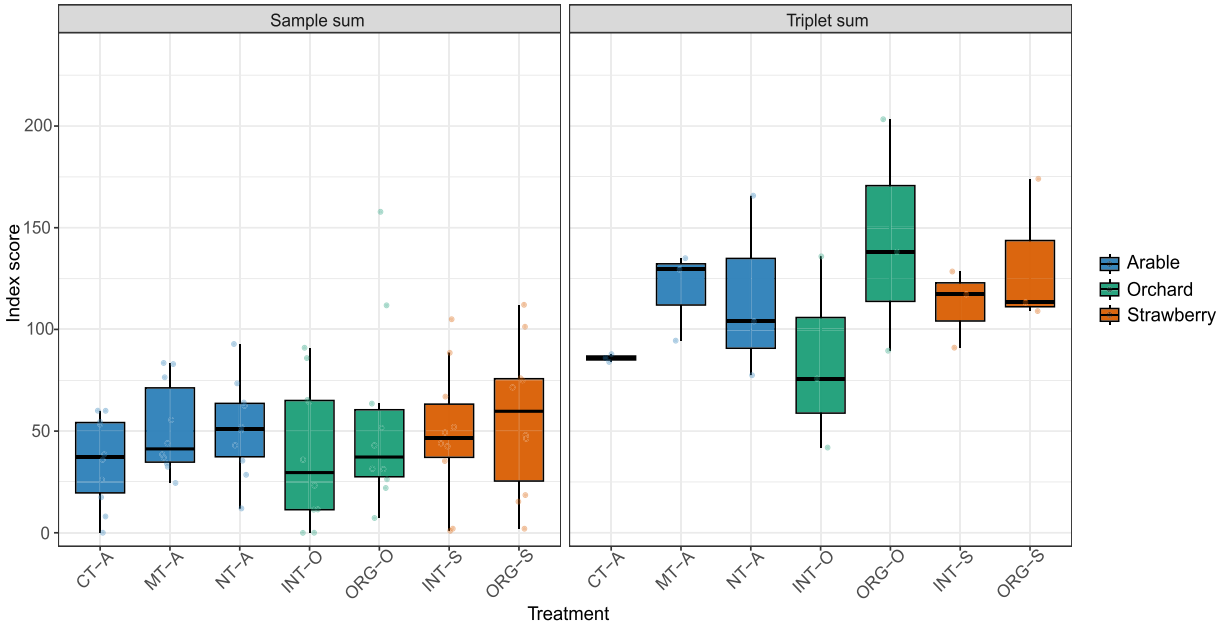

Fig. 6. Boxplots show the DNA-derived index calculated for each treatment, shown for sample-level sums (left panel) and triplet-level sums (right panel). Management treatments are abbreviated as follows: CT-A = conventional tillage (arable), MT-A = minimum tillage (arable), NT-A = no-tillage (arable), INT-O = integrated management (orchard), ORG-O = organic management (orchard), INT-S = integrated management (strawberry), and ORG-S = organic management (strawberry).

4. Discussion

4.1. Contrasting richness and community composition from DNA- and BF-based approaches

We found that patterns in α diversity depended strongly on the method used. Amplicon sequence variant (ASV) richness was markedly higher than the number of biological forms (BFs) extracted by the Kempson apparatus, yet DNA-based richness showed little separation among our production systems, while BF richness distinguished only weakly between them. Such discrepancies between DNA-based richness estimates and BF counts are expected and reflect methodological differences, as metabarcoding detects intragenomic and intraspecific sequence variation and retrieves DNA from multiple life stages (e.g. eggs and nymphs), as well as extracellular or relic DNA (Köninger et al., 2025; Young and Hebert, 2022), whereas a single organism yields just one BF in the QBS framework. Molecular datasets might also include DNA from surface-active or transient taxa whose material falls into the soil, and extracellular DNA can persist for weeks or months (Carini et al., 2016; Young, 2021); therefore, richness estimates combine live and historical signals. ASV richness should therefore be interpreted as sequence diversity rather than true organismal richness, as metabarcoding detects DNA from both active and inactive organisms (and may produce multiple ASVs per species). By contrast, BF counts only record live microarthropods actively moving downward during extraction; individuals unable to crawl into the collecting fluid or killed during processing are under-represented. In contrast to richness, community-level ordination analyses showed clearer separation among production systems in DNA data, while BF-based ordinations grouped systems more broadly and overlapped across management treatments. Despite these differences in resolution, strong concordance between molecular and morphological ordinations indicates that both approaches capture similar underlying environmental and management gradients. The limited agreement between richness measures therefore reflects not only methodological constraints, but also fundamental differences in ecological scope (Basset et al., 2022; Oliverio et al., 2018). Similar incongruence has been reported elsewhere: a recent study of flying insect communities found that about half of all species were recovered by both morphological and metabarcoding approaches, with broadly comparable community patterns (Remmel et al., 2024). An additional consideration when interpreting these results is the spatial scope of the study. Our experiment was conducted at two long-term experimental sites within the same temperate climatic region. This design minimised climatic variability to enable a clearer methodological comparison between morphology-based QBS indices and DNA metabarcoding approaches. However, the restricted geographic scope means that the observed relationships between QBS indices and metabarcoding-derived diversity metrics should be interpreted within this pedoclimatic context. Future studies should therefore evaluate QBS–DNA relationships across broader pedoclimatic gradients to determine whether region-specific calibration of trait-based indices is required.

4.2. Taxonomic composition and indicator taxa

Taxonomic composition differed markedly between methods. BF samples were dominated by mites and springtails, whereas metabarcoding recovered a wider taxonomic spectrum, including abundant Hymenoptera and Hemiptera in arable and orchard soils, and predatory Coleoptera and soil-associated springtail orders (Entomobryomorpha, Neelipleona, Poduromorpha) in organic strawberries. This dominance in BF samples but not in DNA relative read abundance is consistent with the global prevalence of mites and springtails, as they account for more than 95% of soil arthropod individuals but contribute only ~20% of total arthropod biomass (Rosenberg et al., 2023). Consistent with the methodological constraints described in the previous paragraph, differences in the DNA dataset largely reflect known molecular biases, such as the fact that small-bodied mites and springtails often yield little DNA, are frequently under-amplified, and have overlapping intra- and interspecific COI variation that limits reliable assignment below family level (Arribas et al., 2021). Conversely, DNA from epedaphic or surface-active taxa is readily detected and can enter soil through faeces, shed fragments, or carcass deposition, potentially inflating their representation in molecular datasets (Chua et al., 2023; Epp et al., 2012). Similar over-representation of surface-active taxa has been reported in other

terrestrial eDNA surveys, indicating that this is a general feature of soil and litter eDNA rather than a system-specific artefact (Beng et al., 2016; Gossner et al., 2016). These limitations however do not invalidate the study, because our main inferences are based on community-level differentiation, concordance between ordinations, and treatment-associated indicator taxa rather than on quantitative comparison of relative abundances alone.

Indicator taxa analyses nevertheless demonstrate the added ecological resolution provided by metabarcoding. Litter-associated springtail genera characterized reduced tillage plots (Parisotoma, Ceratophysella), bristletails (Rhabdura) distinguished orchard systems and herbivorous weevils (Polydrusus), were characteristic of strawberry fields. These associations might reflect differences in litter dynamics, trophic structure and habitat use that are directly shaped by management, rather than EMI score as defined in the QBS. As such, indicator taxa capture management-specific ecological responses that are not directly aligned with the eco-morphological gradients summarised by QBS. Also, because QBS aggregates taxa into coarse functional categories, such fine-scale, management-specific associations are not resolved by morphology alone. Incorporating DNA-derived indicator taxa into soil bioassessment frameworks could therefore enhance ecological interpretation and provide early warning signals of management-induced change, particularly as improvements in COI DNA marker choice, reference databases, and long-read sequencing continue to refine taxonomic resolution (Le Cadre et al., 2024). In the future, integrating RNA-based analyses may further help distinguish living organisms from relic or transient DNA, improving ecological resolution.

4.3. Implications for QBS indices and trait-based bioindication

Our results indicate that the limited sensitivity of the classical QBS-ar index to management effects is driven less by the small sample size inherent in the standard protocol than by the simplified assumptions embedded in its scoring logic. QBS assigns high weights to euedaphic taxa (e.g., blind, unpigmented Collembola) on the premise that these forms are more disturbance-sensitive than epigeic species, yet such structural traits reflect adaptation to life in soil rather than physiological tolerance or reproductive strategy. In this sense, QBS already represents a directional composite trait describing edaphicity, i.e., the degree of adaptation to life in the mineral soil as opposed to the organic surface layers. Consequently, QBS scores should not be interpreted as proxies for species richness or as direct indicators of management intensity. Replication constraints remain a practical issue because the official protocol defines the QBS index from a composite of three soil cores. Although many studies report QBS values as the mean of several such composite replicates with associated variability, increasing replication still requires additional sampling and processing effort and may constrain statistical power when the number of replicates is limited (Fusco et al., 2023; Perinelli et al., 2025; Rodríguez-Pajares et al., 2025). However, even when additional composite scores were generated by statistical resampling, correlations between QBS scores and ASV richness varied in magnitude and direction across production systems and index variants, underscoring that taxonomic diversity and eco-morphological adaptation respond to partly decoupled ecological drivers. Such decoupling is expected because QBS intentionally compresses taxonomic information into functional groups; its values are more responsive to successional or recovery gradients – where euedaphic forms become dominant – than to changes in species richness or the presence of rare taxa (Madej et al., 2011). Previous studies likewise show that morphological and molecular approaches recover complementary fractions of soil fauna and that primer bias or relic DNA can inflate molecular diversity estimates, particularly in disturbed soils (Basset et al., 2022; Nagler et al., 2018; Young, 2021). Adding abundance weighting (QBS-ab) rarely improves these associations, as abundance weighting seldom changes overall QBS patterns (Gardini et al., 2025; Mantoni et al., 2021). Overall, our findings reinforce that QBS remains a valuable trait-based indicator of soil biological quality but should not be interpreted as a surrogate for DNA-derived richness. Metabarcoding can refine the QBS framework by linking taxa to additional ecological traits – such as trophic guilds, dispersal capacity, and reproductive mode – and by incorporating indicator taxa identified through DNA analyses, as demonstrated in recent trait-weighted nematode indices (Ghaderi et al., 2025). Trait databases therefore provide a pathway to refine EMI assumptions by providing explicit information on edaphicity, trophic role and life-history traits for taxa that are currently absent from, or coarsely represented in, the QBS framework.

4.4. Toward DNA-enabled trait indices

We implemented a DNA-derived QBS-DNA index by adapting community-level metabarcoding data to the eco-morphological categories (with some modifications) used in QBS-ar. The resulting scores correlated positively at the treatment-mean level with QBS-ar and QBS-ar_BF and ranked treatments similarly, indicating that the core eco-morphological attributes underlying QBS are captured by community-level metabarcoding data. This community-level implementation differs from previous molecular trait assessments, which targeted single indicator species (e.g. Lee et al., 2022) or integrated traits after morphological identification (Yan et al., 2012). Yet our results should not be interpreted as validation of the original EMI weighting scheme: epedaphic taxa may act as positive indicators of litter layer functioning, and soil quality may not increase monotonically with the proportion of euedaphic forms. Moreover, COI sequences often lack a clear barcoding gap, complicating species-level assignments and the mapping of functional traits. We therefore consider QBS-DNA as a proof of concept rather than an endpoint. Assigning EMI scores to taxa detected through metabarcoding inevitably involves assumptions regarding edaphic affinity and life-stage representation. In several cases, intermediate or expert-based scores were applied where species-level ecological information was incomplete, introducing uncertainty into the index calculation. In addition, the index was calculated only from ASVs assigned to species level, so most detected arthropod ASVs were excluded from scoring, likely biasing QBS-DNA values through incomplete representation of the underlying community. Nevertheless, its correlation with morphology-based QBS index suggests that it retained ecologically meaningful signal despite this limitation. Future developments could reduce this uncertainty through curated functional trait databases linking taxa to edaphic specialization and ecological traits. In the future, trait-based indices should embrace the capabilities of metabarcoding by integrating multiple genetic markers together with DNA- and RNA-based metabarcoding and long-read sequencing to improve taxonomic resolution. A unified invertebrate soil quality index, combining arthropods, nematodes, and other soil fauna within a single DNA-enabled framework, could provide a more comprehensive and scalable tool for monitoring soil health across diverse agroecosystems.

5. Conclusion

This study shows that QBS-based and DNA-based approaches capture complementary aspects of soil microarthropod diversity. The QBS index remains a practical and robust indicator of soil biological quality, directly linking community structure to functional adaptation, yet it lacks the taxonomic detail required to detect finer management effects. DNA metabarcoding, in contrast, provides high taxonomic resolution and detects cryptic and juvenile forms, revealing compositional and indicator-taxon differences among production systems that BFs alone cannot discern. Despite methodological biases and incomplete reference libraries, both approaches captured major production-system gradients, while evidence for within-system management effects was stronger in the DNA community data than in QBS indices. The DNA-derived QBS-DNA index developed here serves as a proof of concept, illustrating a first step toward embedding trait-based perspectives into molecular

biodiversity assessments. By assigning QBS-derived scores to metabarcoding taxa, we demonstrate how sequence data can, in principle, be translated into functional indicators of soil adaptation. With further refinement, through expanded barcode coverage, curated trait databases, and calibration against morphological benchmarks, such indices could form the foundation of next-generation biomonitoring frameworks. Overall, combining QBS and DNA-based approaches provides a more comprehensive view of soil biological quality: morphology anchors assessments in functional adaptation, while metabarcoding exposes hidden taxonomic structure and management-specific bioindicators. Their integration provides a realistic path forward for developing scalable, trait-informed soil-health indices that unite functional and taxonomic perspectives within a single monitoring framework.

Glossary

- Biological form (BF)

An operational unit that blends higher taxonomic classification with morphospecies style traits distinctions to reflect edaphic adaptation (morphotaxonomic groups). A BF is assigned a score based on adaptation to life in soil.

- Eco-morphological index (EMI)

A score assigned to each BF group reflecting its degree of adaptation to the soil environment.

- Microarthropods

$<$ 2 mm soil-dwelling arthropods, predominantly from groups of mites (Acari) and springtails (Collembola).

- Production system

A broad land-use category reflecting crop type and long-term management context, including arable cropping systems, perennial orchards, and strawberry systems.

- Treatment

A specific management regime applied within a production system, such as conventional, minimum, or no tillage, integrated and organic management.

- QBS-ar (Qualità Biologica del Suolo – arthropods)

A trait-based soil quality index based on the presence of soil microarthropod biological forms (BF). For each taxonomic group, only the highest eco-morphological index (EMI) score is retained, and the index is calculated as the sum of these scores, independent of abundance.

- QBS-ar_BF

A variant of QBS-ar in which all distinct biological forms (BFs) observed in a sample are summed, rather than retaining only the highest EMI per taxonomic group.

- QBS-ab

An abundance-weighted extension of the QBS framework in which EMI scores are weighted by log-transformed abundances of BFs, integrating both soil adaptation and dominance patterns.

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CRediT authorship contribution statement

Vid Naglič: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tijana Martinović:** Writing – review & editing, Methodology, Data curation. **Nataša Šibanc:** Writing – review & editing, Methodology, Investigation. **Tine Grebenc:** Writing – review & editing, Resources, Funding acquisition. **Paul Henning Krogh:** Writing – review & editing, Supervision, Methodology, Data curation. **Rumakanta Sapkota:** Writing – review & editing, Methodology. **Anne Winding:** Writing – review & editing, Resources. **Robert Leskovšek:** Writing – review & editing, Resources. **Irena Bertoncelj:** Writing – review & editing, Supervision, Funding acquisition, 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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2026.107118.

Data availability

The data has been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1399753 and code is publicly available at https://github.com/VidNaglic/qbs-dna-ase

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