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Original Research

Soil mesofauna diversity in agricultural systems of Slovenia using the QBS index and its modifications

Vid Naglič [1*], Nataša Šibanc [2], Tine Grebenc [2], Irena Bertoncelj [1]

Abstract

Soil mesofauna plays a key role in maintaining soil health by supporting the decomposition of organic matter, nutrient cycling and the maintenance of soil structure. In this study of Slovenian agricultural ecosystems, we used four modifications of the QBS index, a soil biological quality index based on soil mesofauna. We compared diversity in arable fields under different tillage intensities, a strawberry field and an orchard, managed with either organic or integrated pest management methods (IPM). The results show significant differences in the mesofaunal communities in the soil. Minimum tillage promoted higher biodiversity, especially of Collembola, compared to conventional tillage. In fruit production systems, the ratio of Collembola to Acarina differed from that of arable fields, skewing in favour of Collembola, possibly related to the use of copper-containing pesticides in organic orchards and systemic herbicides in IPM systems. The QBS index values for soil health varied considerably between systems. Only QBS modifications considering the abundances of organisms (QBS-ab and QBS-a) were able to distinguish between different system-management groups. This study provides insights into the limitations of the originally proposed QBS-ar index to discern the effects of farming intensity on the soil mesofaunal community. Results suggest that minimum tillage and organic management practices can promote healthier soil ecosystems, emphasizing the importance of sustainable soil management for the promotion of soil biodiversity. Future research should aim to incorporate a broader range of agricultural practices and assign fauna to a higher taxonomic rank to further explain the effects on soil mesofauna diversity.

Keywords

Soil health, Soil microarthropods, Biodiversity, Agroecosystems, Tillage intensity, Organic farming

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Citation: Naglič, V., Šibanc, N., Grebenc, T., Bertoncelj, I., (2024). Soil mesofauna diversity in agricultural systems of Slovenia using the QBS index and its modifications. Acta Biologica Slovenica 68 (1)

Received: 11.09.2024 / **Accepted:** 28.11.2024 / **Published:** 29.11.2024

https://doi.org/10.14720/abs.68.01.19787

Raziskava raznovrstnosti talne mezofavne v kmetijskih ekosistemih Slovenije z uporabo QBS indeksa in njegovih izpeljank

Izvleček

Talna mezofavna z opravljanjem ekosistemskih storitev razgradnje organske snovi, kroženja hranil in vzdrževanju strukture tal igra ključno vlogo pri ohranjanju zdravja tal. V tej raziskavi slovenskih kmetijskih ekosistemov smo uporabili štiri različice QBS indeksa, ki so bile razvite za namen preučevanja mezofavne v tleh. Raznolikost te skupine živali smo preučevali na njivah z različnimi intenzivnostmi obdelave tal, v nasadu jagod in sadovnjaku, kjer se uporabljajo ekološke ali integrirane metode varstva rastlin (IPM). Rezultati so pokazali statistično značilne razlike v talni mezofavni preučevanih kmetijskih ekosistemov. Pri minimalni obdelavi tal v primerjavi s konvencionalnim oranjem je bila biodiverziteta višja, zlasti pri skupini Collembola. V sistemih pridelave sadja se je razmerje med Collembola in Acarina v prid Collembola razlikovalo od tistega na njivah, kar je verjetno povezano z večjo občutljivostjo Acarina na bakrove pesticide v ekoloških sadovnjakih in sistemske herbicide v IPM sistemih. Vrednosti QBS indeksa so se med sistemi razlikovale. Le QBS različici, ki upoštevata številčnost organizmov (QBS-ab in QBS-a), sta zaznali razlike med različnimi skupinami sistemov in načinov upravljanja. Ta študija kaže na omejitve prvotno predlaganega QBS-ar indeksa za zaznavanje vplivov intenzivnosti kmetovanja na mezofavno v tleh. Rezultati nakazujejo, da lahko minimalna obdelava tal in ekološko upravljanje spodbujata bolj zdrave talne ekosisteme, kar poudarja pomen trajnostnega upravljanja za spodbujanje biodiverzitete v tleh. Da bi lahko natančneje pojasnili vplive kmetijskih praks na raznolikost talne mezofavne, bi morali v prihodnje raziskave vključiti širši spekter kmetijskih ekosistemov ter določiti talne živali do višjih taksonomskih kategorij.

Ključne besede

Zdravje tal, Talni členonožci, Biodiverziteta, Agroekosistemi, Intenzivnost obdelave tal, Ekološko kmetijstvo

Introduction

The topic of soil health and its indicators has gained considerable research interest in the past 20 years, and as soil mesofauna are responsible for several ecosystem services, they have been proposed as a potential soil quality and soil health indicator (Menta & Remelli, 2020). Soil mesofauna plays a key role, being responsible for numerous essential functions such as organic matter decomposition, nutrient cycling, and soil structure maintenance. As such, they are increasingly recognized as valuable indicators of soil quality and health (Menta & Remelli, 2020). The dominance and diversity of specific taxa, notably *Acarina* (mites) and *Collembola* (springtails), in agricultural soils have been extensively studied (Behan-Pelletier, 2003). These taxa are not only most abundant but also sensitive to environmental changes, with the potential to serve as reliable bioindicators. Previous research (Behan-Pelletier, 2003) has documented different proportions of *Acarina* and *Collembola* in various agricultural settings, highlighting their response to different farming practices. For instance, higher abundance of *Acarina* in organically managed systems compared to integrated pest management (IPM) (Gagnarli et al., 2015) and changes in *Collembola* abundance as a response to different tillage practices (Vignozzi et al., 2019). Among various indices proposed for assessing soil health, there is the QBS (Qualità Biologica del Suolo) index, which is based on the presence of soil arthropod communities and their level of adaptation to living in soil (Parisi et al., 2005). The QBS index has gained considerable attention in some parts of the world, particularly in Italy, where it originated, and has been applied at the regional level (Albertazzi et al., 2021) to highlight the importance of soil biodiversity for ecosystem services (Menta, Conti, Pinto, et al., 2018). What makes this index advantageous is that it does not require species-level identification, making it a practical tool for large-scale ecological assessments and non-taxonomy specialists.

The use of indices for describing ecosystems stems from the challenge of grasping the full complexity of these systems, and such indices may offer a simplified view aimed at addressing specific research questions. As a result, these indices may not always be effective in detecting significant differences in soil quality between various management practices, especially in ecosystems that are heterogeneous or highly disturbed (Tabaglio et al., 2009). Recent advancements in molecular metabarcoding offer more precise tools for assessing soil biodiversity (Orgiazzi et al., 2015; Guerra et al., 2020). These methods enable more detailed identification, moving from the class-level identification necessary for the QBS index to genus or even species-level identification. Studies confirmed a strong positive correlation between molecular and classical identification tools applied to insects (Jin et al., 2013), and some studies have already been done specifically for soil fauna (Basset et al., 2022). Although genetic studies have provided valuable insights into soil mesofauna, they often miss crucial information about the abundance of these organisms. Additionally, relying solely on DNA analysis may be less effective at detecting recent changes in soil communities, as the persistence of relict DNA from soil organisms can distort our understanding of the current structure of the soil fauna community (Foucher et al., 2020).

The QBS index and its variations are employed to assess the impact of agricultural practices on soil biodiversity and health, a need that has become increasingly important due to the new Soil monitoring law proposed by the EU (General Secretariat of the Council, 2024). In arable fields, the intensity of tillage is a critical factor influencing soil mesofauna. Conventional tillage, which disrupts soil structure, often negatively impacts soil biota, whereas no-till practices tend to enhance soil biodiversity and ecosystem services (Betancur-Corredor et al., 2022). In fruit-growing systems like orchards and strawberry fields, organic farming is generally associated with higher biodiversity due to reduced chemical inputs and a focus on ecological balance. However, the specific contributions of various agricultural practices and soil management approaches influence soil fauna diversity, and consequently, soil health and biodiversity remain unknown.

In this study, we aim to explore the diversity and abundance of soil mesofauna across three different agricultural ecosystems with two contrasting soil management systems by applying four different variants of the QBS index. The QBS-based approach was selected as an affordable and simple approach. The experiment was set in Slovenia, where highly preserved (extensive) agricultural lands and intensive soil management intermix at small spatial scales. Arable fields, strawberry fields, and orchards were selected as test cultures affecting the soil fauna community, with a focus on varying tillage intensities and the distinction between organic and IPM production methods.

Materials and Methods

Study sites

The experiment was conducted at two locations of the Agricultural Institute of Slovenia's field research stations: an arable field at the Infrastructure Centre Jablje (46.141204, 14.571509) and a strawberry field and orchard both located at the Infrastructure Centre Brdo (46.166927, 14.680106). The sampling sites within each location were on the same geological base, namely clay gravel and mixed origin gravel soil for Jablje, and clay gravel, sandy loam and clay for Brdo, respectively; same climate conditions Cfbw' (moderate warm humid climate with warm summers and peak precipitation in one of the autumn months) (Ogrin et al., 2023) according to the Köppen climate classification; with comparable micro-location on flat surface; similar average annual precipitation of 1300-1400 mm and average annual temperatures 10-12°C (ARSO, 2023).

The arable field in Jablje was cultivated for five years prior to sampling (since 2018) using three tillage methods: no-tillage without any soil disturbance (0.43 ha), minimal tillage using a disc cultivator or a ripper to a depth of 8-10 cm (0.96 ha), and conventional tillage with ploughing to a depth of 20-25 cm (0.96 ha). The same three-year crop rotation of winter cereal with a cover crop, maize, and soybeans has been practised for all three tillage methods. Fields were fertilized with mineral fertilizers and adapted to crop requirements with average values of 80-100 kg P_2O_5, 100-130 kg K_2O and 160-180 kg N per hectare. Herbicides were applied on average once per year, and fungicides 2-3 times per year in winter wheat crops. All fields were under a large share of crops in the rotation, and no significant pest damage like maize, crimson clover, or soybeans was observed. For the past 50 years, insecticides were used only occasionally, with the frequency once every 4-5 years. The last insecticide application (7.5 g/ha of Lambda-cyhalothrin) was 2 years ago. Lambda-cyhalothrin half-life is around 30-60 days, and considering soil and environmental conditions at the study site (warm and microbiological active soils), insecticide was degraded in a couple of months at the latest (Hornsby et al., 1996). The study area was not known to be actively exposed to plastic materials; however, since microplastics and pesticide residues have not been analyzed in this area to date, we cannot rule out their potential presence or influence on mesofauna.

Fruit production at the Infrastructure Center Brdo is based on organic and IPM production methods. The strawberry field was divided into an organic and an IPM section, planted with strawberries (*Fragaria x ananassa* Duch.; cultivar Clery) two years prior to sampling (in August 2021); four ridges were made, covered with black polyethene and equipped with drip irrigation system. The organic section transitioned through various crops before planting strawberries in 2021, where no fertilizers were used and only plant protection products approved for organic production were used (*Pravilnik o ekološki pridelavi in predelavi kmetijskih pridelkov oziroma živil.*, 2018). In the IPM section, where strawberries were grown according to the recommended crop rotation for eight years before sampling (since 2015), systemic herbicides and only a few fungicides were used before planting.

The orchard in Brdo covers 14.9 ha and is also divided into an organic and an IPM section. The Topaz apple variety (*Malus domestica* var. Topaz) has been cultivated according to the rules of organic production since 2009 (*Pravilnik o ekološki pridelavi in predelavi kmetijskih pridelkov oziroma živil.*, 2018) without the use of herbicides. To remove the weed vegetation under the trees, a rotary tiller is used in combination with a weed brush, which mechanically disturbs the soil to a depth of up to 5 cm. For control of diseases, only solutions approved for organic farming were used, which are mostly copper or sulfur-based (Lešnik et al., 2016; *Pravilnik o ekološki pridelavi in predelavi kmetijskih pridelkov oziroma živil.*, 2018). The Gala apple variety (*Malus domestica* var. Gala) has been grown according to IPM management guidelines with biennial herbicide application according to the Technical guidance (*Pravilnik o integrirani pridelavi poljščin, zelenjave, hmelja, sadja in oljk ter grozdja*, 2023) in the herbicide strip under the trees for control of the weeds.

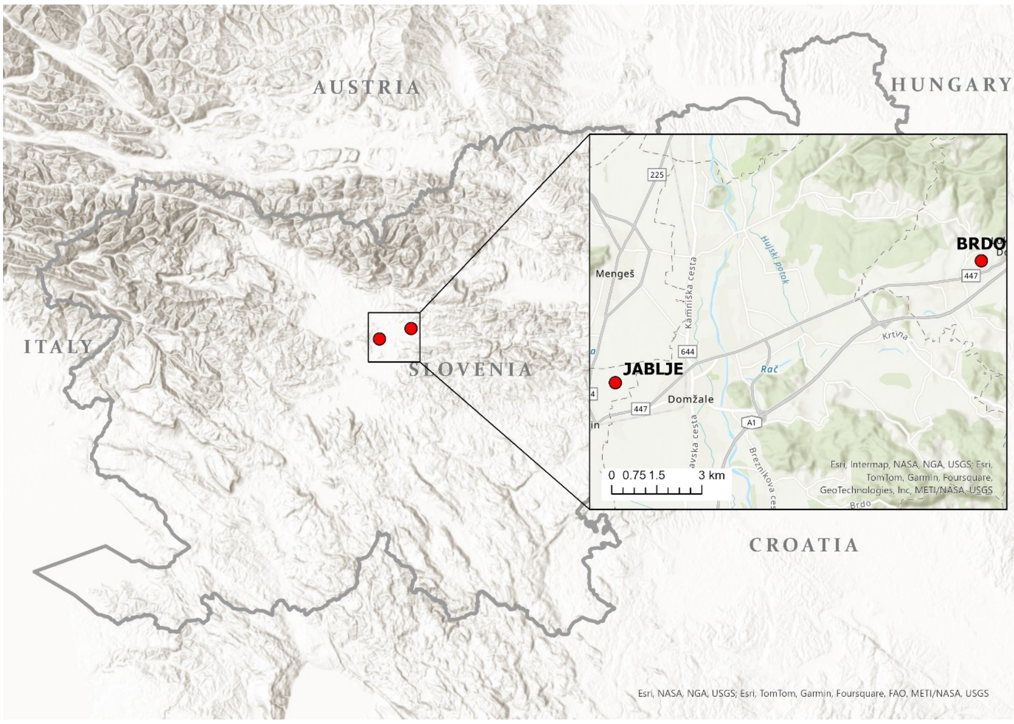

Figure 1. Location of study sites Jablje and Brdo.

Slika 1. Lokacija vzorčnih območij Jablje in Brdo.

Experimental setup and soil mesofauna sampling

Sampling was conducted in three agroecosystems: an arable field, a strawberry field, and an orchard. In the arable field, samples of soil were taken from three tillage methods: no-tillage, minimal tillage, and conventional tillage, with nine samples of each type, giving a total of 27 samples from the arable ecosystem. In the strawberry field, nine samples were taken from each of the organic and IPM sections, giving a total of 18 samples. Two-thirds of the samples were collected from the ridges next to the roots of plants, and one-third of the samples were collected from the interrow spaces between the ridges. In the orchard, we collected nine samples from both the organic and IPM sections, totalling 18 samples, with one-third taken from the root zone of the apple trees and two-thirds from the inter-row areas covered with grass. However, for data analysis, all samples were pooled, resulting in a combined sample size of n=9 for each agricultural system-method group.

Standardized soil samples were taken following the QBS sampling protocol (Parisi et al., 2005) using a soil corer with an 11.3 cm diameter to a depth of 10 cm to collect 1 litre of soil in each soil sample. Prior to sampling, the top layer of vegetation was removed using scissors. The soil was collected in plastic bags and transferred to the Kempson extractor (ecoTech, Bonn, Germany) (Kempson, 1963), where the soil mesofauna was extracted by air drying the samples for 10 days at 30 °C. Extracted animals were preserved in 70% ethanol.

Laboratory analysis of soil mesofauna

Extracted animal individuals were identified and counted using stereomicroscope and classified into taxonomic groups (on the level of class or order) and into eco-morphologic groups according to the protocol established for the calculation of the QBS index (Parisi et al., 2005). Eco-morphological groups are based on taxonomic groups (class or order level), which are assigned an eco-morphological index (EMI) value between 1 and 20 (possible values are 1, 2, 4, 5, 6, 8, 10, 15, and 20) according to their level of adaptation to the soil environment, with higher values indicating higher adaptation to soil life. The level of adaptation is determined by morphological characteristics such as body size, pigmentation, length of appendages and presence of eyes. The total number of eco-morphological groups available according to the QBS methodology is 53. Three categories of eco-morphological groups have been proposed by the QBS index authors: epiedaphic (values 1, 2, 4), hemiedaphic (values 5, 6, 8, 10) and euedaphic (values 15, 20) (Parisi et al., 2005).

QBS-ar was calculated by summing the highest EMI values of taxonomic groups. For example, Collembola were divided into seven eco-morphological groups with EMI values between 1 and 20. For QBS-ar (Parisi et al., 2005), only the highest EMI value recorded in the sample for Collembola was considered. The second modification was QBS-ar BF (D'Avino et al., 2023), which summarized the EMI values of all present eco-morphological groups in the sample. The third modification, named QBS-a (proposed in this article), considered information on abundance, which was ignored by the previous two, by multiplying the EMI value with the abundance of each eco-morphological group in the sample. The result was divided by 100 for readability. The fourth modification, named QBS-ab, was proposed by Mantoni et al. (2021), where the abundance of each eco-morphological group was logarithmically transformed before being multiplied by its EMI value. This was done to reduce the influence of the most abundant groups (Acarina and Collembola).

Data analysis

Cumulative abundance and log-transformed cumulative abundance of taxonomic and eco-morphological groups in all samples were calculated. Species richness rarefaction curves for each agroecosystem were used to determine whether our sampling was thorough with enough collected samples.

Principal component analysis (PCA) was used for soil mesofauna community analysis using QBS eco-morphological groups as species. Sample scores on the first and second PCA axes were compared among the seven system-method groups using ANOVA with Tukey post-hoc tests conducted in R software version 4.4.0 ("stats" library). Average species scores (loadings) of epiedaphic, hemiedaphic and euedaphic groups were calculated.

In our statistical analysis, we categorized our samples into seven system-method groups, each with nine samples: Arable field-conventional (n=9), Arable field-minimum tillage (n=9), Arable field-no-till (n=9), Strawberry-organic (n=9), Strawberry-IPM (n=9), Orchard-organic (n=9), and Orchard-IPM (n=9). Four modifications of the QBS index were calculated to estimate soil degradation. QBS methodology foresees computation of one QBS value based on EMI values of organisms collected in three samples. Therefore, for each system-method group, three QBS index values were obtained out of nine samples. QBS index values as a proxy for soil health were compared between the seven system-method groups using ANOVA with Tukey post-hoc tests.

Results

Examination of the cumulative abundance of taxonomic groups showed *Acarina* were the dominant group in all three arable field management systems, but their dominance was less pronounced in orchard and strawberry fields (Figure 2 A). Log transformation of the abundance improved visualization and statistics for the less abundant groups. The more pronounced differences were for *Hymenoptera* (ants), which were more abundant in the orchard but not in other systems, and larvae of *Coleoptera* and *Diptera*, which were more abundant in arable fields but very few were found in other systems (Figure 2 B).

Overall, 63 soil samples from three agroecosystems and 56.6 % (30) soil-adapted eco-morphological groups were recorded from a total of 53 as defined by the QBS index classification of mesofauna. In orchards, rarefaction curves of morpho-taxonomic groups showed sufficient sampling effort, but for arable and strawberry fields, the rarefaction curves did not reach an asymptote (Figure 3). Considering the rarefaction curve slope for the last five samples, we would gain approximately 0.35 and 0.25 additional eco-morphologic groups with each sample in strawberry and arable fields, respectively (Figure 3 A). As QBS methodology foresees the collection of three samples per sampling site we analysed the richness of eco-morphologic groups in the first three samples of each system-method groups. According to the confidence intervals of these rarefaction curves, the first three samples would detect between 30.5 and 78.0 percent of eco-morphologic groups (Figure 3 B).

PCA analysis was used to visualize differences in the community composition of mesofauna (based on eco-morphologic QBS index groups) for all three sampled agricultural ecosystems, with the first axis explaining 15.8 % of the variability and the second axis explaining 14.7 % of the variability (Figure 4). The first PCA axis was mostly determined by the abundance of very numerous *Collembola* (Figure 4 B) and showed large variability in the community structure of arable fields, especially among minimum tillage samples and lower variability in strawberry field and orchard samples. The seven system-method groups differed significantly ($F=5.3$, $df=6$, $p<0.001$), with post-hoc tests indicating statistically higher values of PCA scores in arable field under minimum tillage compared to arable conventional tillage and all orchard and strawberry field samples under both organic and IPM management. The average species score for the three groups (epiedaphic, hemiedaphic, euedaphic) had a similar length and direction (Figure 4 A).

The second PCA axis separated samples according to less abundant groups such as *Isopoda*, *Diplura*, *Coleoptera* and *Symphyla* (Figure 4 B). PCA sample scores on the second axis were less variable with no statistical differences between the seven system-method groups ($F=1.8$, $df=6$, $p=0.106$), with one of the orchard samples as a clear outlier (Figure 4 A).

In different systems, we observed between 16 (IPM orchard) and 21 (organic orchard) eco-morphologic QBS groups of soil mesofauna (Table 1).

The results of the QBS index varied considerably between different modifications of the index. According to both QBS-ar and QBS-ar BF, which do not consider the abundance of organisms, the highest values were observed for both the organic strawberry field (Strawberry - eco) and the integrated pest management (IPM) strawberry field (Strawberry - int), as well as the organic orchard, with the lowest values detected in the arable field and IPM orchard (Figure 5). Differences between the seven system-method groups were only marginally significant for QBS-ar ($F=3.2$, $df=6$, $p=0.034$), with no significant difference detected by post-hoc tests, and were not significant for QBS-ar BF ($F=2.1$, $df=6$, $p=0.122$).

On the contrary, the two modifications of the QBS index, which consider the abundance of eco-morphological groups, showed higher values for arable fields compared to fruit production agroecosystems (Figure 5). According to the QBS-ab system method, groups differed significantly ($F=8.5$, $df=6$, $p<0.001$), with the post-hoc test indicating higher values for all arable samples compared to strawberry fields under both organic and IPM management (Figure 5). Post-hoc tests also detected significantly higher values in arable minimum tillage compared to both organic and IPM orchard. The QBS-a also differed significantly between the agroecosystems ($F=10.2$, $df=6$, $p<0.001$), where arable minimum tillage received significantly higher QBS-a values than both fruit production systems under organic and IPM management (Figure 5). Furthermore, the QBS-a index of the arable field under minimum tillage was also significantly higher than the conventionally tilled arable field (Figure 5).

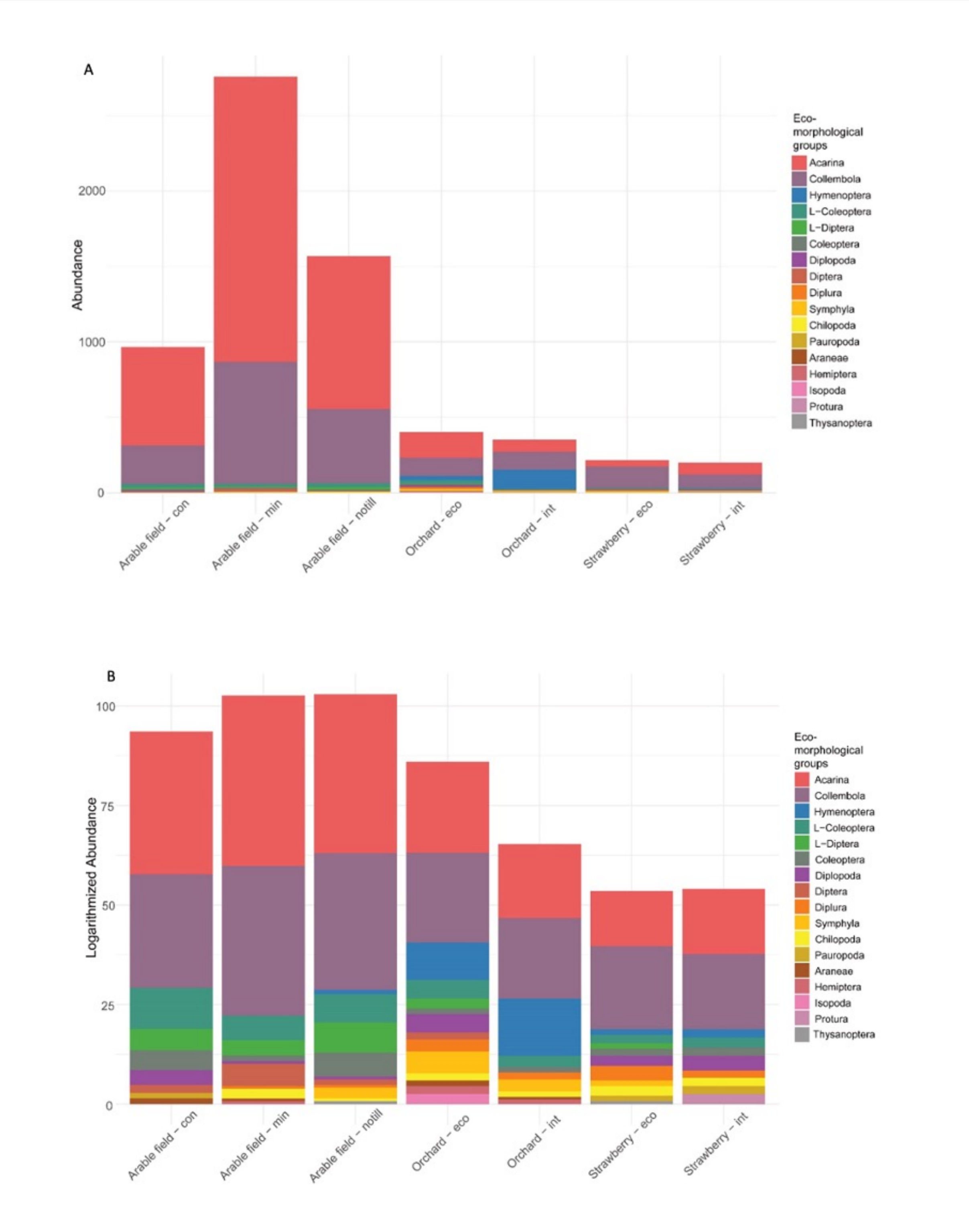

Figure 2. Cumulative abundance (A) and log-transformed cumulative abundance (B) of 17 taxonomic groups of soil mesofauna in arable field, orchard and strawberry agroecosystems of Slovenia with different management regimes. L indicates larval stages.

Slika 2. Kumulativna številčnost (A) in logaritmično transformirana kumulativna številčnost (B) 17 taksonomskih skupin talne mezofavne v njivi, sadovnjaku in nasadu jagod z različnimi režimi upravljanja. L označuje larvalne stadije.

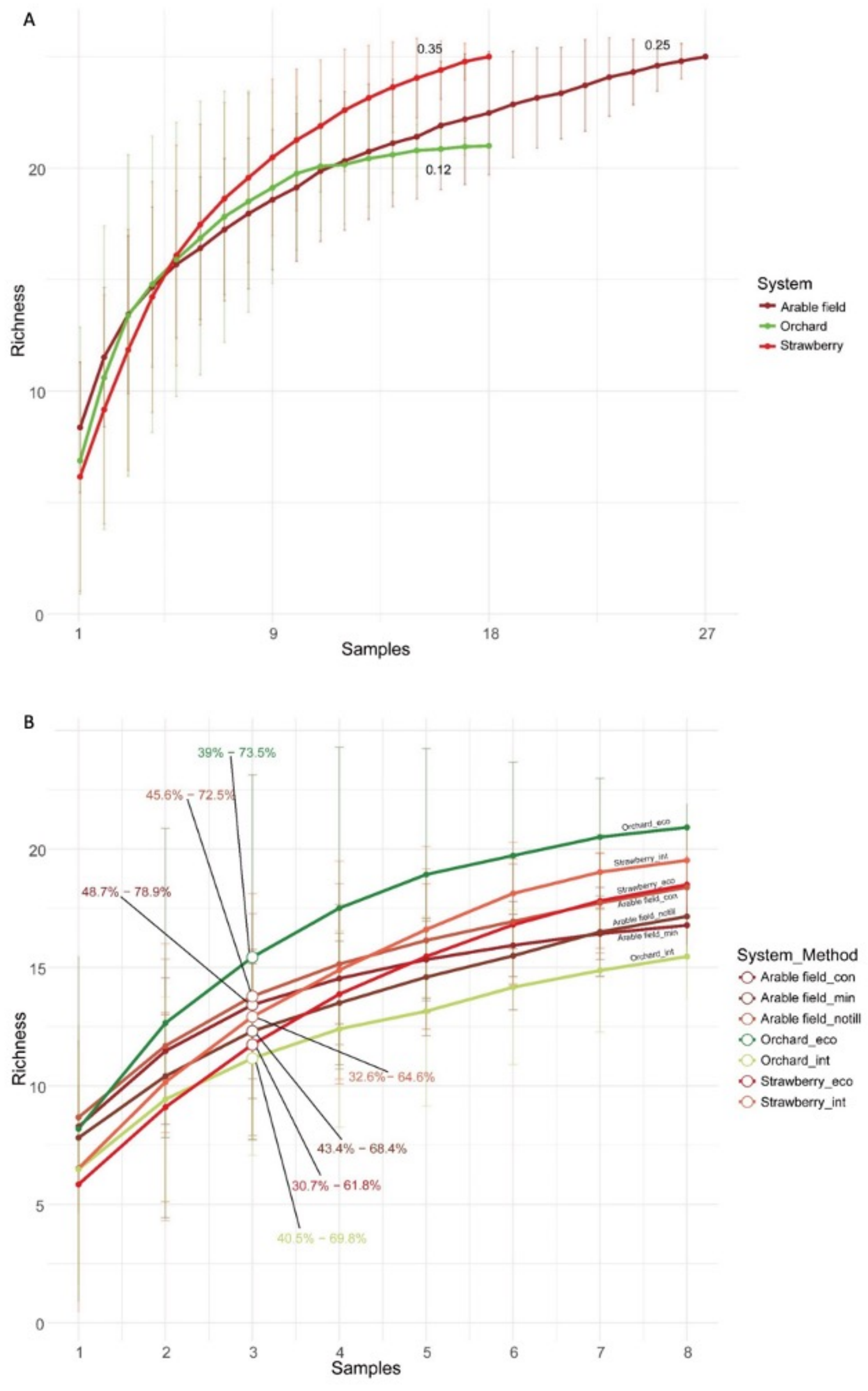

Figure 3. Rarefaction curves with 95% confidence intervals of morpho-taxonomic groups of soil mesofauna according to QBS methodology in A) three agroecosystems with numbers indicating slope for the last five samples and B) seven system-method groups with numbers indicating the percentage of morpho-taxonomic groups detected in the first three samples per sampling site, as required by the QBS methodology.

Slika 3. Rarefakcijske krivulje s 95 % intervali zaupanja za morfo-taksonomske skupine talne mezofavne po metodologiji QBS v A) treh agroekosistemih s številkami, ki prikazujejo naklon za zadnjih pet vzorcev, in B) sedmih sistemsko-metodoloških skupinah s številkami, ki označujejo odstotek morfo-taksonomskih skupin, zaznanih v prvih treh vzorcih na vzorčnem mestu, kot zahteva metodologija QBS.

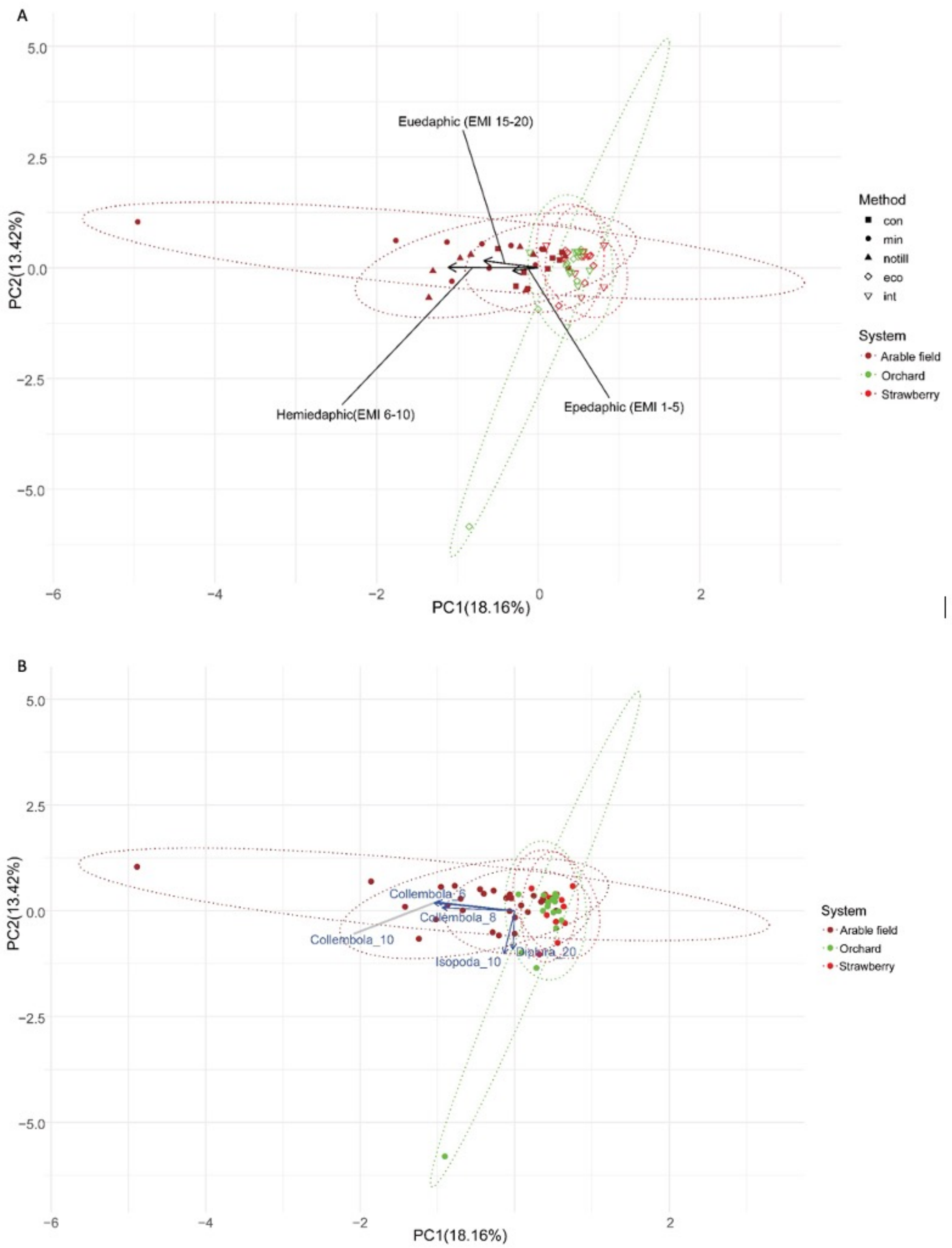

Figure 4. Principal component analysis (PCA) scores on the first two axes of A) arable field, strawberry field, and orchard under different management systems (int = IPM; eco = organic; con = conventional tillage; min = minimum tillage; notill = no tillage) with arrows indicating average species scores of epiedaphic, hemiedaphic and euedaphic groups and B) arable, strawberry field and orchard samples with arrows indicating species scores of 5 eco-morphologic groups with the highest species scores on both axes.

Slika 4. Rezultati analize glavnih komponent (PCA) na prvih dveh oseh za A) njivo, nasad jagod in sadovnjak pod različnimi režimi upravljanja (int = integrirano varstvo rastlin; eco = ekološko kmetovanje; con = konvencionalna obdelava tal; min = minimalna obdelava tal; notill = brez obdelave tal) s puščicami, ki kažejo povprečne rezultate vrst epiedafičnih, hemiedafičnih in euedafičnih skupin, ter B) vzorce njive, nasada jagod in sadovnjaka s puščicami, ki označujejo rezultate vrst za 5 ekomorfoloških skupin z najvišjimi rezultati vrst na obeh oseh.

Table 1. Number of detected eco-morphological groups for soil samples collected in three agroecosystems under different management.

Tabela 1. Število zaznanih ekomorfoloških skupin za vzorce tal, zbrane v treh agroekosistemih pod različnimi režimi upravljanja.

Agricultural ecosystem	Management	Number of eco-morphological groups
Arable field	Conventional tillage	17
Arable field	Minimum tillage	18
Arable field	No-till	19
Strawberry field	Organic	19
Strawberry field	IPM	20
Orchard	Organic	21
Orchard	IPM	16

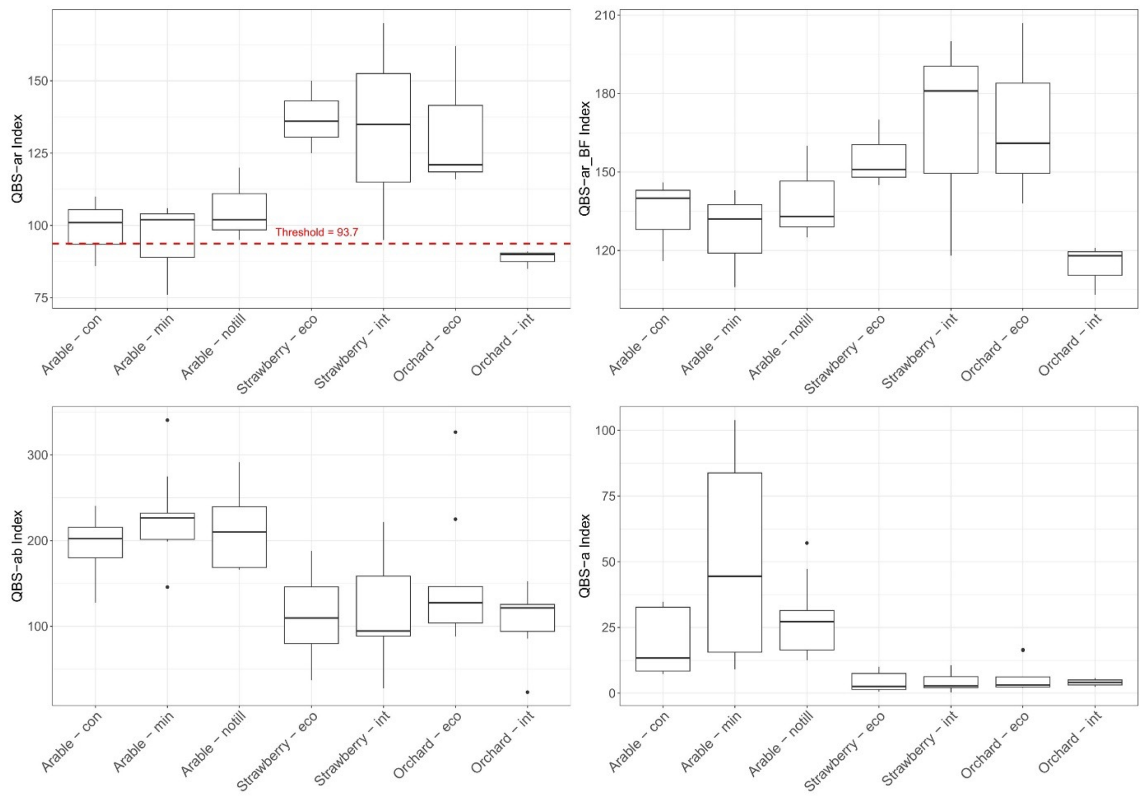

Figure 5. Median and quartile values of four QBS modifications: two without considering animal abundances (QBS-ar, QBS-ar BF), and two considering abundances (QBS-ab and QBS-a) for soil samples from seven system-method groups. The red line indicates a tentative threshold value of 93.7 for the QBS-ar index, separating high-quality soils above the threshold from poor-quality soils.

Slika 5. Mediana in kvartilne vrednosti štirih različic QBS za talne vzorce iz sedmih sistem-metoda skupin. Dva ne upoštevata abundance živali (QBS-ar in QBS-ar BF), dva pa abundanco upoštevata (QBS-ab in QBS-a). Rdeča črta predstavlja predlagano mejno vrednost QBS-ar (93,7) nad katero so tla visoke kakovosti, pod pa tla nizke kakovosti.

Discussion

Across all studied sites, *Acarina* and *Collembola* were the most dominant in samples. These two groups are the two most abundant and diverse taxa of soil mesofauna, which have also been the most investigated (Menta & Remelli, 2020). Their dominance in our samples was unsurprising as it has been reported in similar studies in Europe with the proportion of *Acarina* between 48-57 % and *Collembola* around 30 % in vineyards (Gagnarli et al., 2015), with similar proportions in olive orchards (Vignozzi et al., 2019). Similar proportions with dominant *Acarina* (65 %) and fewer *Collembola* (27.6 %) were reported in forest sites. However, these proportions were inverted in favour of *Collembola* (56.7 %) compared to *Acarina* (39.8 %) in cropland and meadows in a systematic study in France (Cluzeau et al., 2012).

In our study, the dominance of *Acarina* over *Collembola* was much more pronounced in arable fields under all three tillage methods but less pronounced in strawberry fields and orchards. This reduced abundance of *Acarina* in the strawberry field and orchard could be due to the higher sensitivity of *Acarina* to soil contaminants such as heavy metals compared to *Collembola* (Menta et al., 2008), although some experimental studies contradict this (Joimel et al., 2017). Considering the heavy use of copper-based pesticides in fruit production, the lower relative abundance of *Acarina* compared to *Collembola* in orchard and strawberry production could indicate their sensitivity to soil contaminants, although further chemical analysis of soil would be needed to confirm this. Additionally, the presence of black polyethene mulch in both the organic and IPM sections of the strawberry plantation at the Infrastructure Center Brdo may have contributed to a background level of microplastics in these soils. While no specific analysis of microplastics was conducted in this study, it is worth noting that microplastics are an emerging concern for soil health and may influence mesofaunal communities (Jemec Kokalj et al., 2024). Studies indicate that microplastics can adversely affect soil biodiversity and the health of mesofauna, including groups such as *Acarina* and *Collembola* (Shafea et al., 2023). Although assessing microplastic impacts was beyond the scope of this study, future research should consider this factor, particularly in systems where plastic mulching is commonly used.

Collembola, on the other hand, responds more to the soil perturbation associated with agricultural practices such as tillage and fertilization (Cluzeau et al., 2012). Reduced tillage had a positive effect on *Collembola* abundance compared to conventional tillage, with effect varying due to depth, climate, soil texture, but also tillage method and frequency and concurrent herbicide application (Betancur-Corredor et al., 2022). The positive effect of reduced tillage on *Collembola* abundance was also confirmed in our study of arable fields using different tillage methods.

PCA analysis showed that tillage intensity had a much greater effect on soil mesofauna community in arable fields than did the type of agroecosystem or the production method (organic or IPM). Variability in the abundance of different eco-morphological groups of *Collembola* seemed to be the most important driver of discrimination between our samples, as indicated by the first PCA axis. This is in line with the results of Chassain et al. (2024), who examined the effects of cropping systems on soil mesofauna density and diversity in 21 fields using practice intensity indicators and indexes and found that the tillage intensity index showed a major impact. In the case of our tested sites, the differences between organic and IPM fruit production varied in more than one management practice, and it is consequently impossible to disentangle their individual effects on soil biota. In the case of the orchard, the two methods differ in the types of pesticides used on trees (synthetic pesticides in IPM and copper-based pesticides in organic), in types of fertilizer (mineral fertilizers in IPM and organic fertilizers in organic) and in the application of herbicides for weed suppression under trees (IPM) and mechanical disturbance for weed removal (organic). In their recent review of below-ground arthropod diversity in conventional and organic vineyards (Di Giovanni et al., 2024), they pointed to unclear management aspects of organic versus conventional farming as the reasons for conflicting responses in soil biota. The same authors also stressed the importance of assessing individual management practices for soil biota functional groups.

The QBS-ar index values in our analysis (76–170) were comparable to those reported in other agroecosystem studies (Menta et al., 2018). A tentative threshold value of 93.7 was proposed for QBS-ar to distinguish high-quality soils from poorer ones (Menta et al., 2018). According to this criterion, all sampled management groups except the IPM orchard could be categorised as good-quality soils, with the strawberry field having the highest values. However, the QBS-ar index was not able to distinguish between the different tillage systems due to the high variability, resulting in no statistically significant differences between the farming systems. This suggests that while the QBS-ar index is sufficient for distinguishing environments with very different practices, it is not sensitive enough to detect subtle changes resulting from specific management practices such as tillage. Interestingly, the strawberry field — a highly disturbed environment covered with plastic mulch had high QBS-ar values. This could be due to the low number of organisms in the samples, which made it easier for the analyst to identify new ecomorphological groups (EMI) and thus increase the overall score. This highlights a potential limitation of the QBS-ar index: it may overestimate soil quality in disturbed environments where, due to sampling artefacts, low organism diversity coincides with an apparently high diversity of EMI. Conversely, environments with a high number of mesofauna — possibly due to high input of organic matter in disturbed environments — do not necessarily reflect better soil health. High numbers of mesofauna feeding on introduced organic matter may be a response to disturbance rather than a sign of a healthy, stable ecosystem. When looking at organism abundance, the QBS results were reversed, with arable areas with different tillage methods showing the highest values. Only the indices that included abundance (QBS-ab and QBS-a) were able to distinguish between different tillage practices, which is consistent with the PCA analysis. These indices, which are sensitive to changes in population density due to agricultural interventions such as tillage, were indeed able to detect abundance-related differences. These results emphasise the complexity of assessing soil health using these indices. Non-abundance indices (QBS-ar) provide information on overall soil quality and ecological stability but may not accurately reflect the nuances of disturbed environments or the effects of organism abundance. Indices with abundance (QBS-ab and QBS-a) provide a more detailed insight into the effects of management actions on soil mesofauna but do not necessarily correlate with improved soil health. Our study emphasises the need for more comprehensive methods to assess soil biodiversity and health. Although QBS indices provide valuable information, they can only capture the dynamics of the soil ecosystem to a limited extent. New techniques such as DNA metabarcoding could provide deeper insights by enabling more accurate identification and quantification of soil organisms and thus improve our understanding of soil health in different agroecosystems. We recommend that researchers carefully consider their objectives and the limitations of each index when selecting a soil biodiversity assessment method. For general assessments of soil quality or ecological stability in very different environments, the QBS-ar may be appropriate but should be used with caution in disturbed environments with low organismal diversity. To detect subtle changes and examine the effects of agricultural practices where organism diversity varies, QBS-ab and QBS-a are more suitable, although they may not fully reflect soil health. Ultimately, a multifaceted approach that combines traditional indices with advanced molecular techniques may be necessary to gain a comprehensive understanding of soil biodiversity and health. This integrated strategy would contribute to better-informed agricultural management and conservation efforts by enabling a more accurate assessment of the impact of different practices on soil ecosystems.

Originally, the QBS methodology foresees the collection of three samples per sampling site, but we increased the effort to nine samples. Rarefaction curves showed that nine samples were sufficient for orchards but not for arable and strawberry fields, where the asymptote was not reached. According to the rarefaction curves, only between 30.5 and 78 percent of eco-morphologic groups would be detected by collecting three samples. This indicates that spatial heterogeneity of soil mesofauna differs between the agroecosystems, and the number of samples should be higher than the three originally proposed in more heterogeneous ecosystems.

Although this study was limited to three agroecosystems with only one sampling site, it gives a valuable first insight into the diversity of soil mesofauna within and between agroecosystems. A comparison of different tillage and production methods identified tillage as an important factor determining the soil mesofauna community. However, the complexity of agricultural practices in organic and IPM fruit production makes it impossible to disentangle the environmental factors affecting this community.

Supplementary Materials

Suppl. Table S1. Abundances of collected taxonomic groups of mesofauna and their proportion of the total sample.

Author Contributions

Conceptualization, V.N, I.B., N.Š., T.G.; methodology, V.N., I.B., N.Š..; formal analysis, V.N.; writing—original draft preparation, V.N. and I.B.; writing—review and editing, N.Š. and T.G.; visualization, V.N.; All authors have read and agreed to the published version of the manuscript.

Acknowledgement

We would like to thank Agricultural Institute of Slovenia colleagues Dr. Nika Cvelbar Weber, Dr. Robert Leskovšek and Roman Mavec for establishing and maintaining long term experiments which we sampled. We thank Monika Gričnik for producing the map of the study sites.

Funding

This study was co-funded by the Slovenian Research Agency (research programs P4-0431 and P4-0107, and research project J4-3098), Slovene Ministry of Agriculture, Forestry and Food (project CRP V4-2221) and Slovenian Research Agency (project CRP V4-2221).

Data Availability

Data supporting this study will be made available upon request to the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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