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Estimating the microarthropod diversity in cropping systems by comparing ecological indices across Europe[☆]

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ABSTRACT

Microarthropods are pivotal components of the soil mesofauna, significantly contributing to the enhancement of soil quality and structural properties. The evaluation of microarthropod diversity provides valuable information on soil health, owing to their contribution to specific ecosystem services of soil. To monitor mesofauna diversity, the definition and validation of structural ecological indices capable of comprehensively assessing microarthropod community diversity and functionality is needed. In this work, several ecological indices (Hill's number, Acari: Collembola, QBS-ar, FEMI, QBS-ab) based on microarthropod biological forms (BFs) were applied to evaluate soil mesofauna biodiversity in 17 agroecosystem sites under either organic or integrated management. In addition, a novel calculation of the QBS-ar, which consider all observed BFs, called QBS-ar BF, was proposed and compared to the other indices. The frequency and abundance of BFs highly adapted to soil life differ between management systems (integrated < organic) and crop duration (pluriannual > annual). Overall, the indices that best discriminated the different management in cropping systems were those based on the QBS approach (QBS-ar and QBS-ar_BF) . Moreover, the newly developed QBS-ar_BF index highlighted a higher discrimination capacity due to its inclusion of all observed BFs, i.e. all microarthropod community spectrum information. This study confirmed the pivotal role of edaphic mesofauna in highlighting the differences between soil management and crops across Europe, opening perspectives to new monitoring frameworks to detect shifts in community structure and functional traits.

1. Introduction

The loss of soil biodiversity is a global threat for soil health and novel policies need to be timely implemented as adequate countermeasures (Van der Putten et al., 2023). The European Union set ambitious policies aimed to restore soil ecosystems by increasing 10 % high-diversity agricultural land by 2030, and by improving ecological status of

degraded soils (EU, 2021). To this purpose, the development of a well-structured biodiversity monitoring at European level, will become fundamental to achieve those goals (European Union, 2024). However, to be able to monitor soil health – and in particular biodiversity losses – it is of utmost importance to define and validate multiple structural ecological indices which recapture the diversity of soil biota at multiple levels (Harris et al., 2022; Ritz et al., 2009; Stone et al., 2016).

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The evaluation of mesofauna diversity provides valuable information on soil health (Menta and Remelli, 2020), owing to the connection between community diversity and a role in the provision of specific soil ecosystem services (George et al., 2017). More specifically, following the integrative framework described by Creamer et al. (2022), the soil mesofauna might be framed as contributing to diverse soil functions. Microarthropods, i.e., Arachnida, Crustacea, Myriapoda, Hexapoda, with body width smaller than 2 mm, are considered significant components of the soil mesofauna (Coleman et al., 2018), able to enhance soil quality and soil structural properties. Microarthropods are primarily involved in Carbon and Climate Regulation and Nutrient Cycling functions. They sustain the Carbon and Climate Regulation function by carrying out the fundamental process of decomposition and litter transformation (Culliney, 2013). Furthermore, they can regulate CO₂ respiration performed by microorganisms because of their microbial grazing (Scheu et al., 2005). As such, they act as one of the first steps of the soil trophic chain, allowing resources to be assimilated by the food web. Consequently, incorporating soil nutrients into their biomass also performs a paramount role in the Nutrient Cycling function. Additionally, they are also involved in "Disease and Pest Regulation" as microarthropods can reduce soil pathogens and control soil pests via their grazing and predation activities (Innocenti and Sabatini, 2018; Neher and Barbercheck, 2019). These functions contribute to define microarthropods as excellent biological indicator (Pankhurst et al., 1997; Doran and Zeiss, 2000; Bedano et al., 2011; George et al., 2017; Lehmann et al., 2020; Ma et al., 2024). Microarthropods already proved to be useful bioindicators of the impact of different arable land management; practices (e.g. Menta et al., 2017; Menta et al., 2018a; Madzaric et al., 2018; Galli et al., 2020; Fiorini et al., 2020; Menta et al., 2020; Warren Raffa et al., 2021), such as integrated and organic farming (Mantoni et al., 2021). However, the lack of ecological indices that take advantage of these features in monitoring policies, highlights a still limited appraisal of microarthropod functions in legislations, which needs to be addressed at different scales (Geisen et al., 2019; Anthony et al., 2023). Moreover, the implementation of extensive soil monitoring programs is a need at European level, but it can be extremely demanding, especially if multiple levels of soil biota are considered. Difficulties with species-level taxonomic identification led research to also focus on other taxonomic levels instead of species.

Parisi et al. (2005) proposed to group microarthropods in relation to their morphological adaptation to soil life, extending the well-known concept of evolutionary adaptation in Biological Forms (BFs), with no need to perform cumbersome and precise taxonomic classification to the species level. This approach has been successfully applied in numerous studies to describe soil quality through microarthropods presence in many pedo-climatic and environmental conditions worldwide (Yan et al., 2012; Blasi et al., 2013; Galli et al., 2014; Menta et al., 2017, 2018a, 2018b; Galli et al., 2021; Maienza et al., 2022; Tóth et al., 2025). The QBS-ar index was originally conceived by Parisi et al. (2005) and proved to be both sensitive to soil practices and able to discriminate between different land uses and different levels of soil degradation (Menta and Remelli, 2020). The recognition and enumeration of BFs rather than species is one of the strengths of the method. As a matter of fact, BFs identification relies on morphological traits instead of species level identification, fostering the method applicability. In addition, the enumerated BFs, unlike taxonomic species, can be directly connected to ecological aspects of soil life adaptation by the Eco-Morphological Index (EMI), a numerical value assigned to each specific BF (from 1 to 20). The higher the EMI value assigned to a BF, the higher its adaptation to soil life. The BFs that were identified by Parisi et al. in 2005 were 44, while Menta et al. (2018a) reported 48 BFs. To date, 54 BFs (split in frequent and less frequent in European soils) have been identified (D'Avino et al., 2024). Probably, extending the method to global applications, the number of BFs identified will increase. Since this method aims at evaluating the maximal community adaptation level, in cases of microarthropod groups composed by multiple BFs with different EMI score,

such as Collembola or Diplopoda, only the BF with the highest EMI within the group is considered for the QBS-ar calculation. This method, reported in an EU technical report already for several years (Turbé, 2010), has a freely accessible tool/protocol (D'Avino et al., 2024) and is being adopted by the FAO Glosolan network (FAO, 2025). Moreover, it was already used in Italy by public authorities to assess the impact of policies, such as the effect of conditionality measures foreseen by the Common Agricultural Policy 2007-2013 (Landi et al., 2014) or the national plan for sustainable use of plant protection products (D'Antoni et al., 2020). Nevertheless, to the best of our knowledge, a soil mesofauna national monitoring plan has never been implemented, even though the QBS-ar index was used for regional monitoring survey in several Italian regions, (Regione Emilia-Romagna, 2025; SNPA, 2025; ARPA FVG, 2025). Thus, it would be desirable that such an index enters the list of methods foreseen in the shortly expected EU Regulation on soil health monitoring that can be implemented at the national level and later considered among those suitable for the European Union soil health monitoring activities (definition art 3.4 proposal directive on soil monitoring law, European Commission, 2023).

Nevertheless, individuals with lower EMI, i.e. the epigeic forms, also have an ecological role in the trophic chain that should not be underestimated, since all microarthropods are continuously interacting in a complex web of interactions to sustain ecosystem services and support soil health (Neher and Barbercheck, 2019; Magdoff and Van Es, 2021). Thus, since a complete evaluation of diversity should be directed at the whole community, a new formulation accounting for all observed BFs should be considered. This would combine the fairness of whole-community analysis with the positive aspects of a synthetic and reliable index such as the QBS-ar.

Hence, the objective of this study was the evaluation of micro-arthropod diversity by the comparison of several commonly used ecological indices based on BFs, highlighting community diversity features. Specifically, these indices were Hill's numbers (Hs), *Acari:Collembola*, QBS-ar, FEMI (Faunal Eco Morphological Index), QBS-ab. Through this comparison, the development of a novel formulation of the QBS-ar method (here named QBS-ar_BF) considering all the different recorded BFs was established to test a whole-community analysis.

To tackle this goal, soil samples from different European agroecosystems managed with either organic or integrated methods were collected and analyzed to assess the performance of the different indices. We hypothesized that (i) the compared indices differ in their ability to discriminate among microarthropod communities, and (ii) the newly proposed QBS-ar_BF index provides greater discrimination between different soil management systems.

2. Methods

2.1. Sampling sites description

In this study, a total of 17 experimental sites were used. Cropping systems were distributed in 6 European countries (Austria, Denmark, Germany, Italy, Poland, Slovenia), and were under either organic (O) or integrated (I) pest and fertilization management types. Sites under three different cropping systems were included: apple orchard (A) or arable fields intended for planting strawberries (S), or fresh tomatoes (T) (Supplementary Fig. S1). In this study, the 'cropping systems' approach is employed in lieu of the more conventional 'crop types' approach. This is due to the fact that all field trials were part of a planned rotation, and the life span of microarthropods (e.g. Oribatida) can exceed that of arable crop cycle (Lu et al., 2025). The following comparison was thus conducted: permanent cropping systems (i.e. apple orchard, A), semipermanent cropping systems (rotation aimed at the cultivation of pluriannual strawberry, S), and multiple cropping systems (rotation with crop cycles shorter than one year, designed for tomato production, T). The full description of each sampling site is reported in Supplementary Table S1. It includes data about the nomenclature of territorial units

(NUTS), environmental zone (referred according to Metzger, 2018), latitude, longitude (WGS84), elevation (m), climatic indices (obtained by Climate Downscaling Tool ClimateDT - soil type classification WRB (IUSS Working Group WRB, 2022), as well as information about the field management method, crop, and sampling period.

2.2. Sampling, extraction, and identification of microarthropods

Soil sampling was performed between March and June 2021 (Supplementary Table S1), to ensure seasonal consistency across the sites. The spring and the autumn period are considered the most suitable for mesofauna analyses (Menta et al., 2017; Joseph et al., 2014; Parisi et al., 2005; D'Avino, 2002). Periods of fertilizers/tillage application, or periods with extreme climatic events (precipitation or drought, cold or hot temperatures) were avoided, to reduce the risk of population biases due to vertical migration, immobilization, aestivation or activity of soil microarthropods. The sampling area was selected for homogeneity of slope and vegetation and avoiding other factors such as an overabundance of animals (e.g., Formicidae), or the presence of decomposing organic matter (e.g., animal remains), that could affect the microarthropod population. Proximal sensing techniques were also applied to investigate the homogeneity of the topsoil (Loonstra and Van Egmond, 2009; Priori et al., 2016; Van Egmond et al., 2010). Samples were collected in three replicates of 1 dm³ of soil (10x10x10 cm), located at least 10-15 m apart. In apple orchards, two replicates were obtained in the non-compact part of the interrow and one along the row, almost 20 cm from the tree trunk. Overall, 51 soil samples were collected and grouped as described in Supplementary Table S1.

Field collected soil samples were preserved in a cool box during transport. Extraction of soil microarthropods was carried out within 48 h from the sampling for 7-15 days with a 2 mm mesh Berlese-Tullgren, except for Slovenian site n°10 (described in Supplementary Table S1) that used Kempson extractor (Bruckner, 2024). A vessel containing a preservative liquid (75 % ethanol- glycerol 2:1 v:v) was positioned under the funnel to collect the microarthropods. The extracted microarthropods were preserved in the same liquid at room temperature and in the dark. Microarthropod identification was performed using a stereomicroscope at 20× - 40× magnification, classifying each specimen, as previously reported, as biological form according to Sacchi and Testard (1971) as belonging to epigeous, hemiedaphic or euedaphic habitat classes. The precompiled sheet from D'Avino et al. (2024) was used for data collection; it reports a total of 54 biological forms, including 16 which are infrequent in European soils. For each sample, EMIs were recorded on the precompiled sheet together with abundances, which were used to further characterize the population biodiversity.

2.3. Biodiversity indices

As briefly previously introduced, the commonly used diversity indices for the study of the microarthropod community, employed at the biological form level, were the followings:

- The Hill's numbers (H values), focusing in particular on q equal to 0, 1, and 2 (Hill, 1973; Chao et al., 2014), and correspond to richness, Shannon diversity, and Inverse of Simpson index of biological forms, respectively. Among the vast multitude of available diversity measures, Hill's numbers are increasingly used (Chiu and Chao, 2020; Mantoni et al., 2021)
- The total abundance of microarthropods per square meter, as the sum of the abundance of all microarthropod BFs, retrieved in a sample, consisting of the mean of the three replicates, 100 cm² each.
- The Acari:Collembola abundance ratio (Bachelier, 1978), known for being correlated to soil disturbance (Tabaglio et al., 2009).
- The QBS-ar index (Parisi et al., 2005), which assigns an EMI value to each biological form measuring the microarthropod community adaptation to soil life. The EMI sum of observed biological forms

- determines the QBS-ar value. In cases of biological forms with multiple EMIs, only the highest is selected.
- The FEMI index (Yan et al., 2012), which considers the Faunal EMI values, the highest abundance value, and the total amount of taxa.
 Specifically:

$$F_{EMI} = rac{S_0}{S} stimes^{rac{S_0}{2}} \left(rac{d_{i_0}}{d_{iMAX}}stimes EMI_i
ight)}{\sum\limits_{i=1}^{S} \left(EMI_i
ight)}$$

Where S is the total BF richness across all samples, S_0 is the BF richness in the sample, d_{i0} is the abundance of the ith BF in the sample, d_{iMAX} is the abundance of the ith taxa across all samples, and EMI_i is the EMI score of ith BF.

- The QBS-ab index (Mantoni et al., 2021), whichaims to weight the different BFs based on the abundance of retrieved animals. To this aim, the QBS-ab index has the same calculation as QBS-ar, but the EMI scores of each BF is multiplied by its abundance priori to index calculation. To reduce the impact of highly abundant groups, abundances were log10-transformed. Indeed, Acari and Collembola frequently exhibit higher levels of abundance when compared to other BFs, often surpassing them by two or three orders of magnitude.
- The newly developed formulation of the QBS-ar, named QBS-ar_BF, which takes into account all the different biological forms observed. The QBS-ar_BF index has the same calculation as QBS-ar but, in cases of taxonomic groups with multiple EMI scores (Supplementary Table S2), the EMI of all BFs is considered in the sum instead of the maximum EMI score only.

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2.4. Data analysis

To better describe the novel index QBS-ar BF proposed here, a more detailed description of the OBS-ar calculation is proposed. The OBS-ar method defines BFs in order to assign an unambiguous EMI, proportional to soil adaptation (Supplementary Table S2). The BFs is defined, in a streamlined manner, as the group that has an EMI value. In many cases the entire taxon exhibits the same level of adaptation to the soil, consequently the BF is identified with a class (e.g., Simphyla, Pauropoda, Protura) or with an order (e.g., Pseudoscorpiones, Opiliones, Isopoda, Psocoptera, Embioptera). In other cases, it is necessary to delve deeper to the life stage (i.e., Holometabolous insects or Cicada nymphs). Otherwise, the BFs depends on size (e.g., spiders or diplopods) or specific traits (e.g. springtails or beetles). The QBS-ar index value of a sample is obtained by the sum of the EMIs of the observed BFs in all (three) replicates collected for a certain soil. However, in the case of BFs belonging to the same taxonomic group (e.g., Chilopoda, Collembola or Coleoptera), only the highest EMI within the group is included in the calculation.

In this setting, it is important to assess whether the three replicates effectively represent a homogeneous community context, since any biological form could be retrieved from a single replicate to be included in QBS-ar calculation. This analysis was conceived by Parisi (2001), but never formalized, and showed that the homogeneity of replicates should be validated through the analysis of the spectrum of the community (so-called Spectral Analysis, SA). A formalization of the SA formula is

proposed in the following equation, considering observed BFs:

$$SA = \frac{\sum_{j=1}^{n} |\alpha 1j - \alpha 2j| + \sum_{j=1}^{n} |\alpha 1j - \alpha 3j| + \sum_{j=1}^{n} |\alpha 2j - \alpha 3j|}{\sum_{i=1}^{3} \sum_{j=1}^{n} \alpha ij}$$

Where: α_{ij} corresponds to the EMI value of j^{th} BF in i^{th} replicate (i from 1 to 3) when the BF is recorded, while corresponds to 0 if the BF is absent. SA is calculated as ratio of the sum of the difference among α_{ij} of all possible pairs of replicates and the sum of all α_{ij} in all three replicates.

Therefore, the more the three replicates are similar (and BFs are the e same in all three replicates) the more the numerator and, consequently, SA tends to zero. According to QBS experience, a threshold value of 1 for SA is proposed to estimate whether the three replicates belong to the same edaphic community spectrum (SA \leq 1); with higher SA, the replicates are considered non-representative, and the sampling area is not homogeneous (Parisi et al., 2005). In this study only those sites that had $SA \leq 1$ were analyzed. All statistical analyses intended for index comparisons were performed in R software v 4.2.1 (R Core Team, 2022). Significant testing and magnitude of index values between samples with different management or cropping system was evaluated using the Wilcoxon rank sum test, as implemented in the ggpubr and rstatix packages, respectively (Kassambara, 2022a; Kassambara, 2022b). The Kruskal-Wallis test was used to perform a cross-comparison between management systems (I, O) and crop types (A, S, T), by building and using a composite variable. However, to limit the "field effect" in different NUTS2 that unpredictably could affect the crossing results, this statical analysis was performed on a data subset. This dataset comprised all combinations, but located in a radius of 13 km in Emilia-Romagna NUTS2 (same climatic and soil region, Supplementary Fig. S1 and Table S1).

The whole set of indices was further explored with multivariate analysis, namely Redundancy analysis (RDA), to evaluate which of them explained the highest variance on the RDA1 dimension, the dimension on which the difference between samples from integrated and organic managed soils is represented. Prior to RDA ordination, indices were standardized using z-score transformation. The RDA ordination and model testing with ANOVA, were performed using *vegan* package (Oksanen et al., 2023). The *rstatix* package was also used to calculate the Wilcoxon rank sum test for testing the difference in abundance of the different BFs and EMI groups between the two soil management types. The Multilevel pattern analysis (De Cáceres and Legendre, 2009) was used to evaluate the association between BFs and the management type, to identify a subset of BFs that could be considered as indicators of the management type (multipatt function in the *indicspecies* R package with 9999 permutations and using option func = "r.g").

3. Results

3.1. Exploring the connection of specific BFs to farm management type and cropping system

In total, 3158 animals were isolated and assigned to a biological form, and possibly grouped into relative taxon. The most abundant taxon at all sites were the subclass Acari (with an estimated value of 3616 individuals per m^2), the class Collembola (1284 per m^2 , out of which approximately 13 % belonged to euedaphic forms), and the order Hymenoptera (673 individuals per m^2 , of which about 90 % belonged to the Formicidae family). Subsequently, the order Araneae (mainly >5 mm length), the order Collember Symphyla and, among the class Collember Insecta, many juvenile stages (larvae) belonging to the orders Collember Insecta and Collember Insecta were identified.

A detailed evaluation of microarthropod community composition first focused on the distribution of the specific BFs in soils characterized by different management practices (Fig. 1A). Diptera larvae (EMI = 10; Wilcoxon rank sum test, p -value = 0.00199), Coleoptera larvae (EMI = 10; Wilcoxon rank sum test, p -value = 0.0317), Diplura (EMI = 20;

Wilcoxon rank sum test, p -value = 0.0460), and *Collembola* (EMI = 8; Wilcoxon rank sum test, p -value = 0.0075, and EMI = 20; Wilcoxon rank sum test, p -value = 0.0430) were significantly more abundant in O than in I managed soils. These results were confirmed when BFs were averaged on their EMI values (Fig. 1B), highlighting a significantly higher abundance of hemi- and euedaphic BFs (EMI 20, 10, and 8; Wilcoxon rank sum test, p -value = 0.0043, 0.0001, 0.0074, respectively) in the O managed sites with respect to those from I management.

Beside abundance, we also evaluated the incidence of BFs between soils from different managements (i.e., frequency of samples in which a BF was observed with respect to the total I or O samples). Generally, all BFs showed a higher frequency in O managed sites, except Formicidae and the very rare *Protura* (Fig. 1C). Euedaphic forms (i.e., Collembola with highest EMI values, *Diplura*, *Pseudoscorpiones*, euedaphic *Diplopoda*, *Pauropoda*, euedaphic *Chilopoda*) were more frequent in O managed sites than in I ones. *Acari* (EMI = 20) were the only BF found in all samples, whose total absence, however, would have emphasised an impairment of soil functionality (Gulvik, 2007; Behan-Pelletier and Lindo, 2023). At the same time, flies and other adult *Diptera* were often present in both I and O sites.

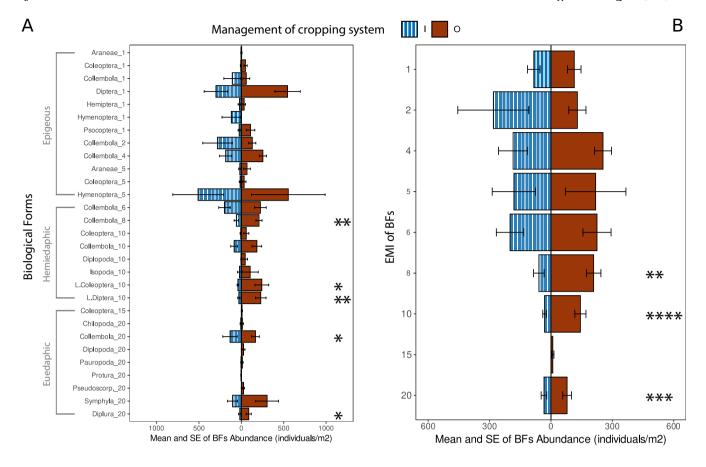
The three cropping systems showed differences regarding the composition of the microarthropod BFs community (Fig. 2A), as well as in the distribution of certain BFs (Fig. 2B): in established apple orchards *Diptera*, *Collembola*, *Hymenoptera*, and *Coleoptera Larvae* were observed with the highest abundance in all samples. On the other hand, fresh tomato sites showed the lowest abundance of these BFs, where only *Diptera* and *Acari* were observed in all samples. In addition, sites where strawberries were planned to be planted showed intermediate BFs values, but none (except *Acari*) were observed in all samples.

3.2. Comparing indices for microarthropod community assessment in organic and integrated farms

The diversity of microarthropod community in the whole dataset was explored using and comparing the applied ecological indices. All indices, except for A/C (p = 0.96) and total microarthropod abundance (p =0.24), were able to significantly discriminate (Kruskal-Wallis test; p <0.05) the microarthropod communities according to the two management systems (See text annotation above boxplots in Fig. 3): The O sites had higher indices values than the I (Fig. 3). However, the indices had different efficiency in differentiation of farm management, with an effect size ranging from 0.55 for H with q = 0 (p-value = 0.026; representing species richness, Fig. 3A) to 0.65 for QBS-ar_BF (p-value = 0.005; Fig. 3F). The effect size was 0.56 for FEMI index (p-value = 0.021; Fig. 3B) and 0.61 for H with q = 1 (p-value = 0.011; representing Shannon's diversity, Fig. 3C), while it was similar (0.63) for both QBS-ab (p-value = 0.008; Fig. 3D) and QBS-ar (p-value = 0.011; Fig. 3E). While H discriminated the microarthropod community of O and I managed soils when the q value was ranging from 0 to 1, this discrimination was no longer evident when the q value rose above 2 (representing the inverse of Simpson index) with a plateau being reached at q = 8 (Fig. 3G).

The indices did not clearly discriminate against microarthropod community diversity according to the three cropping systems (Supplementary Fig. S2); indeed, only H with q=1 (i.e., Shannon diversity) showed a significant difference based on the cropping system. Overall, diversity tended to be higher in permanent cropping systems (A), intermediate in semi-permanent cropping systems (S) and the lowest in multiple cropping systems (T).

The cross-comparison of the Emilia-Romagna data subset (Supplementary Table S3) confirmed that QBS-ar and QBS-ar_BF discriminated between crops and management most effectively ($p \le 0.01$). These were subsequently followed by QBS-ab, FEMI, H with q=0 ($p \le 0.05$). Conversely, H with q=1, total abundance, H with q=2 and Acari: Collembola were found to be non-significant in differentiating between the six groups identified through cross-comparison. Redundancy analysis (RDA) was employed on a set of 6 indices (QBS-ar_QBS-ar_BF, QBS-



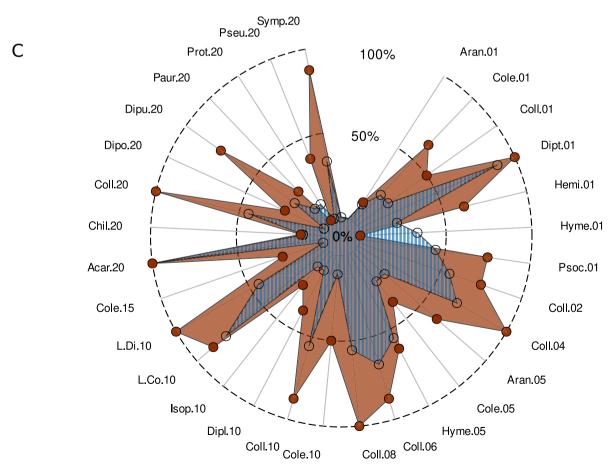


Fig. 1. Mean \pm SE abundance (individuals/m²) of microarthropod community biological forms (BF) in cropping systems managed with integrated (I, dashed azure) and organic (O, brown) methods, except *Acari*, excluded in the Figure for better visualization. Panel (A) reports abundance for all BFs included holometabola larvae (L.) and except *Acari*, removed to allow for a better visualization of the data, due to their high abundance, see text for details; panel (B) reports BFs grouped by their ecomorphological index (EMI), abundance of *Acari* were excluded from EMI 20 for a better visualization of the data. Statistically significant differences based on Wilcoxon rank sum test are reported as notation on the plot (* = p-value <0.05, ** = p-value <0.001, *** = p-value <0.001, *** = p-value <0.0001). Panel (C) shows the percentage of samples in which BFs were observed in integrated (I, dashed azure shade) and organically (O, brown shade) managed cropping systems (values in radar plot expressed as fraction respect to total O or I samples).

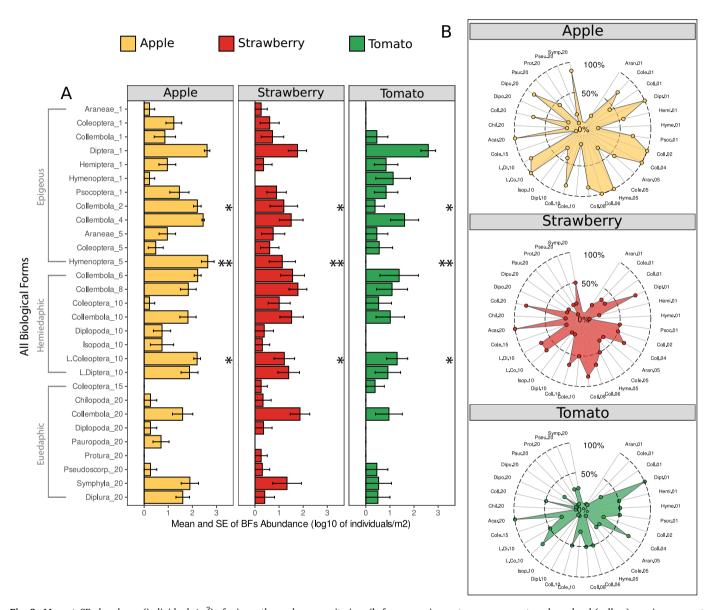


Fig. 2. Mean \pm SE abundance (individuals/m²) of microarthropod community in soils from cropping systems: permanent apple orchard (yellow), semi-permanent intended for strawberry production (red), and multiple intended for tomato production (green). Panel (A) reports data of all biological forms (BFs), *Acari* were excluded for better visualization, due to their high abundance, see text for details. Statistically significant differences based on Wilcoxon rank sum test are reported as in Fig. 1. Panel (B) shows the percentage of samples in which BFs were observed in permanent (apple orchard, yellow), semi-permanent (including strawberries, red), and multiple (including tomato, green) cropping systems (values in radar plot expressed as fraction respect to total samples from each cropping system).

ab, FEMI, *Acari:Collembola*, and 11 different q values for the Hs calculation) using the management (I, O) and cropping system (A, S, T) as constraints to verify whether a multivariate approach yielded the same evidence as the univariate one, and to determine which index/indices mostly contributed to the differences in a multivariate setting. The overall model showed to be statistically significant (ANOVA test with permutations; p-value = 0.018, applied model = indices \sim Management + Cropping system). However, upon the evaluation of each term, only the management system term of the model resulted significant

(Management: $F_{1,12}=5.459$, p-value = 0.004; Cropping system: $F_{2,12}=1.357$, p-value = 0.286). Moreover, the differences between O and I management were captured by the first dimension of the analysis (RDA1), which was indeed significant ($F_{1,12}=7.339$, p-value = 0.009) and explained 36.4 % of constrained variance, while second axis (RDA2) was not significant ($F_{1,12}=0.690$, p-value = 0.797). Fig. 4 reports the biplot representation of RDA results. All the variables (indicated with arrows and text) except for A:C index, point towards the same directions (towards negative values of RDA1), confirming that the indices were

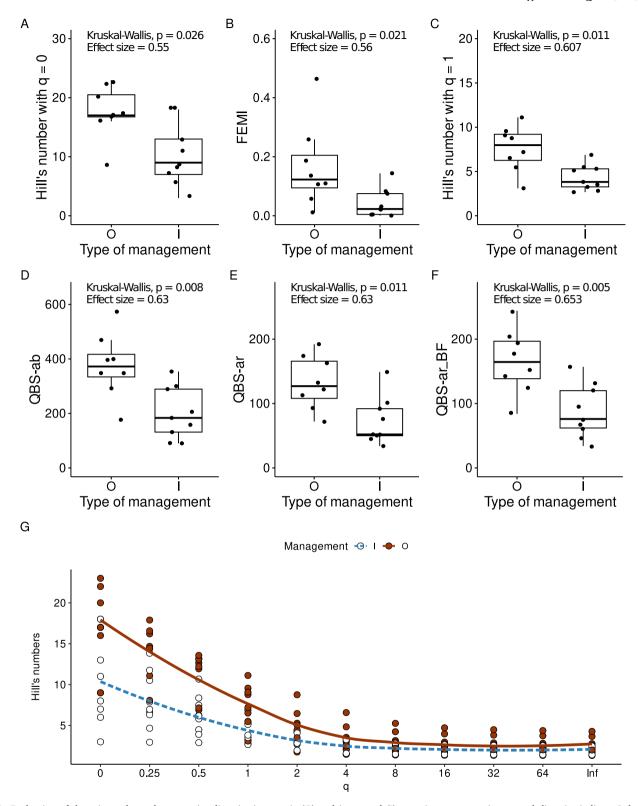


Fig. 3. Evaluation of the microarthropod community diversity in organic (O) and integrated (I) cropping systems using several diversity indices. Only indices showing significant difference between I and O (p value <0.05) were reported, sorted by Wilcoxon rank sum test effect size on management factor: Hill's number with q=0 (A), FEMI (B), Hill's number with q=1 (C), QBS-ab (D), QBS-ar (E), and QBS-ar_BF (F). Panel (G) reports Hill's number for each sample at increasing q values for O (brown) and I (dotted azure) cropping system.

always higher in soil samples from organically managed sites. Investigation of the score of all variables on the first component (RDA1, Supplementary Table S4) showed that H with q values of 0.5, 0.25, 1 and 0 had the highest contribution (10.8 %, 9.5 %, 9.5 %, and 7.3 %,

respectively) followed by the two QBS-ar variants (7.1 for QBS-ar and 7.0 % for QBS-ar BF) and the QBS-ab (6.6 %). A valuable contribution in explaining the variations derived also from H with other q values, while FEMI and *Acari:Collembola* had the lowest contribution (3.0 % for FEMI,

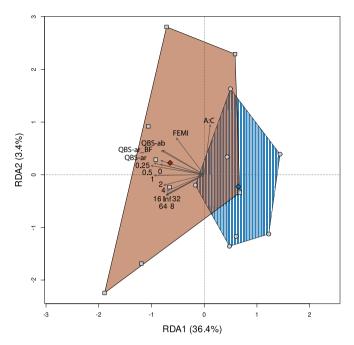


Fig. 4. Redundancy Analysis (RDA) ordination plot. Points are represented in the ordination space formed by the first RDA component (RDA1) and the second RDA component (RDA2). Each grey point represents a sample, with shape mapping to the management (square = organic; circle = integrated). Arrows represent the variables of the RDA ordination, that is, the FEMI, QBS-ar, QBS-ab, QBS-ar_BF, Hill's numbers with increasing q (0, 0.25, 0.50, 1, 2, 4, 8, 16, 32, 64, Inf), and *Acari:Collembola* (A:C) indices. Dashed azure and brown polygons represent the distribution of all integrated and organic cropping systems in the ordination space, respectively. For both groups the centroid is highlighted by a diamond point.

0.1 % for Acari:Collembola).

Finally, the multi-level pattern analysis was applied to investigate the associations between BFs and management. The analysis found 4 BFs ($Collembola_8$, $Diptera\ larvae_10$, $Coleoptera\ larvae_10$, and $Diptura_20$) associated with the O management group (p < 0.05). No specific BF was significantly associated with the I management (Supplementary Table S5). The analysis confirmed results obtained with univariate approach (Wilcoxon's rank sum testing), except the $Collembola_20$ BF, in pointing out which were the most valuable BFs for discriminating between O and I management.

4. Discussion

4.1. Power of different indices to define microarthropod community diversity

The responsiveness of soil fauna communities to soil management practices of diverse agroecosystems is extremely complex (Macchio et al., 2024). Nevertheless, our results showed that certain indices had high suitability to differentiate the impact of various management practices on these communities, despite the inclusion of samples from very different European pedoclimatic conditions. On the contrary, generally low and non-significant differences emerged considering different cropping systems, suggesting that the management method (integrated vs. organic) is a stronger determinant of microarthropod community diversity. The ecological indices based on the microarthropod community's BFs showed in general high and significant discrimination power between the managements methods except for the Acari:Collembola index and Hill's number with $q \geq 2$. This result suggests that the increased weight that relative abundance has in the index value calculation (i.e., the use of abundance in Acari:Collembola ratio, or q

value increase in Hill's number) resulted inversely proportional to the discrimination ability between the soil management methods, irrespective of the cropping systems. (Fig. 3 and Supplementary Fig. S1). The aggregate distribution of soil biota (Clark and Evans, 1954; O'Connell and Bolger, 1998) might explain this evidence, as it is not uncommon to find a high number of individuals of the same species in a sample, particularly for springtails and mites. It is possible to argue that sampling only 10 cm³ of soil may easily result in the hotspot of a certain species (e.g., around deposits of organic matter, close to an anthill) or that some juvenile forms have developed during extraction in the absence of natural predators. Increasing the number of replicates may overcome the aggregated distribution issues but would result in losing the characteristics of appropriateness of any monitoring program of soil health (e.g. like the one planned at European scale). Moreover, a high abundance of individuals from a single species, as in the case of pests, generally does not increase biodiversity (Altieri and Nicholls, 2004). For this reason, abundance is not used for the calculation of the QBS-ar index, since the possibility of finding many specimens of a BF/taxonomic group can derive also from their social habits. Moreover, abundance is not necessarily associated with their adaptability to the soil conditions as previously highlighted (Clark and Evans, 1954; O'Connell and Bolger, 1998). This aspect was confirmed by the study results, which showed that QBS-ar_BF and QBS-ar were the indices that better discriminated the soils according to management practices (Fig. 3, Fig. 5), indicating that the QBS approach can be generally preferable compared to indices other indices (e.g. Acari:Collembola ratio or richness), that do not valorize euedaphic forms. Moreover, the newly developed QBS-ar_BF index was more effective in discriminating the soils (higher effect size) likely because it does not exclude any observed BF, thus embedding the whole information about the population composition and the ecological role of each BF. From these results, we would conclude that ecological indices inferring the frequency or the presence of microarthropods are more suitable to describe the microarthropod community, especially when accounting for the level of soil adaptation.

An interesting feature of the new index is that existing results of the QBS-ar index are comparable with results of the QBS-ar_BF index, but only when data of the entire community are reported. Indeed, when QBS-ar_BF is applied and the list of all BFs is reported, the corresponding value of the QBS-ar index can always be calculated. Vice versa, it is not possible to calculate QBS-ar_BF from a previous QBS-ar survey, where only BFs for the QBS-ar calculation have been listed.

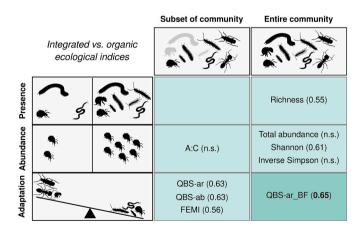


Fig. 5. Graphical view of the results obtained from the comparison of organic vs integrated fields from diversity indices applied in this study. Teal green boxes report the effect size of each index (in parentheses; n.s. stands for not significative, A:C stands for Acari:Collembola), classified according to the grade of community observation (presence/absence, abundance, level of soil adaptation) and whether it is a subset of the totally of observed BFs. The darker boxes refer to the index showing the highest discrimination ability in the dataset.

The applied indices might be classified according to whether they included all BFs retrieved in the sample or only a subset, and according to whether they were able to account for different aspects of a community, considering only presence, abundance, or by weighting presence according to the level of soil adaptation. This classification, with an overview of the results obtained in discriminating between I and O management, is depicted in Fig. 5. The different versions of the QBS index calculated in the present work showed the highest ability of discrimination, being focus on soil adaptation level. This evidence is in line with literature findings. For instance, recent observations confirmed that the use of certain plant protection products can harm the BFs of epigeous springtails more severely than the euedaphic ones (ISPRA, 2020), which probably tend to escape into the deeper layers of the soil.

4.2. Occurrence of microarthropod groups as affected by cropping system type

Concerning the influence of the cropping system type, results indicate that the microarthropod community diversity can be related to the crop cultivation duration. Soils from cropping systems cultivated with pluriannual cropping system rotation (i.e., apple and rotation including strawberry) compared to those having short-term crops (i.e., multiple rotation including tomato) showed the highest QBS-ar value and richness. Similarly, although not statistically significant, the abundance of euedaphic BFs tended to be higher in soils from cropping systems with pluriannual crops. However, even if both pluriannual cropping systems showed the same BFs richness, the overall number of BFs was different. Apple orchards showed a high number of BFs with maximal EMI (20), confirming the hypothesis that undisturbed permanent crops, with less tillage practices and other anthropic pressure, favor well-adapted microarthropods that live exclusively in the soil (Betancur-Corredor et al., 2022), contributing to the improvement of soil biodiversity (Gardi et al., 2002; Tabaglio et al., 2009; Fiorini et al., 2020). Moreover, our results agree also with the findings of Menta et al. (2020), which showed that the variability of fauna in terms of community composition strongly depends on the crop type as well as crop and soil management.

4.3. Ability of microarthropod community in defining/monitoring overall soil quality and health

Soil health is the soil's capacity to function as a living system (definition art 3.4 proposal directive on soil monitoring law, European Commission, 2023). Therefore, using soil biota as a proxy for monitoring soil health is receiving an increased interest, particularly if it involves an interdisciplinary approach that copes with different scales (Faber and Van Wensem, 2012; Creamer et al., 2022; Van Straalen, 2023; Brunetti et al., 2024).

Soil quality is known to be strongly associated with the occurrence of microarthropod groups that show a high adaptation to soil habitats (Blasi et al., 2013; Menta et al., 2018a). Concerning agricultural soils, as a first approximation, a soil with QBS-ar index value higher than 93.7 can be considered "rich and biodiverse" with a rich microarthropod community, whereas scores lower than 90 can be considered "poor soils", with low microarthropod abundance and low biodiversity. However, the type of landscape can have a significant impact on the microarthropod population: the QBS-ar increases from pasture to forestry, but its value is always low ($\langle 100 \rangle$ in arable land (Menta et al., 2018b). The same relationship between QBS-ar value and land use emerged in the present study. Moreover, previous studies proved that conversion from intensive to organic management, reclaiming soil or landfill restoration can encourage the re-establishment of soil microfauna, which supports using this group of organisms as an indicator of soil quality in terms of biodiversity (L., 2000; Battigelli, 2011; Ashwood et al., 2022).

Despite the eminence of soil microbial biodiversity (bacteria and fungi) in defining soil health, it is noteworthy to highlight also its

drawbacks (Fierer et al., 2021), which would support their combined use with microarthropod indices. These are mainly related to the high spatial diversity in microbial colonization spheres (e.g. drilosphere and rhizosphere) (Uteau et al., 2022), the presence of relic DNA, that does not allow the distinction between active and inactive biomes (Carini et al., 2016), as well as the limited amount of analyzed soil from which its biodiversity is inferred. Mesofauna sample size fits more with farm operation field size, although individual distances still do not interfere with the sampling tile. This is actual especially for micro- and mesofauna, however, some assumptions about population distribution at the level of individuals are recommended (Parisi, 1974).

5. Conclusion

The results of the present study confirm that the differences between the microarthropod communities can be easily and reliably evidenced through a BFs analysis approach. Among all the tested indices, the QBSar can be efficiently applied to assess soil quality and biodiversity loss under different agricultural conditions, as its application proved to be useful in comparing and evaluating sites located in different pedoclimatic regions and managed with diverse farming methods, i.e. organic and integrated. OBS-ar was more discriminant than abundance indicators and classic biodiversity indices. Nonetheless, the outstanding performance of the newly developed QBS-ar BF index highlighted that small adjustments to the former may be helpful to increase the discrimination power of microarthropod communities, thus being appropriate for assessing soil health and its dynamics. Considering that the QBS-ar BF index makes use of data from all BFs, its use would improve a whole-community analysis approach, implementing information about the edaphic community. The study provided evidence of the relationship between soil management, cropping system length, and the complexity of microarthropod community structure. However, further investigations would allow to further clarify the impact of different agricultural soil management practices on soil faunal dynamics and microarthropods' role in regulating soil health (Janzen et al., 2021). The whole method for sampling and extracting the microarthropods, though achievable at a low cost, could benefit from a future automation of the BFs identification process through specifically developed imageanalysis software, further simplifying the implementation of the analysis and making it applicable on a broader scale (D'Avino et al., 2021).

QBS-ar was confirmed to be an optimal approach to discriminate between fields managed with organic and integrated methods, being more discriminant than abundance indicators and classic biodiversity indices. Nonetheless, the outstanding performance of the newly developed QBS-ar_BF index highlighted that small adjustments to the QBS-ar index may be helpful to increase its discrimination power between microarthropod communities, thus being appropriate for assessing soil health and its dynamics. The use of the QBS-ar_BF index considering all retrieved BFs would improve a whole-community analysis approach, implementing information about the edaphic community, better discriminating differences and avoiding any data loss.

The study provides evidence of the strong relationship between soil management, crop persistency, and the complexity of microarthropod community structure. However, further investigations are needed to clarify the impact of different agricultural soil management practices on soil faunal dynamics and microarthropods' role in regulating soil health.

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CRediT authorship contribution statement

Gaia Bigiotti: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Francesco Vitali: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. Stefano Mocali: Writing – review & editing, Supervision,

Resources, Funding acquisition, Conceptualization. Giovanni L'Abate: Writing - review & editing, Writing - original draft, Investigation, Formal analysis, Data curation. Eligio Malusà: Writing - review & editing, Supervision, Resources, Project administration, Data curation. Dawid Kozacki: Writing - review & editing, Writing - original draft, Investigation, Formal analysis. Irena Bertoncelj: Writing - review & editing, Writing - original draft, Investigation. Morgane Ourry: Writing - review & editing, Writing - original draft, Investigation, Formal analysis, Data curation. Massimo Pugliese: Writing - review & editing, Supervision, Resources, Investigation, Data curation. Heinrich Maisel: Writing - review & editing, Supervision, Resources, Investigation, Data curation. Expedito Olimi: Writing - review & editing, Writing - original draft, Investigation, Formal analysis, Data curation. Maria Grazia Tommasini: Writing - review & editing, Writing - original draft, Supervision, Resources, Investigation, Formal analysis, Data curation. Carlo Jacomini: Writing - review & editing, Writing - original draft, Methodology. Lorenzo D'Avino: Writing - review & editing, Writing original draft, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Author contributions

GB, LD and FV conceived the idea of the manuscript and wrote the first draft. IB, HM, EM, EO, MO, MP, MGT selected the study sites and developed the experimental design. IB, GB, LD, DK, GL, HM, MO, MP and FV carried out the sampling. IB, GB, LD, OE, MO, MP and FV carried out the extractions. GB, LD, GL and FV collected the field data. FV processed and analyzed the data. SM collaborated to conceive the idea and obtained the funding. All authors revised and approved the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

Data are provided via the following link: doi:https://doi.org/10.5281/zenodo.10843513

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