DEPARTMENT OF AGRICULTURAL, FOOD AND ENVIRONMENTAL SCIENCES

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Assessment of biological soil fertility using Quality Soil Indexes (QBS-ar and QBS-e)

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Dedications

I dedicate this thesis to my mother Gabriella and my sister Alicia, who have supported me during these hard weeks. Despite the distance between us, they have managed to support and hearten me. To my uncle Vincenzo, who has always been by my side throughout my life, filling a large and difficult void. To my uncle Salvatore, always ready to bestow wise advice and suggestions for my future career. To my beloved girlfriend Viola, who put up with me during these hard days, filled with work, nervousness, and thesis writing, with few free moments to reciprocate her affection and patience. Thanks also to professors Stefania Cocco and Sara Ruschioni for the support and availability shown to me during this work, and to all the supervisors who followed me in this project, with special thanks to my supervisor Dominique for her professionalism and immense patience shown to me these days.

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Abstract

Healthy soils provide services that are fundamental to human life and although the 95% of our food comes from soil, its importance is too often underestimated by the food industry. Indeed, a healthy soil can ensure a good quality product on the contrary, a scarce soil can lead to poor and food-unsafe products. The aim of this study was to assess the biological fertility of soils under different agricultural management by using the Biological Quality Indexes specific for arthropods and earthworms (QBS-ar and QBS-e, respectively), as valuable indexes to predict the quality of the final food products. In addition, morphological and physicochemical soil analysis was carried out to obtain a complete soil quality evaluation. The study was settled in the "Società Agricola La Mandriola s.s." in Marche region, Italy. The study site consisted of two different organic management: alfalfa and blackberry fields, in which 3 soil profiles per crop field were opened, described, and analyzed for how concern particle-size distribution, textural class, pH, and total organic carbon. For the QBS-ar and QBS-e, 10 soil samples were collected (5 each crop field) to extract arthropods and earthworms. Results of soil analyses have shown that, despite the different management, morphological and physicochemical soil properties are statistically identical. The total score of the QBS-ar obtained from the average between the two management classified the quality of the soil as “good”. The QBS-e reported a lower score than QBS-ar, including the studied soils in a “sufficient” qualitative class, the assessment was also worsened by the impossibility of finding specimens in all the soil replicates of blackberry field. In conclusion, QBS-ar and QBS-e are valid indexes of soil biological quality, in line with the results of morphological and physicochemical soil analysis. To obtain a complete and exhaustive assessment of biological soil quality, it is recommended to repeat the study in different seasons throughout the different crop cycles.

1. Introduction

The food industry often underestimates the importance of healthy soils in providing vital services that are essential to human life. According to the data available in FAOSTAT (FAO, 2022) soils play an essential role in producing over than 95% of food items such as vegetables, fruits, oils, nuts, eggs, dairy products and livestock meats (FAO, 2015). This high percentage underscores the importance of maintaining fertile and healthy soils in the food production sectors, directly impacting food security and sovereignty (FAO, 2023). One of the main goals of the "Farm to fork" strategy in the EU-Green Deal is to ensure food security, nutrition, and public health for everyone in a sufficient and sustainable way, combining food safety, plant and animal health, dietary needs and preferences. Food quality is a fundamental aspect of food security, which includes the safety and nutritional value of food products. Healthy soils will provide the necessary macro and micro elements for plant growth, contributing to the production of high-quality food and livestock feed for wholesome human and animal nutrition. On the other hand, scarce soil fertility contributes to a worldwide issue affecting over two billion people called "hidden hunger", characterized by a chronic lack of micronutrients leading to severe health problems and diseases (GSP et al, 2023). According to the EU "Farm to fork" strategy, soil health should be a primary mission of the EU Member States to guarantee food quality and to make the EU food system resilient (E.Commission, 2023) (E.commision, 2020). The term "healthy", referred to the soil, is a synonym of "fertile" or "quality"; but how can we define the soil as "healthy"? The Food and Agriculture Organization (2015) (FAO, 2015) proposed the definition *"Soil health has been defined as the capacity of soil to function as a living system. Healthy soils maintain a diverse community of soil organisms that help to control plant disease, insect and weed pests, form beneficial symbiotic associations with plant roots, recycle essential plant nutrients, improve soil structure with positive effects for soil water and nutrient holding capacity, and ultimately improve crop production. A healthy soil also contributes to mitigating climate change by maintaining or increasing its carbon content".*

Nowadays, one of the main limitations is the deficiency of comprehensive and consistent data on soil health obtained by monitoring procedures. Several EU Member States have adopted soil monitoring, but they are dispersed, unrepresentative, and unharmonized, constituted by various sampling, measurement, and analytical techniques (EU Commission, 2023). Several physical, chemical, and biological soil aspects can be adopted as indicators for soil fertility. Chemical indicators are the most commonly used for assessing soil health, but there is an increasing emphasis on the role of pedofauna and its biodiversity (Lehmann et al, 2020) as an essential parameter to understand soil ecological functions and networks, with arthropods being an important tool for understanding soil-living ecosystems responsible for soil quality. They are involved in several processes, such as the breaking down, decomposition, and transportation of organic matter (through the digestion process), cycling nutrients, contributing to the development of soil structure, and water management (Lavelle et al,2006) (Saha et al, 2020). Furthermore, since they are incredibly suited to soil conditions and because they live, eat, and reproduce into the soil, some groups are particularly sensitive to changes in soil quality and characteristics (Parisi et al, 2005). In the last decades, the Soil Biological Quality Index based on arthropods (QBS-ar), created by Parisi (2001), has been increasingly used and implemented. The QBS-ar metric is determined by counting several groups of microarthropods that are morphologically suitable to soil niches. The soil quality improvement is directly correlated with a higher number of well-adapted groups to specific soil conditions. The QBS-ar was also used as a starting model to develop another biological indicator: the Soil Biological Quality Index based on earthworms (QBS-e) (Paoletti et al, 2013). The importance of earthworms for soil fertility is well-known since they can ingest large amounts of soil through their digestive tracts and expel it added of nutrients (N, P, K, Ca) able to improve soil fertility and organic compounds able to improve the soil granular structure (USDA, 2009). This enhanced fine structure may then be used as a seedbed to develop plants. The production of binding agents by earthworms is what creates macro-aggregates that are stable in water. Through soil mixing and burrowing, they increase soil porosity, improve soil aeration and water percolation (reducing erosion phenomena). Earthworms are involved in the cycling of nutrients, the breakdown of plant residue, and the redistribution of nutrients across the soil profile while they eat. Their castings are a source of nutrients, as are decomposing or dead earthworms. These advantageous effects encourage root development and proliferation far into the soil to meet water and nutritional needs. Furthermore, certain earthworm species are particularly important due to their supposed ability to design ecosystems, meaning they can directly affect their surroundings and make available resources to other organisms (Ashwood et al, 2022).

The aim of this work was to assess the biological fertility of soils under different agricultural management by using the QBS-ar and QBS-e bioindicators, as valuable indexes to predict the quality of the final products.

To test the hypothesis, the biological indexes were evaluated in two different soil management: blackberry (BB) and alfalfa (AA) fields in the “Società Agricola La Mandriola s.s.”, Marche region (Italy). Furthermore, the impact of anthropic activities and the influence of intrinsic soil morphology and physicochemical properties were considered to explain the variability of QBS-ar and QBS-e indexes in the two management.

2. Materials and Methods

2.1. Description of study site

The study was run in the “Società Agricola La Mandriola s.s.” located in a hilly area (42 m a.s.l.) 2 km far from the Adriatic Sea in Marche region (Italy) (Figure 1), on soil formed on soil formed on terraced alluvial deposits prevailing gravels associated with subordinate sands, silts, and clays (Carta geologica regionale, 2000).

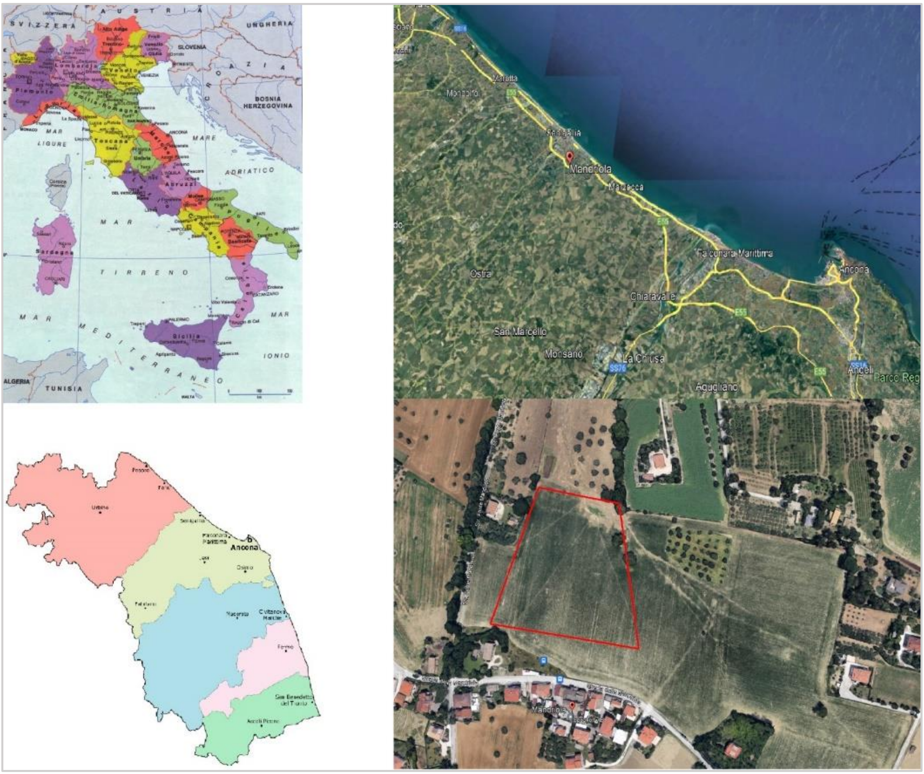

Figure 1. Study site location in "Società Agricola La Mandriola s.s.", Marche region, Italy.

Following the Köppen-Geiger climate classification, the climate of the studied area is mediterrean, characterized by mild wet winters and warm to hot dry summers (Lionello et al, 2006; Kottek et al, 2006; Belda et al., 2014). The studied field is subdivided into 1 ha of alfalfa and 0.75 ha of blackberries (*Var. Chester and Loch Ness*). The alfalfa field was settled on March 2021, while the blackberries field was settled on December 2021, following both organic and minimum-tillage management. The blackberry field has a planting layout of 1 × 2 m and it is micro-irrigated.

2.2. Soil, QBS-ar and QBS-e sampling

In June 2023, a geomorphological and soil survey was run to select sampling sites with similar north exposure and slope (10%).

To evaluate soil characteristics, a total of 6 profiles representative of the study site condition were opened, 3 profiles per field (Figure 2).

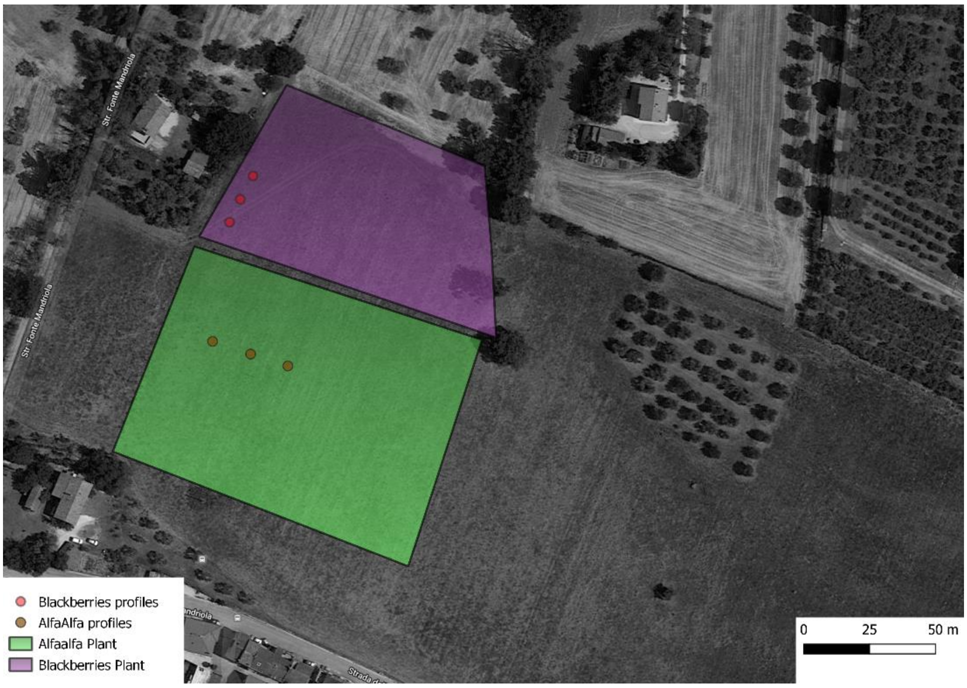

Figure 2. Blackberry (BB) and alfalfa (AA) fields with soil profile localization in "Società Agricola La Mandriola s.s.", Marche region, Italy.

In each crop field soil profiles were excavated at approximately 20 m from each other, until the depth of ≈1 m and then morphologically described using Schoeneberger et al. (2012). Representative soil samples of about 1 kg were collected from each genetic horizon and maintained inside a portable fridge and, once in the laboratory, they were air-dried, sieved at 2 mm to remove the skeletal particles. To extract arthropods for the assessment of QBS-ar, 10 mini-pits of soil samples (5 x each crop field), each with a volume of 10 cm^3, were excavated at approximately 10 m from each other (Figure 3). The soil samples were then placed, sealed, and transported inside a portable fridge to the laboratory for the extraction.

To extract earthworms, a spade fork was used to dig 30 ×30 cm holes and to hand-sort specimens (Paoletti et al., 2013) in 10 sample points (5 x each crop field) (Figure 3). The specimens were preserved in plastic tubes with 90% ethanol and transported inside a portable fridge to the laboratory for the identification.

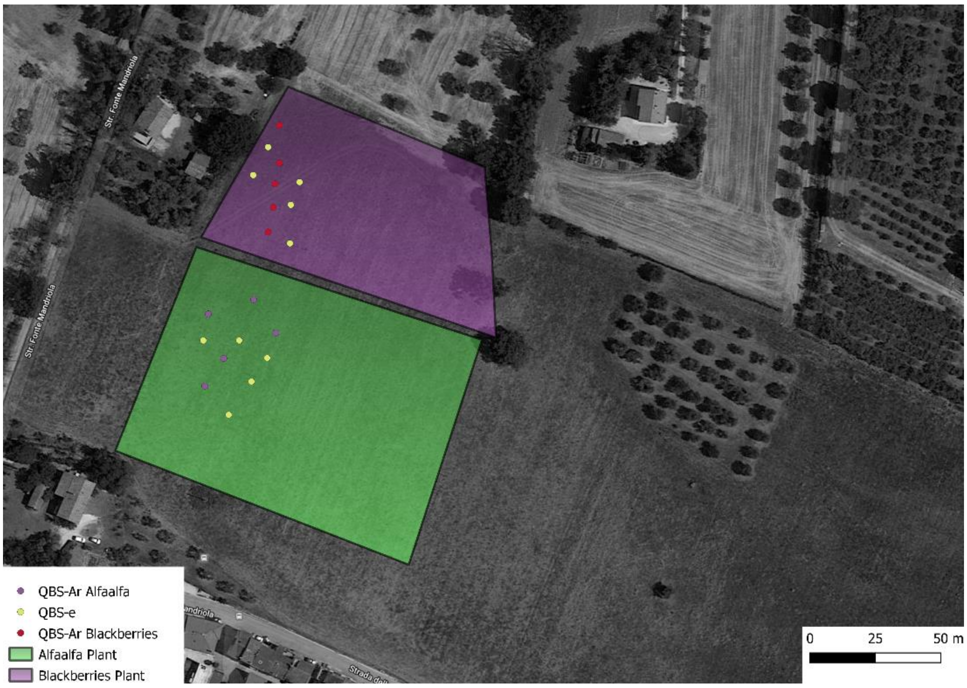

Figure 3. Blackberry (BB) and alfalfa (AA) fields with mini-pits localization for QBS-ar and QBS-e evaluation in "Società Agricola La Mandriola s.s.", Marche region, Italy.

2.3. Soil analyses

To determine the particle-size distribution were used an aliquot of 15 g of fine earth, then sand was retrieved by wet sieving at 0.053 mm, while silt and clay were obtained by column sedimentation at 19–20 ◦C (Day, 1965). The pH was measured potentiometrically in water (1:2.5 solid:liquid ratio), using a combined glass-calomel electrode immersed into the suspension. The content of total organic C (TOC) was estimated by K-dichromate digestion, heating the suspension at 180 ◦C for 30 min (Allison, 1965).

2.4. Data treatment and statistical analysis of soil properties

For each horizon, a single determination was performed for particle-size distribution, pH, and TOC. Results obtained from each horizon in the three profiles for BB and AA were considered replicates and used to calculate average and standard deviation in BB and AA. R program (March 1, 1093, R Core Team, 2014) was used for statistical analysis, that was run for soil physicochemical properties among soil profiles. To analyse the results, ANOVA was applied to enhance significant differences in soil profile properties between BB and AA. Data were tested for normality and homoscedasticity by performing the Shapiro-Wilk statistical test (stats R package) and by the Levene's test (car R package), both at 5% of significance level, respectively. If the transformed data were normally distributed, a post-hoc Tukey's Honest Significant Difference (HSD) test with $P \leq 0.05$ was used to compare the means. When normality was not respected, the Kruskal-Wallis test was applied. In the case of heteroscedasticity, the Welch one-way ANOVA test was performed.

2.5. Microarthropods extraction and specimens identification

The samples were transported to the laboratory, and the microarthropods were extracted using the Barlese-tullgren met hod (Figure 4) (Menta et al, 2015) which consists of a mesh above a funnel together with the soil sample.

Figure 4. Extraction of the microarthropods using Barlese-tullgren method

A bottle containing a preservative solution (composed of a high percentage of ethanol) is placed under the funnel to collect the microarthropods. The time of extraction should not be less than 5 days. The microarthropodes collected, were observed at low magnification (stereo-microscope at a 5-100x range) (Figure 5).

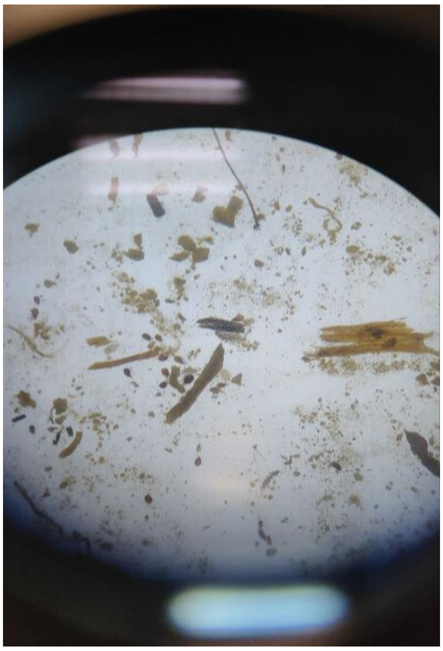

Figure 5. Observing microarthropods under stereo-microscope

The defining of the biological forms (morpho type) is based on the recognition of the different adaptation levels to the soil environment for each systematic group. Once the microarthropods are observed, an Eco-morphological score (EMI) will be assigned, proportionate to its adaptation level (Table 1).

Table 1. Guidelines to recognize the arthropods-morpho type. (Aspetti et al, 2009)

Group	EMI score
Protura	20
Diplura	20
Collembola	1-20
Microcoryphia	10
Zygentomata	10
Dermaptera	10
Orthoptera	1
Embioptera	1-20
Blattaria	10
Psocoptera	5
Hempitera	1-10
Thysanoptera	1
Coleoptera	1-20

Hymenoptera	1-5
Diptera (Larvae	10
Other holometabolous insects(larvae)	10
Other holometabolous insects(adults)	1
Acaria	20
Araneae	1-5
Opiliones	10
Pseudoscorpiones	20
Isopoda	20
Chilopoda	10-20
Diploda	10-20
Pauropoda	20
Symphyla	20

2.6. Specimens identification and QBS-e assessment

Once in the laboratory, the collected earthworms were observed under a stereomicroscope (range 5-100 x) for how concern anatomical features like length, body pigmentation, type of prostomium, distance of setae, position of clitellum and number of spermathecae. Morphological and anatomical identification of the specimens were carried out by using LOMBRI software (Paoletti and Gradenigo, 1996) and especially Mršić (1991). Species names were upgraded following Blakemore (2008) and Csuzdi (2012) and the website http://taxo.drilobase.org.

To assess the QBS-e index (Paoletti et al., 2013) an ecological category was attributed to each sampled earthworm among five categories: endogeic, epigeic, deep-burrower, coprophagic, and hydrophilic, established on the ecology, ethology and anatomic features of each living specimen, and the age recognition between immature and adult (without or with clitellum) (Fusaro et al., 2018). An EcoMorphological score (EMI) was attributed to each ecological category and age (Table 2).

Table 2. EcoMorphological Index (EMI) scores attributed to each ecological category and age (Paoletti et al., 2013).

Ecological category	Age	EMI score
Hydrophilic (IDR)	Immature (Im)	1

Hydrophilic (IDR)	Adult (Ad)	1
Coprophagic (COP)	Immature (Im)	2
Coprophagic (COP)	Adult (Ad)	2
Epigeic (EPI)	Immature (Im)	2.5
Endogeic (END)	Immature (Im)	2.5
Epigeic (EPI)	Adult (Ad)	3
Endogeic (END)	Adult (Ad)	3.2
Anecicus (ANE)	Immature (Im)	10
Anecicus (ANE)	Adult (Ad)	14.4

In detail, ecological categories present the follow characteristics:

- Hydrophilic earthworms, living and feeding in damp soils, river bottom and shallow water-table soils (i.e. *Eiseniella tetraedra*);
- Coprophagic earthworms, living and feeding in manure or compost and closely associated with a high content of raw OM (i.e. *Eisenia fetida*);
- Epigeic earthworms, dorsally pigmented, living and feeding in the litter or in A horizon of soil profile, with scarce digging capacity (i.e. *Lumbricus castaneus*);
- Endogeic earthworms, usually less pigmented, living and feeding between A02 and A1 horizons, able to dig mainly horizontal burrows (i.e. *Aporrectodea caliginosa*);
- Anecic and/or deep-burrower earthworms, even larger in size than other ecological categories, can reach A2 and B soil horizons, are able to dig vertical burrows up to a few meters in depth (i.e. *Lumbricus terrestris*), but often rise to the surface to feed on litter (i.e. *Octodrilus complanatus*).

The formula proposed by Fusaro et al. (2018) was used to assess the QBS-e Index:

*QBS-e = (HYD Im, Ad score * N) + (COP Im, Ad score * N) + (EPI Im score * N) + (END Im score * N) + (EPI Ad score * N) + (END Ad score * N) + (ANE Im score * N) + (ANE Ad score * N)* where N = n° individuals/m^2, therefore it is important to determine the density of each ecological category in order to compare data. In this study, due to the number of replicates, the coefficient has a value of 2.2.

3. Results and discussion

3.1. Soil morphology and physicochemical properties

In the two crop fields (BB and AA) the soils belonged to the order of Inceptisols according to Soil Survey Staff (2014), due to the presence of A (ochric) and Bw (cambic) horizons (Table 3). In general, A and Bw horizons showed from light olive brown to light yellowish brown colours, and a good degree of aggregation made generally of blocks from very fine to very coarse. Such good state of aggregation and the fine texture indicated these soils as moderately drained and, consequently, with good water-holding capacity (Neeteson, et al., 1997). Roots were rather abundant, from very fine to coarse in size. Skeleton were everywhere under 2% of abundance. The Bw horizons showed slickensides and cracks, due to the presence of 2:1 clay mineral in fine soil fraction produced by the parent material weathering.

Table 3. General features and morphology of the soils under blackberries and alfalfa. Società Agricola La Mandriola s.s., Marche, Italy. For symbols see legend.

Horizon[a]	Depth	Thickness	Boundary	Color[b]	Structure[c]	Roots[d]	Skeleton	Observation
	cm				Grade Type Sz	Qty Sz	%	
Blackberry field								
Soil classification: Inceptisols (Soil Survey Staff, 2014).								
BB_P1 - Soil cover: 100%, grassed								
Ap	0-17	9-14	CW	2.5Y 4/4	2 sbk vf,f,m,co,vc	3 vf,f	< 2% mm	
Bw1	17-36	20-22	CW	2.5Y 4/4	2 sbk vf,f,m,co,vc	2 vf,f	< 2% mm	Presence of slickensides
Bw2	36-42+	-		2.5Y 4/4	2 sbk vf,f,m	3vf	< 2% mm	Presence of mottles
BB_P2 - Soil cover: 100%, grassed								
Ap	0-10	5-10	CW	2.5Y 4/4	2 gr vf,f; sbk vf,f,m,co	3 vf,f	< 2% mm	
Bw1	10-38	26-28	CW	2.5Y 4/4	1 sbk vf,f,m,co,vc	2 vf,f	< 2% mm	
Bw2	38-40+				1 sbk f,m,co,vc	2 vf,f	< 2% mm	
BB_P3 - Soil cover: 100%, grassed								
Ap	0-15	8-15	CW	2.5Y 4/4	2 sbk-abk vf,f,m,co,vc	2 vf,m	2% mm	
Bw1	15-29	10-11	CW	2.5Y 4/4	2 sbk-abk f,m,co,vc	2 vf	2% mm	Lithochromic features (blue clay)
Bw2	29-43+	-		2.5Y 4/4	1 sbk-abk f,m,co,vc	1vf	2% mm	Lithochromic features (blue clay)
Alfalfa field								
Soil classification: Inceptisols (Soil Survey Staff, 2014).								
AA_P1 - Soil cover: 100%								
Ap	0-20	15-20	CW	2.5Y 4/4	2 sbk vf,f,m,co,vc	3vf,f; 1m, co	< 2% mm	
Bw1	20-30	10-17	CW	2.5Y 5/4	2 sbk vf,f,m,co,vc	2vf,f; 1m	< 2% mm	Presence of cracks: with 0.5 cm
Bw2	30-45+	-	-	2.5Y 5/4	2 sbk vf,f,m,co,vc	3vf,f; 1m,co	< 2% mm	Presence of cracks: with 0.5 cm
AA_P2 - Soil cover: 100%								
Ap	0-18	12-16	CW	2.5Y 5/4	2 sbk vf,f,m,co,vc	3 vf,f; 1 m,co	< 2% mm	
Bw1	18-32	13-16	CW	2.5Y 5/4	2 sbk vf,f,m,co,vc	2 vf; 2m	< 2% mm	Presence of slickensides
Bw2	32-42+	-		2.5Y 6/4	2 sbk vf,f,m,co,vc	2 vf; 2m	< 2% mm	Presence of slickensides
AA_P3 - Soil cover: 100%								
Ap	0-15	15-19	CW	2.5Y 4/4	2 sbk vf,f,m,co	3 vf; 1f	< 2% mm	
Bw	15-32+	-		2.5Y 4/3	2 sbk vf,f,m,co	3 vf; 1 m,co	2% mm	

[a]Horizon nomenclature according to Schoeneberger et al. (2012).
[b]mixed at field humidity, according to Munsell Soil Color Charts
[c]1=week, 2=moderate, 3=strong; f=fine, m=medium, co=coarse; vc=very coarse; gr=granular, abk=angular blocky, sbk=subangular blocky
[d]1=few, 2=common; 3=abundant; vf=very fine, f=fine, m=medium, co=coarse.
[e] C=clear, W=wavy

The texture analysis identifies the studied soils mainly as silty loam, clay loam, and clay (Table 4) which means that fine dominate on coarse particles. However, statistical analysis does not enhance significant differences in particle-size distribution between alfalfa and blackberry fields. The pH values of soils in BB and AA fields range from 8.1 to 8.4, with no significant differences between the two management, defining soils as alkaline. Those values are explained by the common limestone parent material from which they originated (Carta Geologica Regionale, 2000). For how concern TOC results, soils in blackberries field show values ranged from 10.35 g kg^{-1} to 17.70 g kg^{-1}, while soils in the alfalfa field show values ranged from 7.20 g kg^{-1} to 16.35 g kg^{-1}. Statistical analysis does not underline significative differences in TOC between the two different management. Despite the anthropic impact, soil carbon contents are moderate with the tendency to slightly decrease along the profile. TOC values show that a proper soil management preserved organic matter and therefore organic carbon, being the TOC a reliable proxy to assess the soil quality and fertility. Furthermore, clay-rich soils shield organic matters from decomposing by stabilizing molecules that adhere to clay surfaces. (Jien et al, 2015)

Table 4. Single values and mean values of particle-size distribution, pH, and total organic carbon (TOC) of the soils in blackberries (BB) and alfalfa (AA) fields, considering the three soil profiles as replicates (Px). Società Agricola La Mandriola s.s., Marche, Italy. Numbers in parentheses are the standard deviations. No statistical differences were detected in soil physicochemical properties between BB and AA fields, at $P \leq 0.05$ level of significance.

Profile	Horizon	Particle size distribution			Textural class	pH	TOC
		Sand	Silt	Clay			
			g kg^{-1}				g kg^{-1}
BB_P1	Ap	535	310	155	Sandy loam	8.4	13.20
	Bw1	362	272	366	Clay loam	8.4	17.70
	Bw2	417	235	348	Clay loam	8.3	10.50
BB_P2	Ap	177	369	454	Clay	8.4	11.40
	Bw1	340	473	187	Loam	8.4	10.35
BB_P3	Ap	524	281	195	Loam	8.1	14.40
	Bw1	119	335	545	Clay	8.4	13.50
	Bw2	179	613	209	Silt Loam	8.3	12.60
AA_P1	Ap	393	133	473	Clay	8.3	12.00
	Bw1	105	793	101	Silt Loam	8.3	8.70
	Bw2	227	512	261	Silt Loam	8.3	7.80
AA_P2	Ap	405	217	378	Clay	8.1	10.35
	Bw1	407	389	204	Loam	8.3	10.20
	Bw2	92	667	241	Silt loam	8.4	7.20
AA_P3	Ap	167	772	61	Silt Loam	8.2	16.35
	Bw1	175	373	452	Clay	8.3	12.15
avg_BB[1]		332(160)	361(125)	307(143)	Clay loam	8,3(0.1)	12.96(2.40)
avg_AA[1]		247(135)	482(248)	272(153)	Clay loam	8,3(0.1)	10.59(2.94)

[1] averaged numbers of soil physicochemical properties in blackberries (BB) and alfalfa (AA) field; standard deviation in parenthesis (n=8).

3.2. QBS-ar

According to the QBS-ar results (Table 5), the highest score was achieved regarding the AA sites of the project (127).

Table 5. Result of the QBS-ar regarding the alfalfa (AA) and blackberry (BB) fields managed as organic (O) agricultural system, the Biological Forms (BF) recognized and the QBS score, Marche region, Italy.

Crop field	Agricultural system	BF	QBS
AA_1	O	11	122
AA_2	O	9	106
AA_3	O	12	117
AA_4	O	11	127
AA_5	O	9	109

BB_1	O	14	118
BB_2	O	10	99
BB_3	O	11	101
BB_4	O	14	104
BB_5	O	8	121
avg*_AA	O	10.4	116.2
avg*_BB	O	11.4	108.6
avg*_Total	O	10.9	112.4

* avarage of the replicates

This result is certainly related to the fact that alfalfa required much less agricultural tillage compared to blackberries, providing less stress to the biological component of the soils, and allowing the development of edaphic forms of highest biological quality. Considering the soil as a whole, the final QBS score is 112.4 which, according to the reference data related to the QBS score (Menta et al,2011) provided a characterization and a score of the soil regarding the land use and the QBS-ar score, includes the studied soil in the "good" category, recognizing the soil fertility and quality (Table 6).

Table 6. Soil quality related to the QBS-ar score and the intended use of that land.

Arable Lands and herbaceous crops		Tree crops and vineyards		Natural environments, woods, meadows, pastures	
QBS-ar Value	Quality	QBS-ar value	Quality	QBS-ar value	Quality
>120	Optimal	>160	Optimal	>200	Optimal
101-120	Good	141-160	Good	171-200	Good
81-100	Decent	121-140	Decent	151-170	Decent
61-80	Sufficient	101-120	Sufficient	131-150	Sufficient
41-60	Moderate	81-100	Moderate	111-130	Moderate
31-40	Poor	61-80	Poor	91-110	Poor
<30	Insignificant	<60	Insignificant	<90	Insignificant

3.3. QBS-e

A total of 36 earthworms were counted and recognized from the replicas collected in AA and BB fields during the QBS-e assessment (Table 7). While the replicates performed on AA all yielded specimens, only 2 of the 5 BB replicates showed earthworms. This is certainly linkable to the tillage activities applied on the BB field, which have disturbed earthworms' ecosystem and activities (Gavinelli et al., 2018). According to the QBS-e references (Paoletti et al 2013), the quality class of all soils is within the "sufficient" range (Table 8), taking into consideration that the AA field site provided a score within the "decent" range, while the BB field, due to the lack of specimens, is classified as "poor". It was expected to obtain a higher QBS-e score, thanks to the silty-loam texture of the soil, suitable for earthworms (Vanvoren et al., 2015), and the organic C content but, due to the high sensitivity of earthworms to high temperatures, the earthworm population underwent through a collapse of adult individuals (Salwam-al Maliki et al., 2021). This can be confirmed also by our assessment: about 90% percent of collected earthworms were recognized as young individuals, proving the short-life span of the adults during the sampling period (June). Anecicus and coprophicus edaphic forms were not found during the assessment.

Table 7. Specimens, ecological categories, and QBS-e (Paoletti et al, 2013) evaluated in soil replicates of alfalfa (AA) and blackberry (BB) fields, Marche region, Italy.

		Alfalfa			
Replicates	**Taxon**	**SdS**	**EC**	**Ad**	**Im**
AA_1	*Aporrectodea Rosea*	Adult	EN	1	
AA_1	*Allelobophora chlorotica*	Adult	EN	1	
AA_1	*Aporrectodea Rosea*	Young	EN		3
AA_1	*Murchieona Minuscula*	Young	EN		2
AA_1	*Dendrobaena cognetti*	Young	EN		1
AA_2	*Aporrectodea Rosea*	Adult	EN		1
AA_3	*Murchieona Minuscula*	Young	EN		8
AA_3	*Dendrobaena cognetti*	Young	EP		2
AA_4	*Dendrobaena cognetti*	Adult	EP	2	
AA_4	*Murchieona Minuscula*	Young	EN		1
AA_5	*Eumenoscolex Antipai*	Adult	EP	1	
AA_5	*Murchieona Minuscula*	Young	EN		6
AA_5	*Eisiniella tetraedra bernensis*	Young	HY		1
HYj	1	1	22,22	22,22	
Epj	2,5	5	111,11	277,78	
Enj	2,5	24	533,33	1333,33	
Ena	3,2	0	0,00	0,00	
	Ad	**Im**	666,67	1633,33	

N.es	5	25	**es/m2**	**QBS-e**	
es/m^2	55,56	277,78	**133,33**	**326,67**	
		Blackberries			
BB_1	*Dendrobaena cognetti*	Young	EP		1
BB_1	*Murchieona Minuscula*	Young	EN		1
BB_2	*Eumenoscolex antipai*	Young	EP		1
BB_2	*Aporrectodea Rosea*	Adult	EN	3	
EC	**EC**	**n.es**	**es/m2**	**N**	
HYj	1	1	22,22	22,22	
Epj	2,5	2	44,44	111,11	
Enj	2,5	5	111,11	277,78	
Ena	3,2	0	0,00	0,00	
	Ad	**Im**	177,78	411,11	
N.es	0	3	**es/m2**	**QBS-e**	
es/m^2	0,00	33,33	**35,56**	**82,22**	
Avg QBS-e				**204,44**	

Table 8: QBS-e soil quality classes (Paoletti et al, 2013)

QBS-e score	Quality class
QBS-e > 1000	Excellent
600<QBS-e<1000	Good
300<QBS-e<600	Decent
100<QBS-e<300	Sufficient
0<QBS-e<100	Poor

4. Conclusion

The results of QBS-ar assessment demonstrate the undoubted fertility of the soil as well, with varied micro-arthropod communities, synonymous of the hospitality demonstrated by the soil environment, both relative to AA and BB fields. Lower values than expected were found using the QBS-e, ascribable to earthworms' susceptibility to high temperatures and soil tillage, as demonstrated by the quality gap between the QBS-e score in AA and BB. Despite this, the average value is still sufficient to attribute a fair quality to the soil. The AA and BB fields, according to the results, have similar soil morphologies and statistically equal physicochemical properties. These characteristics, together with the endowment of TOC, allow us to safely state that the soil turns out to be of quality, and that it can generate healthy products for human consumption. In conclusion, it can be said how the assessment of soil characteristics, coupled with QBS-e and QBS-ar assessment were found to be valid indexes of soil quality and health and thus valid for the recognition of soils capable to produce healthy food. To obtain a complete and exhaustive assessment of biological soil quality, it is recommended to repeat the study in different seasons throughout the different crop cycles.

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