

## Article

# Soil Microarthropods as Tools for Monitoring Soil Quality: The QBS-ar Index in Three European Agroecosystems

Filippo Gallese <sup>1</sup>, Laura Gismero-Rodriguez <sup>2</sup> , Anton Govednik <sup>3</sup> , Laura Giagnoni <sup>4</sup> , Erica Lumini <sup>1</sup> , Marjetka Suhadolc <sup>3</sup> , Francesco Primo Vaccari <sup>1</sup>  and Anita Maienza <sup>1,\*</sup> 

<sup>1</sup> National Research Council (CNR), 00185 Roma, Italy; filippo.gallese@ibe.cnr.it (F.G.); erica.lumini@ipsp.cnr.it (E.L.); francesco.vaccari@ibe.cnr.it (F.P.V.)

<sup>2</sup> Instituto de la Grasa (IG-CSIC), 41013 Sevilla, Spain

<sup>3</sup> Biotechnical Faculty (BF), University of Ljubljana, 1000 Ljubljana, Slovenia; marjetka.suhadolc@bf.uni-lj.si (M.S.)

<sup>4</sup> Department of Civil, Environmental, Architectural Engineering and Mathematics (DICATAM), University of Brescia, 25121 Brescia, Italy; laura.giagnoni@unibs.it

\* Correspondence: anita.maienza@ibe.cnr.it

**Abstract:** The QBS-ar, based on the study of microarthropod community structure, is well known as a quick and low-cost indicator to monitor soil biological quality at the farm scale. Temperature fluctuations and other climate factors in European countries may indirectly influence soil microarthropod communities by altering resource availability and microhabitat conditions. In the context of the climate crisis, along with drought and erosion threats, especially in southern Europe, it is essential to define the limits and advantages of the QBS-ar index. We applied the QBS-ar index along a warm temperature gradient at three long-term experimental sites. Our results underlined that the QBS-ar is very sensitive for detecting soil quality and treatment effects. The results suggest that the choice of sampling season is a particularly vulnerable phase, especially for southern Mediterranean sites. Air temperature and cumulative precipitation, even in the months prior to sampling, are critical factors to consider when applying the QBS-ar index in European countries. Drought periods can negatively influence the results for soil microarthropod relative abundance; however, the presence of biological forms seems to provide useful information about the effects of treatments on soil quality. This paper lays the groundwork for scaled-up QBS-ar applications considering soils and several environmental characteristics of agroecosystems in Europe. The work can contribute to the development of applications of the index, facilitating and improving the monitoring of soil biology at the field scale. Furthermore, this study can open future perspectives for the application of QBS-ar on a larger scale thanks to the implementation and updating of an open-source database.

**Keywords:** soil; biological indicators; conservative agriculture; climate



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## 1. Introduction

Conservative soil management helps to protect soil in the long term, avoiding threats and having a strong impact on minimizing biodiversity loss and soil erosion [1] while aiming to safeguard soil as a resource. Agronomic practices such as cover crops, crop diversification, reduced tillage and organic amendments are biodiversity-based solutions that aim to generate sustainable and resilient agroecosystems, which could enhance the supply of ecosystem services [2].

Several efforts invested in soil monitoring in Europe did not lead to a comprehensive and updated body of knowledge or methodology for identifying healthy soils, especially

under conservative management [1]. In agricultural experiments, soil organic carbon (SOC) is the most studied property for establishing soil quality and is a widely agreed-upon indicator [3]. Therefore, considering the inestimable value of soil as a reservoir of global biodiversity [4–6], there is an urgent need to include and harmonize soil biological monitoring methodologies to avoid biodiversity loss in Europe. Moreover, there is an EU vision for soil by 2050, which is anchored in the EU Biodiversity Strategy for 2030 (European Commission, 2020). The European Joint Programme on Agricultural Soil Management (EJP SOIL, <https://ejpsoil.eu/>) of the EU provides a framework for the necessary research. Within EJP SOIL, the project MINOTAUR (Modelling and mapping soil biodiversity patterns and functions across Europe) seeks the harmonization of soil biodiversity indicators at a European level, and the project Energy Link (Linking crop diversification to microbial energy allocation and organic carbon storage in soils) *studies the link between crop diversity and the soil biome across a pan-European pedo-climatic gradient. Combining the objectives of these projects, our study aims to evaluate the application of a soil biological index: the QBS-ar (based on microarthropod communities) in three agroecosystems with different geographical locations in Europe.* Microarthropods are directly linked with above- and below-ground functions of soil and include taxa that play essential roles in maintaining soil physical and chemical qualities, such as structure and consequent hydrology, the decomposition and humification of organic matter and the direct stimulation of the microbial mineralization of soil nutrients [7,8]. Currently, soil biological indicators lack reference systems to diagnose the quality of soils. The study of soil microarthropod communities and the QBS-ar index has become largely widespread in recent years, increasing the available data and helping to improve protocols, with value related to soil use and management [8].

The QBS-ar index was developed in Italy and over time has become largely widespread, especially in the Mediterranean area [8]. The index was also recently suggested in the standard operating procedures (SOPs) of the network FAO GLOSOLAN, which aims to globally harmonize soil standard operating procedures.

In addition, the QBS-ar has been also proposed as a methodological framework to infer biodiversity spatial distribution, providing a map that could represent a basis for validating hypotheses on the mechanisms driving biodiversity indicator patterns at regional scales [9]. However, limiting factors of its application at the farm scale across geographical locations and meteorological contexts still need to be assessed and defined. Over more than 20 years of application, the QBS-ar index has become a user-friendly, rapid and low-cost method sensitive to catching soil quality changes even in the short term [8,10,11]. On the other hand, the index is closely tied to its specific application conditions: (a) primarily in Italian soils, (b) limited to isolated/single cases and (c) used in only a few international studies [12–14]. The existing studies on QBS-ar applications have focused on areas with a Mediterranean climate, where this method has been applied in different environmental contexts (forestry, grassland, agricultural management, restored sites) [8,10]. Considering its high potential impact on European soil biological monitoring, we consider an urgent need to apply the index in different geographical contexts. In fact, warming and changes in precipitation amounts are factors that strongly influence the community of microarthropods [15–17] and directly impact the usefulness of the QBS-ar for monitoring soil biological quality.

In our study, we apply the QBS-ar in three different agroecosystems along a warm temperature gradient [18].

The three long-term experiments (LTEs) observe conservative soil management and are respectively a loam soil vineyard (Slovenia), a clay loam soil vineyard (Italy) and a silt loam soil olive orchard (Spain). In each LTE, soil was sampled to estimate the abundance and presence of microarthropods, ecological indices, the QBS-ar index and the relative biological forms in two different seasons. Our results may be useful for identifying patterns

linking the QBS-ar index to environmental features, providing accurate technical and methodological information on the application of protocols for the QBS-ar index. The results can help to provide an overview for ranges of QBS-ar values depending on the characteristics of the sites and the geographical location and to pinpoint reference systems for assessing soil quality in European countries.

## 2. Materials and Methods

### 2.1. Experimental Sites and QBS-ar Sampling Procedure

The three study areas along a warm temperature gradient [18] are shown in Figure 1.

The first one, the Slovenian site, is an experimental vineyard of a total of 570 ha located in Koper (Obalno-kraška, Western Slovenia) (Lat 45°34'22.8" N, Long 13°46'26.4" E) 50 mt above sea level and managed since 1997 with different interrow management: permanent natural vegetation cover (TREAT) and bare cover with tillage to control weeds (CTRL). The experimental farm has some plots 2.2 m wide and 60 m long for a total of sampling surface of 3168 m<sup>2</sup>. The climate of the site is classified as a warm oceanic climate (Cfa) [18].

The Italian site is an organic vineyard located in San Casciano Val di Pesa (Tuscany, Central Italy) (Lat 43°66'88" N, Long 11°19'94" E). The altitude is 165 m above sea level, and the vineyard comprises 4 ha of surface area. The vineyard has been managed since 2019 with mixed interrow vegetation cover (CTRL) in combination with biochar treatment application (TREAT) at doses of 30 tha<sup>-1</sup>. The sampling field has a total surface of 1500 m<sup>2</sup>. The climate of the site is classified between a warm Mediterranean climate (Csa) and warm oceanic climate (Cfa) [18].

The Spanish site is an experimental olive orchard located in Benacazon (Andalusia, Southern Spain) (Lat 37°20'24" N, Long 6°13'44.4" E), started with the objective to combat soil erosion. This site elevation is 92 m above sea level, and it has longitudinal plots 8 m wide and 60 m long for a total of sampling surface of 4300 m<sup>2</sup> with a slope of 11%, where the interrow area has been managed since 2009 with either tillage (CTRL) or with mixed natural cover (TREAT). The climate of the site is a warm Mediterranean climate (Csa) [18].

Meteorological parameters were collected at automatic weather stations close to the experimental fields. To better understand the differences between sites, we report in Table 1 a description of crops, management and texture during the mesofauna sampling period. The main soil properties are reported in Table 2.

**Table 1.** Main field information related to crops, experimental design, pedological properties and meteorological average values of the Slovenian, Italian and Spanish sites during the sampling campaign/period.

Trait	Koper (Slovenia)	San Casciano V.P. (Italy)	Benacazon (Spain)
Crop	Vineyard	Vineyard	Olive Orchard
Experimental design	Bare soil vs permanent vegetation cover	Mixed cover vs mixed cover + biochar	Bare soil vs mixed cover
Start experiment	1992	2019	2009
Soil texture	Loam	Clay loam	Sandy loam
Vegetation cover	Ctrl = absent Treat = permanent	Ctrl = permanent Treat = permanent + biochar	Ctrl = absent Treat = permanent
Site climate classification [18]	Csa Warm oceanic	Csa–Cfa Warm oceanic–warm Mediterranean	Cfa Warm Mediterranean
Average air temperature	12.2 °C	17 °C	18 °C
Cumulative precipitation	570 mm	55 mm	0 mm

**Table 2.** Main soil properties within the sampling period (October 2022–June 2023) in CTRL (control) and TREAT (treatment) plots in Slovenian, Italian and Spanish LTEs.

Soil Parameters	CTRL Koper (Slovenia)	TREAT Koper (Slovenia)	CTRL San Casciano V.P. (Italy)	TREAT San Casciano V.P. (Italy)	CTRL Benacazon (Spain)	TREAT Benacazon (Spain)
Clay (%)	32 ± 2.6	33.2 ± 1.3	34 ± 0.01	34 ± 0.01	71.5 ± 3.2	67.89 ± 6.69
Silt (%)	43.7 ± 46.3	46.3 ± 1.5	30 ± 0.01	30 ± 0.01	16.9 ± 3.11	21.97 ± 4.24
Sand (%)	23.4 ± 3.1	20.5 ± 2.5	36 ± 0.01	36 ± 0.01	11.55 ± 0.07	10.12 ± 2.60
BD (g/m <sup>3</sup> )	1.33 ± 0.14	1.44 ± 0.14	1.2 ± 0.1	1.1 ± 0.1	1.24 ± 0.0	1.13 ± 0.04
SOC (%)	1.22 ± 0.022	1.88 ± 0.20	1.56 ± 0.45	0.99 ± 0.17	0.77 ± 0.06	0.80 ± 0.13
N (%)	0.10 ± 0.01	0.17 ± 0.02	0.14 ± 0.02	0.14 ± 0.01	0.07 ± 0.00	0.09 ± 0.01

Soil sampling is the most sensitive step for QBS-ar application. QBS-ar samples were collected twice, following the sampling protocol of the Energy Link project and the toolbox proposed to standardize the QBS-ar procedure [19]. In each field experiment, 3 undisturbed soil subsamples (depth: 0–10 cm) were collected at the center of both control and treated plots, avoiding the limit between the plough layer and subsoil; the soil moisture at sampling time was between 5 and 45%. One sample was located in the center of the plot, and the remaining two were located 5 mt away. Since soil microarthropods are sensitive to temperature [19], we sampled in two different seasons. In Slovenia and Italy, the first core samples were collected in November 2022, and in Spain, they were collected in January 2023 (Season 1), with an average air temperature between 10 °C and 14 °C. The second QBS-ar sampling was carried out in June 2023 for Slovenia and Spain and in May 2023 for Italy (Season 2), with an average air temperature between 18 °C and 22 °C. Soil sampling was performed with special PVC cylinders (10 × 10 cm), with samples being fresh shipped (to maintain natural condition) within 48 h to the laboratory of the Soil Biological Quality of the Institute of Bioeconomy of CNR in Sesto Fiorentino (Florence) for extraction and analysis.

Vegetation cover was cut to eliminate any possible escape route for microarthropods. The total number of core samples analyzed in three sites was 120, divided as follows:

1. Koper (Western Slovenia): a total of 48 samples (3 subsamples for 4 bare-soil control plots and 3 subsamples for 4 permanent natural vegetation plots) × 2 sampling events (November 2022 and June 2023);
2. San Casciano VdP (Central Italy): a total of 36 samples (3 subsamples for 3 control plots with cover crops and 3 subsamples for 3 plots with cover crops and biochar) × 2 sampling events (November 2022 and May 2023);
3. Benacazon (Southern Spain): a total of 36 samples (3 subsamples for 2 bare-soil controls and 3 subsamples for 4 mixed-cover plots) × 2 sampling events (January 2023 and June 2023).

Microarthropods were extracted using a Berlese–Tullgren funnel over seven days, and an incandescent lamp (60 W) was placed 30 cm above the soil to gradually create an inhospitable condition for the arthropods. During the extraction phase, we continuously monitored the internal soil temperature with moisture and temperature sensors (TEROS 12) to avoid a temperature spike during the extraction and leave the soil at an average internal temperature of 25 °C. Under this condition, the mesofauna was completely extracted within 7 days and conserved in liquid (ethanol glycerol mixture, ratio 2:1) at 5 °C. Extracts were then taken back to the laboratory for identification under a stereomicroscope at 80× magnification (Zeiss), analysis of the abundance and presence of 21 taxa [10] and calculation of ecological indices such as the Acari/collembola ratio (A/C) [20], Shannon index (H) and Simpson index (S).



**Figure 1.** Geographical representation of the field experiment sites in the study. Slovenia (yellow) and Spain (red) are the extremes of the warm temperature climate following the Koppen–Geiger climate classification in Europe [20]. The Italian site (Central Italy) has a mild and intermediate temperature condition compared to the others (orange).

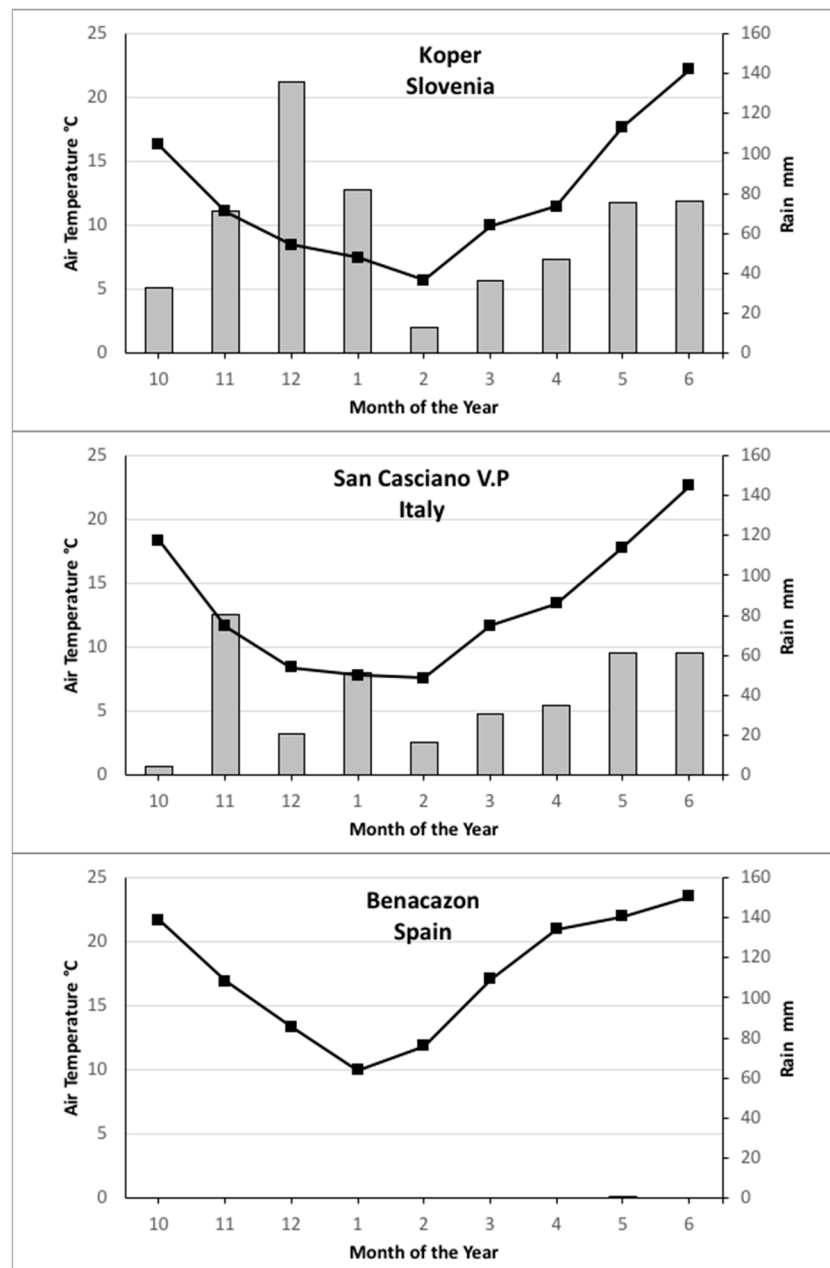
The QBS-ar index is based on the identification of biological forms and the relative attribution of the ecomorphological index (EMI) to the degree of adaptation of edaphic life following the procedure described [10,19]. The EMI score is the sum of the total EMI for each subsample; when more biological forms are present for the same group of organisms, a higher EMI score is taken into consideration [10]. The QBS-ar index value is obtained from the sum of the EMI of all collected groups [14]. The QBS-ar results were also integrated with the QBS index based on biological forms (QBS-BF) proposed in [19]. The QBS-BF index considers every biological form that occurs in the calculation of the QBS-ar, regardless of whether it belongs to the same group (i.e., class or order). The total number of edaphic forms of microarthropods (as the number of microarthropod groups adapted to the soil habitat) were calculated using the tool proposed in [19].

## 2.2. Statistical Analysis

A one-way ANOVA followed by the Tukey’s HSD test (post hoc test) was applied to test for differences among treatments. The homogeneity of variance and normal—it was checked using Bartlett’s and Shapiro–Wilk tests, respectively. All tests were performed using RStudio version 1.3.1093 (R Development Core Team, Vienna, Austria, 2021).

## 3. Results

Figure 2 shows the monthly average air temperature and precipitation from October 2022 to September 2023 (sampling campaign period) in the three LTEs. Meteorological data showed comparable values for monthly average air temperature in Slovenia (Koper) and Italy (San Casciano), as shown in Figure 2. At both sites, the warmest temperatures were recorded in June, and the coolest month was registered as February. In Spain (Benacazon), the average air condition registered warmer values in four months: October, April, May and June, while the coolest average month was January. Cumulative precipitation patterns were more randomized, although for both Slovenia and Italy, the rainiest month was found to be December, and in Spain, the recorded monthly cumulative daily precipitation was 0.

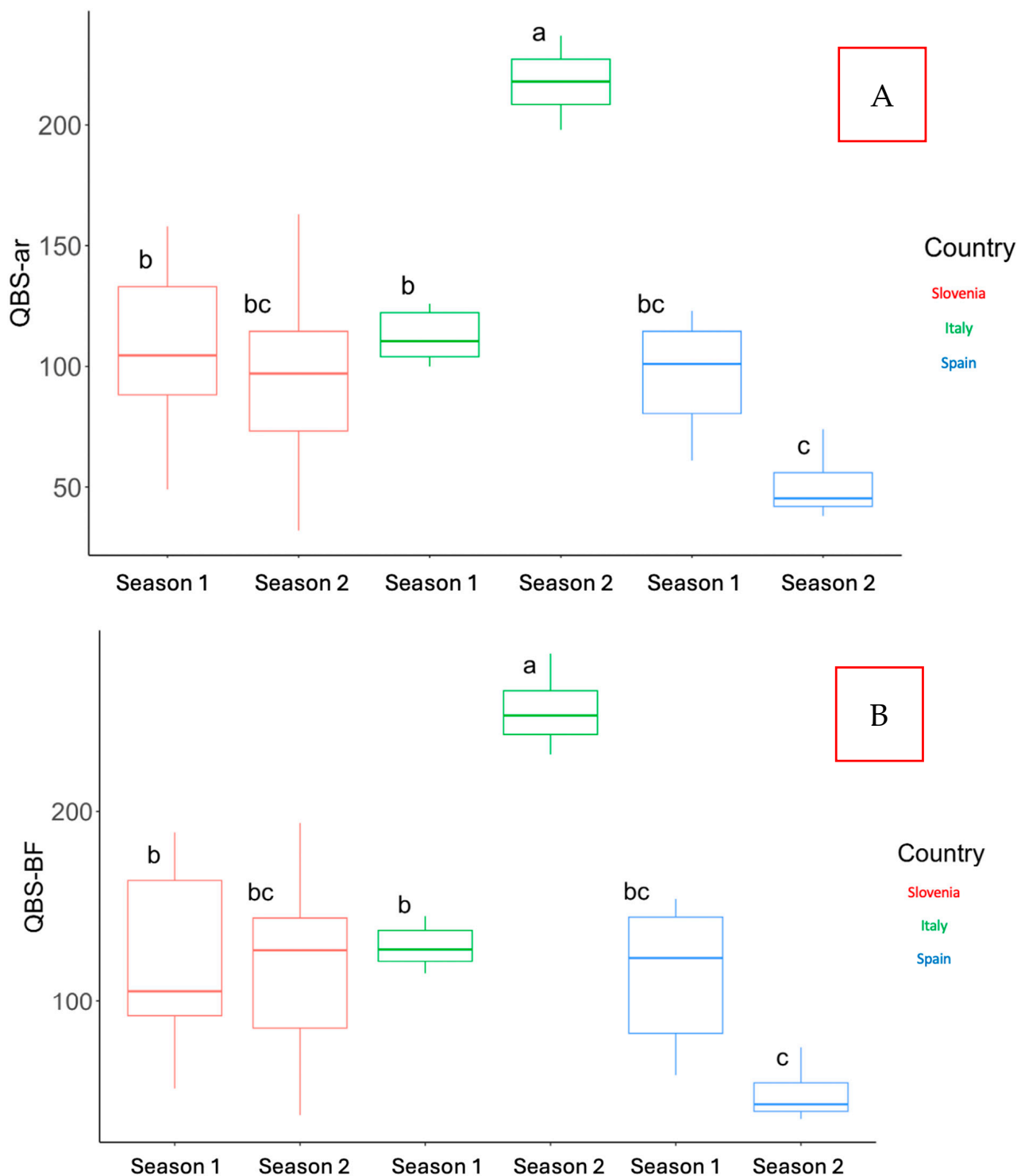


**Figure 2.** Weather parameters during the sampling campaign from October 2022 to June 2023 for each LTE.

The microarthropod relative abundance (%) in each LTE is shown in Table 3. In Koper, Collembola was the most abundant group, with an average total presence of 40%, while Acarina reached 34%. In San Casciano and Benacazon, Acari was the predominant group, with an annual average of 64% and 55% for total abundance, respectively, followed by Collembola, which reached around 30%. San Casciano showed the highest number of taxa detected (19) and the highest average number of edaphic organisms (average of 3.5 per sample), and Benacazon's species richness value was the lowest (taxa found = 12) (Table 3).

Concerning the QBS-ar and QBS-BF indices, the Koper site (Slovenia) did not show differences between values from season 1 and season 2 (Figure 3A,B), while in San Casciano and Benacazon, there was a marked seasonal effect. In San Casciano, both QBS-ar and QBS-BF values significantly increased ( $p < 0.001$ ) from autumn to spring (season 2) (Figure 3A,B). In Benacazon, the QBS-ar and QBS-BF values were higher ( $p < 0.05$ ) in season 1 than

in spring samples (Figure 3A,B), as were the number of edaphic forms and % of total abundance, as reported in Table 3.



**Figure 3.** Seasonal value (spring–autumn/winter) for each site using the QBS-ar index (A) and QBS-BF index (B); letters denote significant differences between treatments based on Tukey's HSD test (post hoc test) at  $p < 0.05$ . Season 1 represents sampling performed in November 2022 in Slovenia (Koper site) and Italy (San Casciano V.P.) and in January 2023 in Spain (Benacazon); season 2 represents May in Italy (San Casciano V.P.) and June Slovenia (Koper) and Spain (Benacazon).

**Table 3.** Relative abundance (%) of the total arthropod taxa within seasons at each experimental site. Season 1 indicates autumn/winter; Season 2 indicates spring.

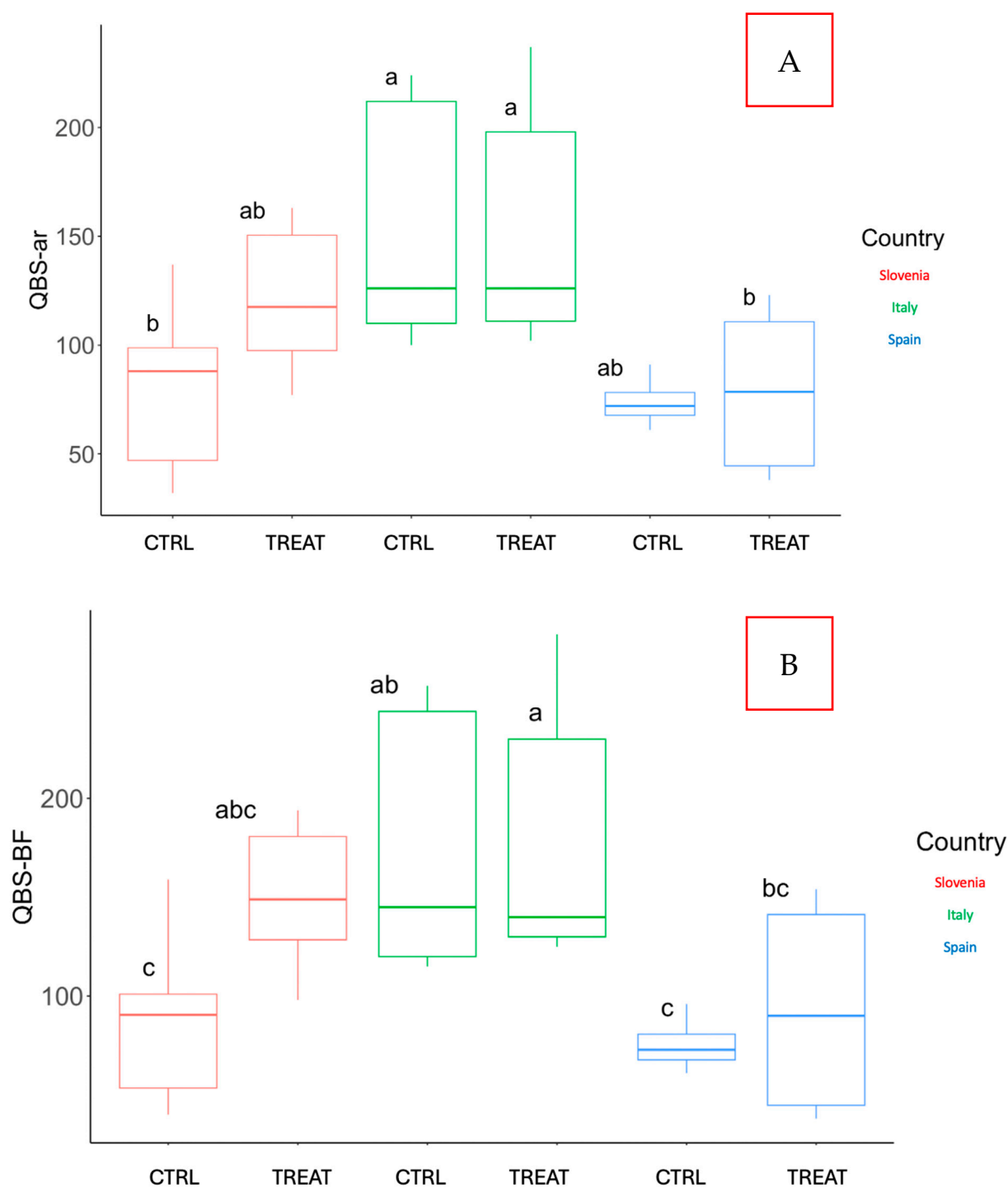
Subphylum		Koper (Slovenia)				San Casciano V.P (Italy)				Benecazon (Spain)			
		CTRL Season 1	CTRL Season 2	TREAT Season 1	TREAT Season 2	CTRL Season 1	CTRL Season 2	TREAT Season 1	TREAT Season 2	CTRL Season 1	CTRL Season 2	TREAT Season 1	TREAT Season 2
Chelicerata	Acarina	64.75	34.75	28.24	41.94	53.97	66.10	44.81	64.66	73.91	100.00	45.74	66.13
	Aranea	-	0.25	-	-	0.84	0.13	0.55	0.39	-	-	-	1.61
	Opiliones	0.36	-	0.09	0.13	1.26	-	1.09	-	-	-	-	-
	Pseudoscorpiones	-	-	-	-	-	-	-	0.24	-	-	-	-
Crosteacea	Isopoda	-	-	-	-	0.42	0.13	1.64	0.29	-	-	-	-
Myriapoda	Chilopoda	0.36	-	-	0.39	0.42	0.20	-	0.29	0.48	-	0.17	-
	Symphyla	-	0.50	-	1.18	-	0.39	-	0.24	0.48	-	2.17	1.61
	Diplopoda	-	-	-	0.13	-	0.92	1.09	2.46	-	-	-	-
Hexapoda Entognatha	Paupoda	0.36	0.25	0.23	0.92	-	1.11	-	0.48	2.42	-	3.51	-
	Collembola	21.58	19.75	51.90	24.90	31.38	27.42	34.97	26.71	18.36	-	38.73	3.23
	Diplura	0.72	0.75	-	0.52	1.26	0.26	1.09	0.82	-	-	0.50	-
	Protura	-	-	-	0.52	-	0.79	1.64	0.96	-	-	-	-
Hexapoda Insecta	Hymenoptera	3.96	40.25	12.04	22.41	4.60	0.85	9.29	0.58	-	-	0.33	8.06
	Thysanoptera	1.08	-	0.23	0.39	-	0.13	-	0.24	-	-	0.17	6.45
	Psocoptera	-	0.50	0.05	0.13	-	-	1.64	0.05	-	-	-	-
	Hemiptera	-	0.25	1.58	4.33	-	0.46	-	-	-	-	1.50	-
Larvae	Diptera	3.24	0.75	2.17	1.83	3.77	0.39	1.09	0.34	2.42	-	5.51	4.84
	Lepidoptera	-	-	0.05	-	-	-	-	-	-	-	-	-
	Coleoptera	3.60	2.00	3.44	0.26	2.09	0.39	1.09	0.96	1.93	-	1.67	8.06

Considering the mean QBS-ar values in each LTE, Koper showed an average QBS-ar value of 101, San Casciano had a QBS-ar value of 154, and Benacazon's QBS-ar value was 73. The QBS-BF index followed the same trend, further stressing the differences. Considering the effectiveness in detecting soil quality in relation to treatments, QBS-ar showed a sensitive response to Koper soils (Figure 4A,B), as well as ecological indices such as the A/C ratio and the number of edaphic groups in TREAT soils compared to CTRL (Table 4). Furthermore, QBS-BF exhibited increased significance between treatments. At San Casciano, no significant differences were found in TREAT soils compared to CTRL (Figure 4A), although there was a slight increase in the Shannon index (Table 4). Finally, at the Benacazon site, no differences were found in QBS-ar (Figure 4A) or in the other traditional ecological indices, while a slight increase in QBS-BF values was found in soils subjected to permanent mixed cover (Figure 4B).

**Table 4.** Treatment comparisons at each site using traditional ecological indices. Asterisks denote significance between treatments ( $p < 0.05$ ).

Country	Treatment	Shannon	Evenness	Simpson	A\C Ratio	Number of Edaphic Forms
Slovenia	Control bare soil	1.19	0.49	0.57	2.16 *	1.9 *
Slovenia	Permanent grassland	1.29	0.62	0.66	1.14 *	2.8 *
Italy	Control mixed cover	1.05	0.44	1.05	2.17	3.78
Italy	Mixed cover + biochar	1.17	0.50	1.17	2.18	3.86
Spain	Control bare soil	0.81	0.63	0.54	3.94	2.75
Spain	Mixed cover	1.09	0.61	0.59	3.99	1.83





**Figure 4.** Control (CTRL) and treatment (TREAT) comparison for each site using the QBS-ar index (A) and QBS-BF index (B). Letters denote significant differences between treatments.

#### 4. Discussion

The QBS-ar index was confirmed to be a very fast and sensitive method for soil biological monitoring at the field scale in European countries.

Particular attention has to be paid to the climate characteristics of sites, especially those subject to low average annual rainfall or an increase in drought threats because this could affect the values of the abundance in the samples. In fact, as found in [21], droughts reduce the number of soil invertebrates by around 35%, and rainfall change effects depend on fauna size. Mesofauna groups like collembola and mites are more severely affected

than smaller animals like nematodes or larger animals such as beetles [21]. In our study, while no significant differences in average air temperature were recorded between LTEs (Figure 2), the cumulative precipitation was considerably different between sites. Koper and Benacazon exhibited the maximum rainfall and drought values, respectively. Benacazon registered 0 mm of cumulative precipitation within the sampling period, with a consequent drastic decrease in the relative abundance of microarthropods in the second season, as reported in Table 3, confirming the sensitivity of microarthropods to moisture [21,22]. This also led to a significant decrease in the QBS index in spring, which was much lower than that in the first sampling season. This result highlights the importance of the careful application of the index, especially in fragile zones under a warm Mediterranean climate with risks of reduced water availability, increased drought and a severe loss of biodiversity. However, one should note that the QBS-ar and QBS-BF indices are based on microarthropod presence, biological forms (not on abundances) and the related morpho-ecological characteristics of edaphic life. This guarantees effectiveness in detecting soil quality status and treatment effects, as reported in the second part of our results.

The choice of sampling season is very important in relation to geographical area. In fact, while in Central Italy, the QBS-ar and QBS-BF values decreased during autumn in accordance with the literature [8–10,15], in Western Slovenia and Southern Spain, we found maximum values in the autumn/winter season.

Higher values of soil biological quality (QBS-ar and QBS-BF) were found in the San Casciano V.P. experiment compared to the other two. This result is first connected to the experimental design; in fact, the permanent presence of vegetation cover in control soil (mixed cover) is positively related to the presence of microarthropods [22,23]. The QBS-ar values for the control soil at the Koper site/vineyard are comparable with the results reported by [24,25] in a conventional vineyard, while in Benacazon, the experimental results are lower than those for other orchard crops found by [9].

Considering [8], QBS-ar values under 100 define degraded or low-fertility soils, and the cover of grass over the soil is strictly related to the QBS-ar values. The lower average values found in our study in Benacazon suggest a need to define the QBS-ar thresholds for European geographical areas, land use and management.

QBS-ar and QBS-BF effectively discriminated the treatment effects in each LTE. In fact, when the sites with bare soil were compared to those with permanent soil cover (Koper and Benacazon), the increase in soil biological quality was underlined. Specifically, the joint use of the QBS-ar and QBS-BF helped to highlight the differences in the Benacazon site. The use of biological forms could be a valid and useful method to monitor soil biological quality in warm Mediterranean agroecosystems. No differences between treatments were observed in San Casciano, suggesting that the beneficial presence of soil vegetation cover affected soil biological quality more than biochar application, confirming [23].

The QBS-ar and QBS-BF, which is based on the analysis of biological forms, resulted more significant than the traditional ecological indices such as the Shannon or Simpson index, in accordance with previous works [21,24–26].

This is the first study using this approach and may help to understand the limitations and the potential of applying this index in view of the climate crisis in southern Europe. The protocol should also consider environmental differences, agronomic management and climate for each country.

Moreover, we underline as well as always having to perform double seasonal sampling to avoid underestimating the QBS-ar values. The combined use of the QBS-ar and QBS-BF is a useful tool for monitoring soil quality in different geographical zones.

## 5. Conclusions

The QBS-ar is based on the presence and the morpho-ecological characteristics of edaphic microarthropods. This guarantees an effective method to detect the quality status of soil and treatment effects in climatic fragile zones in Europe. The results can be useful for identifying patterns linking soil microarthropods and biological forms to soil use and climatic factors, providing accurate technical and methodological information on the application of the QBS-ar index. Future studies and applications of the QBS-ar and QBS-BF should also consider the environmental differences, air temperature and cumulative rainfall specific to each country in order to create site-specific thresholds.

The work can also contribute to improving an updated open-source database, refining the outcomes and helping to monitor soil biodiversity in view of the EU-wide proposal for a soil monitoring and resilience directive.

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**Data Availability Statement:** The data presented in this study are available on request from the corresponding author. The dataset generated for this study will be available in the Minotaur dataset repository.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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