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Soil and legumes of the Sibillini for high quality pasta

Application of the arthropod-based Biological Soil Quality Index (QBS-ar)

for soil health assessment

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“L'uomo, dall'inizio dei tempi, ha sempre rivolto lo sguardo al cielo.

*Le luci delle stelle hanno sicuramente destato curiosità
e sarà sembrato un ottimo posto per credere che potesse essere
la dimora degli Dèi o il luogo in cui ci ricongiungeremo alla fine dei nostri giorni.*

La terra, invece, no.

La terra è cruda, è tangibile, e per questo motivo può aver suscitato meno interesse e poche fantasie.

*Ma la terra è donna, la terra è madre, la terra dona,
e che il suolo fosse un universo così popolato non lo avrei creduto mai...”*

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1 ABSTRACT

Although progress has been made in recognizing the importance of soil, the crucial role that soil biota plays in providing essential ecosystem services is still largely overlooked and underestimated. Among the most abundant soil organisms, soil microarthropods are closely linked to plant health, crop production, and soil fertility. They contribute to organic matter decomposition and nutrient cycling, making them effective and reliable indicators of soil health. As a result, these organisms are increasingly being used in global assessments of soil quality and monitoring programs. The main objective of the study was to evaluate soil quality across various agroecosystems using the arthropod-based soil quality index (QBS-ar). Three distinct projects were presented, each focusing on a specific research question regarding the application and functionality of the QBS-ar index in different contexts within the Marche region, encompassing both protected and unprotected sites.

Specifically, the QBS-ar index was used to: i) explore the relationship between soil quality and the quality of crops and food, with an emphasis on organic legume cultivation in the Sibillini Mountains; ii) examine and compare soil quality and the conditions of the soil arthropod community across different organic agroecosystems – arable lands, vineyards and olive groves - within Conero Park (AN); and iii) assess the impact of agricultural practices over time, in the framework of the Sentina Reserve in San Benedetto del Tronto (AP).

The QBS-ar index has demonstrated its usefulness for various applications. Notably, a potential link was identified between soil quality, the chemical and physical composition of the soil, and the centesimal composition of chickpeas. Furthermore, higher soil quality was observed in arable land, followed by olive groves and vineyards. Similarly, soil quality was found to be higher in less intensive agricultural systems that exhibit higher landscape diversity and specific chemical and physical attributes, such as higher sand and potassium (K) content.

Nonetheless, to evaluate and compare performance and results, the QBS-ar index has been used alongside various metrics based on specific groups of soil microarthropods including, abundance,

density (ind/m² and ind/m³), community composition, the number of biological forms (BFs) and euedaphic forms (EFs), the Acari/Collembola (A/C) ratio, the percentage of Oribatid mites out of the total mite population (OA%), and Collembola morphological traits. Additionally, the QBS-ar index was also applied in conjunction with other soil bioindicators, such the Soil Biological Quality index based on earthworms (QBS-e), soil rhizospheric bacteria (16S), fungi (ITS), and protists (18S), as well as ecotoxicological tests, including earthworm avoidance tests (ISO Standard 17512-1:2008) and soil functional test, including bait lamina tests (ISO Standard 18311:2016).

Through this study, we aim to enhance the understanding of the QBS-ar index and provide valuable information that can contribute to both theoretical discussions and practical applications across various fields.

Key words: Soil biodiversity; soil microarthropods; agroecosystems; agriculture; food quality.

2 INTRODUCTION

2.1 SOIL BIODIVERSITY

Beneath our feet is an ecosystem so astonishing that tests the limits of our imagination. It is estimated that a tablespoon of fertile soil can contain more than 300 metres of fungal hyphae, more than a billion bacterial cells, and thousands of other different taxa. These include about one million cells of protists, and several hundred of nematodes. In addition to microorganisms and microfauna, soil also harbours different species of meso, macro and megafauna, including arthropods, earthworms, and mammals (FAO, 2020) (Fig. 1).

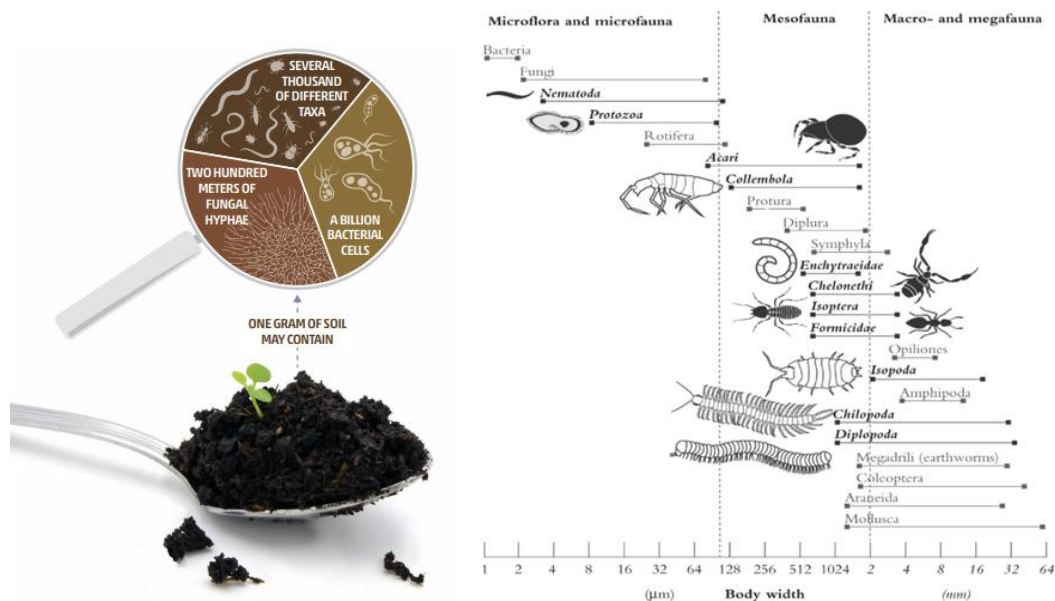


Fig. 1 - A teaspoon of soil contains more living organisms than people in the world. (FAO, 2020) (left). A schematic classification of soil biota based on body size. Modified from Swift, Heal and Anderson, 1979 (right).

Previous attempts to enumerate soil biodiversity estimated that it constituted approximately 25% of the Earth's total biodiversity (Decaëns et al., 2006). However, recent studies have advanced the hypothesis that soil biodiversity is numerically higher than previous estimates, constituting more than 60% of the total biodiversity on planet Earth (Anthony et al., 2023). However, the importance of soil biodiversity and its conservation is still partially neglected in most global biodiversity assessments, conservation actions and European policies, and underestimated by the public (Zeiss et al., 2022).

2.2 ECOSYSTEM SERVICES AND SOIL BIOTA FUNCTIONS IN SOIL

A healthy soil functions as a vital ecosystem for plants, animals, and humans (Bünemann et al., 2018), thanks to the activities that are performed or regulated by the soil biota. Soil biodiversity plays a crucial role in numerous ecosystem functions and soil functions. These include organic matter decomposition, nutrient cycling, plant productivity, soil formation, water retention and purification, climate regulation, bioremediation, and maintenance of health for plants, animals, and humans (Fig. 2) (Lavelle & Spain, 2001; Decaëns et al., 2006; Brussaard et al., 2007; de Bello et al., 2010; Mulder et al., 2011; Da Silva et al., 2016).

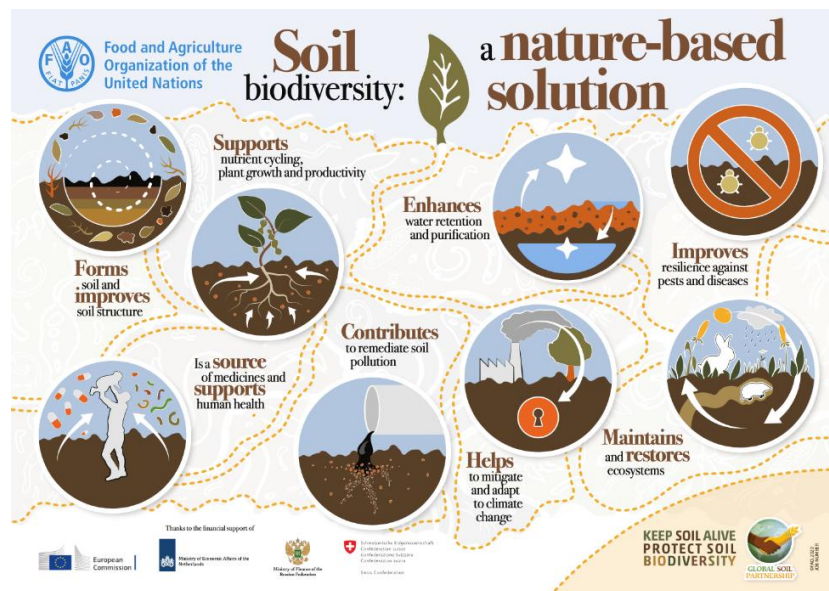


Fig. 2 - Soil biodiversity: a nature-based solution (FAO, 2020).

Protecting soil biodiversity is vital for addressing current environmental and social challenges, such as climate change and food security (Montanarella & Panagos, 2021). Healthy soils are essential for life. When managed sustainably, they provide food, clean water, habitats for biodiversity, and other vital services, while also enhancing climate resilience. We often take these services for granted, but soils are, in fact, a non-renewable and threatened resource. It is estimated that 60-70% of soils in the EU are unhealthy, primarily due to unsustainable management practices. Soil organisms play a crucial role in the health and functioning of soils, but they are at risk from poor soil management. Sustainable land use and soil quality improvement are suggested by FAO and United Nations to achieve lots of Sustainable Development Goals related to soil.

2.3 FOOD PRODUCTION

Soil biodiversity is essential for ensuring a secure food supply. According to the FAO, 95% of the food we consume originates from the soil, either directly or indirectly (FAO, 2015). This means that soil is the matrix in which food production takes place. However, soil is a non-renewable resource, making food security inextricably linked to soil conservation. Additionally, since plants obtain all their nutrients from the soil, both the quality of the food we eat, and its nutrient content depend on soil health. Thus, without healthy soil, we cannot produce healthy food. This idea was clearly highlighted in the title of the Horizon Europe Mission proposed by the Soil Health and Food Mission Board, titled *Caring for soil is caring for life*. However, our food system is known to be the primary driver of biodiversity loss (Benton et al., 2021). As agriculture impacts directly and severely on soil biodiversity (Orgiazzi et al., 2016). What is clear is that to maintain the health and fertility of soil and its ecosystem services, soil biodiversity cannot be sacrificed for the sake of ensuring a high primary production (Köninger et al., 2022).

2.4 UNSUSTAINABLE AGRICULTURAL PRACTICES

Unsustainable agricultural practices pose a threat to soil health, resulting in the loss of soil biodiversity and vital ecosystem services (Benton et al., 2021; Köninger et al., 2022). Negative effects are especially expected in intensively managed systems with simple cropping sequences (Eisenhauer, 2016). To foster sustainability, soil fertility, biodiversity and nutrient supply from the soil, organic farming uses diverse crop rotations, which include different leguminous crops, and rely on organic fertilisation (Moss et al., 2020). Land use changes have been shaping soil biodiversity during the last decades. At the regional level, across different European eco-regions, agriculture intensification, as well as afforestation or reforestation, are among the main factors determining patterns of soil fauna diversity (Lavelle et al., 1997; Bengtsson, 2002). Much of these changes have led to a decline in ecosystem quality and soil resources, with the consequent loss of biological diversity (Vandewalle et al., 2010).

2.5 SOIL-RELATED POLICY

The European Union (EU) placed the concept of "healthy soil" at the centre of the European Green Deal (EGD) adopted in 2019, which includes a strategy for climate change aimed at achieving climate neutrality by 2050. There are several soil-related policy instruments relevant to soil protection (Köninger et al., 2022). For instance, the EU Biodiversity Strategy 2030, recognised soil as: "one of the most complex and diverse ecosystems, and as the habitat of an extraordinary variety of organisms that regulate and control essential ecosystem services such as fertility, nutrient cycling and climate regulation". Although awareness of soil's significance is growing, there is still no specific EU legislation for protecting and monitoring soil health. In this regard, given the increasing degradation of European soils and biodiversity loss, the European Commission called for the development of a Thematic Strategy on Soil Protection within the Sixth Environment Action Programme (6EAP) with the objective to protect soils and its sustainable use across the EU. In July 2023, the European Commission proposed a soil monitoring and resilience directive (Halleaux, 2024). The goal of this proposal is to establish the first soil protection legislation applicable throughout the entire EU, known as the Soil Health Law (EU Commission, 2023; Köninger et al., 2022). The Soil Monitoring Law aims to monitor soil quality and restore healthy soils by 2050. This legislation will help the EU achieve its goals related to climate change, biodiversity loss, and food security. The objectives are to establish a monitoring framework for all EU soils, to define what constitutes "healthy soil" based on physical, chemical, and biological parameters, to identify and investigate contaminated sites, and to promote sustainable soil management in the EU. In addition, the proposal calls for the definition of conditions determining good soil ecological status and the improvement of soil quality monitoring through the application of new bio-indicators. Currently, several steps are still required to adopt the law. The European Parliament adopted its position on the Commission's proposal in April 2024 (Halleaux, 2024). This proposal outlines a framework for systematically assessing soil health. Effective measures to achieve these goals are currently under review and negotiations are ongoing. If an agreement is reached, the Council will approve the text.

2.6 SOIL HEALTH AND SOIL QUALITY

While the terms "soil health" and "soil quality" are often used interchangeably, there is a nuance between them. The term **soil health** emphasises the importance of soil in meeting societal needs, such as food production, and is linked to environmental sustainability, which encompasses economic, social, and environmental issues (Bünemann et al. 2018). In contrast, **soil quality** refers to the extent to which soil properties fulfil expected functions for the plants, animals, and human well-being (Schjønning et al. 2004), and it can be measured by using soil organisms.

2.7 SOIL MICROARTHROPODS AND THE ARTHROPOD-BASED BIOLOGICAL SOIL QUALITY INDEX (QBS-AR)

Among soil organisms, soil microarthropods are tiny organisms, which range from 0.1 to 2 mm in dimension, and live in the first 10-20 cm of the soil (**Fig. 3**).



Fig. 3 – Soil microarthropods. Photos by Andy Murray. A chaos of delight. Exploring life in the soil (<https://www.chaosofdelight.org/>).

They are considered key actors of agricultural soil functioning (Parisi et al., 2005; Yin et al., 2020; Ashwood et al., 2023; Chassain et al., 2024) and sensitive, promising and reliable indicators of both soil quality and sustainability of the agricultural practices applied (Reis et al., 2016; Joimel et al., 2017; Parisi and Menta, 2008). Soil quality can be assessed by using soil microarthropods and specifically the arthropod-based Biological Soil Quality Index (QBS-ar). The index is based on a coarse taxonomic identification and a classification into functional groups based on specific eco-morphological traits (EMI) (1-20) of adaptation to the soil habitat (Parisi, 2001). The morphological

traits reflect the performance of organisms in the soil environment and their contribution to soil functions. It is assumed that the better the soil quality, the higher the number of microarthropod groups that are well adapted to the soil (Parisi 2001, Parisi et al., 2005; Menta et al., 2018). The QBS index represent a numeric value calculated by summing the EMI values of the different microarthropod groups found in the soil sample according to their “biological forms” (BFs) (Sacchi and Testard, 1971), which indicate groups characterised by having the same convergent morphological features (Parisi, 2001). The highest value (20) is attributed to “euedaphic forms” (EFs), referring to microarthropod groups that exhibit morphological traits of strong adaptation to the soil environment, such as depigmentation, the absence of a flight or visual apparatus, and reduced appendages (Parisi et al., 2005). For example, Pauropoda, Symphyla and Diplura represent EFs in **Fig. 3**. In this regard, the method overcomes the challenges of species identification, which is rather time-consuming and requires specific taxonomic training (Parisi et al., 2005). The main phases for obtaining the QBS index are: (1) soil sampling; (2) soil microarthropods extraction; (3) preservation of the collected specimens; (4) stereoscopic analysis, determination of BFs and assignment of EMI values; (5) QBS index calculation for each soil sample (**Fig. 4**) (Parisi, 2001; Parisi et al., 2005).



Fig. 4 – Phases for QBS-ar index determination.

With reference to the soil sampling phase, at each selected site, one QBS-ar sample consists of three subsamples of soil (A, B, C) collected at a depth of 10 cm, each with a volume of approximately 1000 cm³, collected using a core drill or spade (**Fig. 5**). In detail, in the presented case studies, an adaptation has been made, a cylindrical steel soil corer ($\varnothing = \sim 10$ cm) was used to collect approximately 800 cm³ of soil at a depth of 10 cm. Soil microarthropod extraction was performed using a Berlese-Tüllgren funnel, equipped with 25 cm diameter funnel, 2-mm sieve mesh, and 25 W

light bulbs (~220 lm) suspended about 25 cm above each sample. The extraction processes lasted for 7 days. The extracted specimens were preserved in a solution of 75% ethyl alcohol and 25% glycerol by volume and were examined using a stereomicroscope (Olympus SZ30) with magnifications between 20x and 40x.

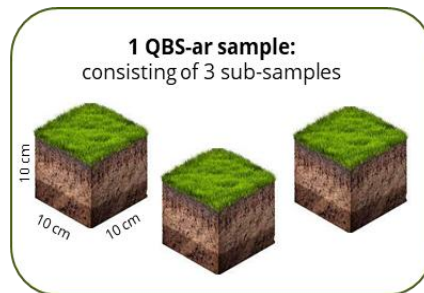


Fig. 5 - Representation of a QBS-ar sample.

Depending on the type of environment or agricultural system considered, soil quality judgments can be assigned to QBS-ar numerical values (**Tab. 1**) (Menta et al., 2011).

ARABLE LANDS AND HERBACEOUS CROPS		TREE CROPS AND VINEYARDS		NATURAL ENVIRONMENTS, WOODS, MEADOWS-PASTURES	
QBS-ar	Quality judgement	QBS-ar	Quality judgement	QBS-ar	Quality judgement
>120	Excellent	> 160	Excellent	>200	Excellent
101-120	Good	141-160	Good	171-200	Good
81-100	Discreet	121-140	Discreet	151-170	Discreet
61-80	Sufficient	101-120	Sufficient	131-150	Sufficient
41-60	Modest	81-100	Modest	111-130	Modest
<30	Null	<60	Null	<90	Null

Tab. 1 - Subdivision of QBS-ar values into intervals with corresponding quality ratings based on the environment (Menta et al., 2011b).

When the QBS was developed by Parisi (2001), a classification of the Italian soils, based on the index, was provided. This classification considered a wide range of environments, such as forests, pastures, and different types of farming systems, distributed across lowlands, hills and mountains. They aimed to provide a basis for soil quality cartography through quantitative measures (Parisi et al., 2005). Having regional maps relating to the QBS-ar index would be a significant advancement in the implementation and recognition of national-level databases and policies. Nonetheless, it is imperative that the standardisation of the methodology is prioritised as a fundamental initial step. In line with this, below is a SWOT analysis of the QBS-ar index based on D'Avino et al. (2021).



2.8 OTHER SOIL ORGANISMS AS BIOINDICATORS OF SOIL QUALITY AND ABIOTIC VARIABLES

To obtain a complete understanding of the soil quality, a part from the QBS-ar index (Parisi, 2001), other indicators based on soil microarthropods can be used, including soil microarthropod abundance, density (ind/m² and ind/m³) and community composition, number of biological (BFs) and euedaphic forms (EFs), Acari/Collembola (A/C) ratio (Bachelier, 1978), percentage of Oribatid mites out of total mites (OA%) (Aoki, 1967; Aoki et al., 1977). Or it is possible to focus on specific microarthropod groups, such as Collembola (Collembola morphological traits and QBS-c). Or it is even possible to consider other organisms, such soil and rhizosphere bacteria (16S), fungi (ITS) and protists (18S) communities or earthworms, using the Soil Biological Quality index based on earthworms (QBS-e). In addition, ecotoxicological tests can also be used, including avoidance test with earthworms (ISO Standard 17512-1:2008) (ISO, 2008) or soil functional test, such as the Bait Lamina Test (BLT) (ISO Standard 18311:2016) (ISO, 2016).

Nonetheless, to further assess soil quality, abiotic variables should also be considered. In many studies soil chemical-physical properties are mentioned, but there are also other abiotic variables that can affect soil microarthropods, including: soil chemical-physical properties, soil environmental parameters, agricultural practices, climatic data, and landscape features (Le Provost et al., 2021).

3 AIM OF THE THESIS AND RESEARCH QUESTIONS

The main objective of the present thesis was to evaluate soil quality across various agroecosystems using the arthropod-based Biological Soil Quality Index (QBS-ar). The research aimed to test the functionality of the index by exploring its applications across various contexts, including different agroecosystems and agricultural management practices in both protected and non-protected sites at a local scale, in the Marche region.

In the following chapters, three separate projects will be presented (**Fig. 6**), each focusing on a specific research question. Specifically, the QBS-ar index was used to 1) explore the relationship between soil quality and crop and food quality, 2) examine and compare soil quality and soil arthropod community conditions in different organic agroecosystems, and 3) assess the impact of agricultural practices over time.

Through this exploration, the thesis seeks to deepen the understanding of the QBS-ar index and provide valuable insights that can inform both theoretical discussions and practical applications in diverse fields.



Fig. 6 – Three main projects and research questions.

Nonetheless, to evaluate and compare performance and results, the QBS-ar index has been used alongside various metrics based on specific groups of soil microarthropods including, abundance, density (ind/m² and ind/m³), community composition, the number of biological forms (BFs) and euedaphic forms (EFs), the Acari/Collembola (A/C) ratio, the percentage of Oribatid mites out of the total mite population (OA%), and Collembola morphological traits. Additionally, the QBS-ar index was also applied in conjunction with other soil bioindicators, such the Soil Biological Quality index based on earthworms (QBS-e), soil rhizospheric bacteria (16S), fungi (ITS), and protists (18S), as well as ecotoxicological tests, including earthworm avoidance tests (ISO Standard 17512-1:2008) and soil functional test, including bait lamina tests (ISO Standard 18311:2016).

4 CHAPTER 1: SOIL AND LEGUMES OF THE SIBILLINI FOR HIGH QUALITY PASTA



Project ID: Aree Interne DOT1306935

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*“E che pensieri immensi,
che dolci sogni mi spirò la vista,
di quel lontano mar, quei monti azzurri
[...]”*

Giacomo Leopardi (I Canti – XXII Le Ricordanze, 1829)

4.1 ABSTRACT

The 95% of the food we consume originates from the soil, making soil health a crucial factor in determining food quality. However, soil is a non-renewable resource, thus ensuring food security relies on its preservation. Despite this importance, many aspects of the “soil-food link” require further investigation. The goal of the present study was to explore the relationship between soil and food, focusing on the agricultural soils of the Sibillini Mountains and locally produced legumes. To shed light on this relationship, a holistic approach was adopted. The soil quality was assessed using various indicators, including soil microarthropods and multiple abiotic variables. Additionally, functional traits and rhizospheric bacterial communities were analysed to define crop quality. For evaluating food quality, chemical-physical and technological parameters of chickpeas, chickpea flour, and chickpea paste (produced specifically from the agricultural fields studied) were assessed. The results indicated that biological soil indicators, particularly the QBS-ar index, could

potentially predict crop characteristics and overall food quality. However, many aspects of this study warrant further investigation, and additional data are needed to validate and confirm these findings. Since the study involved actual producers and farms, several challenges arose that resulted in a significant reduction in the experimental sample size. Nevertheless, this is the first study to examine the relationship between soil quality and food quality.

4.2 INTRODUCTION

Sibillini mountains, known as *monti azzurri*, are a mountain range located in the heart of the Italian Apennines, straddling the regions of Marche and Umbria, including the provinces of: Ascoli Piceno, Fermo, Macerata, and Perugia. This massive limestone rock has always stirred the imagination of people because of the uniqueness of its landscape, marked by peaks, narrow gorges and dense forests. Numerous legends (*La Sibilla Appenninica* and *il Lago di Pilato* to name a few) still live on in these mystical and contemplative environments and revive in the imagination of those who enter them, making the Sibillini Mountains one of the most mysterious and fascinating places in Italy.

My Ph.D. project focused on this area as the scholarship, through which it was funded, is part of the *Piano Stralcio* for Research and Innovation 2015-2017 and the National Strategy *Aree Interna*. The main objective of which is the recovery, and the enhancement of marginal area, which are characterized by the loss of essential services (education, health, mobility) and population decline. The project was focused specifically on the *Area Interna Piceno* located in Ascoli Piceno (AP) province (**Fig. 7**). The area is composed of 15 municipalities that particularly experienced damage following the earthquakes that struck central Italy in 2016 and because of which population decline was further exacerbated. The project stems from the idea of promoting the revitalization of these marginal areas. Azienda Agricola Monte Monaco S.r.l. (www.montemonaco.bio), a local company specialized in the production of organic legume pasta, was our partner in the study. The company produces products with a marked territorial

connotation, high added value and capable of satisfying broad market sectors: organic, vegan, gluten-free, with a clearly rising trend in Italy and abroad. The gastronomic speciality *pasta di legumi* is the latest innovation in the pasta market segment and responds to modern consumer demands for health/nutritional properties and ‘new taste’ in products already present in Italian gastronomic culture.

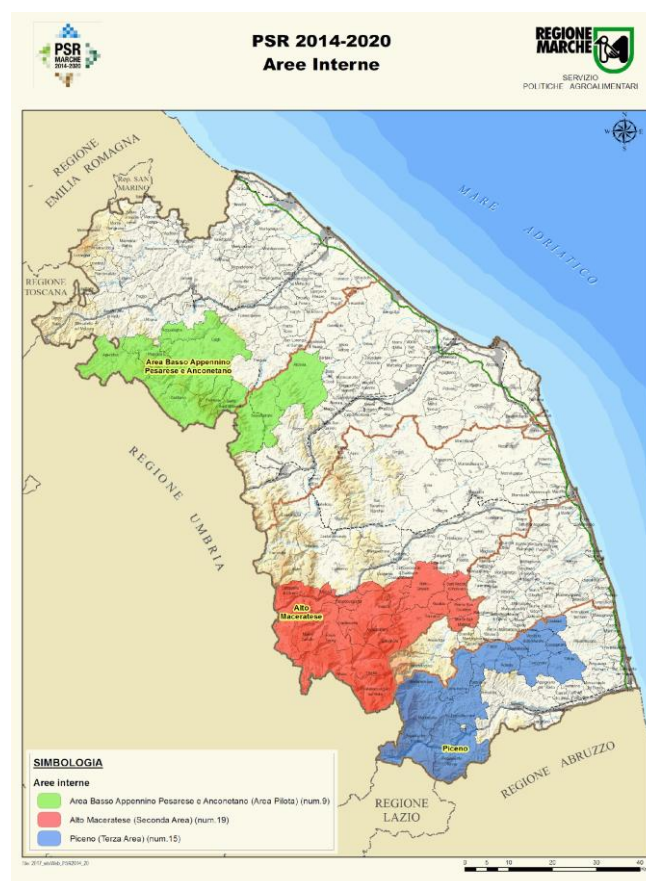


Fig. 7 – The “Area Interna Piceno” which comprises the following municipalities: Acquasanta terme, Arquata del Tronto, Carassai, Castignano, Comunanza, Cossignano, Force, Montalto delle Marche, Montedinove, Montegallo, Montemonaco, Offida, Palmiano, Roccafluvione, Rotella.

The participation of Azienda Agricola Monte Monaco, supports and raises awareness of activities that have remained in marginal areas. In fact, the project focuses on the innovation of a traditional Italian product (pasta) with a view to eco-sustainability, qualitative and nutritional improvement, as well as enhancement of the ecological and gastronomic-cultural healthiness of Marche region marginal areas. As soil is the matrix in which food production takes place, food quality is significantly influenced by the condition of the soil.

4.2.1 Traditional and contemporary uses of chickpeas in food

The crop selected for the study was chickpea (*Cicer arietinum* L.). Chickpeas are nutrient-rich legumes, composed mainly of carbohydrates, proteins and lipids, as well as vitamins and minerals. In 100 grams of dried chickpeas, there are approximately 343 calories, 20.9 grams of protein, 6.3 grams of lipids and 46.9 grams of carbohydrates, including sugars and fibre. They are also a good source of B vitamins and vitamin A, as well as minerals such as iron, selenium, magnesium, calcium, phosphorus, potassium, copper and zinc (CREA, 2019).

Chickpeas are used in both traditional cuisine and innovative processed products, making them suitable for a wide range of nutritional and dietary needs. In the Italian cuisine, chickpeas are mainly used in traditional preparations (soups and broths, cold salads, purees and veloutés, stews or baked dishes, toasted snacks). In addition, chickpea flour derivatives, obtained from dried seeds, is widely used in *farinata* and *panelle*, typical regional dishes; crepes and vegetable fritters, batter and dough for bread, baked goods. In recent years, there has been a growth in innovative chickpea-based processed products, including chickpea pasta, biscuits, confectionery products, and aquafaba, the liquid from cooking chickpeas, used as a substitute for egg white in mayonnaise and vegan desserts.

Particularly, chickpea flour is a useful ingredient in the production of gluten-free pasta. It helps to replace the role of gluten, improving the structure, texture and cohesion of the pasta thanks to the formation of a protein-starch matrix. It contributes positively to physical-chemical properties such as starch gelatinisation and water absorption. Although chickpea pasta has longer cooking times and greater losses during cooking than traditional pasta, it offers significant nutritional advantages: it is more digestible, has greater antioxidant activity, a lower glycaemic index and benefits the intestinal microbiota thanks to its prebiotic compounds (Smuda et al., 2024; Llavata et al., 2020). Furthermore, compared to other gluten-free pastas, it has a better taste and texture, making it a more popular choice, especially for those following diets for coeliac disease, diabetes or obesity.

4.2.2 Chickpeas cultivation in the Marche region

Chickpeas play an important role in both agriculture and culinary traditions in Italy. Chickpea cultivation is primarily found in the southern regions of Puglia, Sicily and Basilicata. There has been renewed interest in chickpea cultivation in recent years thanks to their nutritional value, low water requirements, and the potential benefit they offer to soil fertility as nitrogen fixers (Di Donato et al., 2022).

In the Marche region, for example, the area cultivated for chickpeas increased from 58 hectares in 2022 to 116 hectares in 2023, with an average yield of 18 quintals per hectare (Clementi, 2023). Chickpeas are an ideal crop for hillside cultivation, accounting for 53% of the region's land area (Marche Region, 2025), as they are susceptible to waterlogging. However, chickpea plants do not thrive in overly clayey soils, which are characteristic of the Marche hills. They prefer semi-arid, poor, light and well-drained soils. For this reason, the inner and mountainous areas of the region, such as the Sibillini Mountains, are ideal for chickpea cultivation. In these areas, sowing takes place in spring, as chickpeas are not very cold-resistant, and harvesting takes place in summer.

Nonetheless, chickpea cultivation can have positive implications for local communities and the rural economy of inner areas. Promoting this crop can encourage mountain farming, which combats land abandonment and supports the protection of the territory. Chickpeas can become a driving force for the diversification of agricultural income, including in terms of organic farming and short supply chains. By encouraging the revival of the rural economy, it is possible to develop a series of local micro-economies linked to processing (pasta, flour, ready-made products), (as our partner in the study did) direct sales or farmers' markets.

Additionally, it can increase the value of the landscape and identity, contributing to the conservation of the historic agricultural landscape and biodiversity. In this case, it can also strengthen local identity, especially if linked to native varieties or traditional designations, as it has already been done in the Marche region with *Cece Quercia di Appignano* (AMAP, 2024).

4.3 AIM OF THE FIRST PROJECT

The project aimed to explore the **soil-food link** considering the health of the Sibillini agricultural soils and the quality of the locally produced crops and derived food products. For the first time, an attempt was made to shed light on this relationship by applying a holistic approach. Starting with the assessment of soil quality, considering both biotic and abiotic indicators, crossing the entire supply chain, leading to the definition of crop and food quality.

The distinctive and unique aspect of this study is that the products obtained from each site were treated separately from one another, although the processing methods for the final product were consistent across all samples. This approach maintained a clear connection between food cultivation and the final product, enabling an assessment of the potential role of soil in influencing the characteristics of the food (**Fig. 8**). Consequently, it offered an effective and innovative method of analysing the relationship between soil quality and the quality of food produced there. Nevertheless, this was made possible thanks to a partnership with a small local company that had close ties to the area under investigation.



Fig. 8 - The three macro-areas of study involved in the project.

The following section will be divided into three parts:

- a) Soil quality assessment;
- b) Crop quality assessment;
- c) Food quality assessment.

4.4 SOIL QUALITY ASSESSMENT

To evaluate soil quality, various soil chemical-physical and biological indicators were applied, including the arthropod-based Soil Biological Quality Index (QBS-ar) (Parisi, 2001), and other indices based on soil arthropods were determined. In addition, weather, agricultural practices, soil chemical and physical parameters, soil environmental factors and landscape variables were considered.

4.4.1 Materials and methods

The project started in March 2021. The first phase was focused on the selection of organic farms with supply chain agreements with Az. Agr. Monte Monaco, to be involved in the biomonitoring activities. The monitoring plan included both farms located outside and inside *Area interna Piceno* to analyse a transect from the Sibillini Mountains to the Adriatic coast, to better assess any differences in soil health. Due to change of the ownership of the Company and the change in the crop rotations, it was not possible to maintain the same fields for the two years of monitoring. Then, four sites were selected in 2021 (1, 2, 3, 4) and four different sites were selected in 2022 (5, 6, 7, 8), for a total of eight sites considered (**Fig. 9**).

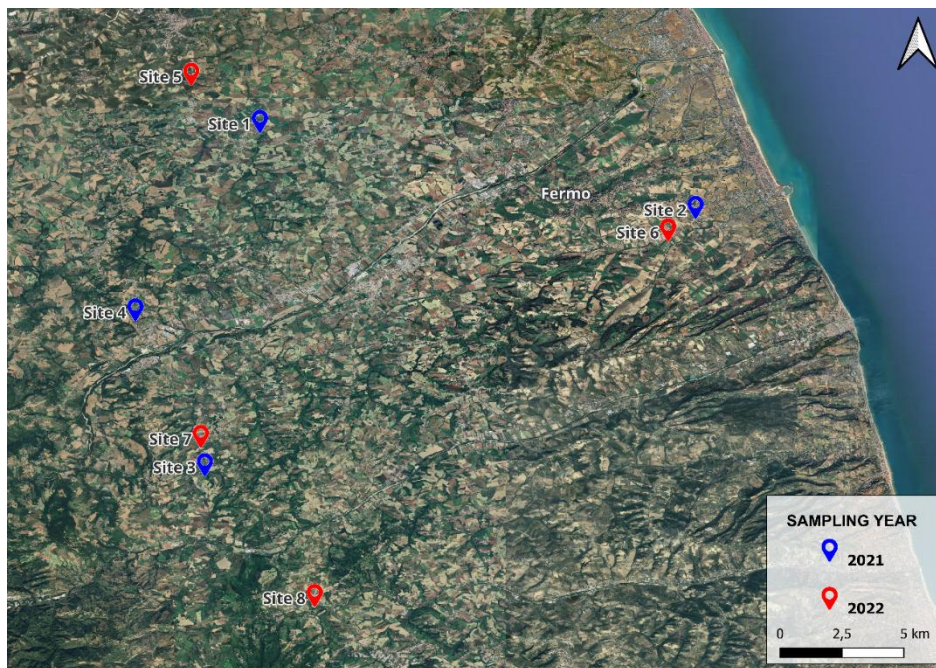


Fig. 9 - Satellite images (QGIS) of the investigated sites. All sites were located within Marche region (Italy). The pin represents the central point of each site, in blue the sites monitored in 2021, in red the sites monitored in 2022.

4.4.1.1 Site selection and agricultural management information

Each farm was contacted with the aid of Az. Agr. Monte Monaco. Those who agreed to participate were asked to make a site available for the soil analysis. Moreover, farmers were interviewed in detail, asked to fill a form to collect general information about the farm, (i.e., year of organic conversion, presence of animal farming), and agricultural practices applied, considering a period of three years. The information required referred to: crop rotation, fertilisation, irrigation, use of herbicides and pesticides, irrigation, ploughing depth (cm), grubbing, harrowing, rolling, sowing, yield, type of agricultural machinery used etc. As the selected farms were under organic management, all relevant information was available in the *Quaderno di campagna*. A total of eight sites, belonging to management system SG1, according to Marche Region MOnitoring SYstem of Soils at multi-Scale Project (MOSYSS Project) classification (Tiberi et al., 2014), and cultivated with legumes at the sampling time were considered. Samplings took place in spring (June) and autumn (October) in both years (2021 and 2022). In spring, all fields were cultivated with the same variety and accession of chickpeas (*Cicer arietinum* L., var. Sultano). Thanks to blockchain technology implemented by the company, each batch of product can be traced back to the farmer and to the site where the chickpeas were grown. This is how we can define the quality of the food.

Site	Farm	Province	Location	Pedological Province	Geographical coordinates	Field dimension (ha)	Yield (Kg/ha)	Time of sowing
1	Az. Agr. Santarelli e Arriva	Fermo	Francavilla d'Ete	5.7.6 – Medie colline fermane tra Ete M.e Tesino.	Lat: 43°11'43.19" Long: 13°32'58.81"	5.8	1417	Spring
2	Az. Agr. Le corti del Frate	Fermo	Contrada Salvano	5.7.6 – Medie colline fermane tra Ete M.e Tesino.	Lat: 43° 9'9.99" Long: 13°45'55.01"	4.0	303	Spring
3	Az. Agr. Antolini Ettore	Fermo	Santa Vittoria in Matenano	5.7.1 – Alte colline ascolane di Santa Vittoria in Matenano	Lat: 43° 1'31.78" Long: 13°31'20.69"	4.0	1193	Spring
4	Az. Agr. Senzacqua	Fermo	Piane di Falerone	5.7.4 – Colline di Loro P. e Falerone tra Fiastra e Tenna.	Lat: 43°06'7.51" Long 13°29'16.91"	3.0	500	Spring
5	Az. Agr. Santarelli e Arriva	Macerata	Corridonia	5.4.2 – Colline interne tra i fiumi Esino e Chienti	Lat: 43°13'07.4" Long: 13°30'56.3"E	6.6	1929	Spring
6	Az. Agr. Monte Monaco	Fermo	Fermo	5.7.2 - Colline interne e litoranee del Piceno dal Chienti al Tronto – Montelparo – Montalto delle Marche	Lat: 43°8'30.03" Long: 13°45'6.16"	1.9	852	Autumn
7	Az. Agr. Antolini Ettore	Fermo	Santa Vittoria in Matenano	5.7.2 - Colline interne e litoranee del Piceno dal Chienti al Tronto – Montelparo – Montalto delle Marche	Lat: 43°2'22.30" Long:13° 31'13.57"	1.5	880	Spring
8	Az. Agr. Simone Fedeli	Ascoli Piceno	Rotella	5.7.2 – Colline interne e litoranee del Piceno dal Chienti al Tronto	Lat: 42°57'38.87" Long: 13°34'36.34"	2.4	1074	Autumn

Tab. 2 – Information related to the sites considered in the study.

Vegetation cover (percentage, height, and type of herbaceous cover) was recorded at each site (**Tab. 3**). For each site, altitude, slope, and exposure were determined at the sampling time using the Compass & Altimeter app (PixelProse SARL, France) and further evaluated using Google Earth Pro and QGIS.

Site	Altitude (m asl)	Esposition	Slope	Herbaceous cover (%)		Herbaceous cover height (cm)	
				Spring	Autumn	Spring	Autumn
1	129	S/E	22.40 %	81-100	1-20	31-60	1-15
2	52	S	10.9-0.9 %	81-100	1-20	16-30	1-15
3	300	S/O	17.4-3.3 %	81-100	<1	16-30	1-15
4	295	E	0.8 %	81-100	<1	31-60	<1
5	150	N/E	3.4-8.2 %	81-100	<1	31-60	1-15
6	30	S	2.6 %	81-100	81-100	31-60	> 60
7	220	O	0.4-18.4 %	81-100	61-80	31-60	31-60
8	358	S/O	19 %	61-80	61-80	31-60	31-60

Tab. 3 – Other parameters recorded at each site.

4.4.1.2 *Soil biological parameters*

Soil samplings took place in June and October in both years (2021 and 2022). At each site, a QBS-ar sample, consisting of three subsamples (A, B, C) with a volume of about 800 cm³ each, was collected using a cylindrical soil corer (Coletta et al., 2025). Subsamples collection was equally spaced along a diagonal in the central part of each site. A homogeneous area of the site was chosen; disturbed areas (e.g., flattened vegetation, waterlogging, tractor tracks, water drainage channels) were excluded. Each year, 24 subsamples were collected, for a total of 48 soil sub-samples. In the laboratory, a Berlese-Tüllgren funnel, equipped with 25 W light bulbs and a 2-mm sieve mesh was used for arthropod extraction (10 days). The extracted specimens were collected and preserved in 3/4 ethanol 75% and 1/4 glycerol by volume. A stereoscopic analysis of the collected specimens was performed to determine biological form (BF) and Ecomorphological Index (EMI) (1 to 20), based on soil living adaptation, according to Parisi *et al.*, (2005). Total QBS-ar and partial QBS-ar values were calculated for each sample (Coletta *et al.*, 2025). Based on the agroecosystem considered, QBS-ar values were compared with a reference table to establish a soil quality judgment (Menta et al., 2011b). A quantitative approach was used; the number of individuals present for each BF was counted in each

subsample. In addition, soil microarthropod abundance, density (ind/m² and ind/m³) and community composition, number of biological (BFs) and euedaphic forms (EFs), and Acari/Collembola (A/C) ratio (Bachelier, 1986) were determined.

4.4.1.3 Soil chemical-physical properties

Soil samples for chemical-physical analyses were collected and analysed in accordance with the official methods for soil analysis of the Italian legislation (DM 13/09/1999 SO No. 185 - *Approvazione dei Metodi Ufficiali di analisi chimica del suolo*). In each site, 10 soil sub-samples were collected by using a pedological auger at a depth of 0-20 cm, uniformly mixed, and reduced to a single composite sample (1 kg). The samples were analysed by the laboratory of the *Agenzia per l'Innovazione nel Settore Agroalimentare e della Pesca "Marche Agricoltura Pesca" (AMAP)*, Jesi (Italy). The analysed parameters were: soil texture (coarse sand, medium sand, fine sand, silt, and clay content expressed in g/kg of soil), pH, electrical conductivity (EC, dS/m); cation exchange capacity (CEC, meq/100g of soil); total organic carbon (TOC, g/kg); organic matter (OM, g/kg); total nitrogen (TN, g/kg); C/N ratio; assimilable phosphorus (P, mg/kg); exchangeable potassium (K, mg/kg). Soil samples for chemical-physical analysis were collected in the different sites during the spring sampling, both in 2021 and 2022.

4.4.1.4 Weather and soil environmental factors

To define the weather within the study area, the meteorological data were provided by *Agenzia per l'Innovazione nel Settore Agroalimentare e della Pesca "Marche Agricoltura Pesca" (AMAP)*, Jesi (Italy). The dataset included: average temperature (°C), total precipitation (mm) and relative moisture (%). For each site, the closest meteorological station was selected for data gathering. In 2021, the Montegiorgio weather station was considered for site 1 and 4, Montelparo weather station for site 3, and Fermo for site 2. In 2022 the Montegiorgio weather station for site 5, Montelparo for site 7, while Montefiore dell'Aso for site 6 and Montedinove for site 8. In addition, soil environmental parameters, including soil moisture (%) and

temperature (°C), were measured at the sites in triplicate using a soil moisture meter (PMS-714 Lutron) and a thermometer (HI-98501; Hanna Instruments, Milan, Italy).

4.4.1.5 Landscape

To assess the influence of the surrounding areas on soil health, land cover classes were analysed in a Geographic Information System (GIS) software, running on QGIS v. 3.40.4-Bratislava. The smallest landscape-level spatial scale was selected: a circle with 500m radius from each site centroid was defined (Coletta et al., 2025; Le Provost et al. 2021), thus an area of approximately 78.5 hectares was described inside each circle for each site. The 2023 Land Cover map (10x10m resolution) provided by ISPRA's National Environmental Information System (SINAnet, 2023) was used as base map for the elaboration. The map is an integration of data from the Land monitoring service of the Copernicus programme, with reference to the Local (Urban Atlas, Coastal Zones, Riparian Zones and Natura 2000) and Pan-European (CORINE Land Cover, CLC+ Backbone) components referred to 2018 and the ISPRA National Land Consumption Map 2023. For each area, the percentage of each land-cover type was calculated to visualize the differences at landscape level between the eight investigated sites. A total of 7 land-cover types have been identified: Periodic herbaceous vegetation (2211), Permanent herbaceous vegetation (2212), Shrubs (2120), Broad-leaved trees (2111), Permanent water bodies (3100), Unconsolidated non-vegetated natural surfaces (1220), Sealed artificial surfaces (1110).

4.4.1.6 Statistical analysis

Data were checked for normality (Shapiro-Wilk test) and homoscedasticity (Bartlett or Levene test, according to distribution), then compared for site (One-way Anova or Kruskal-Wallis test, according to distribution) or for season or year (Paired Student's T test or Wilcoxon test, according to distribution). For the biological parameters, the statistical analysis was based on the evaluation of partial values (referring to the single replicates A, B, C constituting the

sample). Statistical analyses and plots were performed using R software, version 4.4.0 (R Development Core Team, 2021). A *p-value* < 0.05 has been considered significant.

To visualize the grouping of arthropods communities, non-metric multidimensional scaling (nMDS), based on Bray-Curtis dissimilarity index was performed. To associate the community composition with soil characteristics and evaluate the associations between NMDS site scores, the “envfit” function was used. Results were plotted in an NMDS ordination diagram, performed with the “ggplot2” and “ggforce” packages. Permutational multivariate analysis of variance (PERMANOVA) was used to test for differences in assemblages among the different patterns visualized with NMDS. Ordination analysis, PERMANOVA, and SIMPER were performed with “vegan” package. To test the influence of abiotic variables, on QBS-ar index and to understand which of them exerted a greater influence on the index, correlation matrix using *corrplot()*, package “corrplot”; *ggcorrplot()*, package “ggplot2” and *cor()* package “stats”, multiple linear regression (MLR) (*lm()*, package “stats”) and Partial Least Squares (PLS) regression model were used (*pls()*, package “pls”). The data was subjected to an initial exploratory analysis where correlations between variables and QBS-ar index, and the ratio of observations to variables were calculated. Several assumptions were tested, including normality, homoscedasticity (Breusch-Pagan test) and independence of residuals (Durbin-Watson test). In addition, total abundance of microarthropods was graphically represented through heatmaps <http://www.heatmapper.ca/> (Babicki et al., 2016).

4.4.2 Results

4.4.2.1 Soil biological analysis

4.4.2.1.1 Total QBS-ar values

High total QBS-ar values, and thus excellent soil quality (> 120, Menta et al., 2011b), were found in most of the sites, in both year and seasons (**Tab. 4**). Although not significant, higher average total QBS-ar values were found in autumn (172) compared to spring (162), in both years (2021 and 2022) (**Tab. 4**).

Site	Total QBS-ar - 2021			
	Spring		Autumn	
1	129	Excellent	136	Excellent
2	133	Excellent	98	Discreet
3	115	Good	161	Excellent
4	143	Excellent	186	Excellent

Site	Total QBS-ar - 2022			
	Spring		Autumn	
5	206	Excellent	191	Excellent
6	228	Excellent	219	Excellent
7	188	Excellent	188	Excellent
8	155	Excellent	198	Excellent

Tab. 4 - Table of the total QBS-ar values and related soil quality judgment, according to Menta et al. (2011b), in 2021 (left) and 2022 (right).

Nonetheless, in spring the sites monitored in 2022 showed a significantly higher soil quality (194) compared to 2021 (130). Similarly, although not significant, higher average total QBS-ar values were found in 2022 (199), compared to 2021 (145).

4.4.2.1.2 Partial QBS-ar values

Higher partial QBS-ar values were found in the sites monitored in 2022, in both years and seasons (**Tab. 5, 6; Fig. x**). The partial QBS-ar values range from 50 to 120 in 2021, and from 90 to 200 in 2022. In both cases, there was not any specific trend related to the increment or the decrement of the values passing from spring to autumn in the different sites. The average partial QBS-ar values among seasons in the same year was extremely similar (Spring = 110, Autumn = 111). In both seasons, a significantly lower soil quality was found in the sites monitored in 2021 compared to 2022 (**Tab. 5, 6; Fig. 10**). In 2021 site 2 was characterized by the lowest soil biological quality, while in 2022 site 6 by the highest quality in both seasons (**Tab. 5; 6**). This latter site was an uncultivated field, which was cultivated for the first time, after a long time, before the spring soil sampling.

	Partial QBS-ar – 2021								Partial QBS-ar – 2022							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
A	118	Good	77	Sufficient	70	Sufficient	107	Good	167	Excellent	137	Excellent	186	Excellent	137	Excellent
B	76	Sufficient	72	Sufficient	68	Sufficient	62	Sufficient	176	Excellent	196	Excellent	127	Excellent	113	Good
C	62	Sufficient	79	Sufficient	91	Discreet	106	Good	85	Discreet	131	Excellent	107	Good	96	Discreet

Tab. 5 - Partial QBS-ar values in both years in spring.

	Partial QBS-ar – 2021								Partial QBS-ar – 2022							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
A	63	Sufficient	81	Discreet	111	Good	116	Good	145	Excellent	154	Excellent	121	Excellent	122	Excellent
B	86	Discreet	57	Modest	100	Discreet	115	Good	125	Excellent	131	Excellent	146	Excellent	127	Excellent
C	86	Discreet	51	Modest	101	Good	115	Good	97	Discreet	173	Excellent	128	Excellent	115	Good

Tab. 6 - Partial QBS-ar values in both years in autumn.

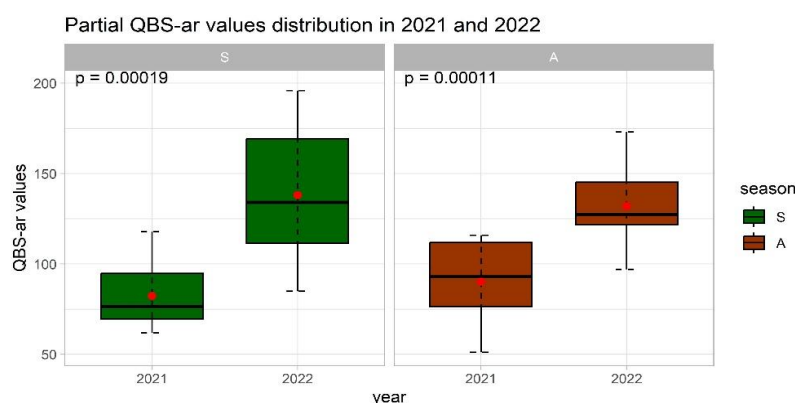


Fig. 10 - Boxplots of the distribution of the average partial QBS-ar values in both years and seasons. Average values for spring: 2021 = 82; 2022 = 138, while for autumn: 2021 = 90; 2022 = 132.

4.4.2.1.3 Soil arthropod abundance

Higher average total arthropod abundance was found in autumn (2003) compared to spring (1448), in both years (2021 and 2022) (**Tab. 7**). However, no differences were found in the comparison among seasons within the same years in both cases. On the contrary, considering soil microarthropods partial abundances, significant differences between the two seasons were found in 2021 (**Fig. 11**).

Year	Site	Spring	Average total abundance in spring	Autumn	Average total abundance in autumn
2021	1	496	814	1023	2082
	2	1092		1861	
	3	831		2056	
	4	837		903	
2022	5	1274	1461	1082	2545
	6	2690		4671	
	7	1734		2720	
	8	2631		1707	

Tab 7 – Soil microarthropods total abundance for each site, and average total abundance for both years and seasons.

Nonetheless, significant differences were found among spring 2021 and 2022 for both soil microarthropod partial and total abundance, while no significant differences were found among autumn 2021 and 2022. In general, a higher total and partial number of soil arthropods was found in 2022, compared to 2021.

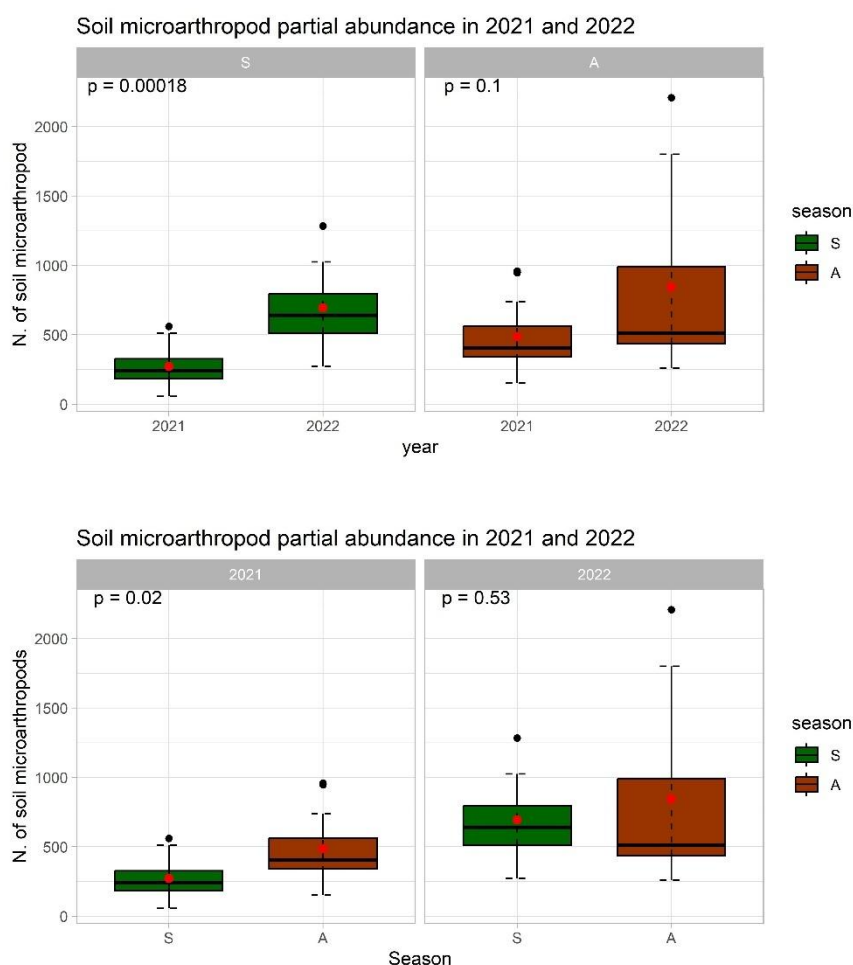


Fig. 11 - Boxplots of the distribution of the average soil arthropods partial abundance in both years and seasons. Average values for spring: 2021 271; 2022 = 694, while for autumn: 2021 = 487; 2022 = 844. Comparison among seasons in different years (above), comparison among seasons within the same year (below).

4.4.2.1.4 Biological forms (BFs) and euedaphic forms (EFs)

The total and partial number of BFs was significantly higher in 2022 than in 2021 in both seasons (**Fig. 12; Tab. 8**). On the contrary, no significant differences were found in the comparison among seasons (spring vs autumn) within the same year, for both partial and total values.

Year	Site	Spring	Average total abundance in spring	Autumn	Average total abundance in autumn
2021	1	13	12	10	11
	2	11		10	
	3	12		12	
	4	12		12	
2022	5	16	16	12	16
	6	19		19	
	7	15		15	
	8	14		16	

Tab. 8 – Total number of BF_s for each site, and average total abundance for both years and seasons.

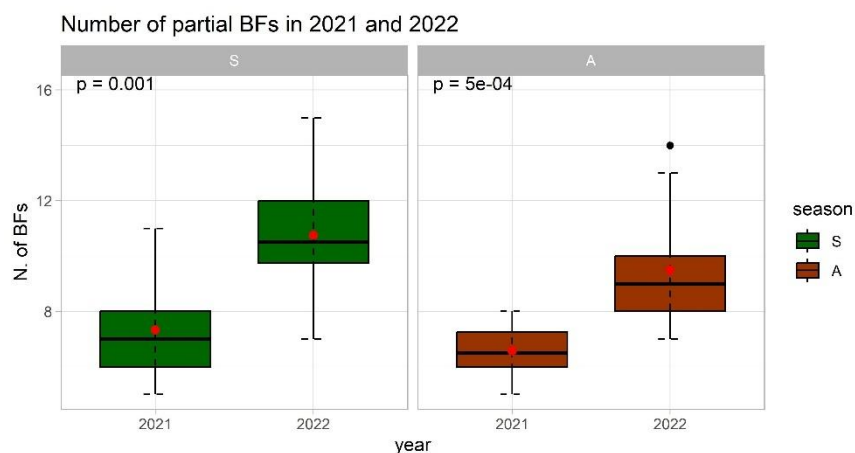


Fig. 12 – Boxplots of the distribution of the partial number of BF_s in both years and seasons. Average values for spring: 2021 = 7; 2022 = 11, while for autumn: 2021 = 7; 2022 = 10.

Considering the total number of EF_s, significantly higher values were found in spring 2022 compared to 2021. Considering the partial number of EF_s they were significantly higher in 2022 compared to 2021, in both seasons (**Tab. 9; Fig. 13**). Whereas, for both total and partial EF_s number there were no differences in the comparison among seasons (spring vs. autumn) within the same year.

Year	Site	Spring	Average total abundance in spring	Autumn	Average total abundance in autumn
2021	1	4	5	5	6
	2	5		3	
	3	4		6	
	4	6		8	
2022	5	7	7	8	7
	6	7		7	
	7	7		7	
	8	6		7	

Tab 9 – Total number of EF_s for each site, and average total abundance for both years and seasons.

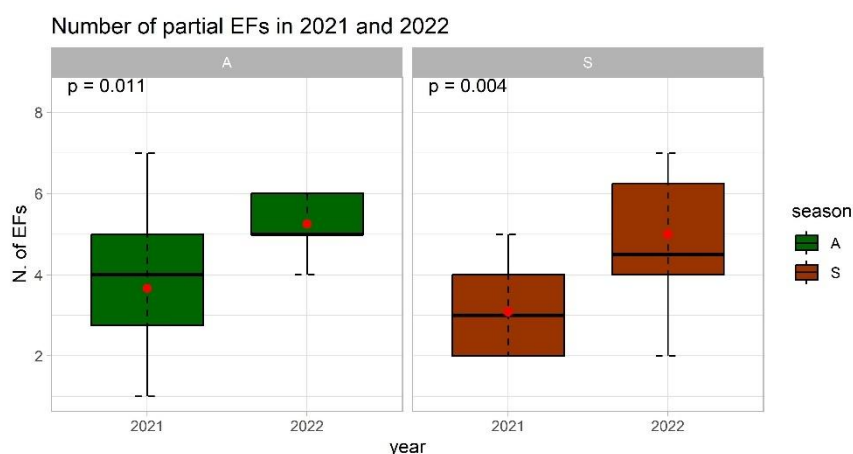


Fig. 13 – Boxplots of the distribution of the partial number of EFs in both years and seasons. Average values for spring: 2021 = 5; 2022 = 7, while for autumn: 2021 = 6; 2022 = 7.

4.4.2.1.5 Representation of soil arthropod abundance and BFs presence through heatmaps

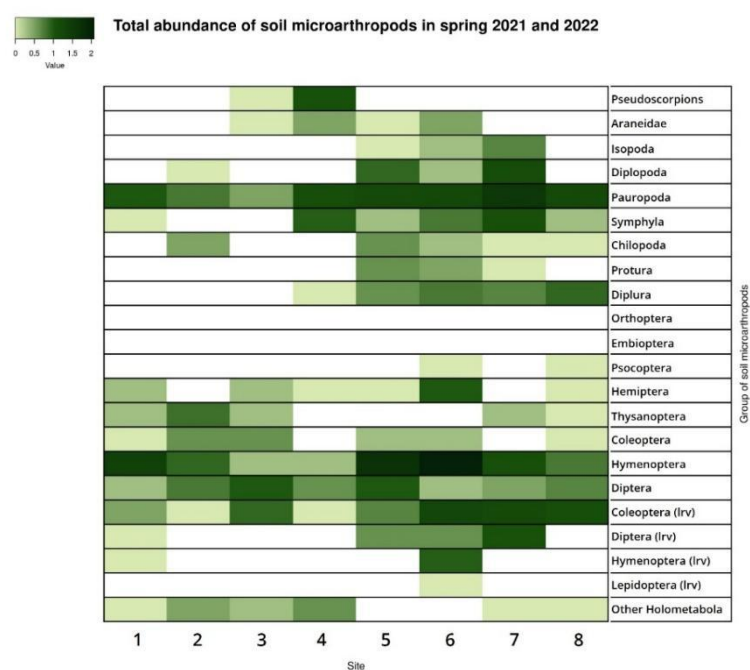


Fig. 14 - Heatmap of the log10-transformed total abundance of each BF in each site in spring. Darker shades indicate high abundance, and light shades indicate low abundance or absence (white). Data concerning Acari and Collembola are not reported. The heatmap was generated using the Heatmapper web server (www.heatmapper.ca).

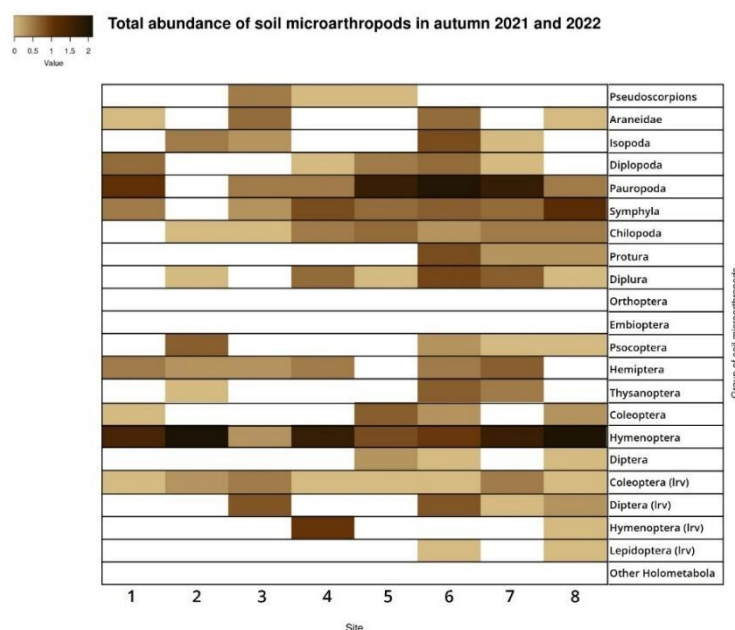


Fig. 15 - Heatmap of the log10-transformed total abundance of each BF in each site in autumn. Darker shades indicate high abundance, and light shades indicate low abundance or absence (white). Data concerning Acari and Collembola are not reported. The heatmap was generated using the Heatmapper web server (www.heatmapper.ca).

Interestingly, Acari, Collembola, Diptera, Hymenoptera, and Coleoptera larvae were found at every site in both years and seasons. In spring, Pauropoda were also present at all sites. Notably, in autumn, Pauropoda and Symphyla were present at all sites except Site 2. Similarly, Chilopoda were present at all sites except Site 1 (**Fig. 14, 15**).

This latter Site showed the lowest number of BFs (10) in autumn together with Site 2. In addition, Site 2 also showed the lowest number of BFs (11) in spring. Orthoptera and Embioptera were absent from all the sites across both years and seasons. Additionally, during autumn, other holometabolous insects were not found in any of the sites. In spring, Pseudoscorpions were only present at the sites monitored in 2021. Meanwhile, Psocoptera, Lepidoptera larvae, Protura, and Isopoda were reported only at sites monitored in 2022. In autumn, Protura, Diptera, and Lepidoptera larvae were exclusively found at the sites monitored in 2022.

Overall, Site 6 exhibited the highest number of BFs (19) in both seasons. In spring, there was a specific association among this Site and the presence of Lepidoptera larvae. In autumn this

BFs was found also in Site 8. Notably, the total number of BFs was significantly higher in 2022 compared to 2021 in both seasons (**Fig. 12, 14, 15; Tab. 8**).

4.4.2.1.6 Acari and Collembola abundance

		1	2	3	4	5	6	7	8
Acari	Spring	398	1042	718	747	1063	2187	1445	2197
	Autumn	925	1682	1966	773	847	3614	2459	1476
Collembola	Spring	49	11	78	39	98	294	164	376
	Autumn	44	33	62	43	159	836	134	82
A/C ratio	Spring	8.1	94.7	9.2	19.2	10.8	7.4	8.8	5.8
	Autumn	21.0	51.0	31.7	18.0	5.3	4.3	18.4	18.0

Tab. 10 – Total number of Acari and Collembola and Acari/Collembola (A/C) ratio for each site and season.

Autumn was characterised for both BFs (Acari and Collembola) by a greater abundance than spring. Similarly, 2022 was characterised by a noticeably higher abundance of both forms compared to 2021. Site 6 was characterised by a greater presence of Acari and especially of Collembola in autumn. This also occurred in spring, for both BFs, in concert with site 8 in this case. Further, the mean A/C ratio was similar between the two seasons (spring = 20.5; autumn = 21.0). While it was generally higher in 2021 than in 2022, in both seasons. Interestingly, the ratio appeared particularly high at Site 2 compared to the other sites, in all seasons (**Tab. 10**).

The results obtained with different soil-arthropod base indices, were in accordance with those obtained with the QBS-ar, the main index applied in the study: significant differences were found between the sites sampled in different years (2022 and 2021). To further evaluate the impact of abiotic variables on the QBS-ar index, an in-depth investigation of the influence of these variables on the QBS-ar index partial values was performed as follows.

4.4.2.2 Soil chemical-physical analysis

All sites had a slightly alkaline pH, ranging from 7.3 to 8.1, a medium to high CEC and they resulted moderately saline (Abrol et al., 1988). The content of exchangeable K was high in all sites, while it was very high in site 1 (598 mg/Kg).

Site	pH	CEC (meq/100g)	C/N ratio	EC (dS/m)	K (mg/kg)	OM (g/kg)	P (g/kg)	TN (g/kg)	TOC (g/kg)
1	8.02	20.7	7.1	0.729	598	16.4	15.6	1.35	9.5
2	7.56	23.6	11.4	0.576	159	4.9	4.4	0.25	2.85
3	7.34	23.5	6.6	0.757	204	11.4	9.4	1	6.6
4	7.28	16	6.7	0.682	230	13.92	32.7	1.2	8.1
5	7.30	23.8	7.3	0.646	218	15	2.6	1.2	8.7
6	7.72	14.2	8.2	0.783	268	15.6	16.6	1.1	9.1
7	8.10	19.3	7.5	0.553	213	9.7	4.1	0.75	5.6
8	7.60	21.3	7.3	0.552	261	16.9	4.1	1.35	9.8

Tab. 11 - Table of the soil chemical parameters: pH, Cation exchange capacity (CEC, meq/100g of soil); carbon/nitrogen ratio (C/N ratio); electrical conductivity (EC, dS/m); exchangeable potassium, (K, mg/kg); organic matter (OM, g/kg); assimilable phosphorus (P, mg/kg); total nitrogen (TN, g/kg); total organic carbon (TOC, g/kg).

The organic matter content and the C/N ratio were generally low at all sites, while TN content was low to medium. In contrast, in site 2 the organic matter (4.9 g/Kg), the TN content (0.25 g/Kg) and TOC (2.85 mg/Kg) were particularly low, while the C/N ratio was high (11.4) compared to the other sites. The content of assimilable P was generally low or very low at all sites, however in site 4 it was particularly high (32.7 mg/Kg), while in site 1 (15.6 mg/Kg) and 6 (16.6 mg/Kg) it was medium (**Tab. 11; Fig. 16**).

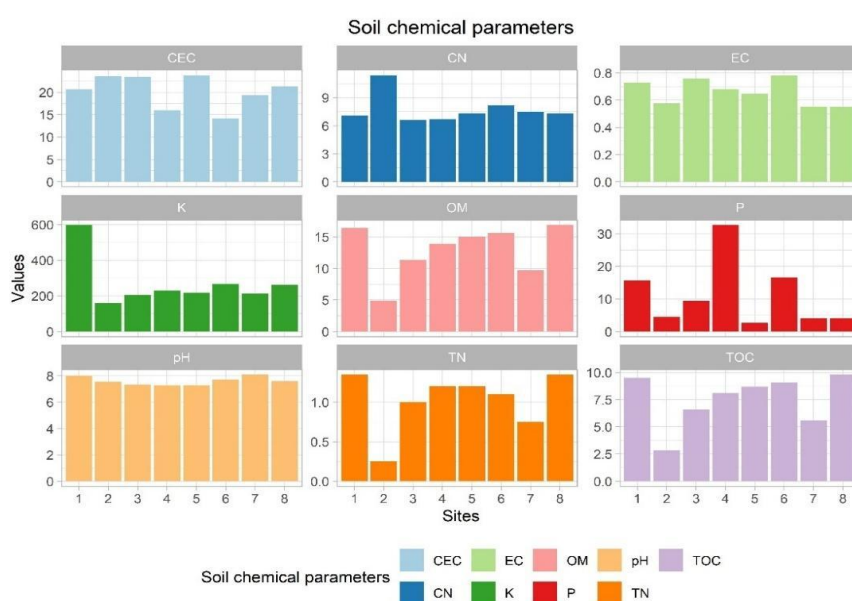


Fig. 16 - Barplots of the soil chemical parameters for each site. Cation exchange capacity (CEC, meq/100g of soil); carbon/nitrogen ratio (C/N ratio); electrical conductivity (EC, dS/m); exchangeable potassium, (K, mg/kg); organic matter (OM, g/kg); assimilable phosphorus (P, mg/kg); pH, total nitrogen (TN, g/kg); total organic carbon (TOC, g/kg).

Site	Coarse sand (g/kg)	Fine sand (g/kg)	Very fine sand (g/kg)	Total sand (g/kg)	Silt (g/kg)	Clay (g/kg)	Textural class
1	25	69	137	231	414	355	Clay loam
2	81	55	37	173	418	409	Silty clay
3	10	63	137	210	453	337	Clay loam
4	32	123	162	317	396	287	Clay loam
5	17	103	147	267	381	352	Clay loam
6	38	211	236	485	349	166	Loam
7	50	97	137	284	404	312	Clay loam
8	27	59	115	201	476	323	Clay loam

Tab. 12 - Table of the soil textural parameters. Coarse sand, fine sand, very fine sand, silt, and clay contents (g/kg) and textural classes are reported for each site.

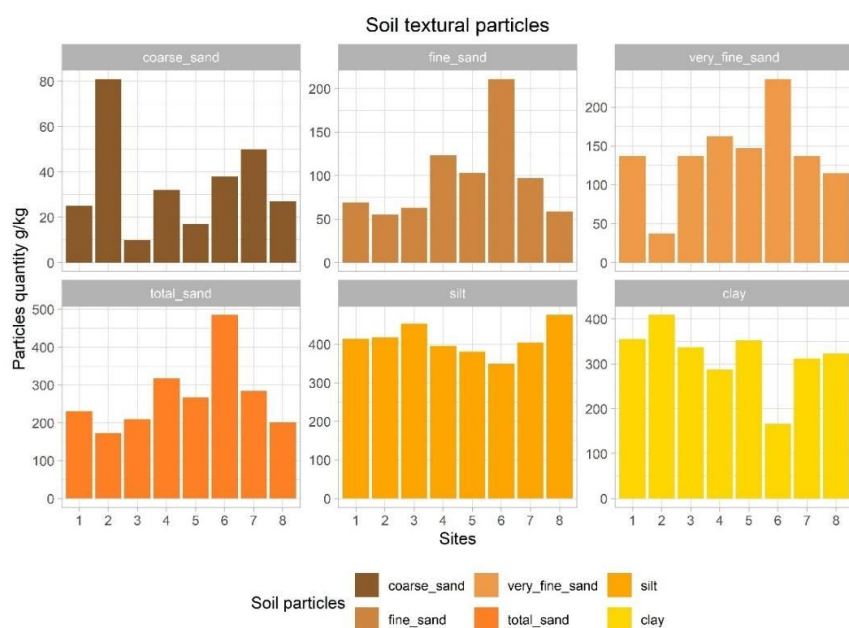


Fig. 17 - Barplots of the soil physical parameters for each site. Coarse sand (brown), fine sand (light brown), very fine sand (salmon), total sand (orange) silt (light orange), and clay (yellow) contents (g/kg).

There were no significant differences in the sand, soil, and clay particle content and resulting texture class (clay loam) among the considered sites, apart from site 2 and 6 which were classified as: silty clay and loam. In fact, site 6 had a slightly higher total sand content (485 g/Kg), as well as a higher fine and very fine sand content (211 and 236 g/Kg), compared to the

other sites. On the other hand, site 2 had a higher coarse sand content (81 g/Kg) compared to the other sites (**Tab. 12; Fig. 17**).

4.4.2.2.1 NMDS soil arthropods and soil chemical-physical properties

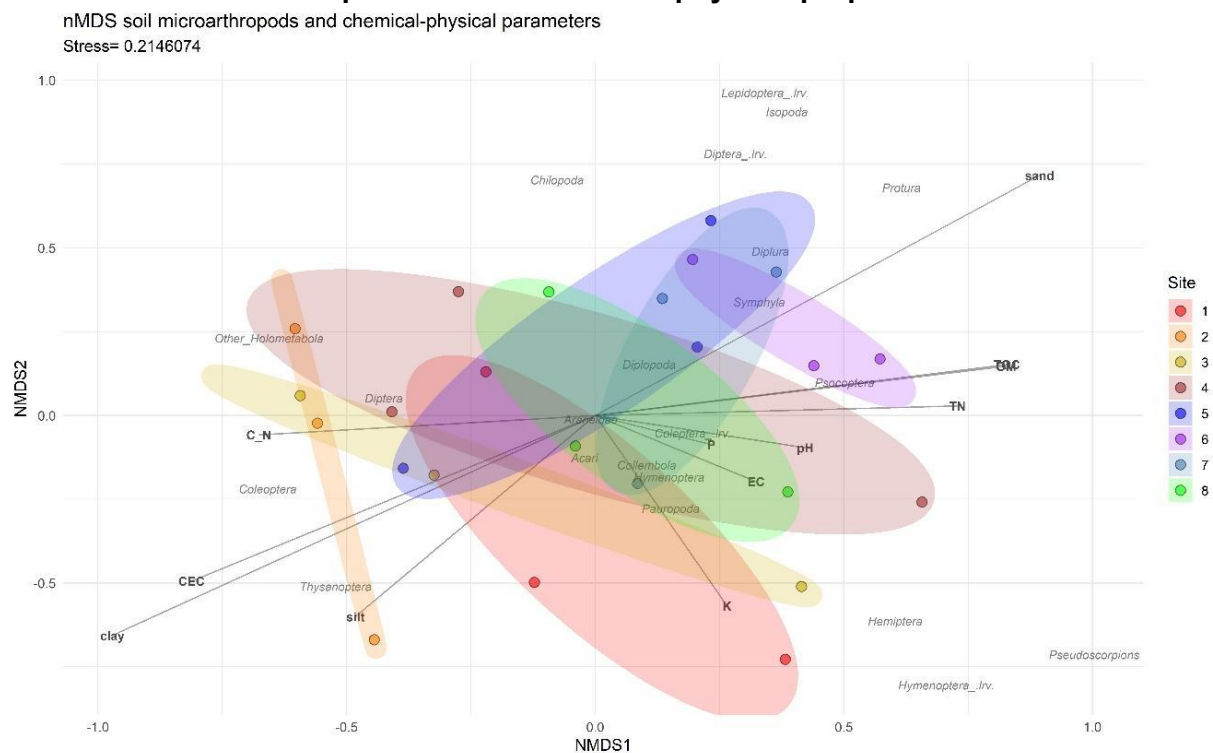


Fig. 18 – Non-metric multidimensional scaling (nMDS) based on Bray–Curtis dissimilarity index of soil microarthropod community composition and soil chemical-physical properties in the various sites in spring 2021 and 2022. Total organic carbon, TOC; soil organic matter, OM; total nitrogen, TN; assimilable phosphorus, P; exchangeable potassium, K; C/N ratio; pH; electrical conductivity, EC; cation exchange capacity, CEC; soil texture (sand, silt, clay).

In **Fig. 18** is reported the total abundance of the different BFs present at the different sites in spring 2021 and 2022. A markedly different spatial arrangement between the soil microarthropod communities present at the sites monitored in one year compared to the other was clearly visible. The communities monitored in the sites during 2021 were on the left side of the graph, while the ones monitored in 2022 were on the right. Interestingly, the soil microarthropod communities collected in the three QBS-ar subsamples appeared very similar to each other at Sites 2 and 6. In contrast, the communities present in the different subsamples from Sites 1, 2, 4 and 5 appeared very differentiated.

In addition, the silt and clay content, CEC and the C/N ratio seemed to be associated with the presence of Thysanoptera, Coleoptera, Diptera and other Holometabola. While sand, N and TOC content to the presence of Pseudoscorpions, Protura, Psocoptera, Diplura, Shymphyla, Isopoda, and Lepidoptera and Diptera larvae. In contrast, K content appeared to be positively related to the presence of Pauropoda, Hemiptera and Hymenoptera larvae. While it seemed to be negatively related to the presence of Chilopoda. The “envit” function underlined a significant contribution of sand, clay and CEC content in the microarthropod assemblages. Furthermore, PERMANOVA showed that nMDS average dimensions differ significantly in the grouping by year ($p\text{-value} < 0.001$). In addition, SIMPER analysis showed that K ($p\text{-value} < 0.018$; cumulative contribution = 0.68) and P content ($p\text{-value} < 0.042$; cumulative contribution = 0.75) played an important role in determining the differences between soil arthropod communities assemblages in the two years.

According to this, the influence of the following parameters (sand, clay, CEC, K and P) on the QBS-ar index was evaluated by using a PLS regression model. The content of K and sand had the greatest effect on the index, with a strong and direct relationship. In particular, K had a negative relationship with the QBS-ar index, while Sand had a positive relationship. The PLS model had a good predictive ability, explaining a significant proportion of the QBS-ar index variance (34.34%).

4.4.2.3 **Weather and soil environmental factors analysis**

The average temperatures recorded in the two years (2021 and 2022) were significantly different, with the only exception of July and August (**Tab. 12**). Generally, 2022 was warmer in most of the months, except for March and September.

Average temperature (°C)	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct
2021	6.2	9.4	9.2	11.9	17.8	24.1	26.3	25.5	21.4	14.5
2022	6.0	8.5	8.0	12.7	20.0	25.3	26.8	25.4	20.4	17.8

Tab 12 – Average temperature (°C) in the different months and years.

Significant differences were found in March and October; in which a higher level of precipitation was registered in 2021. In general, in 2022, precipitations were significantly higher compared to 2021 (**Tab. 13**). Overall, 2022 was characterized by less rainfall, except for September (**Fig. 18**).

Average precipitations (mm)	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct
2021	73.5	30.1	47.3	44.1	17.6	27.7	36.8	26.9	17.1	105
2022	29	89.6	17.0	37.0	15.6	29	39.5	40.0	63.8	10.7

Tab. 13 - Average precipitations (mm) in the different months and years.

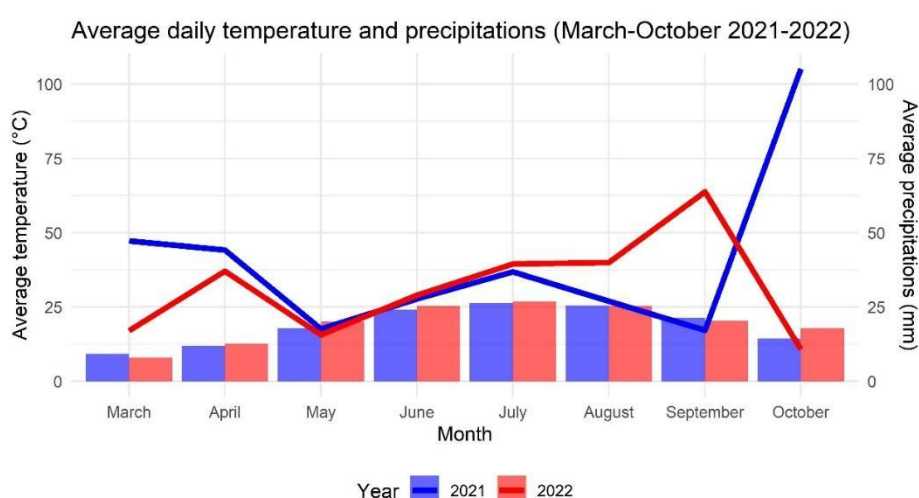


Fig 18 - Average temperature (°C) and precipitations (mm) in the different months and years.

Average relative moisture (%)	Mar	Apr	May	June	July	Aug	Sep	Oct
2021	65.6	68.8	64.8	61.8	61.1	60.8	70.7	82.6
2022	67.2	68.3	71.4	63.1	57	66.3	75.6	80.4

Tab. 14 - Average relative moisture (%) in the different months and years.

Any significant difference was found in the comparison of the relative soil moisture among the two years. Overall, 2022 was characterized by higher relative moisture (68.7%) compared to 2021 (67.0%). To conclude, in the months considered, 2022 was warmer, with fewer precipitations, but more humid compared to 2021 (**Tab. 14**).

The soil environmental parameters collected in each site at the sampling time are reported in **Tab. 15**. The average soil temperature recorded at the time of sampling was lower in autumn (2021 = 19.2 °C; 2022 = 17°C) than in spring (2021 = 27.9 °C; 2022 = 24 °C), in both years. Generally, lower temperatures were recorded in both seasons in 2022. The average soil moisture recorded at the time of sampling was higher in autumn (2021 = 10.1%; 2022 = 14.6%) than in spring (2021 = 9.1%; 2022 = 8.3%), in both years. The year 2022 was characterised by higher average soil moisture in autumn than in 2021. While a higher average value was recorded in spring 2021 compared to 2022.

		1	2	3	4	5	6	7	8
Average soil temperature (°C)	S	23.7	33.5	30.2	24	20.8	21.5	22.4	31.3
	A	21	22.2	20.3	13.3	13.6	16.8	15.9	22
Average soil moisture (%)	S	10.9	8.7	10.9	6	7.6	7.6	10.4	7.5
	A	9.7	10.8	7.8	12	17.8	13	9.8	17.6

Tab. 15 – Soil temperature (°C) and moisture (%) at each site in both seasons. S= spring, A =autumn.

As the weather data showed no correlation with the index, only the environmental soil parameters, which were recorded in the immediate proximity of the area where the QBS-ar samples were collected, were analysed in detail, using a linear regression model. A significant negative relationship between soil temperature and QBS-ar values ($r = -0.68$, $p\text{-value} = 0.029$) was found. On the contrary, soil moisture showed a low correlation ($r = 0.083$) with the QBS-ar index. The MLR model only explained part of the variability in the data ($R^2 = 29.82\%$), but it was not statistically significant.

4.4.2.4 **Soil agricultural practices**

The agricultural practices implemented at the various sites were summarized in **Tab. 16**. As all sites were organically farmed, the main difference lied in seedbed preparation practices, whose effect was noticeable in the spring sampling. In general, ploughing was followed by harrowing and legume sowing. Legume sowing took place in spring (March) for most of the

sites, except for Site 7 and 8, in which it took place in autumn (October). At the time of sampling, all sites were cultivated with legumes (**Fig. 19**). The number of harrow passes varied considerably between the sites, in some cases grubbing and/or soil rolling were also included. Site 8 differed more in the practices applied (subsoiling and combined drilling) than the others. In spring, the number of agronomic practices applied at this site was the lowest. There were also differences in fertilisation, which was in any case very limited on all the sites and was generally applied alternately over the years, depending on the crop rotation. In 2021, fertilization took place only in Site 1 and 2, which were sites characterized by the highest number of activities. It can be noted that in 2021, a higher number of agricultural practices were applied during spring compared to 2022. On the contrary, the practices applied in autumn were equivalent for both years. Harvesting took place at the beginning of July in Site 7 and 8, and at the end of July for all other sites. After the legume harvest, some farmers started harrowing or ploughing, but the majority left the land to rest for a while. Within the model, we focused on the number of practices rather than the intensity of each practice, because the limited data available on the farming equipment used by each farmer prevented a detailed assessment of the soil impact.

Site	Pre- spring sampling (June)	Pre- autumn sampling (October)
1	Ploughing (25 cm), grubbing, harrowing + spring sowing (chickpeas) + soil rolling + fertilization	Combine harvesting.
2	Ploughing (40 cm), grubbing, harrowing + spring sowing (chickpeas) + soil rolling + harrowing.	Combine harvesting.
3	Harrowing x2 + spring sowing (chickpeas) + harrowing + fertilization.	Combine harvesting + harrowing x2.
4	Ploughing (25 cm), harrowing x3 + spring sowing (chickpeas).	Combine harvesting + ploughing (25 cm).
5	Ploughing (25 cm) + grubbing + harrowing + spring sowing (chickpeas).	Combine harvesting + harrowing (for burial of crop residues) + subsoiling.
6	Ploughing (30 cm) + harrowing + spring sowing (chickpeas).	Combine harvesting
7	Harrowing x3 + autumn sowing (chickpeas).	Combine harvesting + harrowing.
8	Subsoiling (35 cm) + combined autumn sowing (combined seed drilling) (green peas).	Combine harvesting

Tab. 16 - Agricultural practices applied in the different sites in the years and seasons. The table also includes the agricultural practices that occurred prior to sampling.

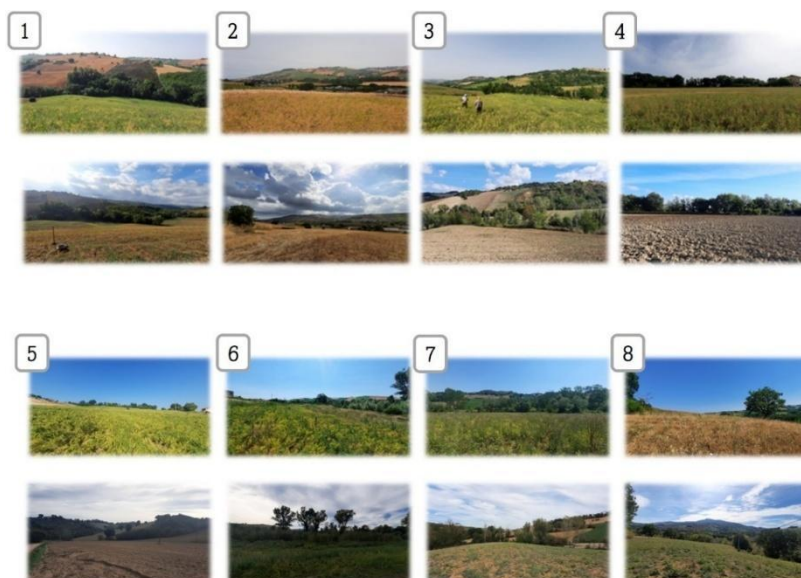


Fig. 19 - Photos of the different sites in the years and seasons. From 1 to 4 sites monitored in 2021, from 5 to 8 sites monitored in 2022.

The impact of the agronomic practices on the QBS-ar index was evaluated for each site considering the two separate seasons. A significant negative correlation ($r = -0.65$) was found only in the spring ($p\text{-value} = 0.006$). The MLR used indicated that agronomic practices can significantly account for approximately 30% of the variance observed in the partial QBS-ar values ($R^2 = 0.31$). In contrast, no correlation was detected in the autumn, suggesting that there may be other variables influencing the index during that season.

4.4.2.5 *Landscape analysis*

The area surrounding the sites was mainly occupied by periodic herbaceous vegetation (arable land) in all cases (**Fig. 20, Tab. 17**). The largest percentage (93%) was present at Site 5. A good number of sites were characterised by the presence of broadleaf trees, particularly Sites 3 (20%), 7 (19%) and 8 (19,5%). On the other hand, in Site 5 broadleaf trees were completely absent. Permanent herbaceous vegetation was present at all sites, especially pasture and grassland, and was particularly present at Site 2 (13%) and Site 6 (10%). The presence of urban or man-made areas was also present at all sites, but particularly characterised Sites 6 (12,5%) and 2 (11%). The presence of permanent water bodies and more fragmented urban areas was only found in Site 6, which was the most diversified site in terms of the types of land cover present. Shrublands

were absent in the vicinity of Site 6, and were only found in Sites 4, 7 and 8, but with extremely small percentages (about 1%).

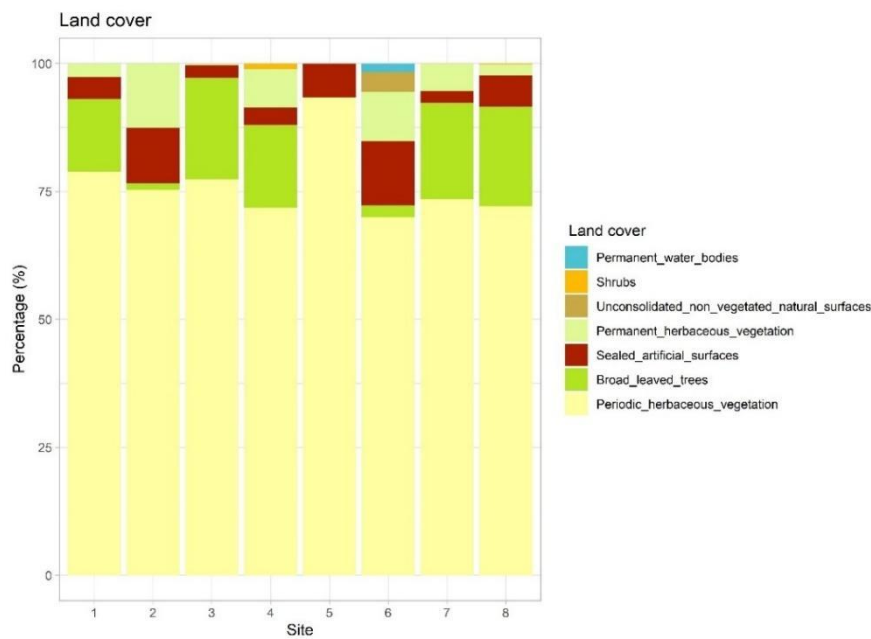


Fig 20 – Land cover types present in the different sites considered.

Site	Periodic herbaceous vegetation (%)	Permanent herbaceous vegetation (%)	Shrubs (%)	Broad-leaved trees (%)	Permanent water bodies (%)	Unconsolidated non-vegetated natural surfaces (%)	Sealed artificial surfaces (%)
1	78.90	2.63	0.00	14.16	0.00	0.00	4.31
2	75.34	12.55	0.00	1.30	0.00	0.00	10.81
3	77.39	0.36	0.00	19.79	0.00	0.00	2.46
4	71.80	7.54	1.06	16.15	0.00	0.00	3.45
5	93.33	0.00	0.00	0.00	0.00	0.00	6.67
6	69.93	9.65	0.00	2.38	1.66	3.86	12.52
7	73.52	5.31	0.09	18.75	0.00	0.00	2.33
8	72.11	2.17	0.14	19.47	0.02	0.00	6.09

Tab. 17 – The percentages of the land cover types found in the 500 m radius-area surrounding the sites considered.

The influence of the land cover types on the QBS-ar index was evaluated by using a PLS regression model. The percentage of periodic herbaceous vegetation and sealed artificial surfaces had the greatest effect on the index, followed by broad leaved tress, permanent herbaceous vegetation, and unconsolidated non-vegetated natural surfaces. The PLS model explained about 23% of the QBS-ar index variability. The influence of the different parameters

is respectively in order of intensity: percentage of periodic herbaceous vegetation, sealed artificial surfaces, and broad-leaved trees. All of them showed a positive relationship with the QBS-ar index.

4.4.3 Discussion

Total and partial QBS-ar values and soil microarthropod abundance were generally higher in autumn compared to spring, in both years (2021 and 2022). Moreover, when comparing the values obtained in the two years, the QBS-ar values and the other biological parameters (abundance, BFs and EFs, A/C ratio) were significantly higher in 2022, in both seasons.

In addition, the occurrence of BFs such as Pauropoda, Symphyla and Chilopoda in most of the sites considered, indicated a high environmental quality. Particularly, the presence of Pauropoda is related to low-input management (Gonçalves et al., 2021). While the presence of BFs such as Acari and Collembola was normal given their ubiquitous presence. The presence of Protura only at sites monitored in 2022 was interesting. They are associated with soil stability (Menta & Remelli, 2020), and their presence seemed to be influenced by soil chemical-physical characteristics (Gonçalves et al., 2021). On the other hand, the presence of Lepidoptera larvae was of no particular interest as these forms only perform part of their life cycle in the soil.

The A/C ratio is an indicator of environmental stability and complexity (Bachelier 1963). High ratios are associated with high soil quality, although there are conflicting opinions (Angelini et al., 2002; Latella & Gobbi, 2015). In the present case, Site 2 was characterized by higher ratios, while Site 6 by the lowest ratios in both seasons. However, all other biological parameters underlined a higher quality for Site 6, underlining a possible inverse meaning of the A/C ratio.

Markable differences were observable in relation to the chemical-physical characterization among the selected sites. In spring, there was a clear indication of a higher soil quality condition for Site 6 than for all the others, with all biological parameters applied. Site 6 had the highest EC and lowest CEC content (**Tab. 11**). On the contrary, Site 2 was characterised by the lowest quality, and the lowest K, N, TOC and organic matter content and the highest C/N

ratio. All sites had clay loam soil texture, whereas site 2 had a silty clay soil texture and site 6 loam soil texture. The chemical-physical parameters that were found to have a significant effect on soil microarthropod community assemblages by the nMDS (**Fig. 18**) in spring were further examined by PLS model. The model showed the significant influence of sand and K content on the QBS-ar index (**Fig. 20**). It can be assumed that the differences in soil quality might depend mainly on the soil chemical and physical parameters of the different sites monitored, as their effect in spring was the strongest ($R^2 = 0.34$) (**Fig. 20**). However, their influence was not measured in the autumn season.

In spring, the effect of the agricultural practices applied was significant, but slightly lower ($R^2 = 0.31$) compared to the one of the soil chemical-physical properties. Spring was probably affected by a higher number of tractor passes for agricultural activities related to seedbed preparation, compared to autumn (**Tab. 16**). Similarly, the soil disturbance due to the tillage activities generally affected the spring sampling rather than the autumn, as such activities took place after the autumn sampling. In fact, the last passage of the agricultural machinery on the field was closer in time to the spring sampling date, than to the autumn one. However, the model did not show a significant result in autumn. In the autumn season, there might be other factors, or a joint effect among factors, influencing the QBS-ar index.

While in spring the level of herbaceous cover at the different sites was similar, due to the ubiquitous presence of chickpea plants (**Tab. 3**). In autumn, the condition was different. It is essential to emphasise the high percentage of herbaceous cover found at Site 6, as well as Site 1, 7 and 8 but with lower percentages. On the contrary, Site 2 was very dry and arid (**Fig. 19**). Sites 3, 4 and 5 were harrowed. There the soil was bare and there were only small hints of wild grass growth. However, the influence of soil temperature and moisture on the QBS-ar index was not detected by the model applied.

Moreover, the weather data and the soil environmental parameters had contrasting results. The year 2022 was warmer, with fewer precipitations, but more humid compared to 2021. On the contrary, the average soil temperature, recorded at the time of sampling, was lower in both

seasons in 2022. While the average soil moisture was higher in autumn 2022 than in 2021, and higher in spring 2021 compared to 2022. Only soil temperature, registered at the sampling point, resulted to have a plausible role in influencing the QBS-ar index, particularly in spring. During this season, a negative relationship between soil temperature and QBS-ar values was visible for Site 5 and 6 only.

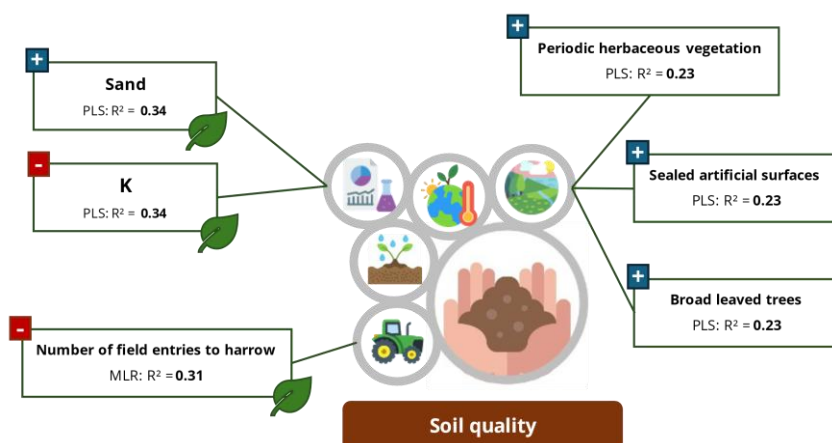


Fig. 21 – More influential variables, according to the models applied (PLS or MLR).

Furthermore, it can be assumed that the differences in soil quality might also depend on landscape features ($R^2 = 0.23$) (**Fig. 20**). Site 2 and 6 were characterized by a high percentage of permanent herbaceous vegetation and sealed artificial surfaces, and a low percentage of broad-leaved trees, compared to the other sites. However, with respect to Site 2, the area surrounding Site 6 had a notable percentage of permanent water bodies and unconsolidated non-vegetated natural surfaces. Overall, Site 6 was characterized by the most diversified environment, even if a high presence of atrophic areas were present. Probably the presence of a complex environment played a role in determining higher QBS-ar index values. Further studies are needed to understand the effect of landscape. To do so more detailed maps, with a higher resolution, of the Marche region and Italy in general are needed to refine the elaboration.

4.5 CROP QUALITY ASSESSMENT

To evaluate crop quality, only Sites 1 to 7 were analysed, which presented chickpeas at the time of spring sampling. Site 8 was excluded as it was the only one presenting green pea. Plant traits and the rhizospheric bacterial communities (16S rDNA metabarcoding) were considered for this part.

4.5.1 Materials and methods

4.5.1.1 *Chickpea plants and rhizospheric soil sampling*

During the spring sampling, 10 chickpea plants, including the root system, were collected at the podding stage from the central part of each site. In total 80 chickpea plants were collected (40 plants each year). Once harvested, the plants were placed in sterile plastic bags and transferred immediately to a polystyrene refrigerated container with eutectic plates previously brought to -40°C. In the laboratory, morphological and productive traits were measured.

The excess soil was removed from the roots by gently shaking them. The rhizospheric soil was then scraped from the roots with a scalpel (McPherson et al., 2018) and immediately transferred to -80°C for subsequent analysis, including DNA extraction and sequencing. From each site, 1 g of rhizosphere soil was collected. Bacterial communities were analysed through the sequencing of 16S genes with a metabarcoding approach.

4.5.1.2 *Chickpeas morphological and productive traits collection*

For each plant, the following plant morphological and productive traits were determined by direct measurements in the laboratory: plant height (cm) (from the collar to the last branch), number of main and secondary branches, number of pods, fresh and dry pod weight, fresh and dry plant weight (without pods). Further, chlorophyll content (mg/m²) was measured using the atLEAF instrument (FT Green LLC, Wilmington, DE). Measurements were taken considering fully expanded leaves located at the top of the plant; 5 leaves were selected and 5

measurements around the primary rib of each leaf were taken for each of them. In addition, site yield data about chickpea seeds were provided by the Azienda Agricola Monte Monaco.

4.5.1.3 DNA extraction and sequencing

Soil rhizosphere DNA was extracted using the DNeasy 96 PowerSoil Pro QIAcube HT Kit (Qiagen, Milan, Italy) according to the manufacturer's instructions. The quality of the extracted DNA was analysed by using a NanoDrop device (NanoDrop, Thermo Scientific, Monza, Italy). Amplification of V3-V4 hypervariable regions of 16S rRNA gene was carried out using the Pro341F (5'-CCTACGGGNNBGCASCAG-3') and Pro805R (5'-GACTACNVGGGTATCTAATCC-3') primers (Takahashi et al., 2014). The amplicons were sequenced on an Illumina MiSeq platform with Paired-End format (2x300 bp) at BMR Genomics (Padua, Italy).

4.5.1.4 Post-run analyses

Sequences obtained were processed using QIIME2 (Bolyen et al., 2019) and R (R Core Team, 2021). Primers were removed using the CUTADAPT plugin (Martin, 2011). Demultiplexing, filtering, cleaning, and merging of the reads and chimeras removal was done using the Divisive Amplicon Denoising Algorithm (DADA2) plugin (Callahan et al., 2016). This step was done setting a quality score threshold of 20 and a max error of 2 for forward reads and a max error of 3 for reverse reads. Taxonomic annotation was performed by first training a classifier the Silva 138.1 (Quast et al., 2012) database, and using the RESCRIPT plugin (Robeson et al., 2021), and then by using the trained classifiers for the mapping of reads. Subsequent analyses were handled with the R software, using *phyloseq* and *microbiome* packages.

4.5.1.5 Biodiversity indices

The following biodiversity indices were determined to provide information on the species community present.

- Shannon - Wiener index (Shannon, 1948): the values range from 0 (presence of only one species) to a maximum value (H' max) represented by the natural logarithm of the total number of species, reached theoretically when all the species are equally distributed. Values normally range from 1.5 to 3.5, rarely exceeding 4.5.
- Fisher index (Fisher, 1943): is calculated from the number of individuals of each species in a sample and considers both richness and uniformity of distribution. It is particularly useful for complex biological communities, where the distribution of species is not uniform, but where there are some very abundant species and others that are very rare. It typically ranges from 0 to higher values but has no defined upper limit. A higher value indicates greater biodiversity (more and/or more evenly distributed species).
- Simpson index (Simpson, 1949): measures the dominance or concentration of species in a community. It is based on the probability that two randomly selected individuals are of the same species, with a lower value indicating greater biodiversity (few dominant individuals). The range of the index goes from 0 (highest biodiversity, uniform distribution of species, few dominant individuals) to 1 (low biodiversity, one species dominates completely).
- Inverse Simpson index (Simpson, 1949): the modified version of the previous one, it directly reflects the diversity of the community. A higher value implies greater biodiversity and a higher species richness.
- Abundance-based Coverage Estimator (ACE) index (Chao et al., 1992): is used to estimate total species diversity in a community, considering not only common species, but also rare species. The index attempts to estimate how many species may have been under-captured in the sample to obtain a more accurate estimate of total biodiversity. The result of the ACE index provides an estimate of the number of species that should be higher than the count of the observed species.
- seACE (standard error of ACE) (Chao et al., 1992): measures the uncertainty associated with the ACE estimate, indicating how much the estimated diversity may vary due to random fluctuations in the sampled data. It is an indication of the reliability of the ACE index. The value of the index can theoretically vary from 0 to very high values. Higher values indicate that the uncertainty is greater, for example when the sample is incomplete, or some rare species have not been caught.

- Chao1 index (Chao, 1984): is an estimate of diversity that considers rare species not found in the sample, particularly those observed only once. The index is useful for estimating the total species richness of a community, considering that some species may be missing from the sample.
- seChao1 (Chao, 1984): is the standard error associated with the estimation of Chao1, which indicates how much the estimation of diversity may vary due to sampling uncertainties. The higher the value of seChao1, the more uncertain the estimate of the total number of species.

4.5.1.6 **Statistical analysis**

Chickpea morphological and productive traits were checked for normality (Shapiro Wilk test) and homoscedasticity (Bartlett test) before analyses. Afterwards, the sites were compared for year (Paired Student's T test or Wilcoxon test, according to distribution). A Principal Component Analysis (PCA) using the built-in R functions *prcomp()* and *princomp()* was used to reduce the dimensionality of trait data. To test the influence of crop traits and biodiversity indices on QBS-ar index (partial values) and to understand which of them exerted a greater influence on the index, a correlation matrix was generated by using *corrplot()*, package "corrplot" and *cor()*, package "stats". The same method was also used to test the influence of the soil chemical-physical properties and weather data on plant traits. Only these abiotic variables were selected because they were the most important factors in influencing chickpea plants. Partial Least Squares (PLS) regression model by *pls()*, package "pls", were used to test the relationship between biodiversity indices and soil chemical-physical parameters.

4.5.2 **Results**

4.5.2.1 **Chickpeas morphological and productive traits analysis**

Generally, taller plants with a higher number of pods, total weight and water content were found in 2022 (**Tab. 18**). In 2022 the average chlorophyll content was lower compared to 2021. In 2022 the values were similar among sites, while in 2021 the values differed the most among sites. Most of the plant traits analysed resulted to be strongly correlated. Thus, in the following analyses the number of traits was reduced to five, including number of pods, main ramifications, plant height, plant fresh weight and chlorophyll content. **Fig. 22** illustrated the

significant correlations ($p\text{-value} < 0.05$) between the selected parameters. The PCA showed the distribution of the different traits; the first three principal components explained about 85.3% of the total variance. PC1 was mainly influenced by the number of pods, height and weight of the plant, while PC2 was influenced by chlorophyll. The contribution of the main ramifications was the lowest.

Site	Pods	Main ramifications	Plant height (cm)	Pod fresh weight (g)	Pod dry weight (g)	Plant fresh weight (g)	Plant dry weight (g)	Plant total fresh weight (g)	Plant total dry weight (g)	Average chlorophyll content (mg/m ²)
1	17.4	1.4	57.4	14.5	5.1	12.4	4.5	26.9	9.6	68.3
2	10.0	2.4	45.1	8.9	4.6	8.5	3.4	17.5	8.0	38.0
3	14.2	1.2	54.4	9.7	3.9	7.7	3.1	17.4	6.9	66.7
4	7.8	1.4	40.2	5.0	1.8	5.7	2.4	10.7	4.2	81.2
5	20.7	1.9	64.3	17.4	6.6	12.9	4.6	30.3	11.2	53.6
6	14.1	2.2	62.3	11.5	5.0	13.8	4.7	25.2	9.7	55.6
7	28.6	1.8	70.3	24.2	10.6	16.7	6.6	40.8	17.2	56.0

Tab. 18. The average values of the crop traits obtained from the measurements of 10 chickpea plants for each site.

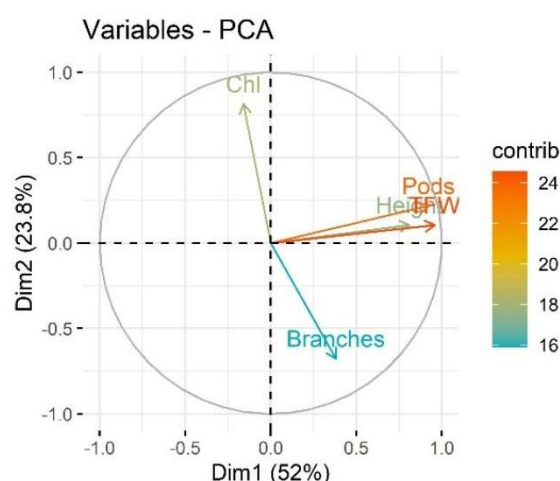


Fig. 22 - PCA plot among the selected chickpea plant traits.

Significant differences among the two years (2021 and 2022) were found for all the selected variables (**Fig. 22**; **Tab. 18**), apart from the number of main ramifications and chlorophyll content. The distribution of the different traits in the different sites can be through violin plots in **Fig. 23**. The variables capable of explaining a large part of the index variability were the

number of pods and main ramifications. Plant height had a moderate impact. On the contrary, chlorophyll content was less relevant, although it seemed to have an effect. The QBS-ar index had a significant positive relationship with plant height ($r = 0.60$). Although not significant, it had also a positive relationship with the number of main branches and chlorophyll content, and a slight negative relationship with the number of pods (**Fig. 23**).

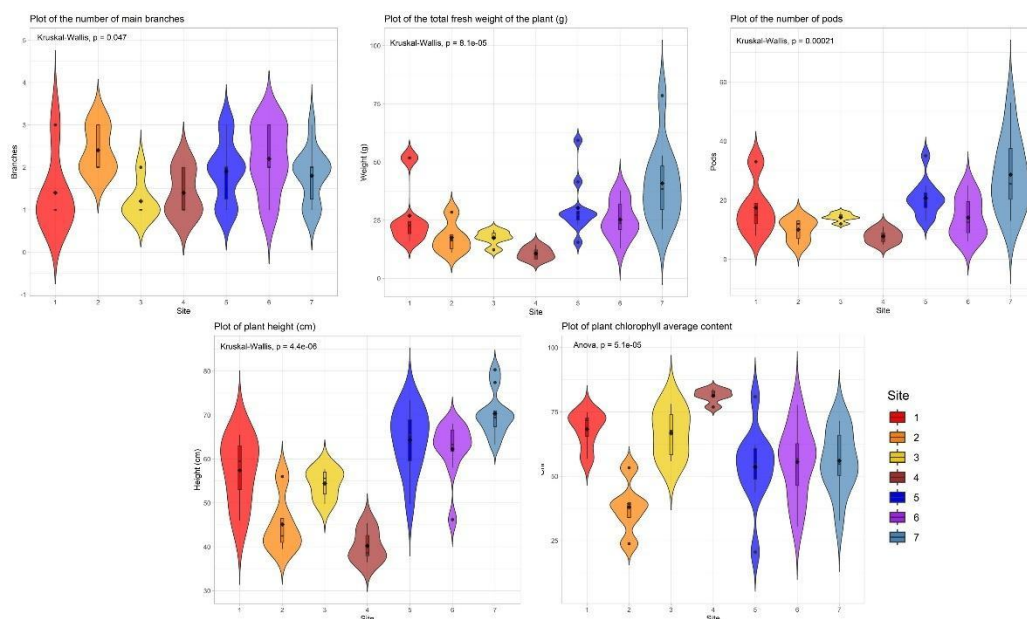


Fig. 23 - The violin plots showed the distribution of the plant traits among the different sites.

4.5.2.2 **Correlations among crop traits and chemical-physical parameters**

Interestingly, pH was found to be positively associated with plant height, number of pods and fresh weight. Chlorophyll levels were positively correlated with EC, K and P content. Similarly, a positive relationship was also found among K content and plant height, number of pods and fresh weight. On the contrary, the number of pods and plant fresh weight were negatively associated with EC content. While the number of main branches was slightly negatively correlated with K (**Fig. 24**).

Plot of correlation among QBS-ar, plant traits and soil chemical-physical parameters

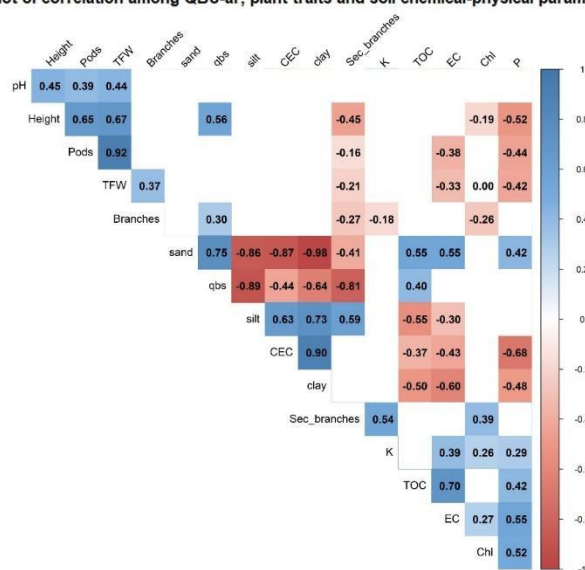


Fig. 24 - Correlation plot (p -value < 0.05) among total QBS-ar index, selected chickpea traits and soil chemical-physical parameters. Soil chemical-physical variables were reduced, due to multicollinearity.

4.5.2.3 Chickpea yield

Site	Year	Yield (Kg/ha)
1	2021	1417
2	2021	1193
3	2021	303
4	2021	NA
5	2022	1929
6	2022	880
7	2022	852

Tab. 19 - Table of the average chickpea yield in each site and year. Yield is understood as Kg/Ha of green grain, gross of pre-cleaning, drying, gluten cleaning. In the case of Site 4, data are missing, as chickpeas were not conferred to Az. Agr. Monte Monaco.

Chickpea yield was higher in 2022 (average yield 1220 Kg/ha) than in 2021 (average yield 971 Kg/ha), but not statistically significant. The yield registered in Site 5 was particularly high. While, in the case of Site 3, the yield was particularly low due to wild boars that did extensive damage to the crop just before harvesting. Although total and partial QBS-ar values were significantly higher in spring, there were no correlations between the two parameters ($r = 0.31$ and $r = 0.10$, respectively).

4.5.2.4 Effect of weather on chickpea plants

To analyse the effect of weather on chickpea plants, a different temporal interval was chosen, compared to soil biological data; from January to July for 2021, and from October 2021 to July 2022 for 2022 (**Fig. 25**). In all sites considered chickpeas were sowed in March and harvested at the end of July. In the case of site 7, chickpeas were sowed in October and harvested at the beginning of July. Significant differences in temperature between the two years were found in all months, except for January and July. Lower temperatures were registered in February and March 2022 compared to 2021, and subsequently higher temperatures from April to June 2022 compared to 2021. In general, there were differences of 1.1 or 1.2 °C. Except in May where the difference was about 2.2 °C. On the other hand, significant differences in precipitation were only found in January, February and March (**Fig. 25**).

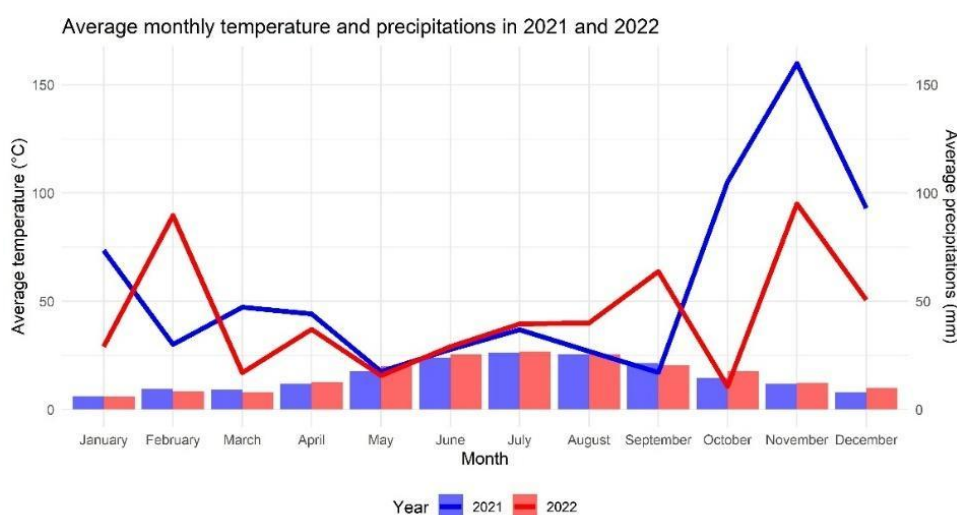


Fig. 25 - Average temperature (°C) and precipitations (mm) in the different months and years.

In 2022, there was more precipitation in February, June and July, but only February was significantly wetter than 2021. In **Fig. 25** a clear different precipitation pattern is visible from January to April among the two years. On the contrary, no differences were found for relative humidity. About the functional traits of the plants, meteorological data suggested that differences in some months may have influenced certain phenological phases of the plant. The chickpea plants may have had a positive effect due to higher rainfall in the months closest to

the 2022 harvest, whereas they may have had a negative effect due to higher rainfall in the periods closest to the 2021 sowing. In addition, slightly higher temperatures in the growing phase could also have had a positive effect.

4.5.2.5 Analysis of the rhizospheric bacterial communities

4.5.2.5.1 Soil biodiversity indices

Site	Year	Observed	Chao1	se.chao1	ACE	se.ACE	Shannon	Simpson	InvSimpson	Fisher
1	2021	301	319.2	8.4	314.5	8.1	1.5	0.49	1.95	46.6
2	2021	117	118.0	1.6	118.1	5.4	1.4	0.45	1.83	15.5
3	2021	135	135.0	0.1	135.2	5.5	1.2	0.36	1.56	18.2
4	2021	99	99.3	0.9	99.4	5.0	0.7	0.24	1.31	12.8
5	2022	276	291.2	10.9	279.6	8.2	1.8	0.45	1.80	42.0
6	2022	251	253.0	2.6	252.2	7.1	2.1	0.52	2.09	37.6
7	2022	240	242.0	2.6	240.9	7.6	1.7	0.41	1.70	35.7

Tab. 20 - Table of the average biodiversity indices of the rhizospheric bacterial communities in each site.

The Shannon, Simpson and Fisher indexes showed higher values for the sites analysed in 2022 than in 2021 (**Tab. 20**). In particular, the highest value was recorded at Site 6 for Shannon and Simpson and at Site 1 for Fisher. The ACE index and Chao1 indicated a probable underestimation of rare species at Sites 1 and 5. Very low values were found at Site 4 for the Fisher, Simpson, Shannon index, indicating the presence of fewer species, than at the other sites, which were more evenly distributed with few dominant species. Interestingly, the QBS-ar index was positively correlated with the biodiversity indices. There was a strong positive correlation ($r = 0.68$) with the Shannon index, and a weak positive correlation with the Simpson and Fisher indices ($r = 0.30$).

4.5.2.5.2 Soil bacterial biodiversity indices and chemical-physical properties

In **Fig. 26** is reported the correlation plot among QBS-ar, soil biodiversity indices and soil chemical-physical variables. All indices (QBS-ar, Fisher and Shannon), except Simpson, were negatively correlated with silt content. QBS-ar is also negatively correlated with CEC and clay compared to the other indices. While pH is positively correlated to all biodiversity indices, but not to QBS-ar. Using a PLS model, the relationship between biodiversity indices and soil

chemical-physical parameters was further evaluated. For the Shannon, Simpson and Fisher indices, P showed a significant inverse relationship. For the Shannon and Fisher indices, the influence of sand and clay content was secondary and positive in both cases. While for the Simpson index, sand and K were influential variables, but with a more moderate impact compared to P, while clay had a negligible influence.

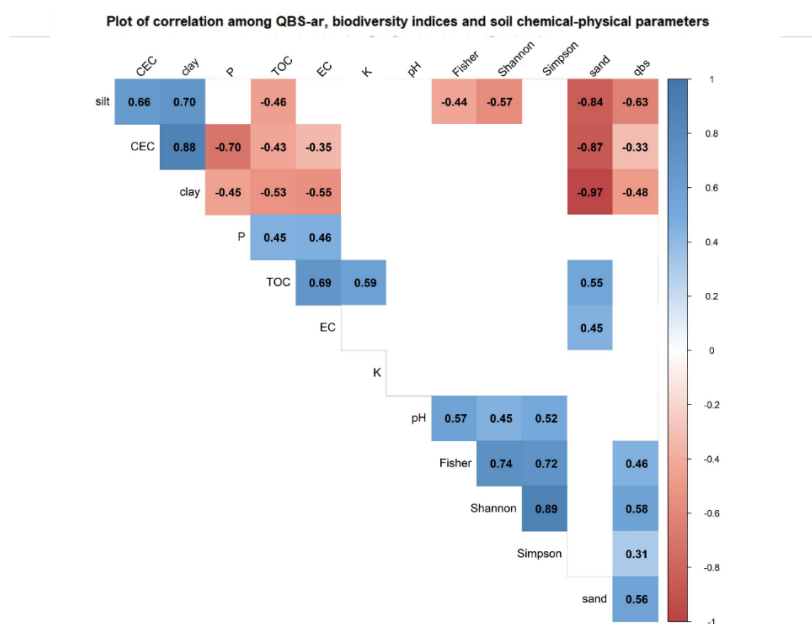


Fig. 26. Correlation plot (p-value < 0.05) among total QBS-ar index, biodiversity indices and soil chemical-physical parameters. Soil chemical-physical variables were reduced, due to multicollinearity,

4.5.2.5.3 Rhizospheric bacterial classes present at each site

The Gammaproteobacteria were the most present bacterial classes at all sites (**Fig. 27**). Especially at the sites monitored in 2021, and among them at Site 1. The presence of Alphaproteobacteria and Actinobacteria was considerable, to a greater extent at the sites monitored in 2022, and similarly between them. While Bacteroidia and Cyanobacteria showed more variable abundances. The former were particularly abundant at sites 5 and 6. The latter at site 4. The Verrucomicrobiae were less present than the former, particularly present in the sites monitored in 2022. Bacilli, on the other hand, were more present in the sites monitored in 2021. Particularly in Site 2. The Sites monitored in 2022 and Site 4 in 2021 compared to the

other sites showed a greater presence of Vicinamibacteria, Thermoleophila and Saccharimonadia.

Site 1 shown to be the least diversified site. In contrast, Sites 5 and 6 were the most diversified.

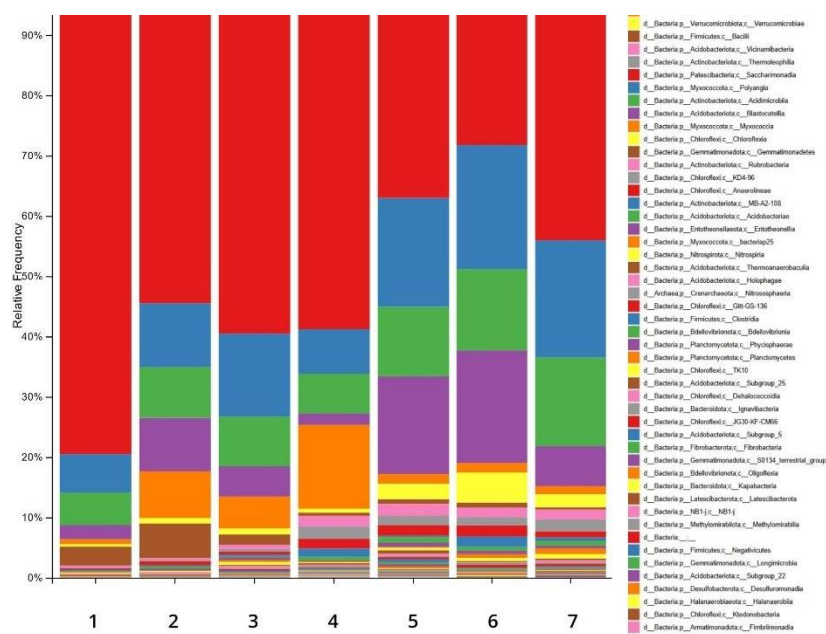


Fig. 27. The bar plots showed the distribution of the bacterial classes present at the different sites.

4.5.2.5.4 Rhizospheric bacterial soil functions

The chickpea, as a leguminous plant, hosted rhizobia in its roots in the form of nodules. It has a diverse bacterial population that plays an important role in its growth and protection (Sahu et al., 2020). Among the most abundant bacterial classes present in the examined samples, Gammaproteobacteria are known to respond chemotactically to exudates and efficiently uses plant exudate products. They can make inorganic nutrients available to plants, promoting their growth (García-Salamanca et al., 2013). Alphaproteobacteria are considered indicator of soil quality (Gazdag et al., 2019). They have a role in the degradation of inorganic compounds and the fixation of N. In the rhizosphere, they can influence carbon metabolism, oxidative photosynthesis, pyruvate metabolism, glycolysis/gluconeogenesis and the tricarboxylic acid cycle (You et al., 2024). While Actinobacteria are involved in the decomposition processes of organic matter, in the turnover of organic matter and in carbon cycling, replenishing the supply

of nutrients in the soil, for the formation of humus (Javed et al., 2021). Similarly, Bacteroidia (synonym Bacteroidetes) contribute significantly to the mobilisation of P in the rhizosphere (Lidbury et al., 2021). Verrucomicrobiae class presence in rhizospheric soil is considered a marker for non-stressed areas (Lucas et al., 2013). Both classes were particularly present in Site 5 and 6. Cyanobacteria can fix C and N in the soil and synthesise exopolysaccharides, which increase soil fertility and water retention and improve soil structure and stability (Chamizo et al., 2018). Cyanobacteria were particularly present in site 4. Saccharimonadia are considered to have a role in N cycling (Shi et al., 2021) and a potential function for seedling N use and growth (Dong et al., 2021). The class Thermoleophilia is known to be abundant in hot springs and soil samples. However, very few species of this class have been cultivated and characterized (Hu et al., 2019). Vicinamibacteria (synonym Acidobacteria) possess many genes involved in different metabolic pathways. They have a role in ecological processes, such as regulation of biogeochemical cycles, decomposition of biopolymers, secretion of exopolysaccharides and promotion of plant growth. Acidobacteria are predicted to possess genes that could promote survival and competitive colonisation in the rhizosphere, leading to the establishment of beneficial relationships with plants (Kalam et al., 2020). They influence nutrient cycling and transportation (You et al., 2024). Firmicutes is one of the most found bacterial phyla. In the class Bacilli, some are known to have plant growth-promoting characteristics and are important in suppressing plant disease (Paul & Frey, 2023).

4.5.3 Discussion

Sites with higher QBS-ar values therefore result in taller and larger plants with a greater number of main ramification and with fewer pods, but greater weight (**Fig. 28**). Soil properties appeared to affect plant growth. Particularly, pH positively influenced plant height, number of pods, and fresh weight (**Fig. 28**). The EC had a negative relationship with the number of pods and plant fresh weight. In addition, soil biodiversity indices were positively related to sand but negatively to silt, CEC, and clay content. pH positively impacted most biodiversity indices except QBS-ar. Similarly, P negatively affected the Shannon, Simpson, and Fisher indices.

Sand and clay had minor positive effects on the Shannon and Fisher indices, while P was the main influence on the Simpson index, along with some impact from sand and K. Yield was not associated with the QBS-ar values and, in general, with the biodiversity indices. A relationship between weather conditions and plant traits can be noted not statistically confirmed. Biodiversity indices showed that Site 6 was characterised by the greatest species richness, but with a few species dominating over the others.

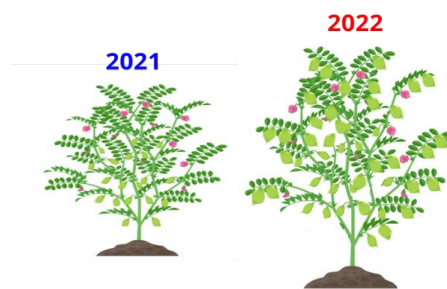


Fig. 28 - Morphological appearance of the plants in the two years.

At Site 1, species richness was equally high, but the species present appeared more evenly distributed. Site 5 emerged as the Site with the lowest rhizospheric microbial biodiversity. However, in these two latter sites that might be some underestimation of the species present. The different bacterial classes found at the sites considered emphasise the presence of numerous positive associations between the plant and the bacterial communities in the rhizosphere. They emphasise the overall wellbeing of the crop. Notably, the presence of Verrucomicrobiae in Site 5 and 6 underlined a positive and good soil condition (Lucas et al., 2013). To conclude, using a correlation matrix, the relationships between the QBS-ar index total values and the various indicators related to crop health were further evaluated, and the interactions that showed statistical significance ($p\text{-value} < 0.05$) were reported in **Fig. 29**.

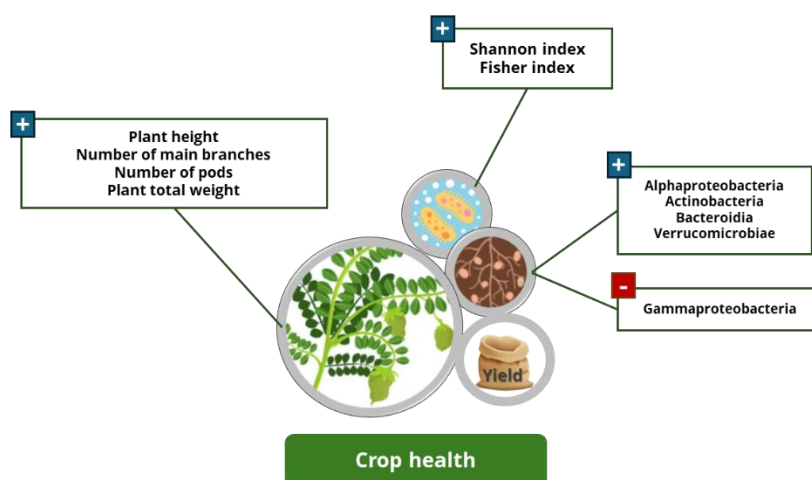


Fig. 29 – The interactions among the QBS-ar index, crop traits and rhizospheric bacterial communities are reported.

4.6 FOOD QUALITY ASSESSMENT

To assess food quality, an analysis of food products was carried out from nutritional and technological perspectives. The particularity of the project is that each food matrix comes from the specific site analysed and the processing of the different matrices took place separately to best preserve the characteristics of the chickpeas (*Cicer arietinum* L., var. Sultano). The physical-chemical properties and the technological functionality of chickpeas, grown in the different farms and locations in Sibillini mountains, and the derived food products (chickpea flour and pasta) were characterized and compared. The different food products (chickpeas, chickpea pasta and flour) were analysed as follows (**Fig. 30**):

- The chemical composition of raw chickpeas, the cooking quality and physical properties of chickpeas cooked to the optimal cooking time (OCT) were determined.
- The physical properties, pasting characteristics, colour, moisture content, and total polyphenol content of chickpea flour were determined.
- The cooking behaviour, physical quality at its OCT, moisture content, texture, and total polyphenol content of chickpea pasta was evaluated.



Fig. 30 - The figure summarises the parameters analysed for each food product.

The food matrices analysed below refer to the 2021 harvest. Thus, chickpeas from three different agricultural plots (1, 2, 3) were analysed. The fourth producer did not deliver the material to the company due to the rescission of the supply contract and the change of ownership of the company, which occurred in 2021. Nonetheless, the 2022 harvest was processed by the company in 2024 and the material was delivered to us in that year.

4.6.1 Materials and methods

4.6.1.1 Raw materials (Chickpeas, flour and pasta)

Azienda Agricola Monte Monaco collected the chickpeas from the different farms and processed the chickpeas into flour and pasta, in the same facility and with the same equipment. The chickpeas were cultivated by three farms (1, 2, 3) in three distinct sites located within the Marche region (**Tab. 21**). The company provided us with 1 kg of chickpeas, 1 kg of chickpea flour and 1 kg of chickpea pasta for each Site. In this latter case, two types of pasta were given: fusilli for Site 1 and 2, and penne for Site 3. All the following analyses were performed in triplicates.

Site	Farm	Province	Location	Pedological Province	Geographical coordinates	Field dimension (ha)	Yield (Kg/ha)	Time of sowing
1	Az. Santarelli e Arriva	Fermo	Francavilla d'Ete	5.7.6 – Medie colline fermiane tra Ete M.e Tesino.	Lat: 43°11'43.19" Long: 13°32'58.81"	5.8	1417	Spring
2	Az. Agr. Le corti del Frate	Fermo	Contrada Salvano	5.7.6 – Medie colline fermiane tra Ete M.e Tesino.	Lat: 43° 9'9.99" Long: 13°45'55.01"	4.0	303	Spring
3	Az. Antolini Ettore	Fermo	Santa Vittoria in Matenano	5.7.1 – Alte colline ascolane di Santa Vittoria in Matenano	Lat: 43° 1'31.78" Long: 13°31'20.69"	4.0	1193	Spring

Tab. 21 - Data on chickpea cultivation for three different farms in 2021. All fields were cultivated with chickpeas (*Cicer arietinum* L., var. Sultano).

4.6.1.2 Characterization of chickpeas

4.6.1.2.1 Physical properties of raw chickpeas

4.6.1.2.1.1 Raw chickpeas morphology, volume and weight

A visual assessment was conducted to determine the morphology of raw chickpea seeds. A total of 100 seeds were considered for each site. The seeds were randomly selected, with the exclusion of broken or damaged ones. The seeds were scanned using a flatbed scanner (Canon CanoScan Lide 400, USA) to obtain images (600 dpi resolution). The images were analysed using Image J software (ImageJ 1.46r, NIH) to acquire chickpea size (area, mm²) and roundness.

To determine the weight of seeds, 50 intact raw chickpeas of each site were randomly selected and weighed (Explorer analytical balance). To determine the average volume of the seeds, the 50 chickpeas were placed in a cylinder containing 25 ml of distilled water. The increase in volume resulting from the insertion of the chickpeas was evaluated and recorded.

4.6.1.2.1.2 Raw chickpea hydration and swelling capacity

To determine the hydration capacity, 50 raw chickpea seeds for each site were weighed and placed in a beaker with 50 ml of distilled water. The chickpeas were kept in the water overnight at room temperature to hydrate. After soaking, the chickpeas were withdrawn from water by using a colander then reweighed. The hydration capacity (%) was calculated as follows (Williams et al., 1983):

$$\text{Hydration capacity (\%)} = \frac{\text{Weight after soaking} - \text{weight before soaking}}{\text{Total seed}} \times 100$$

To determine the swelling capacity, previously soaked chickpeas from hydration were used. The soaked chickpeas were placed in a graduated cylinder containing 50 ml of distilled water.

The increase in volume resulting from the insertion of the seeds was evaluated and recorded.

The swelling capacity (%) was then calculated as follows (Williams et al., 1983):

$$\text{Swelling capacity (\%)} = \frac{\text{Volume of Soak chickpeas} - \text{Volume of dry chickpeas}}{\text{Volume of dry chickpeas}} \times 100$$

4.6.1.2.2 Chemical composition of raw chickpeas

The content of unsaturated fatty acids, lipids, carbohydrates, total sugars, fibre, protein, moisture, and ash were determined in each sample. The samples were analysed by *Agenzia per l'Innovazione nel Settore Agroalimentare e della Pesca "Marche Agricoltura Pesca"* (AMAP) laboratories (Jesi, Italy) in accordance with the methods defined by the Italian legislation for food analysis: Rapporti ISTISAN 1996/34, D.M. 23/07/94 SO GU n. 186 10/08/94, D.M. 11/04/94 SO GU n. 92 21/04/94, PP057 Rev. and UNI EN ISO 2171:2010.

4.6.1.2.3 Chickpeas cooking behavior

4.6.1.2.3.1 Optimal Cooking Time (OCT)

To determine the chickpeas optimal cooking time (OCT), a pot filled with a defined volume of water (approximately five times the volume of the chickpeas) was brought to the boiling point before adding the chickpeas, by using a hotplate (ARGO LAB M2A Magnetic Hotplate stirrer) which was set to 300°C. About 80 g of chickpeas were boiled for different times, ranging from 30 to 145 min, during which two to three chickpeas were collected every 10 minutes and were checked for a white spot in the middle of chickpea. and visually inspected. The cooking time from 80 to 150 min was selected to further evaluate the OCT. The test was repeated two times for each sample to confirm the appropriate interval. Afterwards, 100 to 150 min was further selected for the final assesment of the OCT. Then, raw chickpeas were cooked for 100 to 150 min and 10 chickpeas were sampled at 2-minute intervals. The timing at which the totality of chickpeas has no white spot was considered as the optimum cooking time.

4.6.1.3 *Physical properties of chickpeas cooked to OCT*

4.6.1.3.1.1 *Moisture content*

Chickpeas were cooked to OCT, then collected from water immediately by using a colander. After cooling, the oven-drying method was used to determine the moisture content of cooked chickpeas. Approximately 12-15 cooked chickpeas were placed inside an aluminium container (W1), the total weight was recorded (W2). The samples were placed inside a 105°C preheated oven and dried to constant weight. After drying, the total weight was recorded (W3) and the moisture content (%) was calculated as follows:

$$\text{Moisture content (\%)} = \frac{W2 - W3}{W2 - W1} \times 100$$

4.6.1.3.1.2 *Texture*

The textural properties of chickpeas cooked to OCT were measured by using a texture analyser (AMETEK TA1 Test and calibration instrument LLOYD) (**Fig. 31**). For each site, 30 chickpeas from each cooking batch were used for the measurements. The machine was equipped with a 36 mm diameter cylindrical probe to perform a double-compression test to compress the samples up to a 40% of their depth at a test speed of 1 mm/s. The holding time between two compressions was 5 s. Hardness (N), adhesiveness (J) and springiness were recorded by NexygenPlus Materials Testing Software (Xu et al., 2019).

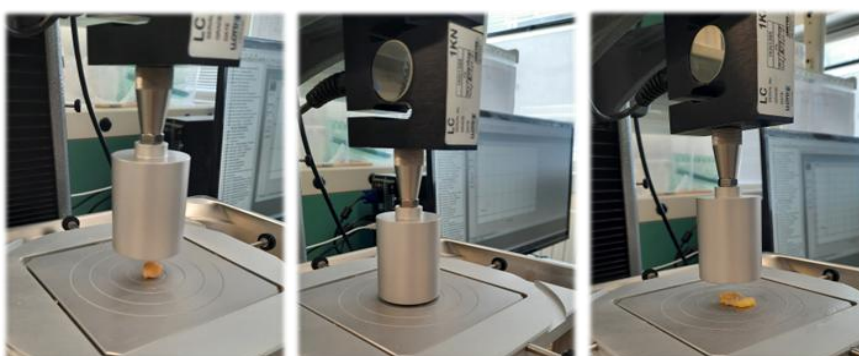


Fig. 31 - The picture showed three steps of a texture analyser-based compression test performed on chickpeas. This setup applied pressure to the chickpeas to evaluate their texture.

4.6.1.4 Characterization of chickpea flour

4.6.1.4.1 Physical properties

4.6.1.4.1.1 Flour particle size

The particle size distribution of the chickpea flour was analysed using a sieve shaker (RETSCH, Germany). Different sieves were mounted on the mechanical stirrer and placed in descending order of mesh size: 425, 300, 125, 53, 38 μ . For each Site, 50 g of flour were measured (Explorer analytical balance), and placed on the first sieve with the largest mesh size (425 μ). The sieves were agitated for 5 minutes at 60 rpm. The amount of flour retained on each sieve and in the container at the bottom of the stirrer were weighed. The different flour fractions (%) were calculated as follows:

$$\text{Flour fraction} = \frac{\text{Sieve with flour weight} - \text{Empty sieve weight}}{\text{Weight of flour}} \times 100$$

4.6.1.4.1.2 Solvents binding ability

To determine the Water Holding Capacity (WHC), which refers to the ability of a flour, or other substances, to retain water within its structure without the application of external force, 0.1 g of chickpea flour was weighed (Explora Scale) and added in a 2 ml conical tube containing 1 ml of distilled water. The mixture was mixed with a vortex (Velp scientifica 2x³) for 10 seconds every 5 min for a total of 30 min. The mixture was centrifuged (using a Megafuge 1.0R) for 15 min at 3000 rpm to separate the free water. The hydrated flour was weighed to determine the amount of water retained. The WHC (%) was calculated as follows (Stone et al., 2019):

$$\text{WHC (\%)} = \frac{\text{Weight of hydrated flour (g)} - \text{weight of dry flour (g)}}{\text{weight of dry flour (g)}} \times 100$$

To calculate the Oil Holding Capacity (OHC), which refers to the ability of a flour, or other substances, to absorb and retain oil, 0.1 g of chickpea flour were placed in a 2 ml conical tube, and 1 ml of sunflower oil was added. The mixture was mixed with a vortex (Velp scientifica 2x³) for 10 seconds every 5 min for a total of 30 min. Then, it was centrifuged (Megafuge 1.0R) for 15 min at 3000 rpm to separate the free (unabsorbed) oil from the flour. The hydrated flour

was weighted to determine the amount of oil retained (Stone et al., 2019). The OHC was calculated as follows:

$$\text{Oil Holding Capacity (\%)} = \frac{\text{Weight of hydrated flour (g)} - \text{weight of dry flour (g)}}{\text{weight of dry flour (g)}} \times 100$$

4.6.1.4.1.3 Pasting property

The pasting properties of flour were investigated by a micro visco-amylograph (Brabender, GmbH, Germany). For each site, 100 g of chickpea flour were measured and deionized water was added to each sample to create a 15% homogeneous suspension (dry weight basis, w/w). Each suspension (115 g) was loaded into the visco-amylograph and then heated, while stirring, from 30 °C to 93 °C at a heating rate of 7.5 K/min, held at 93 °C for 5 min, cooled to 30 °C at 7.5 K/min, and held at 30 °C for 1 min. Onset gelatinization temperature (°C), peak viscosity (BU), breakdown (BU), final viscosity (BU), and setback viscosity (BU) were obtained.

4.6.1.4.1.4 Chickpeas Flour Colour

For each site, 50 g of chickpea flour were homogeneously distributed inside a tray. A colorimeter (Chroma meter CR-400, Konica Minolta Inc., Japan) was pressed on the flour for few seconds and different parameters, including L* (0: black, 100: white), a* (+ : redness, - : greenness), and b* (+ : yellowness, - : blueness) were measured.

4.6.1.4.1.5 Chickpeas Flour Moisture Content

The flour moisture content was measured with the same protocol used for chickpeas cooked to OCT; 2g of chickpea flour was dried in an oven at 105°C to a constant weight. The moisture content was recorded as the weight referred to the flour weight before drying and was expressed as g water/100 g flour (%).

4.6.1.4.2 Chickpeas flour total polyphenols

1 g of chickpea flour was placed into a centrifuge tube with 4 ml of 70% ethanol (v/v). The pH was adjusted to 4 using 2N hydrochloric acid. The sample was mixed using a vortex (Velp scientifica 2x³) for 60 minutes at 80°C, then centrifuged at 5000 rpm for 15 min. 100 µL of the filtered extract was mixed with 5 mL of 0.1M Folin-Ciocalteu reagent. A saturated sodium carbonate (Na₂CO₃) solution (3.5 mL) was added to the mixture. After mixing, the mixture was incubated at room temperature in the dark for 2 hours. A series of gallic acid standard solutions (50, 100, 200, 500, 700 mg/L) was prepared, then 0.5 mL of each standard was pipetted into separate test tubes. The absorbance of each standard and samples was measured at 760 nm using a spectrophotometer.

4.6.1.5 Characterization of chickpea Pasta

4.6.1.5.1 Cooking behaviour and cooking quality

The chickpea pasta provided by each site (site 1 = fusilli, site 2= fusilli, and site 3 = penne) was analysed to determine cooking behaviour and cooking quality. Several parameters have been analysed, including OCT, solid loss (or cooking loss) and pasta weight gain after cooking to OCT.

4.6.1.5.1.1 Chickpea Pasta OCT

The chickpea pasta was boiled in five times ml of water in respect to the grams of the pasta used for cooking. During cooking, every minute, from 5 to 13 min every minute, the texture of the pasta was manually checked. To better define the OCT, a sensory evaluation was conducted using a panel test with 12 participants. They evaluated each pasta sample for hardness, adhesiveness, stickiness and flavour, and overall satisfaction (**Fig. 32**). The cooking time at which the pasta received the highest score was considered as the optimal cooking time.

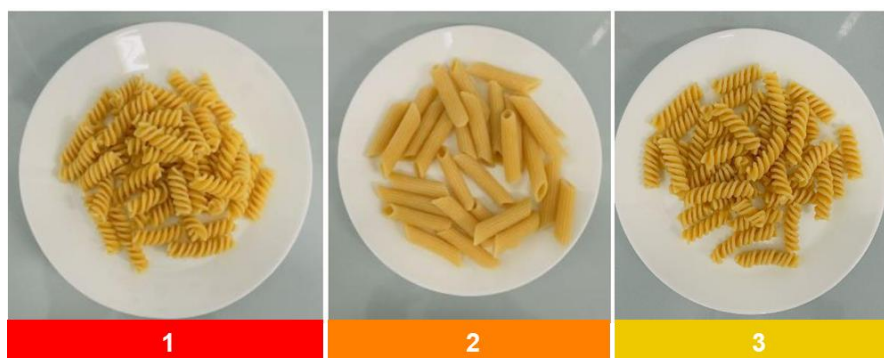


Fig. 32 - The image showed an experimental cooked pasta for optimum cooking time.

4.6.1.5.1.2 Solid loss

Eighteen (18 g) of uncooked chickpea pasta were weighted. A pot of water was brought to the boiling point, and the pasta was cooked to OCT. After cooking, the pasta was drained using a colander, for removing the excess water. The cooking water was collected in separate beakers. The cooked pasta weight and the empty beaker weight were recorded.

To determine the solid loss, the cooking water was dried in oven at 105°C to constant weight. Then solid loss (%) was calculated as follows:

$$\text{Solid loss (\%)} = \frac{\text{Container with dry solids weight} - \text{container weight}}{\text{Raw Pasta weight}} \times 100$$

4.6.1.5.1.3 Pasta weight gain

To determine the pasta weight gain, after cooling for 1 min, the pasta post-cooking weight was recorded (**Fig. 33**). The weight gain (%) was calculated as follows:

$$\text{Pasta weight gain (\%)} = \frac{\text{Cooked pasta weight} - \text{Raw pasta weight}}{\text{Raw pasta weight}} \times 100$$



Fig. 33 - The image showed the pasta cooked to OCT and the cooking water, used to determine the solid loss and the pasta weight gain.

4.6.1.5.1.4 **Pasta dimension increase (length and thickness)**

To determine pasta length and thickness dimensional changes, a raw representative sample (40 pieces) of chickpea fusilli and penne pasta pieces was considered. Lengths and thickness of pasta pieces were measured with a calliper before (raw) and after cooking to OCT. Dimensional change was then calculated as follows:

$$\text{Change in Thickness (\%)} = \frac{\text{After cooked thickness} - \text{Raw pasta thickness}}{\text{Raw pasta thickness}} \times 100$$

$$\text{Change in length (\%)} = \frac{\text{After cooked length} - \text{Raw pasta length}}{\text{Raw pasta length}} \times 100$$

4.6.1.5.2 **Physical quality of pasta cooked to OCT**

All pasta samples were cooked to OCT and then characterized as follows:

4.6.1.5.2.1 **Pasta Colour**

Cooked pasta color was measured using a Minolta colorimeter (Chroma meter CR-400, Konica Minolta Inc., Japan) by acquiring color coordinates of 7 pasta pieces of three cooked pasta batches. L (lightness), a (red/green), and b (yellow/blue) were measured.

4.6.1.5.2.2 **Pasta moisture content**

The method for cooked pasta moisture content is the same as the method already described above for cooked chickpeas moisture content. Data was expressed as g water/100 g cooked pasta.

4.6.1.5.2.3 **Pasta texture**

To determine texture, pasta adhesiveness, and hardness the AMETEK TA1 Test and calibration instrument LLOYD was used (**Fig. 34**). For both analyses, cooked pasta, whose cooking time was optimised based on the previous analysis, was used. For each farm we used 32 pasta pieces. To measure adhesiveness, the instrument was equipped with a 36 mm diameter cylindrical probe to perform a double-compression test to compress the samples up

to a 40% of their depth at a test speed of 1 mm/s. The interval time between two compression was 5 s. Adhesiveness and cohesiveness were calculated from the Texture Profile Analysis (TPA) curves by NexygenPlus Materials Testing Software (modified from Xu et al., 2019).

To measure the hardness of cooked pasta, a cutting test was performed. In this case, the instrument was equipped with a shear blade. The speed was configured to 2 mm/s, and the shearing distance was programmed for 10 mm. A single strand of pasta was positioned on the platform and subjected to brokening. Then the peak force to break pasta samples was taken as hardness (N).

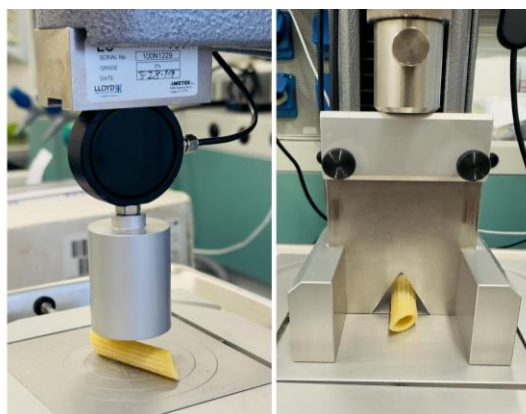


Fig. 34 - The image showed the different probes used to measure chickpea pasta hardness and adhesiveness.

4.6.1.5.3 Total polyphenols of pasta cooked to OCT

1 g of pasta from each site was freeze-dried at -54 °C and 0.05 mbar using a BUCHI Lyovapor™ L-200 freeze-dryer (Büchi Labortechnik AG, Flawil, Switzerland) (**Fig. 35**). The freeze-dried samples were ground before analysis. After that the method for cooked pasta total polyphenols is the same as the method already described above for flour chickpeas total polyphenols content.



Fig. 35 - The image showed sample preparation for chickpeas pasta Total polyphenol content.

4.6.1.6 **Statistical analysis**

For the statistical evaluation, data were first checked for normality of distribution (Shapiro-Wilk test). Differences between experimental data obtained by the three farms were evaluated through One-way variance analysis (ANOVA) and post hoc tests (Tukey's honestly significant difference) were used to determine differences among farms. A p-value < 0.05 has been considered significant. Statistical analysis was performed using R software, version 4.4.0 (R Development Core Team, 2021) and by using XLSTAT 2023.2.1413.1413 with OriginPro 2022. The plots were created by using R software.

4.6.2 **Results**

4.6.2.1 **Characterization of raw chickpea**

4.6.2.1.1 **Physical properties of raw chickpeas**

4.6.2.1.1.1 **Chickpea appearance**

Notable differences between chickpeas appearance were obtained from the various Sites (**Fig. 36**). In particular, the chickpeas from Site 2 were larger in size, exhibited an irregular surface and shape, and the veining was less prominent. Chickpeas from Site 3 were the smallest, with a uniform shape and surface, and a not so evident veining. Chickpeas from Site 1 resulted in a medium size, a regular surface and shape, and an evident veining.

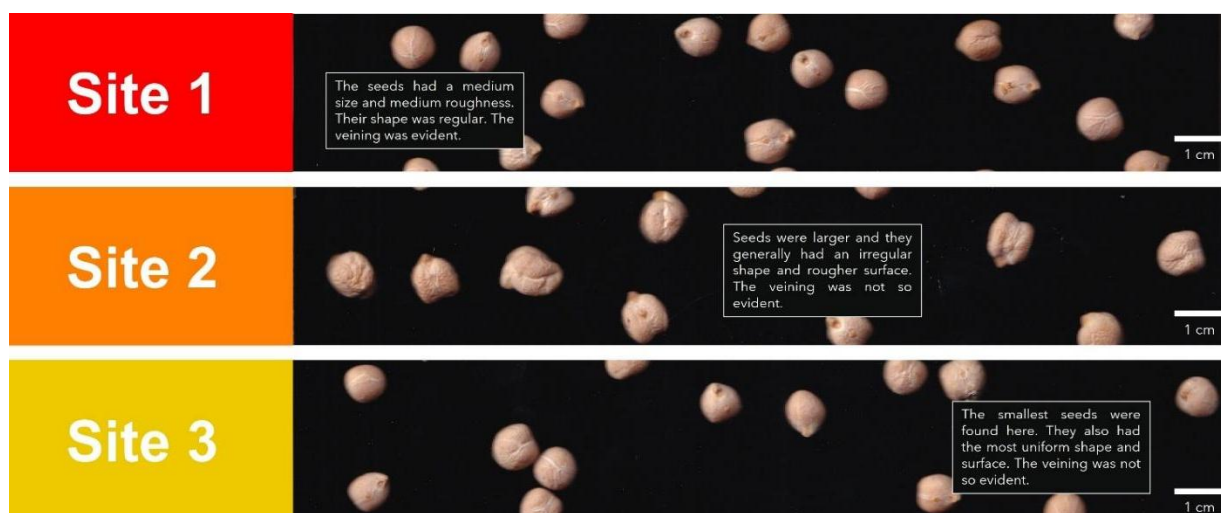


Fig. 36 - Images obtained from raw chickpea scanning.

4.6.2.1.1.2 Chickpeas area and roundness

Chickpea seed from sites 2 and 3 had a significant larger area compared to Site 1 (**Tab. 22**).

On the contrary, chickpeas from Site 1 had the highest average roundness, while chickpeas from Site 2 had an intermediate average roundness, and chickpeas from Site 3 had the lowest roundness.

Site	Area (cm ²)	Roundness (cm ²)
1	0.53±0.03b	0.89±0.05a
2	0.60±0.07a	0.86±0.07ab
3	0.60±0.16a	0.82±0.18b

Tab. 22 – Raw chickpeas area and roundness. In the table mean values ± standard deviation is reported. Lowercase letters represent significant differences between Sites by ANOVA at *p-value* < 0.05.

4.6.2.1.1.3 Chickpeas, volume, size, and weight

Site 2 chickpea seeds had the largest volume (cm³) and weight (g) followed by site 1 and 3 (**Tab. 23**). For both parameters, the results obtained in the three sites were significant. Similarly, site 2 had the highest hydration and swelling capacity followed by site 1 and 3 which, in this case, were similar and didn't show any significant differences among them (**Tab. 23**). Overall, chickpea seeds from site 2 were markedly different from the other two.

Site	Weight (g)	Volume (cm ³)	Hydration Capacity (%)	Swelling Capacity (%)
1	15.65±0.114b	0.24±0b	0.318±0.005b	1.53±0.012b
2	19.13±0.199a	0.28±0a	0.398±0.011a	1.67±0.023 ^a
3	14.83±0.155c	0.22±0c	0.305±0.006b	1.51±0.012b

Tab. 23 – Raw chickpeas weight, volume, hydration and swelling capacity. In the table average values ± standard deviation is reported. Lowercase letters represent significant differences between Sites by ANOVA at *p-value* < 0.05.

4.6.2.1.2 Chemical properties of raw chickpeas

Site 2 showed a notable higher content of total saturated fatty acids, lipids, and proteins (**Tab. 24**). While site 3 had the highest carbohydrates and raw fibre content. Ashes content was higher in site 1. The contents of total sugars and sucrose were similar among Sites. Other sugar content: including fructose, glucose, and maltose were low in all samples.

Site	Total saturated fatty acids (g/100g)	Lipids (% d.m.)	Carbohydrates (g/100g)	Total sugars (g/100g)	Fructose (g/100g)	Glucose (g/100g)	Sucrose (g/100g)	Maltose (g/100g)	Raw fibre (% d.m.)	Protein content (%)	Moisture on grain (%)	Ashes (% d.m.)
1	0.49	3.88	48.43	2.3	< 0.5	< 0.5	2.3	< 0.5	3.44	22.28	10.5	3.07
2	0.61	4.59	45.82	2.2	< 0.5	< 0.5	1.8	< 0.5	3.53	24.5	10.3	2.86
3	0.48	3.53	50	2.1	< 0.5	< 0.5	2.1	< 0.5	3.76	21.74	9.6	2.97

Tab. 24 - Raw chickpeas centesimal composition.

4.6.2.1.3 Chickpeas cooking quality

4.6.2.1.3.1 Chickpeas Optimal Cooking Time OCT

Chickpeas from site 1 (141 min) and 2 (142 min) had very similar average OCTs, while chickpeas from site 3 had a shorter average OTC (126 min).

4.6.2.1.4 Physical properties of chickpeas cooked to OCT

4.6.2.1.4.1 Moisture content

The moisture content of the chickpeas cooked to OCT was similar among Sites (60 %). No significant differences among them were found (**Tab. 25**).

Site	Moisture content (%)
1	59.84±0.73a
2	59.06±2.13a
3	58.73±0.23a

Tab. 25 – Cooked chickpeas moisture content (%).

4.6.2.1.4.2 Cooked chickpea texture (*hardness, adhesiveness, springiness*)

The results suggested that chickpeas from site 3 had a significantly higher hardness, compared to site 1, which had the lowest value (**Fig. 37**). Hardness in chickpeas from site 2 was intermediate and not significantly different from either site 1 and 3. This difference in hardness might be due to the content of row fibre and carbohydrates, which were higher in site 2, and contributed to the harder texture and to the firmer consistency. On the other hand, no significant differences were observed for stringiness and adhesiveness. In the latter case, site 2 and 3 had higher and similar adhesiveness, compared to site 1 (**Fig. 37**). The springiness was relatively consistent across all sites, indicating similar resilience and ability to return to their original shape after compression.

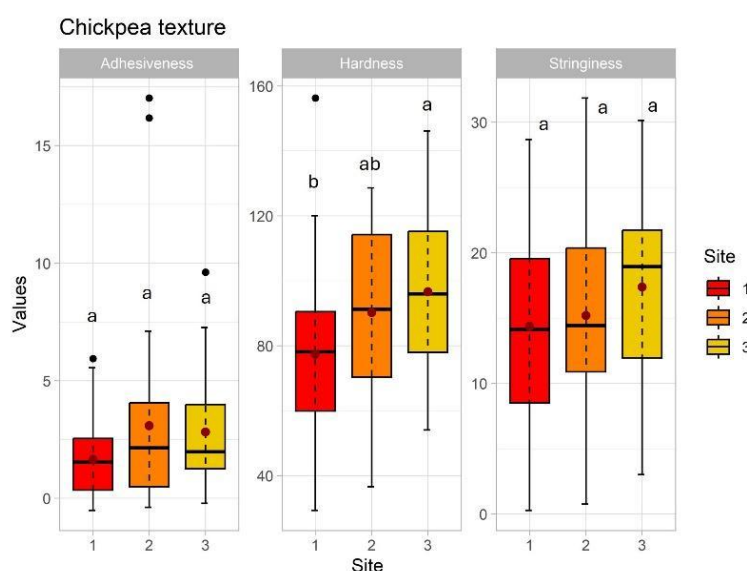


Fig. 37 – The boxplots show the cooked chickpea texture.

4.6.2.2 Characterization of chickpea flour

4.6.2.2.1 Flour physical properties

4.6.2.2.1.1 Flour particle size

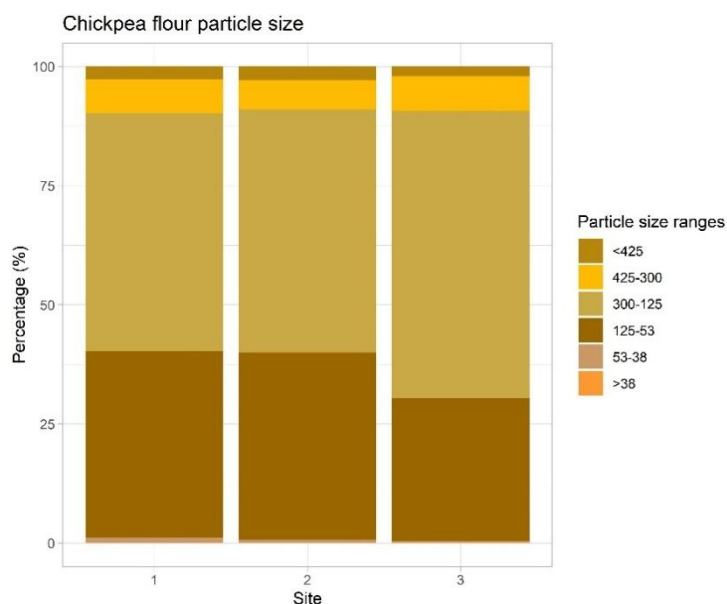


Fig. 38 - The different particle size ranges analysed are represented by different colours. Particle sizes were categorized as follows: < than 38 µm, from 38 to 53 µm, from 53 to 125 µm, from 125 to 300 µm, from 300 to 425 µm, and > than 425 µm.

Most of the flour particles fall within the 125-300 µm and 53-125 µm ranges. Site 3 has a higher proportion of particles in the 300-125 µm range compared to site 1 and 2. Larger particles result in a coarser and grainier dough, with more spaces and a less uniform structure. In addition, the smallest particle size (<38 µm) was almost negligible across all samples, with site 3 showing only a minimal presence.

4.6.2.2.1.2 Solvents binding ability

Any significant differences were found for flour water holding capacity (WHC) (**Fig. 39**). Site 1 had the highest WHC, while sites 2 and 3 had lower and similar WHCs. On the contrary, significant differences were found for flour oil holding capacity (OHC). Site 3 had a significantly higher OHC compared to site 1. In general, a high OHC helps improve the toughness of the dough, resulting in better elasticity.

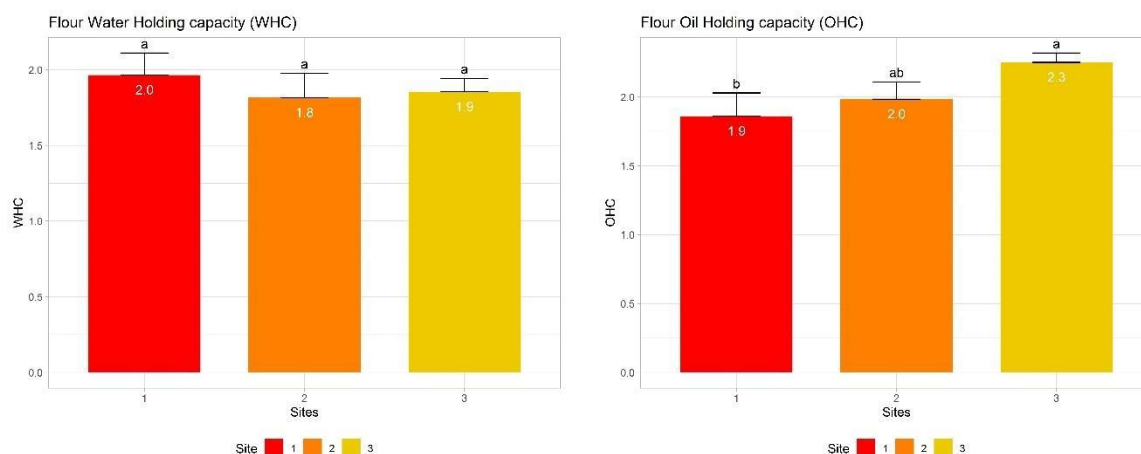


Fig. 39 – Barplots of the flour water holding capacity (WHC) (left) and oil holding capacity (OHC) (right).

4.6.2.2.1.3 Pasting properties

Results showed that all samples had similar gelatinization temperatures, around 75°C (**Tab. 26**). Site 1 showed the highest maximum viscosity and final viscosity, followed by site 2 and 3. Similarly, site 1 displayed the highest breakdown viscosity, indicating more viscosity loss upon heating, while site 3 showed the lowest. Site 2 had the highest setback viscosity, indicating more retrogradation or re-association of starch molecules during cooling, while site 3 had the lowest value for this parameter.

Site	Gelatinization Temperature(°C)	Maximum Viscosity	Final Viscosity [BU]	Breakdown Viscosity	Setback Viscosity
1	75.1±0.67a	150.0±325.0a	237.0±40.5a	2.0±1.4a	81.3±13.1a
2	75.4±0.14a	144.0±0.82a	235.0±2.2a	0.67±0.47a	84.0±1.61a
3	75.0±0.24a	139.0±4.2a	222.7±2.6a	0.33±0.47a	77.3±1.2a

Tab. 26 - In the table average values ± standard deviation is reported. Lowercase letters represent significant differences between farming systems by Student's T test at *p-value* < 0.05.

4.6.2.2.1.4 Chickpeas Flour Colour

In **Fig. 40** the measurements of the various colour parameters (l, a, b) are reported. Any significant differences were found for the redness (a) among the flour samples from different sites. Moreover, all samples had negative values for the present parameter.

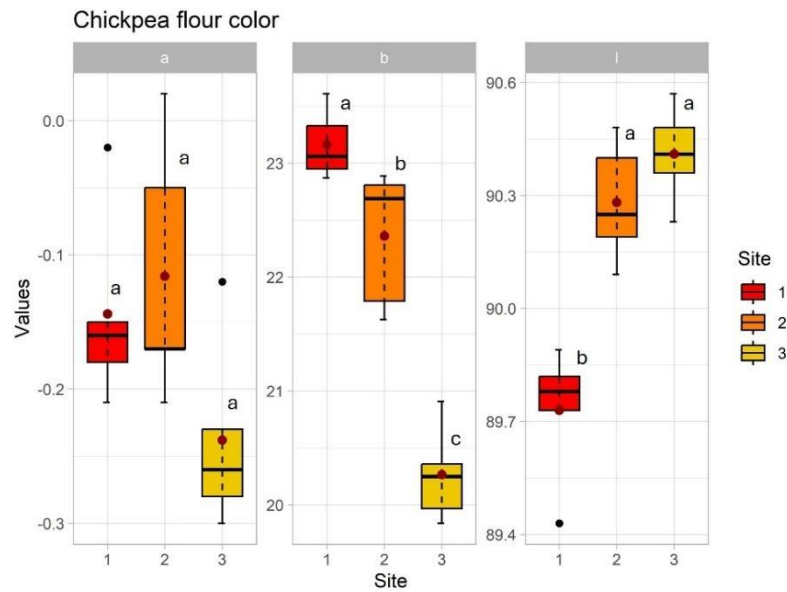


Fig. 40 – The boxplots show the distribution of the values related to flour colour analysis (a = redness, b = yellowness, l = lightness).

In the case of lightness (l), site 1 had a significantly lower value compared to both site 2 and 3, which showed similar values, and no significant differences were found among them. On the other hand, all sites resulted to be significantly different in terms of yellowness (b), and site 1 had a significantly higher value compared to both Site 2 and 3. Generally, site 3 was characterized by lower values for both redness and yellowness, and thus, a higher value of lightness. This may indicate a lower content of pigments, such as carotenoids, in flour from site 3, which resulted in a lighter flour.

4.6.2.2.1.5 Chickpeas Flour Moisture Content

The chickpea flour from site 1 had the highest moisture content (11.0%), and it was significantly different from the other samples. The flour from site 2 and 3 had a similar moisture content (10.0%), and there were no significant differences among them.

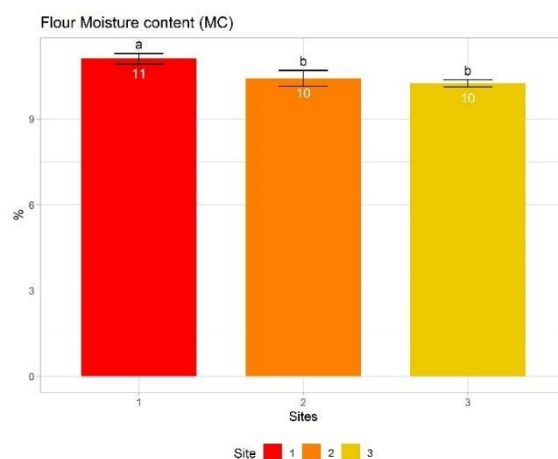


Fig. 41 – Barplot of the chickpea flour moisture content (%) from the different sites.

4.6.2.3 Chickpeas Flour Total Polyphenols

The chickpea flour from site 3 exhibited the highest total polyphenols content (TPC), approximately 112 mg/kg and antioxidant capacity, approximately 51 mg/kg (**Fig. 42**). The chickpea flour from site 1 had a TPC value of 111 mg/kg and an antioxidant capacity of 46 mg/kg. While in site 2 the lowest TPC and antioxidant capacity values were found, respectively 105 mg/kg and 45 mg/kg. Both the TPC content and antioxidant capacity resulted in statistically different results among farms (**Fig. 42**).

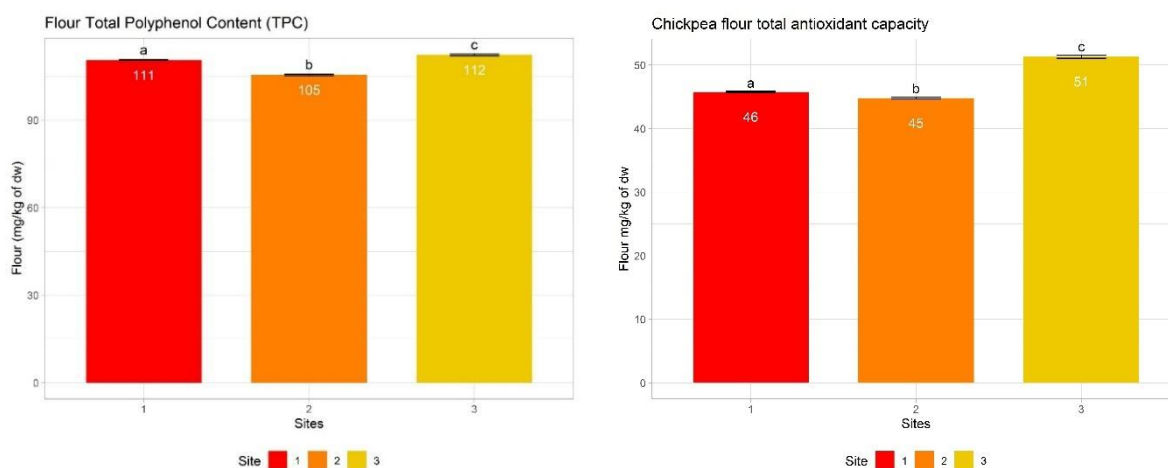


Fig. 42 – Barplot of the chickpea flour total polyphenol content (TPC) (left) and total antioxidant capacity (right) from the different sites.

4.6.2.4 Characterization of chickpea pasta

4.6.2.4.1 Pasta cooking behaviour

4.6.2.4.1.1 Chickpea pasta OCT

The chickpea pasta from site 3 (fusilli) had the highest OCT (8 min and 30 seconds). The sample from site 2 (penne) had a slightly lower OCT (7 min). Whereas the sample from site 1 (fusilli) had the lowest OCT (6 min and 30 seconds).

4.6.2.4.2 Cooking and physical quality of pasta cooked to OCT

4.6.2.4.2.1 Pasta solid loss and weight gain

Chickpea pasta from site 3 had the highest weight gain, site 1 had a slightly lower weight gain, while site 2 had the lowest weight gain. The result obtained from this latter site was significantly different compared to site 3 (**Fig. 43**). However, in general, the solid loss percentages (around 8%) were similar among sites (**Tab. 27**).

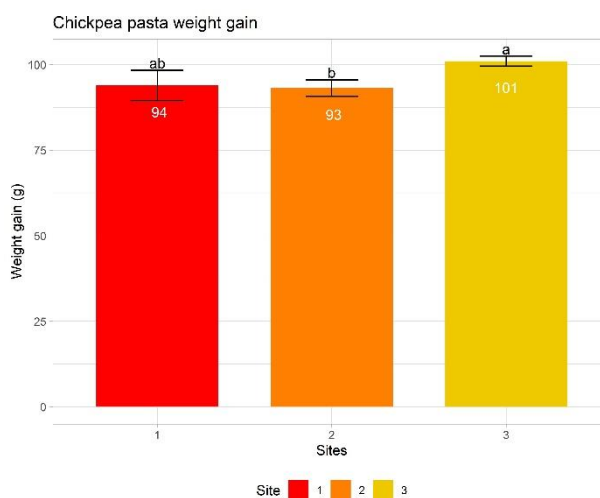


Fig. 43 - Barplot of the chickpea pasta weight gain (g) in the different sites.

4.6.2.4.2.2 Dimension increase (length and thickness)

Pasta from site 3 showed a significant length increase, compared to site 1. Site 2 exhibited a moderate length increase, while site 1 showed the lowest length increase (**Tab. 27**). In terms of pasta thickness increase, site 2 showed the highest thickness increase. However, no significant differences were found among sites (**Tab. 27**).

Site	Moisture content (%)	Length increase (mm)	Thickness increase (mm)	Solid loss (%)
------	----------------------	----------------------	-------------------------	----------------

1	48.41±1.18ab	35.63±5.16b	1.47±0.15a	7.53±0.38a
2	48.22±0.65b	37.55±5.02ab	1.44±0.06a	8.15±0.20a
3	50.26±0.36a	39.50±2.52a	1.41±0.09a	8.22±0.43a

Tab. 27 - In the table average values ± standard deviation is reported. Lowercase letters represent significant differences between sites by Student's T test at *p-value* < 0.05.

4.6.2.4.2.3 *Pasta Moisture Content*

Pasta from Site 3 had the highest moisture content, followed by site 1 and 2, respectively. Significant differences were only found in the comparison among site 2 and 3 (**Tab. 27**). Probably, more water was retained during cooking, this caused a greater dough expansion and more evident post-cooking dimensional changes. Water retention was probably caused by the higher content of fibre and carbohydrates and the higher percentage of coarser flour particles.

4.6.2.4.2.4 *Pasta Texture*

Chickpea pasta from site 3 showed distinct characteristics in terms of adhesiveness and cohesiveness compared to pasta from sites 1 and 2. Specifically, pasta from site 3 had a significantly lower adhesiveness value and a significantly higher cohesiveness value than pasta from the other two sites (**Fig. 44**). Regarding hardness, a significant difference was only observed between sites 2 and 3, with site 3 showing the lowest hardness value (**Fig. 44**).

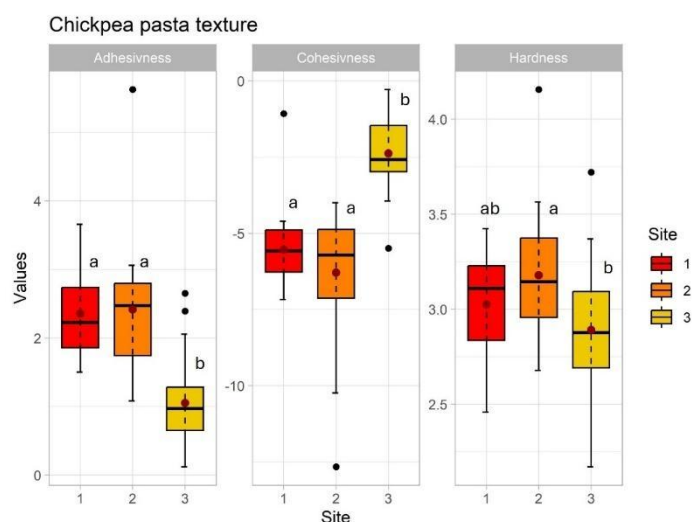


Fig. 44 - The boxplots show the distribution of the values related to the evaluation of chickpea pasta texture.

4.6.2.4.3 Total polyphenols and antioxidant capacity of pasta cooked to OCT

The total polyphenol content and antioxidant capacity in chickpea pasta from site 3 were significantly higher than those from sites 1 and 2. Significant differences were observed among all sites for both parameters (**Fig. 45**), as for chickpea flour (**Fig. 42**).

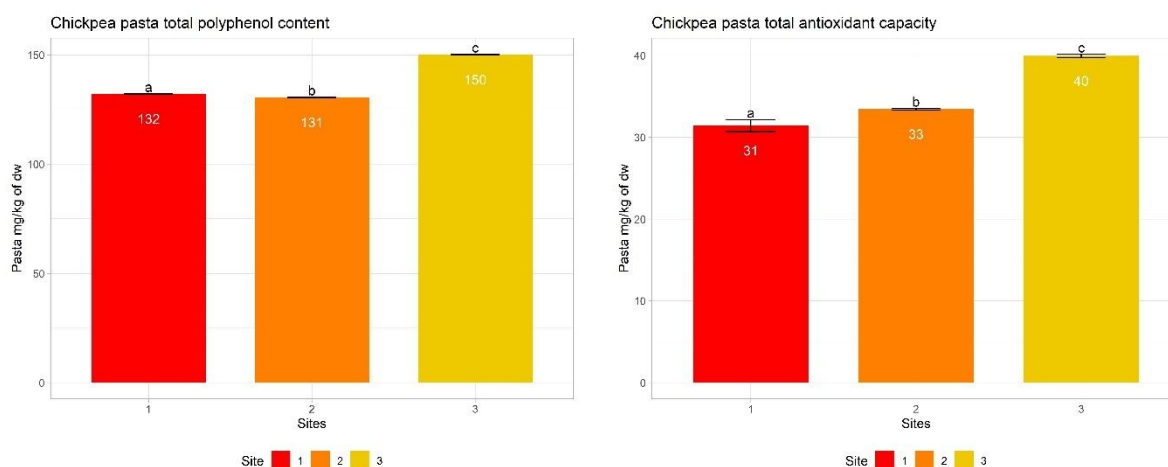


Fig. 45 - Barplot of the chickpea pasta total polyphenol content (TPC) (left) and total antioxidant capacity (right) from the different sites.

4.6.3 Discussion

4.6.3.1 *Chickpeas*

Significant differences in volume, weight, hydration, and swelling capacity were observed among chickpea seeds from the different sites. Chickpea seeds from site 2 were notably larger and differed in all abovementioned parameters compared to the seeds from site 1 and 3. Chickpea seeds from site 2 also had the highest content of total saturated fatty acids (SFA), lipids, and proteins, and the lowest content of carbohydrates. On the other hand, chickpeas from site 3 were characterized by being the smallest, with lower values for all abovementioned parameters. However, they had the highest content of carbohydrates and raw fibre and the lowest optimal cooking time. Considering the textural properties; significant differences were only found for hardness, which was significantly higher in site 3 compared to site 1. The fibre and carbohydrate content of the food likely played a significant role in its texture, which seemed denser and firmer. These nutrients generally not only enhance the structural integrity of the product but also contribute to a more satisfying mouthfeel. A higher fibre content may result in a chewier bite, while the carbohydrates can affect both the hardness and overall texture stability.

4.6.3.2 *Chickpea Flour*

Chickpea flour from site 3 was characterized by a higher proportion of larger particles (300-125 μm) compared to the other sites. The presence of larger particles may result in a coarser and grainier dough, with more spaces and a less uniform structure. Chickpea flour from site 3 was also characterized by a significantly higher oil holding capacity. That may be important in improving the toughness of the dough, resulting in better elasticity. No other significant differences were found in terms of physical and pasting properties among the chickpea flour from the different sites. All samples exhibited significant differences in yellowness. Notably, the chickpea flour from site 3 had a significantly lower yellowness value compared to both site 2 and site 1. Additionally, regarding lightness, chickpea flour from site 1 was the darkest, while sites 2 and 3 showed intermediate and very high lightness values, respectively. Probably, the

content of pigments, such as carotenoids, in site 1 was higher than the other sites, which resulted in lighter flours. Nonetheless, significant differences among flours from all sites were found for total polyphenol content and total antioxidant capacity. In particular, site 3 exhibited higher values for both parameters, indicating a potentially greater nutritional value.

4.6.3.3 Chickpea Pasta

In terms of cooking quality parameters, the chickpea pasta from site 3 exhibited a significantly higher moisture content and weight increase compared to site 2. Additionally, the chickpea pasta from site 3 showed a significantly greater increase in length after cooking than the paste from site 1. Furthermore, site 3 had a longer cooking time than the other sites. This may be attributed to the higher water retention within the pasta, leading to greater dough expansion and more pronounced dimensional changes after cooking. Textural properties indicated that the chickpea pasta from site 3 was the least adhesive and the most cohesive. It was also characterized by the lower hardness compared to the flours from other sites. This suggested the presence of a softer structure with a higher water retention. These findings likely depend on the compositional characteristics of the chickpea seeds, specifically the higher carbohydrate and fibre content found at site 3. Additionally, chickpea flour from site 3 exhibited significantly higher total phenolic content (TPC) compared to the other sites, indicating stronger antioxidant properties.

4.6.3.4 Link between soil and food

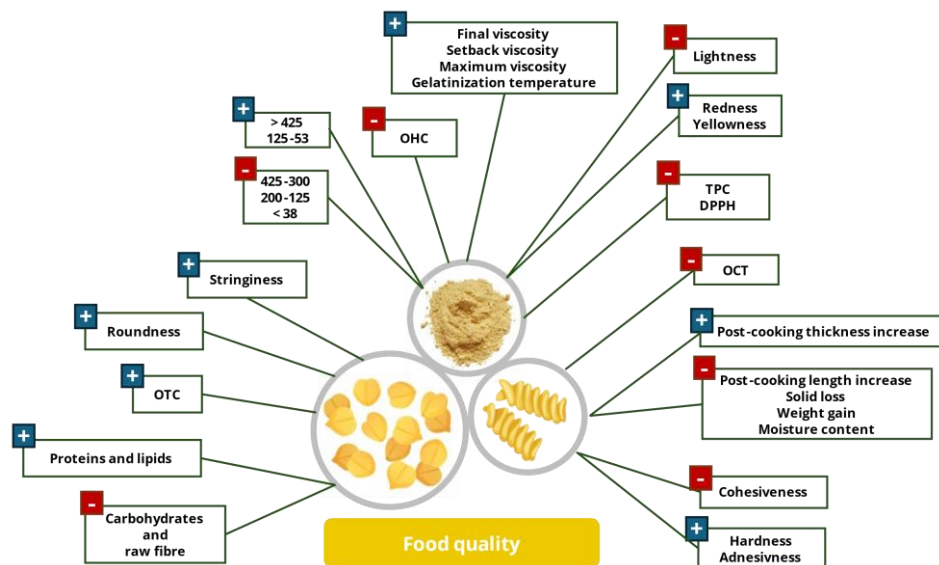


Fig. 48 - The correlations among total QBS-ar index values and food quality parameters. Only significant correlations (p -value > 0.05) were reported in the figure.

In this case, the correlations between the total QBS-ar values and various food quality parameters have been evaluated (**Fig. 48**). It was evident that several technological properties were influenced by the chemical characteristics of the starting raw material: chickpea seeds. For instance, the moisture content and particle size of the flour, as well as the optimal cooking time of pasta, primarily depended on the fibre and carbohydrate content present in the chickpeas.

The QBS-ar index showed noteworthy correlations with the chemical and physical composition of the food products. Specifically, a direct relationship with the content of proteins and lipids, and an indirect relationship with the content of carbohydrates and raw fibre, as well as total polyphenols and antioxidant activity were found (**Fig. 48**).

It can be inferred that there was a connection between the health of the soil and that of the plants, which in turn affected the characteristics of the food produced. A decline in soil health appeared to lead to a decrease in plant health, which results in increased production of polyphenols and higher fibre content—both considered indicators of plant stress (Alves et al., 2024; Jha et al., 2024). Due to the small number of samples, these should be considered

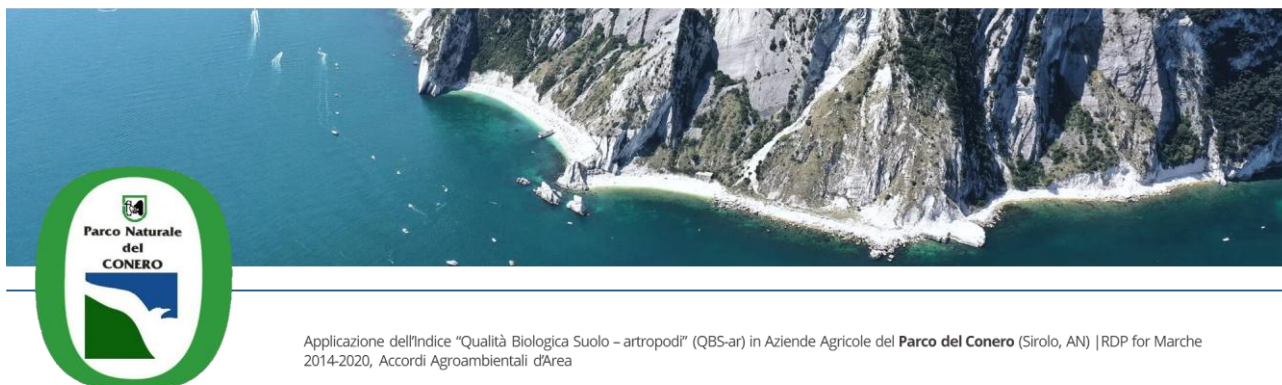
merely assumptions. Nevertheless, it was interesting to identify the type of relationship that may have a strong physiological significance.

4.7 CONCLUSION

This study was the first of its kind to explore the relationship between soil and food quality. Various indicators have been used to assess soil quality, including soil microarthropods and multiple abiotic variables. Additionally, it examined plant characteristics through functional traits assessment and the rhizospheric bacterial communities, while also evaluating the food quality based on its chemical composition and technological properties. Nonetheless, results suggested the potential use of biological soil indicators, specifically the QBS-ar index, to predict crop characteristics and the overall quality of food.

However, many aspects of the study warrant further investigation, and additional data is necessary to validate and confirm these findings. Since this study involved real producers and farms, various challenges arose, leading to a significant reduction in the experimental sample size. By increasing the data set in future studies, it will be possible to assess the overall effects on human health, including other analyses such as resistant starch, expected glycaemic index, starch/amylose ratio, and in vitro starch digestion.

5 CHAPTER 2: MANAGING SOIL TO SUPPORT SOIL BIODIVERSITY IN PROTECTED AREAS AGROECOSYSTEMS: A COMPARISON BETWEEN ARABLE LANDS, OLIVE GROVES, AND VINEYARDS IN THE CONERO PARK (ITALY)



*“Il mare appariva tutto quanto sereno, d'un verde soave,
su cui si movevano, qua e là sparse, larghe macchie violacee,
come ombre di nuvole fuggitive su una prateria novella.*

*Il monte Conero, nel suo semplice e grande lineamento,
appariva appena rosato con non so quale interior luccichio di oro,
quasi un tesoro nascosto da un velario”.*

Gabriele D'Annunzio (Opera omnia, 1887)

5.1 ABSTRACT

Sustainable soil management is essential to conserve soil biodiversity and its provision of vital ecosystem services. The EU Biodiversity Strategy for 2030 highlights the key role of organic farming and land protection in halting biodiversity loss, including edaphic biodiversity. To assess the effectiveness of the proposed measures, a 1-year study was conducted in spring 2022 to determine the soil quality of three organically managed agroecosystems and four sites for each: arable lands, olive groves, and vineyards in the Conero Park, using the arthropod-based Biological Soil Quality Index (QBS-ar) and considering soil chemical-physical

characteristics. Soil microarthropods are sensitive indicators of the impact of agricultural practices on soil quality. Given the diversity of the agronomic practices applied in these agroecosystems, the study aimed to compare the soil quality and identify the system with the least impact on soil biodiversity conservation, with the goal of laying the basis for identifying soil quality benchmarks within each system to be used in monitoring activities in land protected areas. Results showed that organic farming combined with land protection had a positive impact on soil quality. The overall soil quality was excellent, with the highest levels found in arable lands. This is consistent with the intermediate disturbance hypothesis (IDH), which states that slightly disturbed habitats (i.e., arable land with minimum tillage) tend to have higher organism diversity than stable ones. The composition of microarthropod communities in arable land differed from those in stable arboreal crops. Olive groves showed a higher abundance and diversity of microarthropods compared to vineyards, which showed lower values. Promoting the use of QBS-ar, identifying benchmarks for prevalent agroecosystems, and ensuring continuous monitoring of protected areas are thus crucial issues.

5.2 INTRODUCTION

According to the Food and Agriculture Organization (FAO) (2015), 95% of our food comes, directly or indirectly, from soil. Soil organisms play a crucial role in soil processes; they are involved in the nutrient cycle, contribute to soil aggregation and water entry, and promote plant growth and health (Lavelle & Spain, 2001). The soil biota is fundamental in ensuring the long-term functioning of the soil. In fact, soil health is defined as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans (Bünemann et al., 2018). However, unsustainable agronomic practices threaten soil biota and ecosystem services (Köninger et al., 2022). The dominant agricultural production model currently in use is the conventional one, which is characterized by monocultures, excessive tillage, and heavy applications of agrochemicals (Jiang et al., 2021). This type of farming is estimated to be the main driver of biodiversity loss (Benton et al., 2021). To reverse this trend and to counteract the loss of biodiversity, the European Biodiversity Strategy for 2030 has set a goal of increasing

the percentage of the EU's agricultural area under organic farming to 25% and that of protected areas to 30% (Montanarella & Panagos, 2021). The adoption of nature- and biodiversity friendly farming practices, such as organic farming, has been shown to enhance biodiversity in agricultural landscapes (Bengtsson et al., 2005). Additionally, the expansion of protected areas is crucial as these high-quality environmental habitats are critical reservoirs for biodiversity, including soil biodiversity (Mantoni et al., 2021). To test the effectiveness of the proposed solutions on soil fauna, soil microarthropods can be used, as they are considered a sensitive and promising bioindicator. Soil microarthropods have been used for evaluating the impact of human activities on soil functions, including agriculture. They respond sensitively to land management practices and soil disturbances (Parisi et al., 2005; Parisi & Menta, 2008; Reis et al., 2016; Joimel et al., 2017; Yin et al., 2020; Jiang et al., 2021; Ashwood et al., 2023). Nonetheless, the Biological Soil Quality Index based on arthropods (QBS-ar) proved to reliably assess soil health using soil mesofauna (Menta et al., 2018; Parisi, 2001; Parisi et al., 2005). The method has been applied for over 20 years in Italy, as well as in European and non-European countries (Menta et al., 2018). The QBS-ar method describes soil biological quality using numerical values. For example, natural habitats such as forest soils, meadows, and pastures generally have a QBS-ar of over 120 and sometimes even 200. Numerical ranges and thresholds for QBS-ar can vary considerably depending on the environment (Menta et al., 2011, 2018; Parisi, 2001). Most of the QBS-ar collected data concerns agricultural soils and forests in Italy (Menta et al., 2018). However, variations in the QBS-ar values were observed in agroecosystems, depending on the type of crop and management method (e.g., conventional or organic). Specific quality ranges have not yet been concretely defined for the different agroecosystems (arable land, grassland, orchards, olive groves) in Italy. This is necessary because the agricultural practices adopted in each system vary widely. To make a proper comparison, it is necessary to develop quality ranges that are distinct, depending on the agroecosystem considered. Further, in our opinion, the extension of the QBS-ar intervals could be considered key for soils used to grow crops that are widespread and particularly valuable in Italy, such as olive groves and vineyards. We believe that having information on

soil quality in such agroecosystems would add value. Moreover, an integrated approach, including chemical-physical characterization, is needed to evaluate the overall agroecosystem status (Tabaglio et al., 2009). Monitoring the health of these soils is essential for preserving their condition and ensuring highly profitable markets for the Italian economy. In fact, QBS-ar is increasingly used in biological soil quality assessments and soil monitoring programs in Italy, including the MOnitoring SYstem of Soil at multi-Scale MOSYSS Project (Tiberi et al., 2013) that was conducted in Marche Region or the Excalibur (<https://excaliburh2020.eu/en/overview/>) and Minotaur projects (<https://ejpsoil.eu/soilresearch/minotaur>). The objectives of the study were (i) to assess and compare, for the first time, soil health in three organically managed agroecosystems (arable lands, vineyards, and olive groves) in the Conero Park (Italy) using the QBS-ar index and (ii) to characterize the soil microarthropod communities of the different organic agroecosystems and to relate them to the agricultural practices applied and to the chemical-physical characteristics of the soil. In addition, in the long term, we aim to identify the system with the least impact on the conservation of soil biodiversity while promoting the most sustainable agricultural practices to maintain soil health, with the aim of laying the basis for identifying soil quality benchmarks within each system to be used in monitoring activities in protected areas.

5.3 MATERIALS AND METHODS

5.3.1 Study area

The Conero Park (Italy) covers an area of 6011 ha in the central Adriatic side of the Italian peninsula. It includes the municipalities of Ancona, Camerano, Sirolo, and Numana (<https://www.parks.it/parco.conero/Epar.php>). It belongs to the Mediterranean macrobioclimate, with an oceanic pluvisseasonal bioclimate, upper meso-Mediterranean thermotype, and the low subhumid ombrotype (Biondi et al., 2012). While the plant landscape varies in relation to meso- and microclimatic conditions, geolithic and pedological morphology and micromorphology interact in a complex way in a limited area, resulting in the presence of highly differentiated

environments. The Conero promontory is distinguished by carbonate-rich soils, originating from carbonate rocks or other strongly calcareous lithotypes. These soils are susceptible to considerable erosion, attributable to the harshness of the landscape, the inherent erodibility of certain substrates (marl), and human-induced alterations. Consequently, the soils are characterized by a lack of robustness, manifesting as thin thicknesses and a deficiency in humified organic substance content (Calandra & Leccese, 2007). Within the park, three different organic agroecosystems (arable lands, olive groves, and vineyards), and four sites for each, were selected, resulting in a total of 12 sampling sites (Fig. 1, Table 1). Sites were previously identified by the park authority in the framework of the project agroenvironmental area agreement for water protection (RDP Marche 2014/2020). The project has the overall aim of promoting environmentally sustainable agricultural practices capable of minimizing the impact of agriculture on the soil, freshwater, and coastal marine ecosystem of the park. The samplings were conducted in one season, considering an interval of 20 days: from the 13rd of June to the 5th of July. Data related to field management information and agricultural practices applied (use of herbicides, pesticides, and fertilizers, crop rotation, irrigation, plowing/harrowing/subsoiling depth, grubbing, shredding, pruning, hoeing, and sowing) were collected through in-depth interviews with field owners (Tab. S1). Geographic coordinates were recorded (Table 1). Further, soil moisture and temperature were measured at the sites using a moisture meter (PMS-714 Lutron) and a thermometer (HI-98501; Hanna Instruments, Milan, Italy), respectively (data not shown). Due to the limited extension of the area, the influence of climate was assumed to be homogeneous across all sites (Fig. S1).

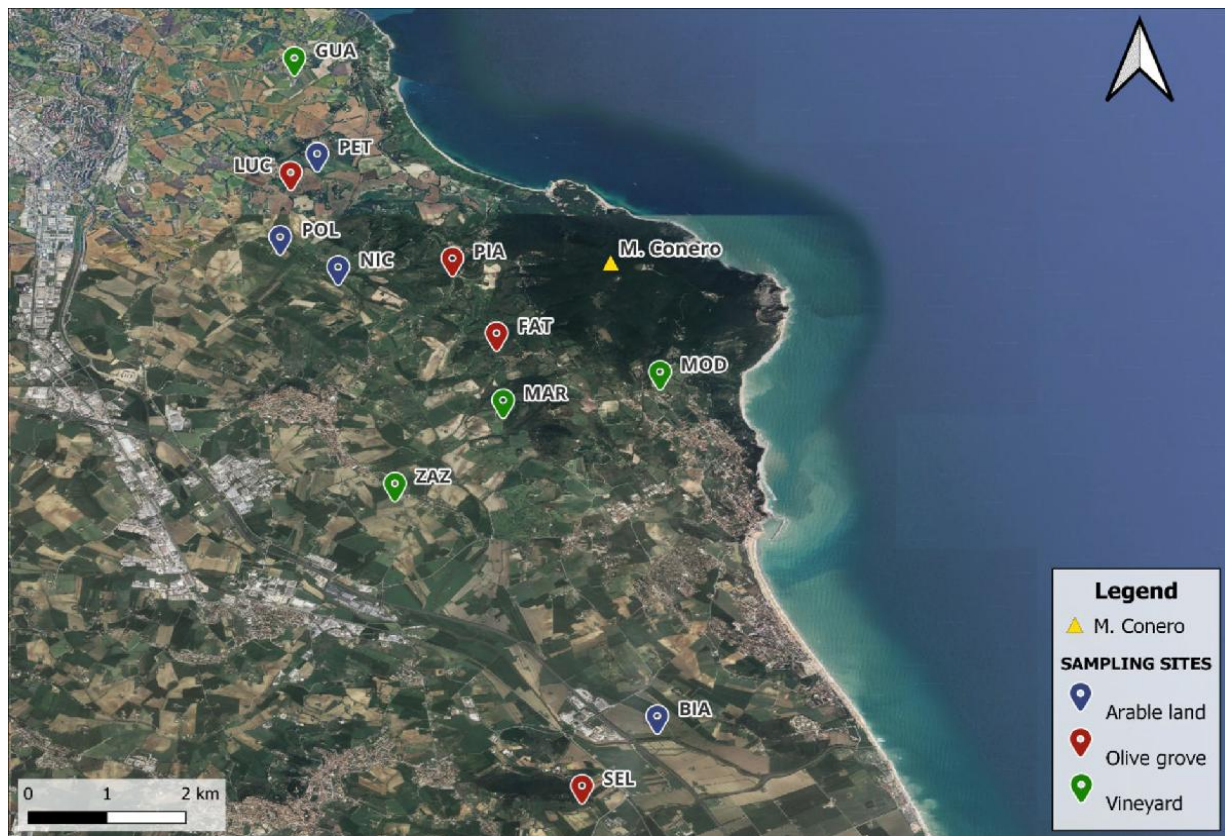


Fig. 1 - Satellite images (QGIS) of the sites considered in the study. All sites are located within the Conero Regional Park, Italy. The pin represents the central point of each site, in blue arable lands, in red olive groves, and in green vineyards.

Number	Site code	Agroecosystem	Locality	Sampling date	Latitude	Longitude	Altitude (m asl)	Slope (%)	Exposure	Herbaceous cover (%)	Herbaceous cover height (cm)
1	BIA	Arable land	Numana (AN)	13/06/2022	43°28'32.95"N	13°36'27.60"E	6 m	1.7	E	81-100	> 60
2	NIC	Arable land	Camerano (AN)	13/06/2022	43°32'55.45"N	13°33'27.60"E	86 m	4.3	N-W	81-100	31-60
3	POL	Arable land	Ancona (AN)	21/06/2022	43°33'13.08"N	13°32'54.89"E	61 m	18.7	S-E	61-80	16-30
4	PET	Arable land	Ancona (AN)	13/06/2022	43°34'1.59"N	13°33'15.80"E	141 m	15.4	S-E	81-100	> 60
5	MAR	Vineyard	Ancona (AN)	05/07/2022	43°31'37.48"N	13°35'0.20"E	140 m	70.4	S-O	81-100	31-60
6	GUA	Vineyard	Ancona (AN)	05/07/2022	43°34'57.63"N	13°33'2.46"E	132 m	6.8	N-W	81-100	16-30
7	MOD	Vineyard	Sirolo (AN)	05/07/2022	43°31'54.16"N	13°36'28.69"E	195 m	27.2	S-E	< 1	1-15
8	ZAZ	Vineyard	Camerano (AN)	21/06/2022	43°30'48.82"N	13°33'59.16"E	84 m	12.2	W/W-S	41-60	31-60
9	SEL	Olive grove	Castelfidardo (AN)	05/07/2022	43°27'52.19"N	13°35'45.09"E	54 m	3.9	N-W	81-100	1-15
10	FAT	Olive grove	Massignano (AN)	28/06/2022	43°32'16.78"N	13°34'56.94"E	189 m	3.4	S	81-100	> 60
11	LUC	Olive grove	Ancona (AN)	28/06/2022	43°33'50.67"N	13°33'0.82"E	126 m	24.9	E	81-100	> 60
12	PIA	Olive grove	Ancona (AN)	28/06/2022	43°33'0.52"N	13°34'31.98"E	247 m	0	N-W	81-100	> 60

Tab. 1 - Sampling sites characteristics. Estimates of herbaceous cover were based on visual observations made by the authors in the field. Altitude, slope and exposure for each site were determined in the field using the Compass & Altimeter app (PixelProse SARL, France) and further evaluated using Google Earth Pro and QGIS.

5.3.2 Soil sampling and QBS-ar determination

Sampling and extraction of soil microarthropods followed the methodology described by Parisi et al. (2001, 2005) and Menta et al. (2020). At each site, three soil subsamples (A, B, C), each with a volume of about 800 cm³, were collected using a cylindrical soil corer at a depth of 10

cm for the QBS-ar index. Each subsample was immediately placed in a plastic bag and transported to the laboratory, within the following 24 h. A homogeneous area was selected in each site, excluding disturbed areas such as flattened vegetation and tractor tracks. The herbaceous cover was removed prior to collecting the soil sample. In arable lands, subsamples were collected at equal intervals (about 10 m) along a diagonal line, in the central part of the field. In both vineyards and olive groves, samples were collected in the interrow. In the latter system, samples were collected in the space between tree canopies. A total of 36 soil subsamples were collected. Arthropod extraction was carried out in the laboratory using the Berlese-Tüllgren funnel, equipped with 25-W light bulbs and a 2-mm sieve mesh. The extraction process lasted for 7 days, and the extracted specimens were preserved in a solution of 75% ethyl alcohol and 25% glycerol by volume. The collected specimens underwent a stereoscopic analysis to determine biological form (BF) and Eco-Morphological Index (EMI) (1–20), according to Parisi et al. (2005). Total QBS-ar and partial QBS-ar values were calculated for each experimental site. The total QBS-ar total was obtained by summing the highest EMI values attributed to each BF in the three subsamples (A, B, C). The partial QBS-ar was derived from the evaluation of the BFs found in the single subsample. A quantitative approach was used, unlike the original protocol (Parisi et al., 2001, 2005), where the number of individuals present for each BF was counted in each subsample, as well as the number of euedaphic forms (EFs). Additionally, total microarthropod abundance and density (ind/m² and ind/m³), microarthropod community composition, Acari/ Collembola ratio (Bachelier, 1963), and percentage of Oribatid mites out of total mites (Aoki, 1967; Aoki et al., 1977) were determined.

5.3.3 Soil chemical-physical analysis

Soil samples for chemical-physical analyses were collected in accordance with the official methods for soil analysis of the Italian legislation DM 13/09/1999 SO No. 185 (Approvazione dei “Metodi Ufficiali di analisi chimica del suolo”). In each site, 10 soil sub-samples were collected in each site by using a pedological auger at a depth of 15 cm and then mixed and

reduced to a single composite sample (1 kg). The samples were analyzed by the laboratory of the “Agenzia per l’Innovazione nel Settore Agroalimentare e della Pesca “Marche Agricoltura Pesca” (AMAP)”, Jesi (Italy). The analyzed parameters were three fraction soil texture (sand, silt, clay; g/kg), total organic carbon (g/kg), organic matter (g/kg), pH, C/N ratio, total nitrogen (g/kg), assimilable phosphorus (mg/kg), exchangeable potassium (mg/kg), field water capacity (%), wilting point (%), total limestone (g/Kg), active limestone (g/Kg), and carbon of humic and fulvic acids (g/kg).

5.3.4 Statistical analysis

To test for differences between the three systems (arable lands, vineyards, olive groves), soil biological indicators (QBS-ar, number of BF and EFs, abundance, Acari/Collembola ratio, percentage of Oribatid mites out of total mites) and total and partial data (obtained from each subsample) were considered. The data were first checked for normality of distribution (Shapiro-Wilk test) and homoscedasticity (Levene’s or Bartlett’s test, depending on the distribution). Differences between agroecosystems were evaluated through one-way ANOVA or Kruskal-Wallis test and post hoc tests (Tukey’s honestly significant difference or Dunn test) depending on the distribution. A p-value < 0.05 has been considered significant. Statistical analysis was performed using R software, version 4.2.0 (R Development Core Team, 2021). Soil chemical and physical properties of the different agroecosystems were statistically analyzed, as described above for biological data, and further, correlations between the different parameters were evaluated by using “ggcorrplot” R package. Non-metric multidimensional scaling (nMDS), based on the Bray-Curtis dissimilarity index was performed to visualize the grouping of arthropod communities. To associate the community composition with soil characteristics and evaluate the associations between nMDS site scores, the “envfit” function was used. Permutational multivariate analysis of variance (PERMANOVA) was used to test for differences in assemblages among the different patterns visualized with nMDS. Ordination analysis, PERMANOVA, and SIMPER were performed with “vegan” and “pairwiseAdonis” R packages,

whereas “ggplot2” was used to generate the plots and the nMDS ordination diagram. The heatmaps of the log10-transformed total abundance of soil microarthropods were generated using the Heatmapper web server (www.heatmapper.ca) (Babicki et al.,2016).

5.4 RESULTS

5.4.1 QBS-ar application

The average total QBS-ar value of the agroecosystems within the Conero Regional Park was 167. The highest average total QBS-ar was found in arable lands, with a corresponding value of 190 (Tab. 2). Within arable sites, the QBS-ar ranged from 155 to 222. Olive groves had an average total QBS-ar value of 176, ranging from 144 to 231, whereas vineyards showed a value of 136, ranging from 84 to 184. No significant differences were found when comparing the total QBS values among the systems. However, when comparing partial QBS-ar values, soil quality in arable lands was significantly higher than in vineyards (p-value = 0.042, TukeyHSD) (Fig. 2).

5.4.2 BF, EF, and abundance

Overall, 24 BFs were found in the agroecosystems considered within the Conero Regional Park. The highest number of BFs was found in olive groves (23), followed by arable lands (20) and vineyards (19) (Table 3). As for BFs, the highest abundance and density were found in olive groves, followed by arable lands and vineyards (Fig. 3). On the other hand, the total number of EFs was the same (9) in the three systems. Any significant differences, in partial and total data, were found in either BFs, EFs, or abundance.

Number	Site code	Agroecosystem	Partial QBS-ar values (A, B, C)	Average partial QBS-ar values (A, B, C) of the system	Total QBS-ar values	Average total QBS-ar values of the system
1	BIA	Arable land	149, 146, 172	135	219	190
2	NIC	Arable land	182, 177, 156		222	
3	POL	Arable land	89, 109, 122		155	
4	PET	Arable land	126, 56, 132		163	
5	MAR	Vineyard	72, 56, 48	99	84	136
6	GUA	Vineyard	99, 86, 138		169	
7	MOD	Vineyard	154, 170, 138		184	
8	ZAZ	Vineyard	75, 82, 75		105	
9	SEL	Olive grove	83, 94, 114	117	144	176
10	FAT	Olive grove	124, 109, 115		155	

11	LUC	Olive grove	139, 101, 154	231
12	PIA	Olive grove	104, 135, 132	175

Tab. 2 - The table shows partial and total QBS-ar values for each site, and average QBS-ar values for each agroecosystem.

Agroecosystem	Total BFs	Average BFs	Average EFs	Total EFs	Average abundance	Average microarthropod density (ind/m ³)	Average microarthropod density (ind/ m ²)
Arable lands	19	16	7	9	1515	504,917	58,036
Vineyards	20	14	5	9	1419	473,083	54,377
Olive groves	23	17	6	9	1656	551,833	63,429

Tab. 3 - The table shows the number of total biological (BFs) and eu-edaphic forms (EFs), and average abundance and density (ind/m and ind/m²) for each site and average total abundance for each agroecosystem.

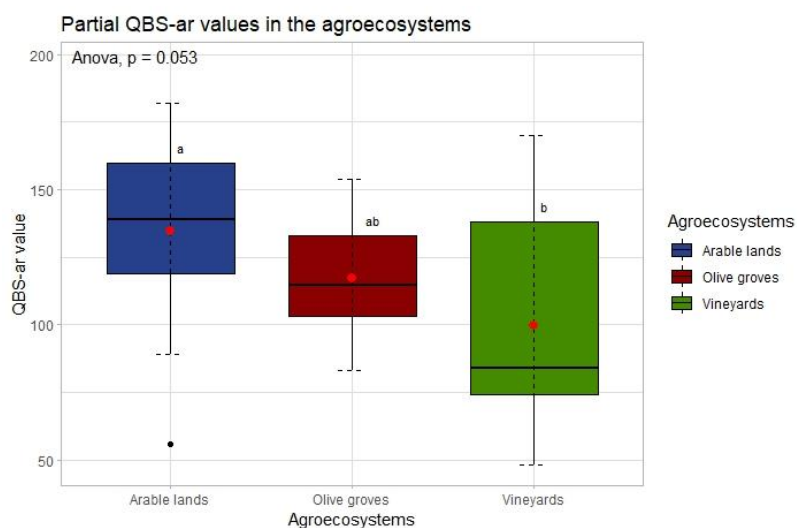


Fig. 2 - Boxplots show the distribution of the partial QBS-ar values of the agroecosystems. The median (horizontal line), mean (red dot), error bars (dashed line) and outliers (isolated black dots) are reported. Lowercase letters represent significant differences between farming systems by Post-hoc Tukey's HSD tests at p-value < 0.05.

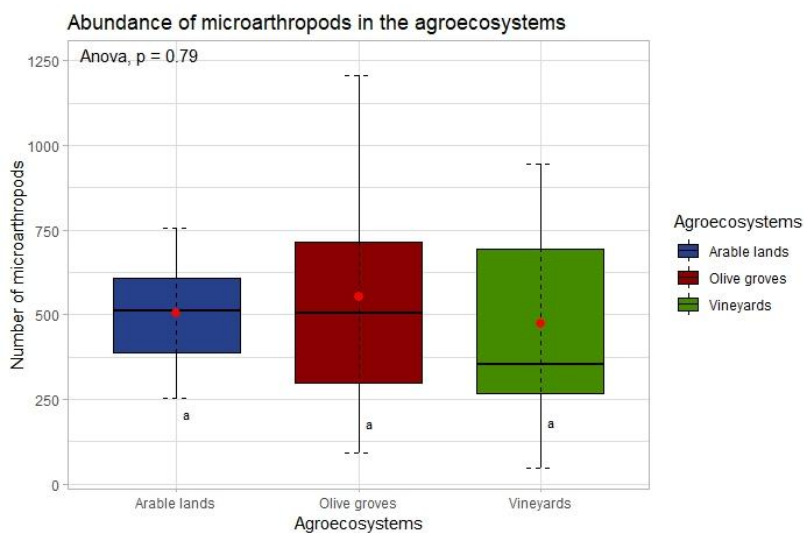


Fig. 3 - Boxplots show the distribution of the partial abundance of microarthropods in the three different agroecosystems. The median (horizontal line), mean (red dot), error bars (dashed line) and outliers (isolated black dots) are reported.

5.4.3 Soil microarthropod community composition

The composition of the soil microarthropod community is shown by the heatmap (Fig. 4). Eighteen BFs were found in all systems: Pseudoscorpions, Araneidae, Isopoda, Acari, Collembola, Diplopoda, Symphyla, Chilopoda, Diplura, Psocoptera, Hemiptera, Thysanoptera, Coleoptera, Diptera, Hymenoptera, Coleoptera and Diptera larvae, and other Holometabola. Among these, Acari, Collembola, and Hymenoptera, due to their ubiquitous presence, are not represented in the heatmap of Fig. 4 because their high abundance tends to mask and unify the presence of BFs with lower abundance. Data referred to these groups will be discussed separately. The presence of Hemiptera, Psocoptera, Thysanoptera, Diptera, and other Holometabola was not so relevant for determining soil quality, as they do not contribute significantly ($EMI=1$) to the QBS-ar value. However, all agroecosystems were characterized by the presence of 9 EFs ($EMI=20$). Acari, Collembola, Pseudoscorpions, Diplopoda (Julidae), Pauropoda, Symphyla, Chilopoda, Diplura, and Coleoptera were found in arable lands and olive groves. The same EFs were found in vineyards, except for Pauropoda, which was not recorded. On the contrary, Protura were found solely in vineyards. Similarly, Hymenoptera larvae (Formicidae) were found in olive groves and vineyards but not in arable lands. Zygentoma, Embioptera, and Lepidoptera larvae were registered only in olive groves. Notably, this latter system was characterized by a high number of Diplopoda and Hemiptera, compared to arable lands and vineyards.

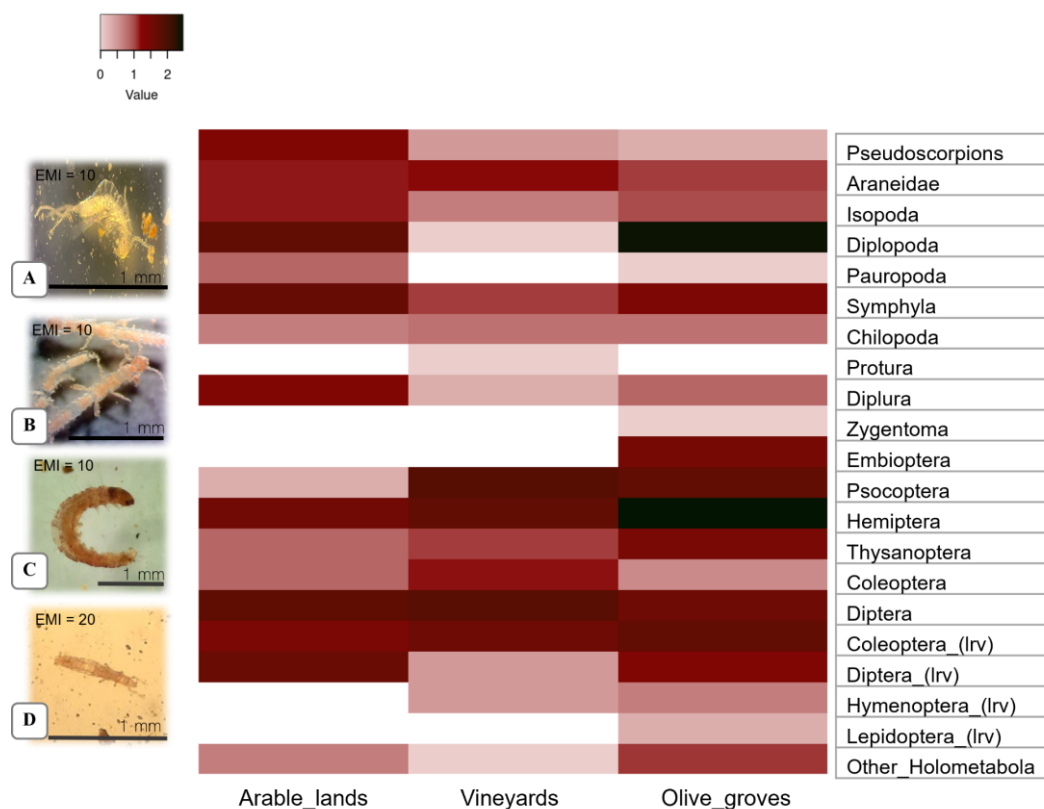


Fig. 4 - Heatmap of the log10 transformed total abundance of each BFs in each agroecosystem. Darker shades indicate high abundance, light shades indicate low abundance or absence (white). Zygentoma (A), Embiopoda (B) and Lepidoptera larvae (C) were found only in olive orchards, while Protura (D) were found only in vineyards. Data concerning Acari, Collembola and Hymenoptera are not reported. The heatmap was generated using Heatmapper web server (www.heatmapper.ca).

5.4.4 Most abundant soil microarthropod groups (Acari, Collembola, and Hymenoptera)

Focusing on the most abundant groups (Acari, Collembola, Hymenoptera), the highest number of Acari was found in arable lands (p-value = 0.030, Kruskal- Wallis) and it was significantly higher compared to vineyards (p-value = 0.037, Dunn) (Fig. 5). The average Acari density in arable land was 49,847 ind/m² while 33,304 ind/m² in vineyards and 37,405 ind/m² in olive groves (Tab. S2). On the contrary, Collembola resulted to be the least abundant group in arable lands, compared to both vineyards and olive groves, which showed similar values. Hymenoptera abundance showed a similar trend to Collembola, with a lower abundance on arable lands. Differently from Collembola, Hymenoptera were higher in vineyards compared to olive groves. Nevertheless, differences were not statistically significant (Fig. 5). The number of the BFs other than Acari, Collembola, and Hymenoptera, resulted significantly higher in olive groves (p-value = 0.040, Kruskal-Wallis), and particularly higher compared to vineyards (p-value = 0.040, Dunn).

5.4.5 Acari/Collembola ratio and percentage of oribatid mites out of total mites

The Acari/Collembola ratio was significantly higher in arable lands (p-value = 0.015, Kruskal-Wallis), particularly compared to olive groves (p-value = 0.011, Dunn) (Tab. 4). Notably, stable systems, such as olive orchards and vineyards, were found to have an identical average Acari/Collembola ratio (3.6), while arable lands were characterized by a five- times higher value (19.2). As for Acari abundance and Acari/Collembola ratio, Oribatid mites were significantly higher in arable lands (p-value = 0.0003, Kruskal-Wallis) compared to both vineyards (p-value = 0.006, Dunn) and olive groves (p-value = 0.0005, Dunn). The density of Oribatid mites in arable land was 19,636 ind/m², while 6006 ind/m² in olive groves and 7816 ind/m² in vineyards (Fig. 6; Tab. S2), whereas the percentage of Oribatid mites out of total mites was 39.4% in arable land, 16.1% in olive groves, and 23.5% in vineyards (Tab. S2).

Number	Site code	Agroecosystem	Total Acari/Collembola ratios of each site	Average Acari/Collembola ratio of the system
1	BIA	Arable land	32.1	19.2
2	NIC	Arable land	13.8	
3	POL	Arable land	28.7	
4	PET	Arable land	13.8	
5	MAR	Vineyard	0.9	3.6
6	GUA	Vineyard	2.0	
7	MOD	Vineyard	5.0	
8	ZAZ	Vineyard	129.7	
9	SEL	Olive grove	7.0	3.6
10	FAT	Olive grove	5.9	
11	LUC	Olive grove	1.8	
12	PIA	Olive grove	8.1	

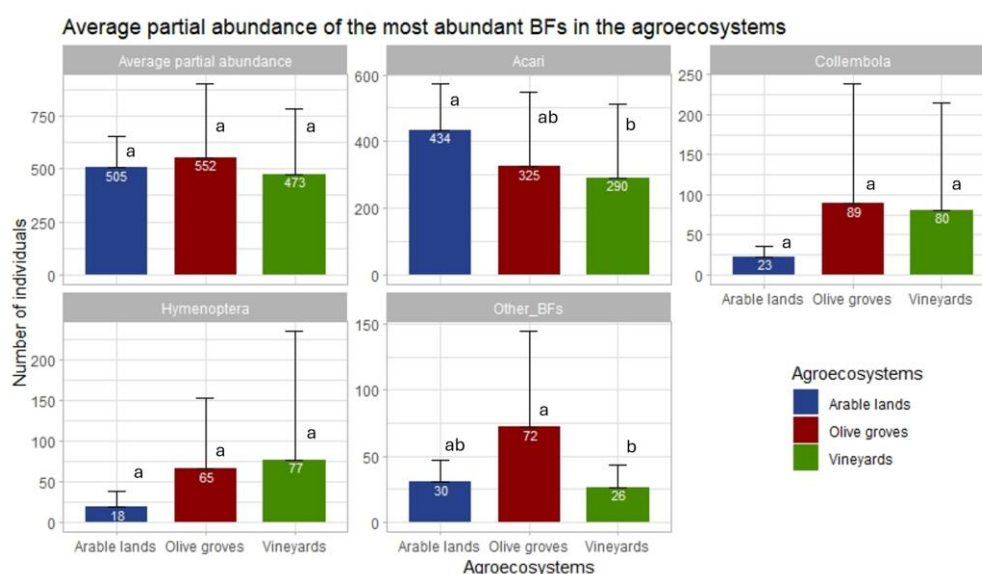


Fig. 5 - The bar plots show the average partial abundance of the soil microarthropods, Acari, Collembola, Hymenoptera, and other biological forms (BFs) apart from Acari, Collembola and Hymenoptera, in each agroecosystem. Numbers represent the average partial abundance, and black lines represent error bars. Lowercase letters represent significant differences between the partial average number of individuals (across farming systems) by Post-hoc Tukey's HSD or Dunn tests at p -value < 0.05 .

5.4.6 Soil chemical-physical parameters and soil microarthropod community structure

Arable lands and vineyards had a silty clay loam texture, whereas olive groves had a clay loam texture (Table 5). However, there were no significant differences in terms of soil particle contents (Fig. S3). All soils were calcareous with a high percentage of active limestone, and soil pH was generally slightly alkaline in all systems. The results were similar in vineyards and arable lands and then in olive orchards. The carbon-to-nitrogen ratio (C/N ratio) was low in all agroecosystems. Olive groves have the highest levels of organic matter (SOM), total organic carbon (TOC), total nitrogen (TN), and C from humic and fulvic acids. In contrast, vineyards have higher levels of assimilable P and exchangeable K. The wilting point (WP) was similar across all systems, while the highest field water capacity (FWC) was found in vineyards (31.9 %) and the lowest in arable lands (21.7%) (Table 5, Fig. S2). Statistical analysis showed that FWC was the only parameter that differed significantly between the three systems (p -value = 0.024, Kruskal-Wallis) (Table 5, Fig. S2). Through correlation analysis (Pearson coefficient), several variables were found to be positively highly correlated, including TN, TOC, SOM, and the carbon content of humic and fulvic acids. Additionally, TL, AL, WP, and FWC exhibited a slight positive correlation, as well as TL, C/N, and clay. Negative correlations were found between the pH and several parameters, particularly TOC and SOM; carbon of humic and fulvic acids and silt; and clay with sand and TL

(Fig. 7). The environmental parameters recorded included soil temperature and soil moisture (data not shown). No significant differences among agroecosystems and sites within the same system were found. The mean soil temperature in arable lands was 24°C, in olive groves 31°C, and in vineyards 30°C, while the mean soil moisture in arable lands was 24.7 %, in olive groves 19.4 %, and in vineyards 20.2%. The composition of the soil microarthropod community was related to the soil chemical-physical properties in a non-metric multidimensional scaling (nMDS) plot based on Bray-Curtis dissimilarity index (stress = 0.12) (Fig. 8). The microarthropod community of arable land differed from the other two systems, while the microarthropod communities in vineyards and olive groves tended to overlap. The results showed that soil textural parameters seemed to have the greatest impact on microarthropod community composition. Additionally, K and FWC were found to be important variables in community assemblages, although their variation was not statistically significant. However, PERMANOVA analyses revealed no statistically significant differences in microarthropod community assemblage between the agroecosystems (p-value= 0.31, PERMANOVA). The SIMPER analysis revealed that the sand and clay contents, as well as K and total limestone (TL), were the parameters that drove the most differences between community compositions, although not significantly.

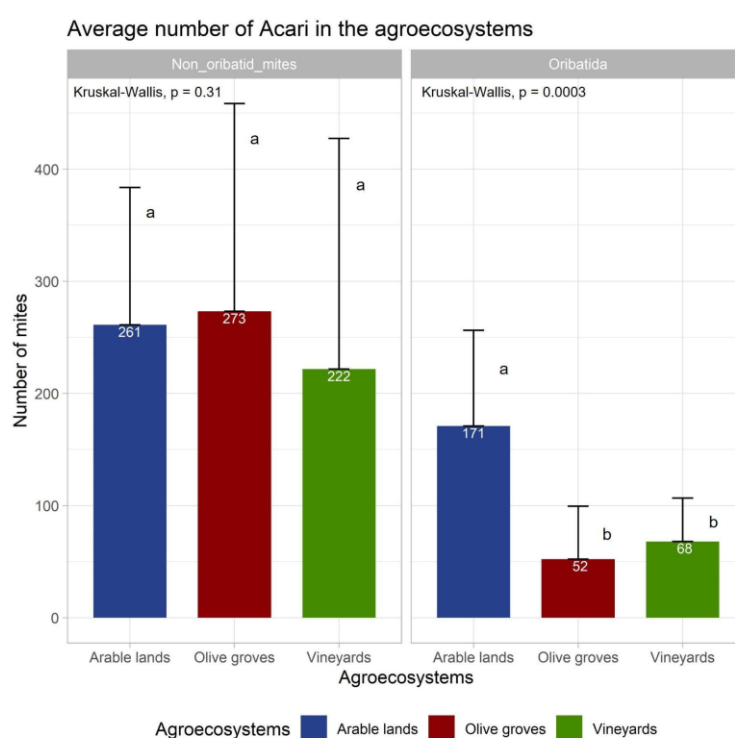


Fig. 6 - The bar plots show the average partial number of non-oribatid mites and Oribatida in each agroecosystem considered (arable lands, olive groves, and vineyards). Numbers represent the average partial abundance values for each management system and black lines represent error bars. Lowercase letters represent significant differences between the partial average number of individuals (across management systems) by Post-hoc Dunn tests at p-value < 0.05.

Soil chemical-physical parameters correlation analysis

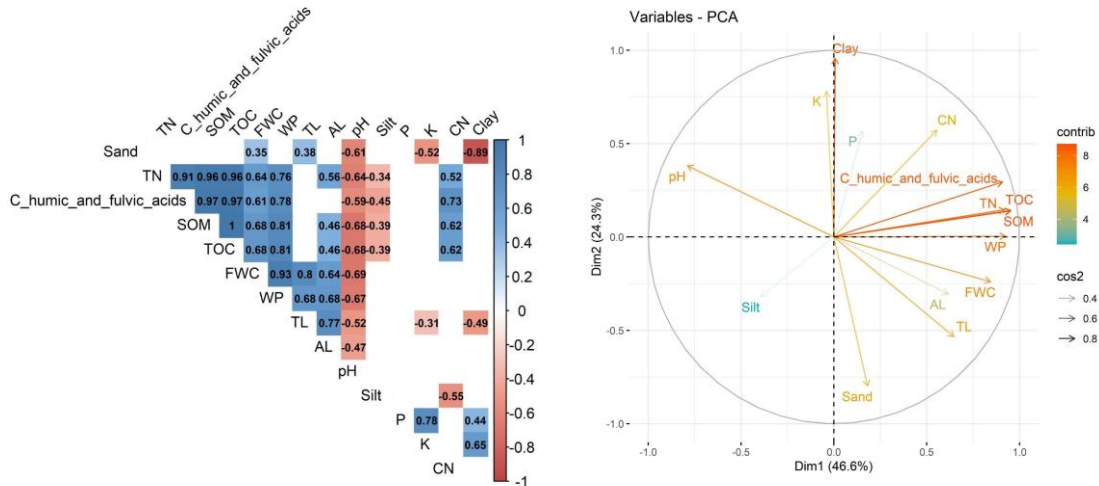


Fig. 7 - The plot presents the results of the correlation analysis (Pearson coefficient). Some variables were found to be positively highly correlated (blue), including TN, TOC, SOM, and the carbon content of humic and fulvic acids. Additionally, TL, AL, WP and FWC exhibited a slight positive correlation, as well as TL, C/N and clay. While negative correlations (red) were found between the pH and several parameters, particularly TOC and SOM; carbon of humic and fulvic acids and silt; clay with sand and TL.

nMDS plot of microarthropod community and soil chemical-physical properties

Stress: 0.1154912

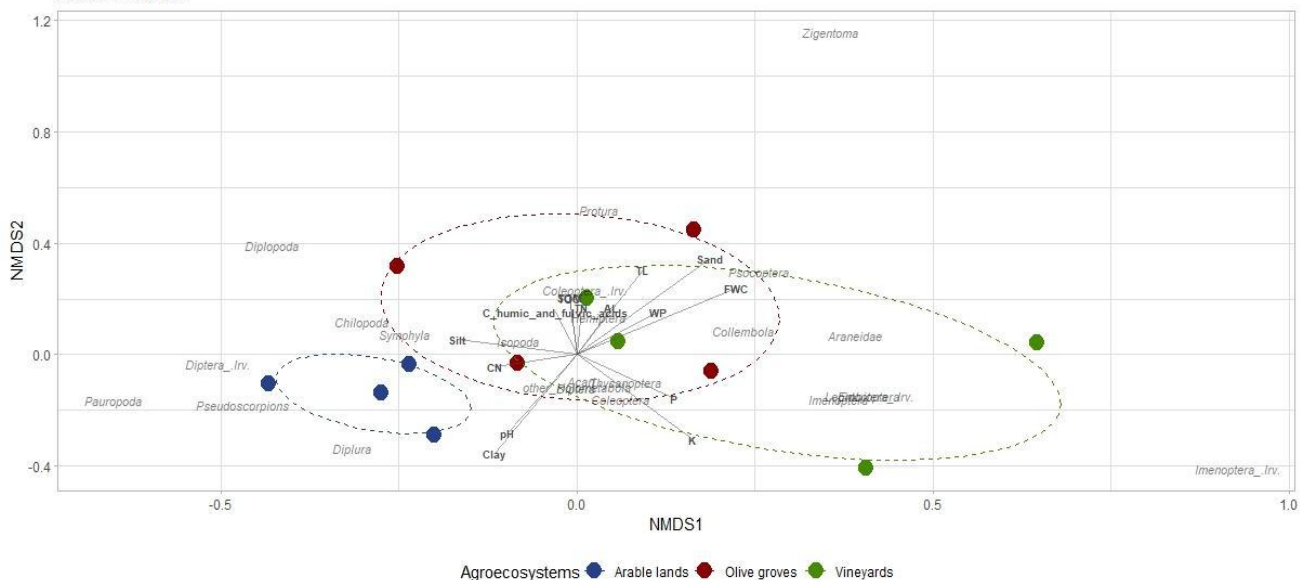


Fig. 8 - Non-metric Multidimensional Scaling (nMDS) based on Bray-Curtis dissimilarity index of soil microarthropod community composition and soil chemical-physical properties in three agroecosystems. Total organic carbon, TOC; soil organic matter, SOM; total nitrogen, TN; assimilable phosphorus, P; exchangeable potassium, K; field water capacity, FWC; wilting point WP; total limestone, TL; active limestone, AL; carbon/nitrogen ratio, C/N ratio; carbon of humic and fulvic acids; pH; soil texture (sand, silt, clay).

Site	Agroecosystem	pH	C/N	SOM (g/kg)	TOC (g/kg)	TN (g/kg)	P (mg/kg)	K (mg/kg)	FWC (%)	WP (%)	TL (g/kg)	AL (g/kg)	C of humic and fulvic acids (g/kg)	Sand (g/kg)	Silt (g/kg)	Clay (g/kg)	Soil texture
BIA	Arable lands	8.03	8	26.9	15.6	2.4	17.5	316	22.6	17.5	237	159	7.4	58	486	456	Silty-clay
NIC	Arable lands	8.08	7.3	24.5	14.2	2.4	11.8	117	22.1	16.9	383	176	7.3	155	526	319	Silty-clay-loam
POL	Arable lands	8.09	8.1	16.7	9.7	1.2	11.3	159	20.2	14.9	158	84	6.5	159	449	392	Silty-clay-loam
PET	Arable lands	8.13	8.1	19.5	11.3	1.4	7.5	165	21.9	17.5	265	155	6.1	120	517	363	Silty-clay-loam
SEL	Olive groves	7.56	7.5	25.7	14.9	2	13.7	140	26.7	16.1	325	139	7.6	324	450	226	Loam
FATi	Olive groves	7.56	8.6	23.7	13.8	2	10.6	147	27.6	18.6	412	171	7.2	219	454	327	Clay-loam
LUC	Olive groves	7.55	7.5	32.3	18.7	2.5	6.5	226	24.5	16.9	345	153	8.1	222	443	335	Clay-loam
PIA	Olive groves	7.28	9.4	67.4	39.1	4.2	15.2	185	41.7	28.2	578	183	19	159	449	392	Silty-clay-loam
MAR	Vineyards	7.37	7.5	33.1	19.2	2.6	14.3	179	46.1	26.3	582	194	8.4	267	473	260	Loam
GUA	Vineyards	8.23	7.9	23.1	13.4	2.1	7.6	245	23.5	14.5	300	125	6.5	184	495	321	Silty-clay-loam
MOD	Vineyards	8.21	6.8	17.6	10.2	1.5	16.9	179	29	17.1	599	169	4.9	182	544	274	Silty-clay-loam
ZAZ	Vineyards	8.28	8.3	25.6	14.9	2.2	29.2	646	27.6	19.3	256	146	9.2	88	448	464	Silty-clay

Tab. 5 - The table shows the soil chemical-physical properties of the three agroecosystems. Total organic carbon, TOC; soil organic matter, SOM; total nitrogen, TN; assimilable phosphorus, P; exchangeable potassium, K; field water capacity, FWC; wilting point WP; total limestone, TL; active limestone, AL; carbon/nitrogen ratio, C/N ratio; carbon of humic and fulvic acids; pH; sand, silt and clay particles content, and soil texture.

5.5 DISCUSSION

5.5.1 Biological soil quality in the Conero Park

The overall soil quality of the agroecosystems in Conero Park resulted to be excellent. The average total QBS-ar value (167) exceeded the threshold set for high biological quality in agricultural soils (93.7) (Menta et al., 2018; Parisi, 2001). The application of organic farming, in conjunction with land protection, seemed to contribute positively to the overall soil quality of the agroecosystems. According to Mantoni et al. (2021), land protection may significantly affect the soil microarthropod community by “rescue effects,” resulting in a possible positive influence on the QBS-ar index in agricultural fields. In several sites, the QBS-ar values even exceeded the threshold assigned to natural environments (Tab. 2), which is approximately 150 (Menta et al., 2018; Parisi, 2001). The soil chemical-physical parameters were similar across the study sites; however, land use and soil management practices varied between them. Considering this, specific soil quality thresholds and reference values to discriminate between high and low soil quality for the prevalent agroecosystems should be determined (Fusco et al., 2023; Menta et al., 2011, 2018; Parisi, 2001). Arable lands had the highest soil quality. The system underwent more frequent and uniform tillage (15–20 cm), compared with the others. These constant and light mechanical operations might create numerous suitable ecological niches for

soil microarthropods, which are maintained for a short period, equivalent to the crop's growth time. As supported by the intermediate disturbance hypothesis (IDH), disturbance plays a crucial role in maintaining biodiversity and ecosystem stability (Connell, 1978; Grime, 1973). In several studies concerning arthropods (Kwon et al., 2013; Wang & Tang, 2019; Swart et al., 2019; Eötvös et al., 2020; Muscolo et al., 2021; Solano-Barquero et al., 2022; Guan et al., 2023), including Isopods (Hassall et al., 2006), Formicidae (Graham et al., 2009), Araneae (Desales-Lara et al., 2013), Diplopoda (Bogyó et al., 2015), and Coleoptera (Fattorini et al., 2020) has been observed that slightly disturbed habitats brought greater benefits for organism diversity than stable ones. Further, arable lands were characterized by a short agricultural cycle (forage crops), while olive groves and vineyards had a long cycle, as stable arboreal crops. Vineyards had the lowest average QBS-ar value (136). Generally, the inter-row vegetation cover was poor, the management of the inter- and under-row was more intense, and the use of Plant Protection Products (PPPs) was frequent. As the QBS-ar intervals resulted similar to those obtained in previous studies in Italy in the same agroecosystems (Costantini et al., 2015; Gagnarli et al., 2015; Ghiglieno et al., 2019; Vignozzi et al., 2019), a review of the existing literature of QBS-ar application at national scale might be very helpful in generating soil quality reference values for the most common agroecosystems. Nonetheless, to obtain a reliable soil quality trend over time, systematic soil biomonitoring is necessary.

5.5.2 Microarthropod overall abundance and number of BFs in the different agroecosystems

Olive groves were characterized by a higher soil microarthropod abundance and number of BFs, compared to arable lands and vineyards (Table 3). Particularly, only in olive groves, bioindicators of stable environments (Menta et al., 2018), such as Zygentoma, Embioptera, and Lepidoptera larvae were found (Fig. 4). On the contrary, vineyards showed the lowest number of BFs and abundance. Protura were found only in vineyards, although with a very low abundance. They are generally associated with soil stability (Menta & Remelli, 2020), and their population density is known to be influenced by soil physicochemical characteristics (Gonçalves et al., 2021). On the other hand, Pauropoda were not found in vineyards. This

group is related to low-input management systems and favourable abiotic soil conditions (Gonçalves et al., 2021). Results suggested that soil microarthropods in vineyards were more disturbed by the agronomic practices adopted, compared to the other systems. Presumably, mowing, frequent under-row and inter-row tillage, copper, and sulfur addition (widely used as PPPs in vineyards) negatively affected soil quality, diversity, and abundance of soil mesofauna in the present system (Oultaf et al., 2022). Soil quality in vineyards was found to be good, although lower than other systems, due to the higher number of inputs. In general, Symphyla occurrence highlighted the presence of non-compacted soils, as they can only use existing soil cracks and tunnels to settle in (Gonçalves et al., 2021; Menta & Remelli, 2020) (Fig. 4). Nonetheless, the presence of generalist predators, such as Pseudoscorpions, Araneidae, Coleoptera, Formicidae, Diplura (Japix), and Chilopoda in all systems, indicated a well-developed trophic food web (Menta & Remelli, 2020). Furthermore, the ubiquitous presence of Isopoda, Diplopoda, Chilopoda, Diplura, Coleoptera, and Diptera larvae under- lined a high environmental quality and confirmed the application of non-intensive agricultural practices (Gonçalves et al., 2021). The occurrence of several groups of organisms well-adapted to soil habitats has demonstrated the sustainability of soil management practices employed in all the monitored agroecosystems of the Park.

5.5.3 Most abundant soil microarthropod groups (Acari, Collembola, and Hymenoptera) and other groups in the different agroecosystems

In olive groves, a higher abundance of microarthropods belonging to groups other than Acari, Collembola, and Hymenoptera was registered (Fig. 5). Presumably, the presence of cover crops positively influenced the abundance of microarthropod groups characterized by a lower number of individuals (Carpio et al., 2018). On the contrary, in vineyards, there was a significantly lower abundance of microarthropods belonging to groups other than Acari, Collembola, and Hymenoptera, compared to olive groves (Fig. 5). The use of increased treatments in vineyards probably resulted in a negative effect on the soil community. Notably, Acari abundance was significantly higher in arable lands compared to vineyards (Fig. 5). Results are partially consistent with Joimel et al. (2017), in which a strong dominance of Acari

was reported in arable lands. In the present study, the average Acari density was 49847 ind/m² (Tab. S2). Contrary to Joimel et al. (2017), a similar density was not found in vineyards which showed a lower Acari density (33305 ind/m²), close to that found in olive groves (37404 ind/m²) (Tab. S2). The burial of crop residues on arable land may have increased the abundance of mites through an increase in the proportion of recalcitrant organic matter. For instance, Oribatids are strongly associated with recalcitrant organic matter (Gonçalves et al., 2021). Further, a high presence of Collembola was reported in olive groves, which is consistent with the findings reported in other studies (Carpio et al., 2018; Gkisakis et al., 2014; Gonçalves & Pereira, 2012). Vineyards and olive groves showed similar values for Collembola abundance, while in arable lands, lower values were registered (Fig. 5). Collembola presence was related to a high content of organic matter, which was found in both olive orchards and vineyards. Collembola are considered prey to generalist predators, and they could enhance predator densities and their impact on biological control (Gonçalves & Pereira, 2012). Focusing on Hymenoptera, Formicidae was the most recorded family. Ants, being social insects with stationary nesting behaviour, are not typically associated with specific farming practices. Their presence is more frequently linked to the presence of a nest in proximity to the sampling area (Menta & Remelli, 2020). There were no statistically significant differences in Hymenoptera abundance between the systems. However, the lowest abundance was observed in arable lands (Fig. 5). Unlike Grime (1973), there appeared to be a negative effect on the abundance of this group of arthropods in the present case.

5.5.4 Acari/Collembola ratio and percentage of Oribatid mites out of total mites

The Acari/Collembola ratio is an indicator of environmental stability and complexity (Bachelier, 1963). High ratios are associated with high soil quality, although there are conflicting opinions (Angelini et al., 2002; Latella & Gobbi, 2015). In the study, arable lands exhibited the highest Acari/Collembola ratio. The system was characterized by a lower prevalence of Collembola in comparison to a high density of Acari. This difference could be attributed to the agricultural practices applied and the nature of the system (in terms of the overall stability of the

environment). In fact, in the case of vineyards and olive groves, the ratio showed similar values. Both are stable systems compared to arable land. On the other hand, the percentage of Oribatid mites out of total mites was significantly higher in arable land than in the other systems (Tab. S2). Almost half of the mites present in arable land were Oribatids (39%), with an average density of 19,636 ind/m² (Fig. 6, Tab. S2). The high abundance of oribatid mites observed in this agroecosystem can be attributed to the feeding habits of this group. Indeed, oribatid mites are frugivorous, and some consume dead woody material or algae (Coddington & Colwell, 2000). In arable land, the preceding crop residues, which are typically rich in recalcitrant organic matter such as lignin, are usually buried (in our case, wheat, or sunflower stubble). Prior research has demonstrated that oribatids benefit from the presence of recalcitrant organic matter in the surface states of the soil (Eissfeller et al., 2013). Their density and diversity appear to be positively influenced by this rather than by the presence of rapidly decomposing organic matter (Gonçalves et al., 2021; Wehner et al., 2016). The average Oribatid density in vineyards (7816 ind/m²) was similar to the one found in other Italian vineyards by Nannelli and Simoni (2002). According to Sommaggio and Paoletti (2007), the percentage of oribatid mites is high in undisturbed soils and decreases in soils with high anthropogenic pressure. In olive orchards, where human intervention is minimal, the lowest percentage of Oribatid mites was found (16.1%). In vineyards, where the agricultural practices applied are quite frequent and intense, the percentage of Oribatid mites is average (23.5%). In arable lands, the frequent burial of crop residues, spread across the whole field, could probably increase the Oribatid mite proportion by increasing the organic matter recalcitrant fraction (Gonçalves et al., 2021). To summarize, the percentage of oribatid mites observed in the different systems may be indicative of the degree of human intervention in the overall stability of the system. In conclusion, the interpretation of the results obtained from this index proved to be challenging, as was the case with the evaluation of the Acari/Collembola ratio, as previously stated in several studies (Jacomini et al., 2000; Santorufo et al., 2012; Joimel et al., 2017; Huang et al., 2020).

5.5.5 Soil microarthropod community, management, and soil chemical-physical parameters

The diversity and structure of soil-dwelling microarthropods seemed to be affected more by soil management practices than soil characteristics (such as texture, pH, or organic matter content). The only soil chemical-physical parameter that differed significantly among systems was FWC, with arable land having the lowest percentage. This parameter is an indicator of soil water holding capacity at saturation and is highly related to agricultural management, as well as soil texture and structure. The nMDS plot in Fig. 7 showed that the microarthropod community of arable lands was different from that of vineyards and olive groves, which tended to partially overlap. This result was supported by the IDH (Grime, 1973, Connell, 1978). In arable lands, tillage is more frequent, and the soil is subject to constant, although lighter, mechanical operations. As previously mentioned, according to the IDH, this can create several ecological niches beneficial to a wide range of arthropods. In fact, several potential positive effects of shallow tillage have been put forth, including improved habitat conditions, reduced disturbance, more abundant and diverse prey, decreased risk of drought, and increased food sources. The minimum tillage for certain cultivations accelerates the decomposition of the crop which favors some microarthropod groups (Betancur-Corredor et al., 2022), particularly mites in our case. Further, reduced tillage can prevent soil compaction and depth stratification of nutrients and organic matter, which could influence the density of soil arthropods in comparison to no-tillage (Xin et al., 2018). Olive groves represent a more stable environment due to the reduced human intervention required for their management. As illustrated in Fig. S1, pruning activities are typically conducted once a year, and the use of organic products is employed to control the olive fly. Grassing is the most common practice, either occurring naturally or through the sowing of specific plant varieties. On the other hand, vineyards require a more frequent and deeper inter-row tillage compared to olive groves, as well as a higher number of treatments (e.g., fertilization and application of PPPs), although there were no marked differences in the textural classes, the soil particle contents of sand and silt appeared to exert an influence on the soil microarthropod community assemblage. Different percentages of soil particles can affect pore size and consequently the habitability of the soil. Additionally,

the composition of microarthropod communities appeared to be influenced by pH and carbonate content. According to several studies, pH seems to profoundly influence soil bacterial communities, which at a lower ecological level could in turn influence soil arthropods (Mo et al., 2021). In addition, different groups of soil arthropods appear to be more or less tolerant to this parameter. Furthermore, the varying impacts of K and P contents, as well as FWC on the community may be more closely linked to management practices, such as fertilizer use and grassing. In fact, it is worth noting that the nutrient content is higher in the vineyards (Tab. 5), where more fertilizer was applied (Tab. S1). A first overview of the agricultural practices applied in the different agroecosystems, with a view to improving soil quality. According to our results, in arable land and olive groves, soil biodiversity and soil health seemed to be well preserved by means of sustainable agricultural practices, such as grassing, the use of combined plant varieties, low inputs, and, generally, the use of a low tillage depth or subsoiling, which is a tillage practice that loosens the subsoil while leaving the surface soil undisturbed and it is primarily used to control plow pans (Tab. S1). On the contrary, vineyards were the system with the lowest soil quality, and it was characterized by the lower percentage of vegetation cover, high inputs, and very frequent activities of hoeing, pruning, mowing, and shredding. In this agroecosystem, to enhance the abundance of soil arthropods and the number of BFs, the growth of spontaneous ground cover or the sowing of mixtures of selected plants (legumes) can be easily implemented (Gonçalves et al., 2020). In this way, the welfare of the crop would be also enhanced by the provision of several ecosystem services by soil arthropods, including pest control, organic matter decomposition, and soil structure implementation (Gonçalves et al., 2020). However, the use of copper hydroxide and sulfur can impact soil invertebrate population (Oultaf et al., 2022). In this sense, it will be of paramount importance to find alternative solutions in fighting vine pathogens, to preserve soil biodiversity. In olive orchards, predatory soil microarthropods provide biological control of the olive fly (*Bactrocera oleae*, Rossi 1790), contributing to increasing Tephritidae pupae mortality (Gkissakis et al., 2014). Despite this, the soil microarthropod community is still poorly studied in olive orchards compared to vineyards and arable fields. To our knowledge, Vignozzi et al.

(2019) is currently the only study in which QBS-ar has been applied in olive orchards. In olive groves, the high soil microarthropod abundance and number of BFs seemed to be favored by the natural wide variety of plant species present, which generated different habitats and provided several food sources. To implement microarthropod diversity, both in arable land and vineyards, the inclusion of ecological infrastructures, such as grassy hedgerows and strips, could be recommended. These natural zones prove to be reservoirs of biodiversity, able to replenish the microarthropod community over time (Mantoni et al., 2021). On the other hand, in arable lands, the low soil disturbance applied (e.g., low depth tillage) proved to positively affect the soil microarthropod community, according to IDH (Connell, 1978; Grime, 1973).

5.6 CONCLUSION

For the first time, the QBS-ar index was applied within Conero Park (Italy). Constantly monitoring biological soil health in protected areas is crucial to take prompt action to preserve soil biodiversity as soon as deteriorating processes start (Fusco et al., 2023). Nowadays, in land-protected areas, farmers no longer have the unique role of primary goods producers, they also have the ecological role of biodiversity conservation and environmental improvement. In pursuing this latter objective, the application of sustainable agricultural practices is mandatory. An increased awareness of protected area managers is necessary to preserve and restore this “hidden” yet functionally essential component of terrestrial biodiversity. Preserving, restoring, and implementing soil biodiversity means maintaining and enhancing the ecosystem services it provides (Robinson et al., 2024). This, in turn, contributes to the health and well-being of plants, animals, and humans. At the European level, it is crucial to achieve sustainable soil management to meet the objectives of the Green Deal, which also states that at least 30% of the EU’s land should be protected by 2050 (Montanarella & Panagos, 2021). This target can only be achieved and be effective for land conservation if soil-dwelling organisms, as well as less charismatic but functionally important organisms, are included in the conservation effort, particularly in protected areas (Zeiss et al., 2024). It is important to note that implementing sustainable agricultural management practices, such as organic

farming, can lead to a resilient and economically productive system that satisfies the production needs, and the protection of the agroecosystem and its functional biodiversity. Furthermore, this study provides knowledge toward an informed use of microarthropods as bioindicators of soil health. It emphasizes the importance of monitoring biological soil health to establish thresholds for the prevalent agroecosystems, characterized by different agronomic practices. This is crucial for promoting the use of the QBS-ar index in national and international soil health monitoring.

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6 CHAPTER 3: HOW DO SOIL CHARACTERISTICS, AGRICULTURAL PRACTICES, WEATHER AND LANDSCAPE AFFECT SOIL MICROARTHROPODS IN THE AGROECOSYSTEM? INSIGHTS FROM THREE YEARS OF MONITORING IN ARABLE LANDS WITHIN THE SENTINA RESERVE (ITALY)



Applicazione dell'Indice "Qualità Biologica Suolo – artropodi" (QBS-ar) in Aziende Agricole della **Riserva Naturale Sentina** (San Benedetto del Tronto, AP) - Comune di San Benedetto del Tronto (AP)

“Già da più notti s'ode ancora il mare,

lieve, su e giù, lungo le sabbie lisce.

Eco d'una voce chiusa nella mente

che risale dal tempo

[...]”

Salvatore Quasimodo (Giorno dopo giorno, 1947)

6.1 ABSTRACT

The arthropod-based Biological Soil Quality Index (QBS-ar) is currently widely used in Italy and is starting to gain international recognition. This index is used to evaluate soil quality across various land uses and management types, typically in conjunction with soil chemical-physical analysis to assess soil fertility conditions. However, the impact of other abiotic factors, such as climate and landscape, on the QBS-ar index remains unclear, and very few studies have investigated this aspect. Understanding these influences is crucial because they can affect both the index and the soil arthropod community. This study aims to fill this knowledge gap by analysing how soil chemical and

textural properties, soil environmental parameters, agricultural practices, weather and landscape factors influence the QBS-ar index using a multiple linear regression model.

The QBS-ar together with other biotic parameters, including soil microarthropod abundance and biological (BF) and euedaphic (EF) forms, were used to assess soil quality in arable lands within the Sentina Reserve in San Benedetto del Tronto (Italy) from 2021 to 2023. Four sites (FE1, FE2, RL1 and RL2), belonging to two different farms (Fattoria Ferri and Selva Giurata), were monitored in spring and autumn each year. The sites were adjacent, shared the same soil origin, climate, and conventional management type, within a comparable landscape. This made them suitable for analysing the influence of the several abiotic variables on the QBS-ar index.

Soil chemical parameters, particularly K content, significantly influenced the QBS-ar. Agricultural practices, especially tillage and harvesting, also had a considerable effect. High landscape complexity contributed to soil quality maintenance, while weather, soil textural and environmental parameters had minimal effects. Overall, high soil quality was observed across all sites and seasons. The Selva Giurata sites showed the highest soil quality, proving that less intensive agronomic practices might effectively preserve edaphic biodiversity. Further research is needed to improve the reliability of the model and to identify the key variables that influence the QBS-ar index and to what extent in different agricultural contexts.

6.2 INTRODUCTION

The global food system is the primary driver of biodiversity loss across the planet (Benton et al., 2021). Intensive agriculture, characterized by monocropping, high chemical inputs, and intensive tillage, has been shown to significantly harm and reduce soil biodiversity in Europe (Tsiafouli et al., 2015). This impact arises from both physical disturbances, such as soil compaction and erosion, and chemical disturbances, including the application of fertilizers and Plant Protection Products (PPPs) (Chassain et al., 2023), particularly herbicides. Conversely, the adoption of sustainable farming practices, that focus on minimizing disturbances to the soil, can significantly improve soil biodiversity (Bengtsson et al., 2005; Brussaard et al., 2007; Sharma et al., 2024).

Techniques such as reduced or minimum tillage, crop rotation, and green manuring, help maintain a healthy soil ecosystem. As a result, soil organisms can thrive, leading to enhanced soil functionality, improving several soil processes, and the overall resilience of agricultural systems (Delgado-Baquerizo et al., 2020).

Among soil fauna, soil microarthropods represent a highly diverse group of invertebrates that play a pivotal role in maintaining soil health. They promote soil health through contributions to organic matter decomposition, nutrient cycling, plant growth and overall ecosystem functioning (Lavelle et al. 2006). They are considered a cost-effective, promising, and reliable indicator of soil quality (Parisi et al., 2005). To date, one of the most widely used soil quality index based on analysis of the soil microarthropod community is the arthropod-based Soil Biological Quality Index (QBS-ar) (Parisi et al., 2001; 2005).

The QBS-ar index is currently a widely used method in Italy and is starting to be used worldwide (Menta et al., 2018a). In fact, at the end of 2024 at the 8th meeting of the FAO network Global Soil Laboratory Network (GLOSOLAN), the Standard Operating Procedures (SOPs) for QBS-ar sampling and calculation were suggested (Gallese et al., 2025) to harmonize the protocol and extend the use of the index worldwide. To date, the QBS-ar index has mainly been used to monitor and compare soil quality in different land uses (Menta et al., 2018a; Machado et al., 2019; Hernández-Tirado et al., 2022) management types (Ghiglieno et al., 2023; Fusco et al., 2023; Kurniawan et al., 2023), agroecosystems (Menta et al., 2011; Gallese et al., 2025; Coletta et al., 2025) and soil types (Fiorini et al., 2020; Leone et al., 2023). Nonetheless, it has been used to detect the effect of a wide range of soil disturbances, from PPPs use (D'Alessandro et al., 2024) to the presence of photovoltaic panels (Menta et al., 2023), from fire impact (Mantoni et al., 2020; Memoli et al., 2022; Çakır et al., 2023) to urban pollution (Horváth et al., 2021; Tóth et al., 2023).

In many of the studies mentioned, data on soil chemical and physical properties are generally collected with the QBS-ar soil samples to define the soil fertility status. However, the extent

to which these and other abiotic variables influence the index has been poorly addressed. For instance, very few studies have attempted to assess the influence of climate (Ghiglieno et al., 2021; Tóth et al., 2025; Gallese et al., 2025) and landscape (Szigeti et al., 2022) on the QSB-ar index.

In this regard, a three-year monitoring was conducted to assess the soil quality in agroecosystems of the Sentina Natural Regional Reserve in San Benedetto del Tronto (AP, Marche region, Italy), using the arthropod-based Biological Soil Quality Index (QBS-ar) and other biotic parameters, including soil microarthropod abundance and biological (BF) and euedaphic (EF) forms (Parisi et al., 2005). Four arable lands (FE1, FE2, RL1 and RL2), belonging to two different farms (Fattoria Ferri and Selva Giurata), were monitored in 2021-2022-2023 in two sampling seasons: spring (June) and autumn (October). Over the three years, data on several abiotic variables, such as agricultural practices, soil chemistry, soil texture, soil environmental parameters, weather, and landscape, were collected and analysed in conjunction with the QBS-ar index.

The main aim of this study was to shed light on the abiotic variables that most significantly impact the QBS-ar index within an agricultural context (at least for the investigated area). Additionally, we believe this research will contribute to enhancing our understanding and application of the QBS-ar index by providing deeper insight into the factors that significantly influence it. To the best of our knowledge, this is the first study to integrate various sets of abiotic variables to examine their effects on the QBS-ar index. In this regard, our hypotheses were:

(i) We expect to find higher QBS-ar values, microarthropod abundance and biological (BFs) and euedaphic forms (EFs) at Selva Giurata than at Fattoria Ferri. Since the former uses less intensive agricultural practices, we assume that the soil microarthropod community would be less affected and the biotic parameters would be higher. Previous studies highlighted that a lower intensity of agronomic practices could lead to a higher QBS-ar index in arable lands

(Parisi, 2001; Parisi & Menta, 2008; Sapkota et al., 2012; Fiorini et al., 2020; Pittarello et al., 2022). Nonetheless, we hypothesize that the intensity of the agricultural practices would be the most influential factor affecting the soil arthropod community (Kladivko, 2001; Köninger et al., 2022).

(ii) Given that the four sites were near one another and shared the same pedological origin (ASSAM, 2006), we assume that the chemical and textural characteristics of the soil would generally be similar. However, we hypothesise that the content of nutrients (N, P, K) may vary based on the intensity of the agricultural practices and could significantly influence the QBS-ar index (Fiorini et al., 2020; Di Giovanni et al., 2024).

(iii) Due to the high sensitivity of soil mesofauna to soil moisture (Parisi & Menta, 2008; Coleman et al, 2024), we expect to find higher QBS-ar values and other biotic parameters during the season with greater soil moisture.

(iv) We assume that the QBS-ar in an agroecosystem will be positively influenced by a more heterogeneous surrounding landscape, compared to a homogeneous one. Soil arthropods are affected by the landscape (Pérez-Bote & Romero, 2012; Franin et al., 2016) and neighbouring areas (Querner et al., 2018; Szigeti et al., 2022).

6.3 MATERIALS AND METHODS

6.3.1 Study Area

The Sentina Natural Regional Reserve in San Benedetto del Tronto (AP, Marche region, Italy) is a complex system characterised by a homogeneous system of land, river, and lake areas, a landscape of water and sand covering an area of 177.55 ha, in the territory of the Municipality of San Benedetto del Tronto (<https://www.riservasentina.it/>). It was established by Regional Decree n. 156 of 14 December 2004. Two farms and two sites for each were selected for the study (FE1 and FE2 for Fattoria Ferri and RL1 and RL2 for Selva Giurata), resulting in a total of four sampling sites (**Tab. 1, Fig. 1, Fig. S1**). The choice was made in agreement with the farmers, the

Municipality and the Reserve Authority. The sites were monitored for three years 2021, 2022, and 2023, in spring and autumn, which represent the ideal seasons for defining the QBS-ar index. The sites were classified under the “MS1 management system”, which refers to arable crop rotation excluding fodder, according to the Marche Region Monitoring System of Soils at Multi-Scale Project (Tiberi et al., 2014; Bharti et al., 2017). Geographic coordinates (**Tab. 1**) and environmental parameters, including soil temperature and moisture, were recorded during soil samplings (**Tab. 2**). The two farms and selected sites were adjacent, shared the same pedological origin (ASSAM, 2006), were exposed to similar weather conditions, within a comparable landscape. In addition, both farms were managed conventionally. This setting provides a suitable framework for analysing the influence of several abiotic variables, such as agricultural practices, soil chemistry, soil texture, soil environmental parameters, weather, and landscape on the QBS-ar index.



Fig. 1 - Aerial photograph of the Sentina Natural Regional Reserve (in green). The perimeter of the four monitored sites is shown in red. The sampling design is also visible with central sampling points.

Farm name	Study sites	Agricultural land type	Farming system	Geographical coordinates	Dimension
Fattoria Ferri	FE1	Arable land	Conventional	42.899119, 13.904068	0,7 ha
	FE2	Arable land	Conventional	42.901360, 13.903035	1,2 ha
Selva Giurata	RL1	Arable land	Conventional	42.90544586, 13.89977890	2,3 ha
	RL2	Arable land	Conventional	42.906988, 13.903888	2,9 ha

Tab. 1 - Sampling sites characteristics.

Season	Year	FE1	FE2	RL1	RL2	Soil environmental parameters
Spring	2021	8.1	18.8	9.9	5.2	Average soil moisture (%)
	2022	8.7	25.6	9.9	3.5	
	2023	31.9	21.0	40.6	19.9	
Autumn	2021	14.3	32.5	8.8	10.9	
	2022	16.8	14.3	22.9	7.8	
	2023	12.0	21.3	21.3	13.3	
Spring	2021	20.6	21.8	23.6	23.9	Average soil temperature (°C)
	2022	26.7	24.3	26.7	29.3	
	2023	21.2	20.6	22.6	22.6	
Autumn	2021	21.2	21.5	22.6	22.2	
	2022	17.0	16.6	18.2	22.5	
	2023	17.2	17.9	19.0	18.7	

Tab. 2 - Average soil moisture and temperature of the different sites (FE1, FE2, RL1 and RL2) measured at the time of sampling.

6.3.2 QBS-ar index and other biotic parameters

Sampling and extraction of soil microarthropods followed the methodology described by Parisi (2001), and Parisi et al. (2005). For three years, in June and October, at each site a QBS-ar sample, consisting of three subsamples (A, B, C) with a volume of about 800 cm³ each, was collected using a cylindrical soil corer (Coletta et al., 2025). A homogeneous area was selected in each site, excluding disturbed areas such as flattened vegetation and tractor tracks. The herbaceous cover was removed prior to collecting the soil sample. Subsamples were collected at equal intervals (10 m) along a diagonal, in the central part of the field. A total of 72 soil subsamples were collected in the three years (12 sub-samples per season). Arthropod extraction was carried out using the Berlese-Tüllgren funnel, equipped with 25 W light bulbs and a 2-mm sieve mesh. The extraction lasted 7 days and the extracted specimens were preserved in a solution of 75% ethyl alcohol and 25% glycerol by volume. A stereoscopic analysis was performed to determine biological form (BF) and Eco-Morphological Index (EMI) (1 to 20), according to Parisi et al. (2005). Total and partial QBS-ar values were calculated for each sample (Coletta et al., 2025). A quantitative approach was used; the number of individuals present for each BF was counted in each subsample. Further, the number of biological (BF), that is the total number of microarthropod groups found, and the number of euedaphic forms (EFs), that is the number of microarthropod groups which were given an Eco-morphological Index (EMI) of 20 (Parisi et al., 2005), were defined. In addition, the total microarthropod abundance was also determined.

6.3.3 Agricultural practices applied

Data related to management information and agricultural practices applied in each site, such as tillage depth, use of PPPs (herbicides) and fertilizers, irrigation, crop succession, and green manuring practices were collected, to the extent possible, through interviews with farmers (**Tab. 3, Tab. S1**). A numerical range from 0 (high impact/disturbance) to 100 (low impact/disturbance) was utilised to define the intensity of the various farming strategies employed at the different sites. The agricultural practices considered were crop rotation; tillage for seedbed preparation

(ploughing and harrowing), use of fertilizers and PPPs (herbicides), irrigation, false sowing, sowing, harvesting, shredding and burial of crop residues (**Tab. 3**).

	Autumn 2020	Spring 2021	Autumn 2021	Spring 2022	Autumn 2022	Spring 2023	Autumn 2023
FE1	Shredding & burial (Corn) + medium tillage (35 cm) + post-tillage harrowing (<15 cm) + fertilization + sowing (Durum wheat) + irrigation (flood)	Fertilization + herbicide use (Durum wheat)	Harvesting + shredding & burial + medium tillage (35 cm) + post-tillage harrowing (<15cm) + fertilization + sowing (Durum wheat)	Fertilization + herbicide use (Durum wheat)	Harvesting + shredding & burial + medium (35 cm) + post-tillage harrowing (<15cm) + fertilization + sowing (Durum wheat)	Fertilization + herbicide use (Durum wheat)	Harvesting
FE2	Shredding & burial (Durum wheat) + medium tillage (35 cm) + post-tillage harrowing (<15 cm) + fertilization + sowing (Soft wheat)	Fertilization + herbicide use (Soft wheat)	Harvesting (Soft wheat) + shredding & burial + medium tillage (35 cm) + post-tillage harrowing (<15 cm) + fertilization + sowing (leek) + fertilization + herbicide use	Harvesting (Leek) + cropping & burial + medium tillage (35 cm) + post-tillage harrowing + fertilization + sowing (Corn) + herbicide use + fertilization + Irrigation (sprinkler) (Corn)	Harvesting + shredding & burial + medium tillage (35 cm) + post-tillage harrowing (<15cm) + shredding + fertilization + sowing (Durum wheat)	Fertilization + herbicide use (Durum wheat)	Harvesting
RL1	Shredding and burial (Durum wheat) + shallow tillage (<15cm) + sowing (horseradish and field beans)	Horseradish and field beans	Shredding & burial + shallow tillage (<15cm) + false sowing + fertilization + sowing (Durum wheat)	Fertilization + herbicide use (Durum wheat)	Harvesting + shredding & burial + deep tillage (50 cm) + post-tillage harrowing (<15cm) + fertilization + false sowing + sowing (Durum wheat)	Fertilization + herbicide use (Durum wheat)	Harvesting + shredding & burial
RL2	Shredding & burial (Durum wheat) + shallow tillage (<15cm) + fertilization + sowing (Soft wheat)	Fertilization + herbicide use + fertilization (Soft wheat)	Harvesting + shredding & burial + shallow tillage (<15cm) + fertilization + sowing (Durum wheat)	Fertilization + herbicide use (Durum wheat)	Harvesting + shredding & burial + deep tillage (50 cm) + post-tillage harrowing (<15cm) + fertilization + sowing (Durum wheat)	Fertilization + herbicide use (Durum wheat)	Harvesting

Tab. 3 - Agricultural practices applied in the different sites in the three years and seasons. The table also includes the agricultural practices that occurred in autumn 2020, prior to the spring 2021 sampling.

6.3.4 Soil chemical and textural parameters

Soil samples for chemical-physical analyses were collected in accordance with the official methods for soil analysis of the Italian legislation DM 13/09/1999 SO No. 185 (*Approvazione dei “Metodi Ufficiali di analisi chimica del suolo”*). In each site, 10 soil sub-samples were collected by using a pedological auger and then mixed and reduced to a single composite sample (1 kg). The samples were analysed by the laboratory of the “Agenzia per l'Innovazione nel Settore

Agroalimentare e della Pesca "Marche Agricoltura Pesca" (AMAP)", Jesi (Italy). The analysed parameters were: five fractions soil texture (coarse, fine, very fine sand, silt, clay; g/kg), total organic carbon (g/kg), organic matter (g/kg), pH, C/N ratio, total nitrogen (g/kg), available phosphorus (mg/kg), exchangeable potassium (mg/kg), cation exchange capacity (meq/100g) and electrical conductivity (dS/m). Samples for soil chemical-physical analyses were collected in spring 2021 (at the beginning of the study). In addition, soil samples for chemical analysis were also collected in autumn 2023 (at the end of the study) to evaluate any changes in the fertility status of the investigated soils.

6.3.5 Weather and soil environmental factors

The Sentina Reserve lies in the temperate climate zone, specifically in a transitional climate between the Mediterranean and European subcontinental sub-types. The climate is generally mild, with moderately hot and dry summers while not very cold, and sometimes foggy winters. According to Sinatra (2014), the average annual temperature in the coastal areas of the Marche region ranges from 13 to 15 °C and Sentina Reserve is one of the hottest areas in the Marche region. The coastline between the municipalities of Cupra Marittima and Porto d'Ascoli is the least rainy portion of the Marche region, with an average of 550-650 mm/year. To define the weather within the Sentina Reserve, the meteorological data provided by "Agenzia Marche, Agricoltura, Pesca" (AMAP) (Jesi, Italy) were analysed. The dataset included the monthly average temperature (°C), precipitation (mm) and relative humidity (%) from 2020 to 2023 recorded by the Spinetoli (AP) weather station. Due to the limited extension of the study area, the climate was assumed to have a uniform influence across all sites. Weather parameters were included in the analysis to investigate their impact on the overall trend of the QBS-ar index across different years and seasons (Fig. 1). In addition, soil environmental parameters, including soil moisture (%) and temperature (°C), were measured on site, adjacent to the sampling point, in triplicate using a soil moisture meter (PMS-714 Lutron) and a thermometer (HI-98501; Hanna Instruments, Milan, Italy)

(Tab. 2). These data were included in the analysis to assess whether these point measurements influenced the index.

6.3.6 Landscape

To analyse the influence of the surrounding landscape on soil microarthropods, land use and land cover classes were mapped in a Geographic Information System (GIS) software, running on QGIS v. 3.34.12-Prizren. The Sentina Nature Reserve map from Santolini et al. (2016) was digitised in the QGIS software based on the land use map (CUS, 2007) and the vegetation map (Vegetation Map, 2009). Data on railways, waterways and roads were retrieved from OpenStreetMap (OSM) and subsequently processed in QGIS. The map was then updated through visual interpretation of Google's 2023 satellite image, available via the Quick Map Services QGIS plugin. As suggested in Le Provost et al. (2021), the smallest landscape-level spatial scale (i.e. 500-m radii from plot centroids) was considered for each site (an area of approximately 78.5 ha for each). In the four areas, 20 different land-use and land-cover types were identified (arable land, continuous buildings, salt marsh vegetation, paved roads, olive groves, unpaved roads, halophytes, scattered buildings, hedges and shrubs, railway, reedbed, pond and small lakes, riparian woodland, gardens and parks, watercourse, grassland, vineyards, psammophilous vegetation, sea, shoreline) were described (**Fig. 2, Tab. 4**).

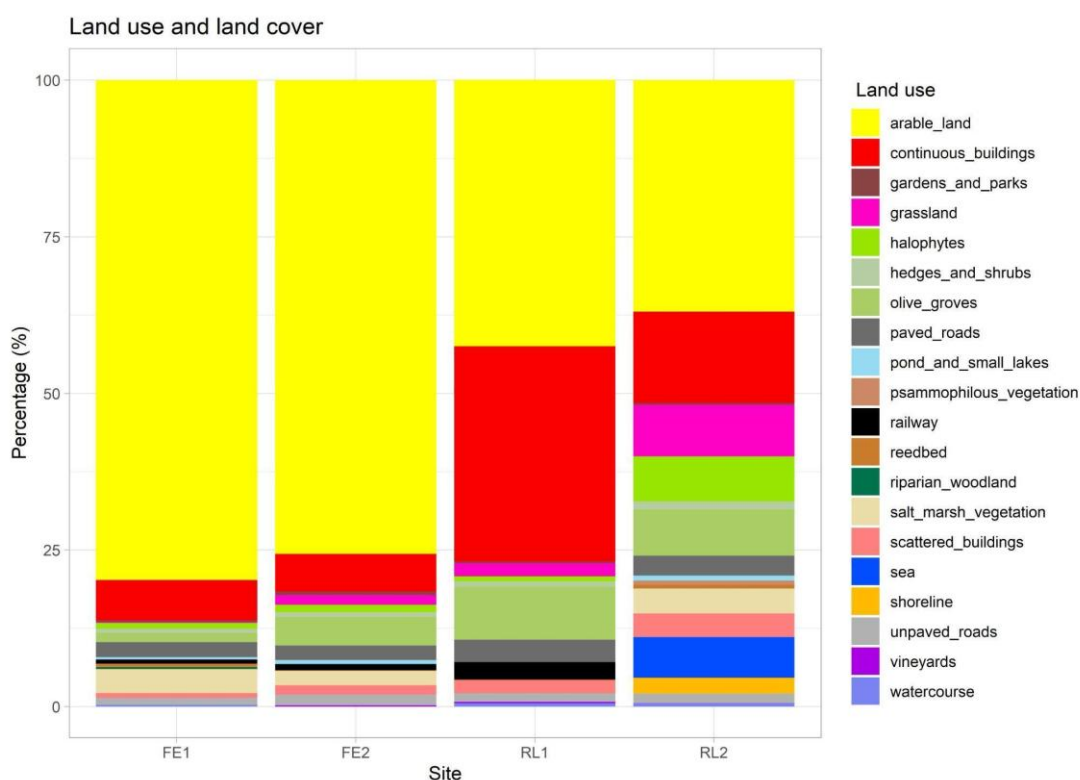


Fig. 2 - Barplot of land-use and land-cover types present in each site (FE1, FE2, RL1 and RL2) considering a landscape-level spatial scale (i.e. 500-m radii from plot centroids).

For each area, the percentage of each land-use and land-cover type was calculated to visualize the differences at landscape level between the four sites (**Tab. 2**).

field	FE1	FE2	RL1	RL2
farm	Fattoria Ferri	Fattoria Ferri	Selva Giurata	Selva Giurata
arable land	79.8%	75.6%	42.6%	36.9%
continuous buildings	6.4%	6.0%	34.4%	14.6%
salt marsh vegetation	3.9%	2.3%	0.0%	4.0%
paved roads	2.4%	2.3%	3.6%	3.2%
olive groves	1.5%	4.5%	8.4%	7.4%
unpaved roads	1.1%	1.7%	1.3%	1.5%
halophytes	0.9%	1.2%	0.8%	7.2%
scattered buildings	0.8%	1.5%	2.1%	3.8%
hedges and shrubs	0.7%	0.8%	0.9%	1.3%
railway	0.6%	1.0%	2.8%	0.0%
reedbed	0.6%	0.1%	0.1%	0.6%

pond and small lakes	0.4%	0.6%	0.0%	0.8%
riparian woodland	0.3%	0.0%	0.0%	0.0%
gardens and parks	0.3%	0.5%	0.3%	0.3%
watercourse	0.3%	0.0%	0.5%	0.5%
grassland	0.1%	1.6%	2.1%	8.2%
vineyards	0.0%	0.2%	0.3%	0.0%
psammophilous vegetation	0.0%	0.0%	0.0%	0.7%
Sea	0.0%	0.0%	0.0%	6.5%
shoreline	0.0%	0.0%	0.0%	2.6%

Tab. 4 - Percentage of the 20 land use and land cover types - derived from the integration of vegetation and land-use maps and photointerpretation of the area surrounding the sites (FE1, FE2, RL1 and RL2), considering a landscape-level spatial scale (i.e. 500-m radii from plot centroids).

6.3.7 Statistical analysis

To test for differences between the two farms, total and partial data (obtained from each subsample) were considered. The data were first checked for normality of distribution (Shapiro-Wilk test) and homoscedasticity (Levene's or Bartlett's test, depending on the distribution). Differences between management systems were evaluated through One-way ANOVA or Kruskal Wallis Test, depending on the distribution, and post hoc tests (Tukey's honestly significant difference or Dunn test, respectively).

To test the influence of the various sets of variables on the QBS-ar index, and to understand which of them exerted a greater influence on it, a multiple linear regression (MLR) model was selected (*lm*, package “stats”). The data referring to the different variables were grouped into six categories: agricultural practices, soil chemical properties and soil textural properties, landscape, weather and soil environmental factors (**Tab. S1**). Each set of variables was subjected to an initial exploratory analysis where correlations between variables and QBS-ar index, the ratio of observations to variables and the Variance Inflation Factor (VIF) were calculated. Highly correlated variables were selected to reduce multicollinearity. Afterwards, several assumptions were tested, including normality, homoscedasticity (Breusch-Pagan test) and independence of

residuals (Durbin-Watson test). Since all assumptions were fulfilled, MLR was adopted. Moreover, to test the reliability of the model, Root Mean Squared Error (RMSE), Mean Absolute Error (MAE) and Akaike Information Criterion (AIC) were calculated (data not shown). When the number of variables to be tested was significant in relation to the number of observations, the selection of variables was performed using a stepwise regression by backward elimination based on AIC. A $p\text{-value} < 0.05$ was considered significant. Statistical analysis was performed using R software, version 4.4.0 (R Development Core Team, 2021).

6.4 RESULTS

6.4.1 QBS-ar results

The average QBS-ar values found in Selva Giurata farm were consistently higher in all years and in all seasons than at Fattoria Ferri (**Tab. 3A**). Nevertheless, results were statistically significant only in spring 2022 (Fig. 3A). Significant differences among the average values registered in the sites were found in all years and seasons, except for autumn 2022 and spring 2023 (**Fig. 4A, Tab. 5**). In general, it can be observed that FE2, from autumn 2021 onwards, consistently showed the lowest average QBS-ar values, which were particularly low in autumn 2021 (69) and spring 2022 (53). In contrast, RL2 exhibited the highest QBS-ar values in both 2021 and 2023. FE1 and RL1 showed similar values, which tended to be relatively constant, particularly in 2021 and 2022. In 2023, FE1 showed higher QBS-ar values compared to RL1 (**Fig. 4A**).

Farm	Site	2021		2022		2023		Average QBS-ar per site	total values	Average QBS-ar per farm	total values
		Spring	Autumn	Spring	Autumn	Spring	Autumn				
Fattoria Ferri	FE1	138	168	154	159	207	172	166		148	
	FE2	174	120	102	113	136	132	130			
Selva Giurata	RL1	158	175	165	125	153	172	158		168	
	RL2	194	163	153	149	209	202	178			
Average total QBS-ar values per season		166	157	144	137	176	170				

Tab. 5 - The table shows the total QBS-ar values obtained in each farm and site in the three years and the average total QBS-ar values across the two farms, the four sites, and according to the season.

Farm	Site	2021		2022		2023		Biotic parameters
		Spring	Autumn	Spring	Autumn	Spring	Autumn	
Fattoria Ferri	FE1	116	119	101	120	135	139	QBS-ar index
	FE2	130	69	56	78	94	92	
Selva Giurata	RL1	130	122	123	103	101	128	
	RL2	173	144	117	91	150	156	
Fattoria Ferri	FE1	2291	1047	867	907	654	1057	Soil micro-arthropod abundance
	FE2	751	76	70	410	392	679	
Selva Giurata	RL1	3883	1849	1787	2208	542	1365	
	RL2	8316	3370	2073	395	805	1073	
Fattoria Ferri	FE1	9	9	10	10	11	9	BFs
	FE2	10	4	4	6	7	6	
Selva Giurata	RL1	11	9	12	6	9	10	
	RL2	14	10	10	7	12	12	
Fattoria Ferri	FE1	4	4	3	4	4	6	EFs
	FE2	5	3	2	2	3	4	
Selva Giurata	RL1	4	4	4	5	3	4	
	RL2	6	6	4	4	5	5	

Tab. 6 - The table shows average partial QBS-ar values, microarthropod abundance, BFs and EFs in each farm and site in the three years.

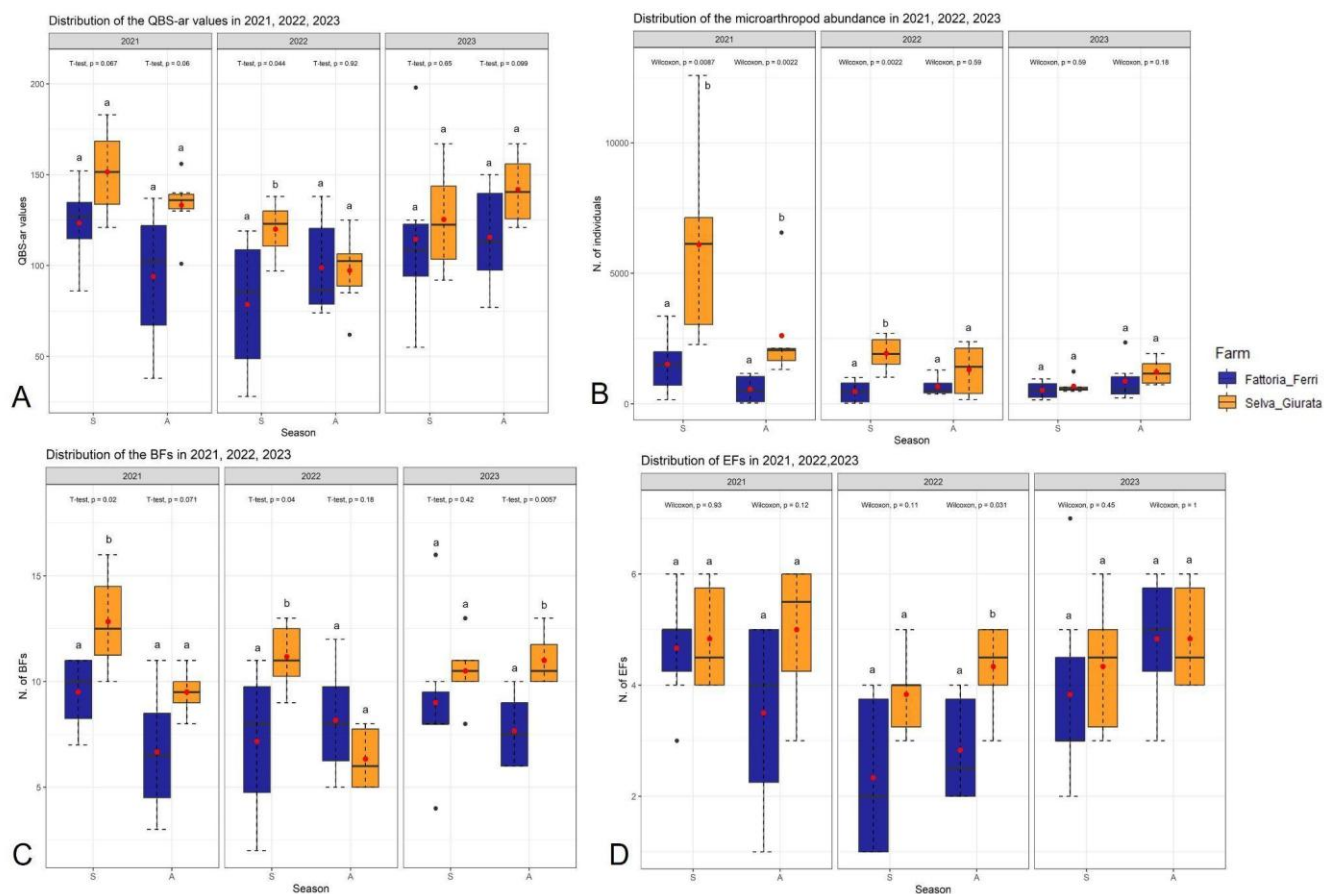


Fig. 3 - The boxplots show the distribution of the partial QBS-ar values (A), average microarthropod abundance (B), number of Biological forms (BFs) (C) and Euedaphic forms (EFs) (D) in the two farms per year and season. The median (horizontal line), mean (red dot), error bars (dashed line) and outliers (isolated black dots) are reported. Lowercase letters represent significant differences among farms by Student's T or Wilcoxon test at p-value < 0.05.

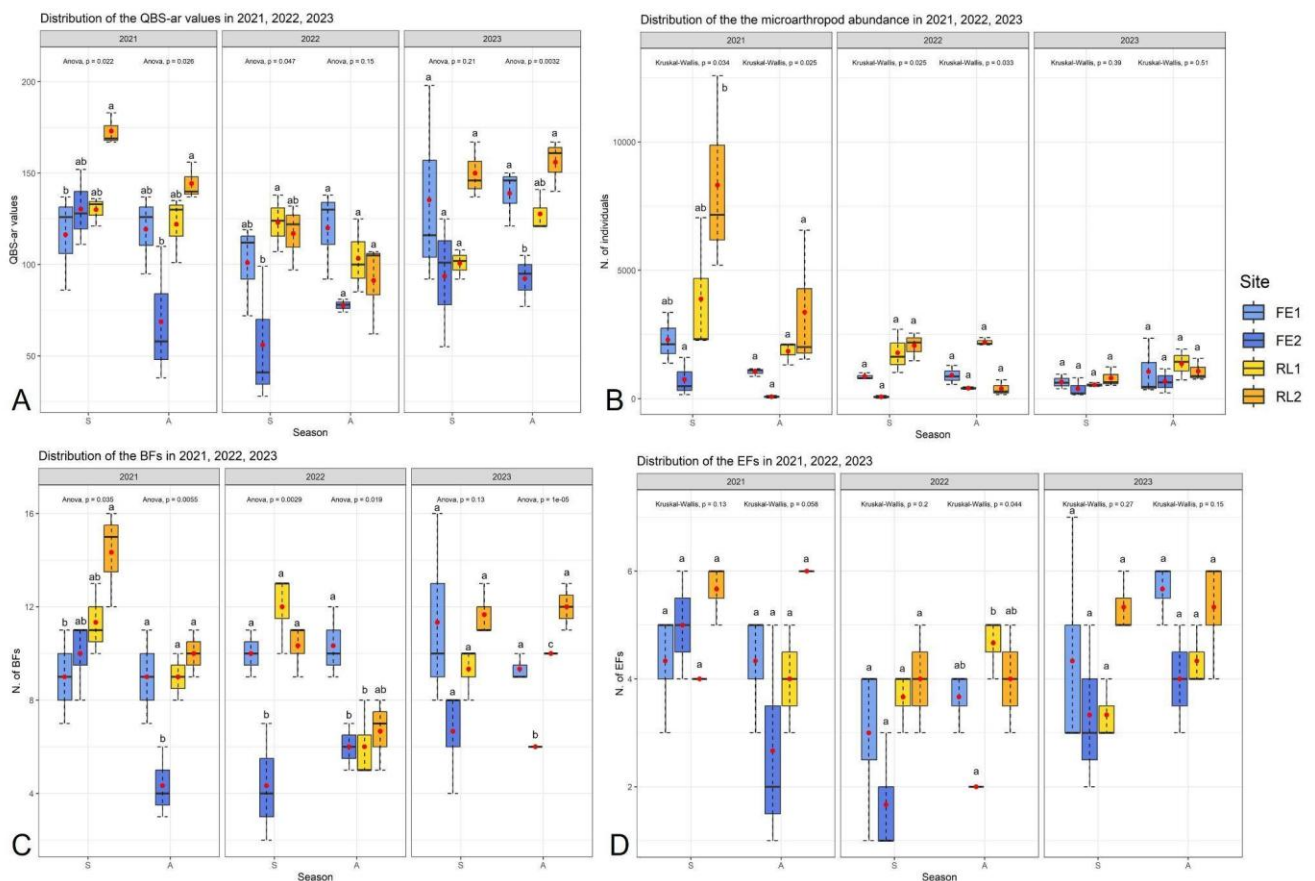


Fig. 4 - The boxplots show the distribution of the partial QBS-ar values (A), average microarthropod abundance (B), number of BFs (C) and EFs (D) in the four sites per year and season. The median (horizontal line), mean (red dot), error bars (dashed line) and outliers (isolated black dots) are reported. Lowercase letters represent significant differences among farms by Tukey or Dunn test at p-value < 0.05.

6.4.2 Soil microarthropod abundance

The average partial soil microarthropod abundance was higher in Selva Giurata in all seasons and years (2306 individuals) compared to Fattoria Ferri (767 individuals) (**Fig. 3B**). However, significant differences between the two farms were only found in spring 2021 and 2022 (**Fig. 3B**, **Tab. 5**). The year 2021 was characterised by the highest average abundance observed, which was significantly higher only compared to 2023. Focusing on individual site behaviour, significant differences were found in spring 2021 and 2022 and in autumn 2022 (**Fig. 4B**). During 2021, FE2 was characterised by the lowest abundance (**Tab. 6**), while RL2 was characterised by the highest abundance, followed by RL1 and FE1. In 2022 the same situation was observed in spring, while in autumn RL1 (2208) was characterised by a highest average abundance compared to all other sites and, moreover, the abundance recorded at RL2 decreased dramatically (395), being even lower than the one found in FE1 (410). In spring 2023, the abundance of microarthropods was

similar across all sites, while in autumn RL1 showed the higher average abundance followed by RL2, FE1 and FE2 (**Tab. 6; Fig. 4B**).

6.4.3 Biological (BFs) and Euedaphic forms (EFs)

Regarding BFs and EFs, the trend was analogous to that observed for the QBS-ar values. In 2022 lower numbers of BFs and EFs were found compared to 2021 and 2023, although the discrepancy was statistically significant only for EFs (**Fig. 3C, Fig. 3D, Tab. 6**). The highest average BFs number was found in all years and seasons in the sites of Selva Giurata farm, except for autumn 2022 where the highest BFs number was found in Fattoria Ferri. However, significant differences among farms were only found in spring 2021, 2022 and autumn 2023. Similarly, the highest EFs number was generally found in Selva Giurata farm, however in spring 2022 and autumn 2023 results were very similar. Significant differences among farms were only found in autumn 2022. Considering individual site performance (**Fig. 4C, Fig. 4D**), F2 was generally the site with the lowest number of BFs and EFs, particularly in autumn 2021 and 2023 for BFs and in 2022 for EFs. In the latter case, the number of EFs generally varied between 6 and 3; FE2 was the only one below this range. RL2 had the highest number of BFs in all years except 2022. Particularly, in autumn 2022, perhaps because of the particularly dry season, there was a notable decline in the number of BFs at all sites, apart from FE1, in which 10 BFs were found (**Tab. 6**).

6.4.4 Agricultural practices applied

In Table 3 were summarized the agricultural practices applied by the different farms in the different sites during the three-year monitoring period (**Fig. S1**). Concerning tillage for seedbed preparation, at Fattoria Ferri there was an extreme consistency in the use of the plough. In both FE1 and FE2 sites, ploughing at 35 cm (medium tillage) followed by refining with disc harrow (post-tillage harrowing) occurred in autumn. On the contrary, in Selva Giurata shallow tillage (< 15 cm) using disk harrow was used more frequently. However, in RL1 and RL2, deep tillage (50 cm) followed by post-tillage harrowing (< 15 cm) was used in autumn 2022. In Selva Giurata, this

practice occurred every four years (RL1) and three years (RL2). The interviews with farmers also gathered information on the farming practices applied in previous years. According to these, the last deep tillage was conducted in 2019 for RL1 and in 2020 for RL2.

Sowing was carried out by means of equivalent agricultural equipment and generally done at the same time for all sites. Unlike the other sites, in FE2 sowing also took place in autumn 2021 because leeks were planted, and the fallow period was absent for this site. Whereas false sowing was used exclusively in RL1. Harvesting occurred in both farms at the same time, with the exception for spring 2022, where leeks were solely harvested in FE2. In autumn 2021, in RL1 no harvesting occurred due to the application of green manure consisting of horseradish and field beans.

In fact, RL1 was designated as an Ecological Focus Area (EFA) for 2021. The site was a set-aside area from farming, it was cultivated with two cover crops: field bean, a nitrogen-fixing legume, and horseradish, characterised by the development of a taproot that exerts a decompaction action in the soil and increasing aeration. Regarding crop succession, durum wheat was the most used crop in both farms. In spring 2023 it was present in all sites. In 2022, it was present on all sites, except for FE2, in which corn was present. In 2021, durum wheat was present only in FE1, while in RL2 and FE2 soft wheat was present.

On both farms there was a considerable use of fertilizers and herbicides. Fertilizers tended to be applied in both the pre-sowing and the crop growing season, except for RL2, in which three fertilizations occurred for soft wheat in 2021. While RL1 had only one pre-sowing fertilisation in autumn 2021 for durum wheat, no fertilizations occurred previously due to the presence of cover crops for green manuring. Herbicides were primarily used in spring, during the growing season. RL1 was the only site not to use them in spring 2021, whereas FE2 was the only site to use them in autumn 2021.

Regarding shredding and burial of crop residues, results were generally similar. Notably, in RL1 in spring 2021 green manuring was applied, due to the burial of horseradish and field beans. Irrigation was applied in FE1 and FE2 when corn was present. In spring 2021 the water was

distributed by flooding, in spring 2022 in FE2 by sprinklers. To summarise, the sites and farms differ in the intensity of the agricultural practices applied (Fattoria Ferri = high intensity, Selva Giurata = low intensity) (**Tab. 1, Tab.3**).

6.4.5 Soil chemical and textural properties

The soil texture analysis showed that FE2 and RL2 had the same sandy loam texture class, while FE1 had a silt loam texture and RL1 had a loam soil (**Tab. 7, Fig. S2**). The soil chemical data demonstrated consistent results between the years (**Tab. 8**). In RL2, greater reductions in certain chemical parameters, including P, K, SOM, and TOC, were observed in 2023 compared to 2021. The K content was the parameter that changed the most among sites in the years. It was notably higher in RL2 compared to RL1, FE1, and FE2 (**Tab. 8**). In fact, K content in 2021 was medium for FE1 and RL2, low in FE1, and high in RL2. In 2023 it was low in all sites except RL2 where it was high. The C/N ratio and SOM were low in all sites and both years. Except for RL2 in 2023, whose soil was found to be alkaline, the pH was slightly alkaline across all sites. All sites appear to have a medium content of N and TOC, except RL2 in 2023, which was low in both parameters. P content was high at all Fattoria Ferri sites in 2021 and 2023, while at Selva Giurata sites in 2021 it was high for RL1 and very high in RL2 in 2021, while for both sites in 2023, it was average. The CEC was similar between sites, it appeared generally medium in all years (ASSAM, 2001). It was only low in 2021 in FE2. In terms of electrical conductivity (EC), there were slight variations in values across the different sites over the years. The soil in FE1 and RL2 was non-saline in both 2021 and 2023. Whereas in FE2, the soil was slightly saline in 2021 and non-saline in 2023. The highest salinity level was observed in RL1, whose soil resulted in moderately saline in 2021 and slightly saline in 2023 (Abrol et al., 1988).

Site	Fine-earth fraction						Soil Texture Class (USDA)
	% Very fine sand	% Fine sand	% Coarse sand	% Total Sand	% Silt	% Clay	
FE1	22.5	7.2	1.7	31.4	51.1	17.5	Silt loam
FE2	26.4	20.0	8.6	55.0	35.5	9.5	Sandy loam
RL1	11.2	13.0	15.5	39.7	39.9	20.4	Loam
RL2	16.2	12.3	3.2	61.3	26.2	12.5	Sandy loam

Tab. 7 - Percentages of sand, silt and clay of each composite sample, texture type obtained from the United States Department of Agriculture (USDA) soil texture triangle and associated labels.

Chemical parameter	FE1		FE2		RL1		RL2	
	2021	2023	2021	2023	2021	2023	2021	2023
pH	8.00	8.02	7.80	8.01	8.04	8.17	8.05	8.26
Available P (mg/kg)	24.4	17	18.4	15.4	23.1	13.9	40	13.8
Exchangeable K (mg/kg)	123	83	77	86	126	83	314	228
CN ratio	6.7	7.5	6.9	6.9	6.8	7.5	7.3	8.5
SOM (g/kg)	18.6	16.7	14.2	17.3	14.1	13.6	17.6	10.2
TN (g/kg)	1.6	1.3	1.2	1.5	1.2	1.1	1.4	0.7
CEC (meq/100g)	18.1	14.6	9.6	13.5	12.2	14.9	13.5	13.5
EC (dS/m)	0.983	1.334	2.425	1.824	4.588	2.473	1.108	0.523

TOC (g/kg)	10.8	9.69	8.2	10.0	8.2	7.9	10.2	5.9
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Tab. 8 - Soil chemical parameters in the four sites in the two sampling periods.

6.4.6 Weather and soil environmental factors

The thermo-pluviometric diagram showed that the weather conditions measured in autumn were similar in the three monitored years (**Fig. S3**). During autumn, a constant relationship between precipitation and temperature was observed, with a consistent water deficit in October. In contrast, the weather conditions measured in spring differed among years. The springs 2021 and 2022 were characterised by dry, water-stressed conditions. In contrast, the spring 2023 was characterised by high precipitation, with rainfall exceeding that of previous years. Notably, RL1 was flooded in the spring period, prior to soil sampling activity. The climatic data for the three years show an upward trend in average temperature and precipitation, which may have influenced the biological component of the soil.

The months with the highest humidity levels were November, followed by December and October. Conversely, the lowest humidity levels were observed in the summer, specifically in July, followed by August, March, and June. Considering the months when sampling occurred, October tended to be more humid than June in all years except for 2023. For the months preceding the sampling, August was extremely dry, while September showed a gradual increase in relative humidity across all years analysed. April and May were dry in both 2021 and 2022, but in 2023, relative humidity levels were higher for both months (**Fig. S4**).

The soil environmental factors, measured with probes at the various sites at the sampling time, showed that the average soil moisture in spring was 16.9%, while in autumn 16.4%. The average soil temperature was 23.7°C in spring and 19.5°C in autumn. The highest average soil moisture was found in spring 2023 (28.3%), and the lowest in spring 2021 (10.5%). The highest average soil temperature was registered in spring 2022 (26.8 °C), while the lowest in autumn 2023 (18.2°C) (**Tab. 2**).

6.4.7 Landscape

The Sentina Reserve could be described as a tiny complex mosaic of different land-use and land-cover types located in a highly anthropized setting. The sites analysed in the study were located in two different contexts within the Reserve (**Fig. 1**). The Fattoria Ferri sites were in the heart of the Reserve, within the most agricultural area. In both sites (FE1 and FE2), the landscape was uniform and predominantly characterised by arable land (**Fig. 2; Tab. 4**). While the percentages of other land-use and land-cover types were very reduced. On the contrary, the area surrounding Selva Giurata sites was more anthropized, but also more heterogeneous (**Fig. 2; Tab. 4**). In fact, RL2 was situated in the most diverse area, while FE1 was in the most uniform. (**Fig. 1**).

Arable land represented the largest percentage of land-use and land-cover types in all sites (**Tab. 4**). In FE1 it represented 80%, in FE2 76%, in RL1 43% and in RL2 37%. The presence of continuous buildings characterized all the sites, but it was particularly high in RL1 (34%). RL1 and RL2 were characterized by a high percentage of olive groves, 8.4% and 7.3%, respectively. While lower percentages were recorded in FE1 (1.5%) and FE2 (4%). Notably, higher percentages of olive groves and grassland (8.2%), psammophilous vegetation and halophytes (7.2%), wetlands and water bodies were found in RL2 (**Tab. 2; Fig. 4**). The presence of salt marsh vegetation was similar in FE1 and RL2 (about 4%), while it was slightly lower in FE2 (2.3%) and absent in RL1. In RL1 there was no presence of a pond or small lakes, compared to the other sites. Only in FE1 there was a very small percentage of riparian woodland, and no vineyards were present compared to the other sites, even if with very low percentages. In FE2 no watercourses were present, and the higher percentage of gardens and parks was found compared to the other sites - even if very low (0.5%). The percentage of paved and unpaved roads was similar among sites (about 2.5% and 1.5%, respectively). The percentage of scattered buildings (4%), hedges, and shrubs (1.3%) was slightly higher in RL2. This site was the only one where the neighbouring landscape recorded the presence of psammophilous vegetation (0.7%), sea (6.5%), and shoreline (2.6%).

6.4.8 Multiple linear regression (MLR)

The influence of the various sets of variables (agricultural practices, soil chemical parameters, soil textural properties, soil environmental factors, weather and landscape) on the QBS-ar index was analysed using a multiple linear regression (MLR) model (**Tab. S1, Tab. 9**). Statistically significant results were found for all the sets of variables tested. The total variance of the QBS-ar index was explained very reasonably by soil chemical variables (R^2 : 0.654, p -value: < 0.0001) and K was the only parameter to have a significant influence on the QBS-ar (p -value: 0.035) (**Tab. 9**). Notably, agricultural practices applied were able to explain a good percentage of the total variance of the QBS-ar index (R^2 : 0.30, p -value: 0.001). Tillage (p -value: 0.020) and harvesting (p -value: 0.002) were the variables which appeared to have a significant influence on the index (**Tab. 9**).

Landscape variables (R^2 : 0.205, p -value: < 0.0001) explained a modest portion of the variance. The QBS-ar index values seemed to be significantly influenced by the percentage of agricultural areas (p -value < 0.020), and forest and semi-natural areas (p -value: < 0.018) (**Tab. 9**). Similarly, soil textural properties (R^2 : 0.210, p -value: < 0.001) explained a similar portion of the variance. Only the content of very fine sand particles resulted to have a significant influence on the index (p -value: 0.004). To conclude, the total variance of the QBS-ar index was not well explained by the weather in the MLR model (R^2 : 0.111, p -value: 0.044), nor by the soil environmental factors (R^2 : 0.102, p -value: 0.0243). However, relative humidity (p -value: 0.006) and temperature (p -value: 0.013) appeared to have a greater influence on the QBS-ar compared to precipitation, as soil moisture (p -value: 0.007) compared to soil temperature (**Tab. 9**).

Weather	Landscape	Agricultural practices	Soil chemical properties	Soil textural properties	Soil environmental factors
Temperature (°C), <i>p-value</i> = 0.012761	Agricultural areas (%), <i>p-value</i> = 0.0207	Tillage for seed bed preparation, <i>p-value</i> = 0.02088	Total nitrogen (TN) (g/kg), <i>p-value</i> = 0.090472	Coarse sand (g/kg), <i>p-value</i> = 0.42722	Soil moisture (%), <i>p-value</i> = 0.00675
Relative humidity (%), <i>p-value</i> = 0.006408	Forest and semi natural areas (%), <i>p-value</i> = 0.0183	Harvesting, <i>p-value</i> = 0.00228	Exchangeable potassium (mg/kg), <i>p-value</i> = 0.000379	Very fine sand (g/kg), <i>p-value</i> = 0.00443	Soil temperature (°C), <i>p-value</i> = 0.55826
Precipitation (mm), <i>p-value</i> = 0.73889	Residual standard error: 30.47 on 69 degrees of freedom Multiple R ² : 0.2046 , Adjusted R ² : 0.1816 F-statistic: 8.875 on 2 and 69 DF, <i>p-value</i> : 0.0003714	Irrigation, <i>p-value</i> = 0.50490 Sowing, <i>p-value</i> = 0.08176 False sowing, <i>p-value</i> = 0.78390 Use of herbicides and fertilizers, <i>p-value</i> = 0.47282 Shredding and burial of crop residues, <i>p-value</i> = 0.20223 Residual standard error: 29.78 on 64 degrees of freedom Multiple R ² : 0.2954 , Adjusted R ² : 0.2184 F-statistic: 3.833 on 7 and 64 DF, <i>p-value</i> : 0.001542	Residual standard error: 19.01 on 21 degrees of freedom Multiple R ² : 0.5415 , Adjusted R ² : 0.4979 F-statistic: 12.4 on 2 and 21 DF, <i>p-value</i> : 0.0002778	Residual standard error: 30.36 on 69 degrees of freedom Multiple R ² : 0.2105 , Adjusted R ² : 0.1876 F-statistic: 9.198 on 2 and 69 DF, <i>p-value</i> : 0.0002877	Residual standard error: 32.37 on 69 degrees of freedom Multiple R ² : 0.1021 , Adjusted R ² : 0.07608 F-statistic: 3.923 on 2 and 69 DF, <i>p-value</i> : 0.02433
Residual standard error: 32.44 on 68 degrees of freedom Multiple: 0.1112 , Adjusted R ² : 0.07201 F-statistic: 2.837 on 3 and 68 DF, <i>p-value</i> : 0.04447					

Tab. 9 - The table shows the results of the multiple linear regression (MLR) applied in the study. When the number of variables to be tested was significant in relation to the number of observations, to exclude multicollinearity, the selection of variables was performed using a stepwise regression by backward elimination based on AIC.

6.5 DISCUSSION

In general, the QBS-ar values registered in the Sentina Reserve showed a very high soil quality, exceeding the threshold set for high biological quality in agricultural soils (93.7) (Parisi 2001; Menta et al., 2018a). In fact, the average total QBS-ar values recorded over the three years was 162 in spring and 155 in autumn (**Tab. 5**).

6.5.1 The influence of agricultural practices on soil microarthropods biotic parameters

In FE2 (Fattoria Ferri) the most intensive management was applied and the lowest values for the QBS-ar index and the biotic parameters were shown. This site experienced the shortest fallow period with the highest use of herbicides, as it is the only site that included two different crops in succession (leek after durum wheat), with the highest number of agricultural practices applied.

Moreover, the crop rotation in both Fattoria Ferri sites, FE1 and FE2, did not include any nitrogen-fixing or cover crops, and medium tillage (35 cm) followed by post-tillage harrowing (< 15 cm) was used (**Tab.3, Fig. S1**).

On the contrary, in both Selva Giurata sites, RL1 and RL2, nitrogen-fixing or cover crops and shallow tillage (< 15 cm) were used more frequently. Notably, in RL2 all the biotic parameters (QBS-ar, soil microarthropod abundance, number of BFs and EFs) were markedly higher throughout the study, particularly in 2021 and 2023.

Concerning the QBS-ar index, the soil microarthropod abundance and the number of BFs, the best performance occurred in RL2 in spring 2021. This outcome could be attributable to a higher level of macronutrients in the soil, particularly K, as detected by chemical analysis. In 2021 more fertilisations occurred at this site compared to the others (**Tab. 3, 8**), with the aim of promoting the growth of soft wheat, which requires more inputs. Di Giovanni et al. (2024) reported that fertilization can significantly affect the biotic components of soil. However, the specific response can vary widely based on the type of fertilizer used, the fertilization regime implemented, and the functional group being considered. The study also found that the impact of fertilization is influenced by climate and soil characteristics.

Only in 2022 there was an exception; the performance of RL1 was better than RL2 which we probably attribute to the beneficial effect of green manure and false sowing practices. Further, the high K content could be probably related to fertilization, which occurred more frequently in RL2 compared to the other sites in 2021. Moreover, the higher K content could result from crop rotation, as this practice may have contributed to better soil nutrient management, by improving soil structure which facilitates the uptake of nutrients (Xing et al., 2025). In fact, RL2 was the only field in which chickpea was cultivated in 2019. Although this leguminous plant does not absorb large amounts of K, its biomass contains potassium, which can be returned to the soil during the decomposition process. Although in RL1, several soil sustainable practices were implemented - such as false sowing, use of horseradish and field beans for green manuring, set-aside practices

(Ecological Focus Area), and a reduced use of herbicides and fertilizers - its performance was generally lower than that of RL2 (**Tab. 3, Fig. 3, 4**).

In autumn 2022, when deep tillage (50 cm) followed by post-tillage harrowing (< 15 cm) was applied, there was a significant decrease in the biotic parameters, in both RL1 and RL2 (**Tab.3; Fig. 3, 4, S1**).

Particularly, during the autumn samplings, in RL1 we observed a significantly altered soil condition compared to the other sites. In 2021, there was no harvesting (which took place in FE1 and RL2), but burial of crop residues occurred. In autumn 2022, unlike FE1 and FE2 deep ploughing followed by post-tillage harrowing was performed, as in RL2. In spring 2023, RL1 was subjected to waterlogging caused by heavy rainfall. In autumn 2023, harvesting occurred at all sites, but in RL1 it was also followed by burial crop residues, shortly before sampling. For this reason, we believe that the time of sampling likely influenced the performance of RL1. In fact, the MLR model indicates that harvesting activities impacted the index, as much as tillage for seedbed preparation activities.

To sum up, our initial hypothesis was confirmed: the QBS-ar and all the biotic parameters were consistently higher in all years and seasons in Selva Giurata compared to Fattoria Ferri. During this three-year analysis, the adoption of less intensive agricultural practices appeared to positively affect edaphic biodiversity and increase soil quality. Similar results were found in previous studies, which emphasised that a lower intensity of applied agricultural practices led to higher QBS-ar index values (Pittarello et al., 2022; Fiorini et al., 2020; Gonçalves et al., 2021; Sapkota et al., 2012; Parisi & Menta, 2008; Parisi, 2001).

According to our results, among the variables related to farming practices, harvesting and tillage were the most influential factors affecting the QBS-ar index (**Tab. 9**). Additionally, the intensity of the agricultural practices emerged as the second most influential factor affecting the soil arthropod community.

6.5.2 The influence of the soil chemical and textural properties on soil microarthropods biotic parameters

The chemical and textural composition of the soil was similar across the four sites and the two farms analyzed. The soil chemical parameters explained a significant portion of the total variance of the QBS-ar index (**Tab. 9**). Additionally, K was the only parameter that seemed to significantly affect the index and exhibited the greatest variation between the sites, being notably higher in RL2 in both years (**Tab. 8**). In line with this, the best performance of all the biotic parameters reported in RL2 in 2023, and particularly in 2021 (**Fig. 3, 4**) might be attributable to the optimal level of K in the soil. Contrary to Mantoni et al. (2021), in the present study, K seemed to have a positive and significant influence on the QBS-ar index. A positive correlation between K content and some soil fauna groups, including Hemiptera Thysanoptera, Formicidae, Diptera, Coleoptera, Blattodea, and especially Collembola was found in previous studies (Fiera et al., 2020; Inkotte et al., 2024). Accordingly, a positive correlation between K and QBS-ar values suggests that this macronutrient can stimulate an ecological network that supports microarthropod presence and diversity in the soil. In fact, optimal K levels promote plant growth by increasing biomass production and providing food sources for decomposers. Additionally, a balanced K content in the soil can positively influence nutrient availability in plants by improving the quality of plant debris, creating a favourable environment for the entire soil food chain, including microarthropods.

The nutrient content (N, P, K) was in general consistent regardless of the intensity of the agricultural practices employed by the two farms (**Tab. 8**). Specifically, there were no significant variations in N levels between the four sites and the two farms between 2021 and 2023. Regarding P content, it was particularly high in RL2 in 2021. This outcome may have contributed positively to the presence of microarthropods that year. In line with this, Ghiglieno et al. (2023) found a positive association between P and the QBS-ar index, although it was not statistically significant. Conversely, Mantoni et al. (2021) found a significant negative relationship between these variables.

We assume that the levels of these nutrients (P and K) might be related to differences in fertilization and crop rotation at site level. In fact, fertilization occurred more frequently in RL2 in

2022 compared to the other sites (**Tab.3, 8**). Similarly, RL2 applied the more varied crop rotation in the previous years, including chickpea. However, unfortunately, due to limited information about the fertilizers used and crop data from previous years, we were unable to draw any specific conclusions.

On the contrary, soil textural properties explained a reduced portion of the variance (**Tab 9**). Only very fine sand particle content seemed to significantly influence the QBS-ar index (**Tab. 7; Fig. S2**). This outcome may be reliable because various studies reported the influence of soil texture on the activity and life cycle of soil mesofauna (Ruf & Beck, 2005; Wolfarth et al., 2013; van Capelle et al., 2012; Domene et al., 2011; Vanolli et al., 2024; Leone et al., 2023). Accordingly, we have evidence that soil texture can significantly influence the distribution of soil fauna (Coletta Marina, Innangi Michele, Marinsalti Silvia, Kumar Santosh, Bharti Daizy, Insom Emilio, Ciabocco Giovanni, Tiberi Mauro, La Terza Antonietta, manuscript in preparation). However, this effect may not have been fully captured in our study due to limited data and soil textural type, which was very similar among the sites considered. A larger number of measurements could have enriched our dataset and enhanced the discriminatory power of the model we used.

6.5.3 **Soil microarthropods response to different weather conditions and soil environmental factors over the years and seasons**

Our hypothesis regarding the sensitivity of soil mesofauna to soil moisture - and thus its dependence on seasonal weather conditions - was not fulfilled. Spring 2023 was the season with the most rainfall (**Fig. S3**). In fact, due to the heavy rain in May 2023, RL1 was flooded prior to spring sampling. This extreme weather condition did not exert a positive effect on the QBS-ar index and the other biotic parameters, probably due to the onset of anoxia conditions. In fact, according to Parisi et al. (2005), soil samples should be collected when the moisture level is between 40% and 80% of the field capacity.

However, in autumn 2023, after the heavy rain, significantly higher values for QBS-ar and BFs were registered compared to the previous autumns (2021 and 2022) (**Fig. 3A, 3C; Tab. 5**). In fact, the 2023 autumn sampling was not affected by a dry summer, with only one dry month. In

contrast, both autumn 2021 and 2022 suffered from a prolonged dry summer, which lasted an average of three months.

Regarding spring sampling, the 2021 showed the best performance for arthropod abundance, BFs and EFs were significantly higher compared to spring 2022 - very dry - and 2023 - extremely rainy (**Fig. 3C, 3D, 4C,4D; Tab. 6**). Similarly, the rainfall in late winter 2020 and in the beginning of 2021 probably had a positive effect. Nonetheless, when comparing QBS-ar values, soil microarthropod abundance and number of EFs among the two seasons (spring vs autumn) no statistically significant differences were found. Only the number of BFs resulted significantly higher in spring than in autumn (**Fig. 3C**). Furthermore, we can assume that the general reduction in all biotic parameters observed in 2022 (**Fig. 3, 4; Tab. 6**) could be attributable to the weather condition. This observation might be supported by the prolonged period of water stress that occurred in that year (**Fig. S3**).

Considering the soil environmental properties, an association was found between QBS-ar values and the environmental soil data collected at the sampling point. The examination of the seasonally averaged total QBS-ar data showed that increasing soil moisture and decreasing soil temperature led to an increase in total QBS-ar values. When examining the autumn mean partial QBS-ar data, the same trend also emerged. However, this assumption was not valid for spring mean partial QBS-ar data, where no clear pattern emerged. When analysing the mean partial QBS-ar data collected at the individual site, an indirect relationship between temperature and soil moisture was frequently observed, but not always a correlation with QBS-ar values.

Summarizing, the weather data and the soil environmental parameters appeared to be the worst predictors to assess QBS-ar index variance in the present study (**Tab. 9**). In any case, we consider them to be important variables to be included in the assessment of soil health based on biotic indicators. Extreme weather events are increasingly frequent, thus monitoring the effects of significant environmental changes on edaphic communities over time therefore is becoming crucial (Gallese et al., 2025; Lakshmi et al., 2021). At this scope, we suggest gathering direct measurements of soil moisture and temperature by using appropriate probes. The same

instrument we used to measure soil moisture was employed in soils with similar texture in Cordero-Vázquez et al. (2021). Compared to that study, which was carried out during a dry season in Mexico in a non-irrigated soil, we observed a wider range of soil moisture values (**Tab. 2**). Nonetheless, we found it important to take several measurements (no less than three) at each sampling point to obtain a more reliable average value.

6.5.4 The influence of landscape on soil microarthropods biotic parameters

The landscape surrounding Fattoria Ferri sites was uniform and predominantly characterized by arable land (**Fig. 1**). The landscape in FE2 was slightly more heterogeneous than FE1 (**Fig. 2; Tab. 4**). Nevertheless, the average values of total and partial QBS-ar were lower in FE2 than in FE1 (**Fig. 4A; Tab. 5**). In contrast, the landscape surrounding Selva Giurata sites was more heterogeneous, even if a higher percentage of artificial buildings was present particularly in RL1. We might suppose that the presence of this more anthropic environment may have reduced the presence of possible reservoirs for soil biodiversity (Mantoni et al., 2021; Franin et al., 2016; Pérez-Bote & Romero, 2012). According to Querner et al. (2018), soil arthropods can move from one habitat to another due to active migration from bordering hedges, forests, or grasslands at small scales (250–1000 m), and due to passive wind dispersal at larger scales (1000–1750 m). Thus, land use, landscape structure and diversity are considered good predictors of mesofauna diversity.

In fact, although more sustainable agricultural practices were applied in RL1, the overall quality of the site was lower compared to RL2. Indeed, RL2 was characterised by the highest percentages of grasslands, halophytes, hedges and shrubs, shoreline, psammophilous vegetation, ponds and lakes compared to the other sites (**Fig. 2, Tab. 4**). Nonetheless, it is in the protection zone of the Reserve while the other sites were in the economic and social promotion zone, where human activities predominate (**Fig. 1**). According to this, we might assume that the QBS-ar was positively influenced by a more heterogeneous landscape, compared to a more homogeneous one. We must highlight that this hypothesis supports the results obtained from the comparison of the Selva Giurata sites, but it did not apply to the comparison of the Fattoria Ferri

sites. In fact, although the landscape in FE2 was somewhat more heterogeneous than FE1, it was still significantly less varied than that of RL2. As a result, the lower percentage of natural elements in FE2 may not have provided the same benefits for soil fauna as observed in RL2. Thus, we can assume that our assumption was partially met.

To conclude, the landscape variables resulted in explaining a small portion of the variance of the QBS-ar in the MLR model (**Tab. 9**). As in the previous case, we faced limitations in the data available for analysing the various land use and land cover types that could help us evaluate the impact of landscape on the index. To simplify our analysis, we grouped these variables into four broad categories (**Tab. S1**). This approach may have led to an underestimation of the landscape's influence on QBS-ar. While the generalizability of the present results might be limited and specific to the investigated area, the effect of the landscape on soil mesofauna has been analysed using detailed, high-resolution cartography at a very small local scale, which represents a key innovative aspect of this study. Nonetheless, we believe that further investigation is required to fully understand the beneficial role of natural elements in this type of agroecosystem, particularly when considering protected areas.

6.5.5 Overall influence of the various variables

Results indicated that multiple variables - including agricultural practices, soil chemical parameters, soil textural parameters, soil environmental factors, weather, landscape - may have exerted an influence on the QBS-ar index in the four sites and in two farms considered in the study. This influence was acceptably, but not entirely, explained by the MLR model adopted. Although data collection took place over three years, the sample size appeared to be small, and this limitation may have led to an underestimation of the variability of the data for most of the set of variables tested.

Although we considered agricultural practices to be the most impactful factor among the ones tested, they emerged as the second driver affecting the QBS-ar index. Nevertheless, we hypothesize that their influence has been underestimated. To accurately measure the impact of agricultural practices, it may be necessary to provide further and even more detailed agricultural

practices data (e.i; type and amount of fertilizers and herbicides used, detailed information on the agricultural equipment employed, etc.). In this sense, we emphasize that the study was not conducted in an experimental context, and one of the most challenging aspects was the interaction with farmers to collect information.

On the contrary, the soil chemical data emerged as the most influential factor, accounting for approximately 54% of the variation for the QBS-ar index (Tab. 9). Among the chemical parameters, K was the most influential. As the content of this macronutrient could be linked to fertilization and crop rotation, this finding may suggest that agricultural practices could have a greater influence on the QBS-ar index, and that its effect may still be overlooked.

The landscape and the soil textural properties accounted for a small portion of the variation in QBS-ar index, while the weather data proved to be the least effective predictor in this study (**Tab. 9**). We believe these factors are important and warrant further investigation, as many studies indicate their influence on soil mesofauna (Vanolli et al., 2024; Leone et al., 2023; Mantoni et al., 2021; Querner et al., 2018; Franin et al., 2016; Wolfarth et al., 2013; Pérez-Bote & Romero, 2012; van Capelle et al., 2012; Domene et al., 2011; Ruf & Beck, 2005):

6.6 CONCLUSION

The promotion of sustainable soil management is a key target of the Biodiversity Strategy 2030, adopted by the European Commission in 2020, and is an essential component of the European Green Deal. We believe that sustainable farming practices are effective in preserving soil biodiversity and overall soil health. However, measuring the impact of the agricultural-related disturbance across various factors - such as agricultural practices, soil chemical parameters, soil textural parameters, soil environmental factors, weather, and landscape - on soil microarthropods, specifically on the QBS-ar index and other biotic parameters has proven to be challenging in the present study.

To further investigate the interactions among these factors and their impact on the QBS-ar index, we consider it necessary to gather extensive metadata on multiple variables and develop robust models that can integrate different sets of data. While we consider the chemical-physical, meteorological and environmental variables easier to assess, the variables related to agricultural practices were more complex to establish. Nonetheless, we find the incorporation of landscape-related variables innovative and noteworthy, especially when applied in land-protected contexts. In fact, the location within a protected area likely aided in the recovery of soil fauna in agricultural ecosystems following soil disturbances. Although weather, soil environmental and landscape variables were not predominant in influencing the QBS-ar index in the present study, we believe that these factors should be considered when applying the index. This consideration could help determine the optimal time for sampling (i.e, soil moisture, proximity to intense rainfall or agricultural activities), enhancing its effectiveness in predicting soil quality. Additionally, establishing specific soil quality thresholds for the different areas analysed will be important for improving the index.

We hope to see the QBS-ar index adopted more widely, as we consider it a reliable tool for predicting soil health through the assessment of edaphic biodiversity. Preserving soil biodiversity is essential due to its intrinsic value and the vital ecosystem services it provides. Therefore, it is important to implement biodiversity-friendly practices and monitor soil conditions over time, rather than relying solely on a one-time survey. This approach will allow for timely interventions and help to prevent the deterioration of soil health.

The present study is currently under revision: Coletta M., Torresi A., D'Alessandro A., Monticelli M., Bharti D., Kumar S., Chelli S., La Terza A. How do soil characteristics, agricultural practices, weather and landscape affect soil microarthropods in the agroecosystem? Insights from three years of monitoring in arable lands within the Sentina Reserve (Italy). Manuscript resubmitted to *Applied Soil Ecology*. Link to preprint: <http://dx.doi.org/10.2139/ssrn.5160488>

7 CHAPTER 4: OTHER SIDE PROJECTS

In the projects described below, the arthropod-based soil biological quality index (QBS-ar) was often used in conjunction with other indicators to obtain a better understanding of the soil health condition. Several indicators based on soil microarthropods have been used, including abundance, density (ind/m² and ind/m³), community composition, number of biological (BFs) and euedaphic forms (EFs), Acari/Collembola (A/C) ratio (Bachelier, 1986), percentage of Oribatid mites out of total mites (OA%) (Aoki, 1967; Aoki et al., 1977). Nonetheless, the functionality of the index has been compared to indices based on specific groups, such as Collembol, including Collembola morphological traits and QBS-c (Parisi, 2001) or other organisms, such as earthworms (QBS-e) or soil and rhizosphere bacteria (16S), fungi (ITS) and protists (18S) communities, or ecotoxicological test, such avoidance test with earthworms (ISO Standard 17512-1:2008) or bait lamina test (ISO Standard 18311:2016). Overall, the results obtained so far show that it is essential to combine complementary information from different bioindicators at different trophic levels to obtain the broadest possible picture of the health status of the soil ecosystem.

Below is a brief description of each project, its objectives and achievements to date.

The objective of the **S.F.I.D.A.** project (Submeasure 16.1 Action 2" Year 2017 – RDP for Marche 2014/2020, ID 29073) was to assess the environmental impact of the distribution of plant protection products through the implementation of precision farming techniques (PFA) together with an examination of the regulatory issues surrounding the aerial application of plant protection products by drones, considering the EU legal framework. As part of this project, we evaluated and compared the effect of an innovative aerial spraying technology (using a drone) and a conventional ground-based spraying technology (using a Casotti® pump mounted on a tractor) for the application of Spintor® Fly in olive groves. We assumed that the use of a drone would have less impact than the conventional method on soil biodiversity. In this regard, to the best of our knowledge, no study has yet evaluated the impact of the bioinsecticide Spintor® Fly on non-

target soil organisms in a field experiment. Furthermore, no study has evaluated and compared the effects of two different delivery systems of Spintor® Fly on soil fauna in olive groves. The QBS-ar and ecotoxicological tests, including the earthworm avoidance test (ISO Standard 17512-1:2008) and the Bait Lamina Test (BLT) (ISO Standard 18311:2016), were applied in this context.

The present study has been published: D'Alessandro, A.*, Coletta, M.*, Torresi, A., Dell'Ambrogio, G., Renaud, M., Ferrari, B. J., & La Terza, A. (2024). Evaluation of the Impact of Plant Protection Products (PPPs) on Non-Target Soil Organisms in the Olive Orchard: Drone (Aerial) Spraying vs. Tractor (Ground) Spraying. *Sustainability*, 16(24), 11302. <https://doi.org/10.3390/su162411302>

OrtoBioStrip (Submeasure 16.1 Action 2" Year 2021 – RDP for Marche 2014/2020, ID 59666, <https://www.arca.bio/en/funded-projects/ortobiostrip/>) aimed to diversify the agricultural system by implementing strip cropping, where different crop species are alternated. In addition, it seeks to assess the role of agroecological service crops and soil management practices that minimize mechanical interventions. The goals are to increase productivity and resilience within the production system, improve soil conditions by protecting it from erosion, and contribute to enriching the landscape. Specifically, we evaluated and compared the effects of strip cropping on soil health against traditional pure stand cultivation over a complete crop rotation cycle of three years, using earthworms (**QBS-erthworm**) and soil arthropods (**QBS-ar**) as indicators.

In the **MIMBT** project (Monitoraggio Integrato Multi-Taxon della Biodiversità presso Montagna di Torricchio Riserva Naturale Integrale dello Stato) we performed a biodiversity investigation applying a multi-taxon approach in the beech forest ecosystem of the Torricchio strict Nature Reserve, part of the International Long Term Ecological Research Network (ILTER). There, 9 permanent plots were sampled from April to October 2024 applying standardised protocols for seven taxa: vascular plants, bryophytes, lichens, saproxylic insects, earthworms, soil arthropods, and fungi. Data on canopy cover and forest structure, and climatic variables were also collected. The activities were carried out in the 52 hectares of habitat 9210 of the Natura 2000 site. The project aimed to: (i) infer patterns and drivers of multi-taxon diversity; (ii) investigate relationships

among the different taxa and between species richness and structural indicators of forests; iii) test new approaches and schemes of biodiversity investigations; (v) obtain a checklist of species, also detecting the occurrences of threatened species for conservation purposes. Within WP2, data on soil microarthropods and earthworms were collected. In this regard, it must be emphasised that in Italy, although earthworms are frequently used as indicators of the biological quality of soils in particular agroecosystems, only very few studies have examined their community structure in natural environments such as forests. Therefore, the activities of WP2 aimed to fill this knowledge gap and may contribute to provide more information on their functional diversity, density and biomass in forests. At the end of the project, we hope to update the Italian earthworm checklist.

In these previous projects, the QBS-ar index was used in conjunction with the study of earthworms, using taxonomic approaches, and using a system for assessing the sustainability of agroecosystem management practices based on earthworms as bio-indicators of soil biodiversity (QBS-e) (Paoletti et al., 2013). In the following two projects the QBS-ar index was applied to assess soil quality considering other food production sectors, including wine and acorns.

In **B.I.O.C.E.R.T.O.** (Submeasure 16.1 Action 2" Year 2017 – RDP for Marche 2014/2020, ID 41377, <https://www.biocerto.it/>) the QBS-ar index, along with rheological and ultrasonic techniques, were integrated with blockchain technology with the aim of ensuring the origin, safety, quality, and the unique characteristics of Marche Region wines for the final consumers by using novel techniques (i.e. ultrasonic spectroscopy), and further to certify the use of sustainable agricultural practices in vineyards, promoting soil biodiversity conservation by using the QBS-ar Index.

- a) Perinelli, D.R.; Coletta, M.; Sabbatini, B.; D'Alessandro, A.; Fabiani, F.; Passacantando, A.; Bonacucina, G.; La Terza, A. A.A.A.Good Wines WANTED: Blockchain, Non-Destructive Ultrasonic Techniques and Soil Health Assessment for Wine Traceability. *Sensors* 2025, 25, 3567. <https://doi.org/10.3390/s25113567>

In the **BioAcornScape** project (Submeasure 16.1 Action 2" Year 2021 – RDP for Marche 2014/2020, ID 59711, <https://www.impresaverdemarche.it/progetto-59711.it>) the aim was that of

bringing back into the limelight a forgotten food resource, represented by oak acorns and derived foodstuffs (flour, bread, biscuits, etc.), to restore the related technical-productive know-how and to re-create a high added-value agri-food chain in the Alto Maceratese area (Marche, Italy). In the project we described the soil ecological aspects, using soil arthropods, earthworms, soil bacteria and several abiotic variables, to promote the revalorization of the wooded crop field with oaks, an ancient agricultural landscape element typical of the Marche region.

In other side projects, presented in the following list, other indicators were applied, such as rhizospheric bacterial (16S) and fungal communities (ITS) and protists (18S), that were analysed by eDNA metabarcoding approaches and crop traits (lettuce and wheat).

National Projects and Networks and related publications

1. **ABRIOPACK** - Submeasure 16.1 Action 2" Year 2017 – RDP for Marche 2014/2020, ID 29057, <https://www.arca.bio/en/abriopack/>
2. **SISTEMA** – La cura ambientale come leva di sviluppo sostenibile, Parco del Conero (Sirolo, AN), Fondazione Cariverona
3. Silverskin and spent coffee: two byproducts of the coffee production chain as promising source of nutraceuticals and ingredients for fertilizing products – Fondo di Ateneo per la Ricerca (FAR) 2019 – Università di Camerino
 - a. Angeloni S., Caprioli G., Cespi M., Acquaticci L., Mustafa A.M., Santanatoglia A., D'Alessandro A., **Coletta M.**, Waris N., La Terza, A. (2024) An Innovative Formulation to Improve Spent Coffee Characteristics as Soil Fertilizer: Nutrient, Chemical Characterization and Effects on Plant Germination. *Biocatalysis and Agricultural Biotechnology*, Volume 61, 103394. <https://doi.org/10.1016/j.bcab.2024.103394>

International Projects and Networks and related publications

1. **ERA-NETs - SUSFOOD2 and FOSC Joint Call 2021 “Innovative solutions for resilient, climate-smart and sustainable food systems” ProgettoAlgaeBrew** | <https://www.ucd.ie/algaebrew/>
 - a. Brooke F., Quinge Ma., Rahimi M., Constantin C., Atilganturkmen B., Silkin A., La Terza A. ... Halim R., The Microalgae *Nannochloropsis* for the Bioremediation of Brewery Side Streams and Co-production of Aquaculture Feed and Bio-Fertiliser: A Comprehensive Review. Manuscript submitted to *Algal Research*.

2. The **GRAPEVINE MICROBIOME** Survey
 - a. Aguiar J.F., Liberal I.M., Muñoz M.L., Cantos-Villar E., Puertas M.B., Archidona-Yuste A., Casimiro-Soriguer A., **Coletta M.**, D'Alessandro A., Gonzáles-Robles A., Gramaje D., Renard D., Roy M., La Terza A., Tarifa R., Vacher C., Veen C., Ochoa-Hueso R. (2025) Development of whole-soil microbial inoculants based on solid-phase fermentation for the regeneration of the functioning of vineyard soils. *Rhizosphere*, 101039 <https://doi.org/10.1016/j.rhisph.2025.101039>
3. The **CROP MICROBIOME** Survey <https://www.globalsustainableagriculture.org/crop-microbiome-survey/>
4. The **GRASS4FUN** Survey <https://www.biodiversa.eu/2024/04/15/grass4fun/>
5. The **EVOLVEBIOME** Survey <https://www.fbbva.es/en/evolbiome-2/>

8 FINAL DISCUSSION

Soil mesofauna plays a crucial role in soil functioning and is highly sensitive to disturbances and agricultural management practices, making it an important indicator of habitat quality (Arboláez et al., 2023). Soil microarthropods are regarded as cost-effective, promising, and reliable indicators of soil health, which has led to their increasing use in biological soil quality assessments and monitoring programs. The QBS-ar index, developed by Professor Vittorio Parisi in Italy in the early 2000s, assesses soil quality based on soil arthropods (Parisi, 2001). After over 20 years of application, this method is now widely implemented in Italy and is beginning to gain traction outside the country (Menta et al., 2018). Notably, last year, the Standard Operating Procedures (SOPs) for the QBS-ar index were discussed at the FAO network Global Soil Laboratory Network (GLOSOLAN) (Gallese et al., 2025). Currently, efforts are underway to standardize the methodology and expand the use of the index globally.

In this thesis, the QBS-ar index was applied to three local-scale projects conducted in the Marche region. The index was utilized for different purposes in each project. The primary objective of the thesis was to explore various aspects related to the applicability of the QBS-ar index, aiming to enhance understanding and highlight additional factors to consider when using it. Below are the results obtained, and the reflections developed in the various projects.

In the first project, for the first time, an attempt was made to examine whether the QBS-ar index (and soil biodiversity in general) could serve as an indicator of crop and food quality. Interesting results emerged, revealing that most sites had high soil quality, particularly those monitored in 2022. Both chemical and physical characteristics of the soil were found to significantly influence the QBS-ar index, along with agricultural practices and landscape features. Strong correlations were observed between soil and plant quality, encompassing both morphological and functional traits, as well as the composition of rhizosphere microbial communities. The study shed light on the relationship between soil and food, with interesting findings related to the chemical and nutritional characteristics of chickpea seeds, including fibre, carbohydrate content, and polyphenols. These components are produced by the plant under stress conditions (Alves et al.,

2024; Jha et al., 2024), and high levels were noted in sites where soil quality was poorer compared to other sites, suggesting a concrete association between these two components. However, several critical issues emerged during the project's course that led to a significant reduction in the amount of data available for analysis. However, the innovation of the project can be largely attributed to the fact that the final product (legume pasta) was traced back to its original source. This aspect is significant and was achieved by focusing on a local spatial scale. Nonetheless, we are aware that further investigation and additional data are needed to confirm our findings. However, the relationship between soil and food has been investigated with experimental evidence for the first time, revealing interesting correlations to support this link.

In the second project, the QBS-ar index confirmed its potential in being used to study the influence of the agricultural practices applied in different agroecosystems. Since the agronomic practices applied vary, to obtain a reliable evaluation we suggest establishing different soil quality thresholds depending on the system considered. The project demonstrated the variation in both soil quality and the abundance and structure of soil microarthropod communities across the various agroecosystems studied. Interestingly, these two parameters did not yield the same results. For instance, arable crops were associated with higher soil quality, while olive groves exhibited greater abundance and diversity of biological forms. Nonetheless, the community structure was more similar among stable tree systems, such as vineyards and olive groves, compared to arable land. However, both indicators (QBS-ar and soil microarthropod abundance) confirmed that vineyards performed the worst, likely due to the specific management practices they underwent, as there were no significant differences in soil chemical, physical, or climatic characteristics among the monitored sites. In summary, the study underlined the need to develop specific soil quality thresholds for the QBS-ar index, that vary according to the specific agroecosystem considered. In fact, in some publications concerning QBS-ar (Menta et al., 2011), vineyards and olive groves were considered in the same category and with the same valuation scales. We consider that, given the diverse management practices in agroecosystems, it is not feasible to compare them using the same criteria. In this regard, we recommend that in studies or reviews where data is assessed on a national or international scale (Menta et al., 2018), individual

agroecosystems should be considered separately. This is crucial for the use of the index and for the future development of soil quality maps, at least on a national level. It is worth noting that this was precisely Parisi's intention when he established the criteria for the index determination (Parisi, 2001).

In the third study, we focused on the abiotic variables that can influence the QBS-ar index. The results indicated that soil chemical parameters had a significant impact on the QBS-ar index, followed by agricultural practices and landscape features. In contrast, meteorological and environmental parameters had minimal effects. For the first time, we proposed an integrated approach to shed light on the influence of abiotic variables on the index. Typically, only the chemical and physical characteristics of the soil are considered in studies applying the QBS-ar. We believe that additional indicators are necessary to achieve a comprehensive assessment of the factors affecting the soil community. This perspective is supported by recent studies highlighting how the surrounding environment can influence repopulation processes that occur after soil disturbance events (Mantoni et al., 2021; Le Provost et al., 2021; Gallese et al., 2024). We believe that considering multiple variables is crucial in the QBS-ar index standardization process. However, we recommend that, during the monitoring phase for determining the index, more samples should be collected across different seasons, which would involve more frequent sampling over time. The soil quality status indicated by the QBS-ar index provides a snapshot of soil conditions at the specific time of sampling. Our study demonstrates that various variables can influence the arthropod community at different times. By having a larger number of samples collected throughout different seasons, we can obtain a more accurate estimate of the health of the soil.

To conclude, in studies in which the QBS-ar method has been used alongside with other soil biological indices, the findings have generally been consistent. Based on our experience, we recommend an integrated approach to evaluate multiple aspects of soil biodiversity and achieve more conclusive and comprehensive results. Although soil is still not extensively studied, and thus both the potential and importance of soil biodiversity are underestimated,

data on the percentage of soil biodiversity present on our planet have been increased from 25% (Decaëns et al., 2006) to 60% (Anthony et al. 2023). Given the importance of the ecosystem services that soil organisms provide, it is crucial to implement monitoring efforts to gather more information about this essential ecosystem. Among the various soil functions promoted by soil biodiversity, they certainly play a key role in ensuring the supply of food. In this sense, it is crucial to study the interactions both between the various soil organisms and between organisms and plants, which are used to produce food.

In this regard, recent studies have shown that, through direct and indirect connections, soil fauna can influence the dynamics of interactions between plants, microbes and soil environments (Levasseur et al., 2025). To date, the description of the mechanisms involved in these processes remains extremely complex to define. It is therefore recommended to study soil to advance the study of soil connections and ecology in both natural and agricultural ecosystems and always keeping in mind that soil is where food begins (FAO, 2023).

9 FINAL CONCLUSIONS AND FUTURE PERSPECTIVES

In the three projects, soil quality was evaluated using the QBS-ar index. The assessment of soil health has been carried out for several purposes, including determining the relationship between soil quality and crop and food quality, estimating the intensity of applied agricultural practices and evaluating the overall influence of various abiotic variables on the index.

The functionality of the index was confirmed by exploring its applications in different contexts, including various agroecosystems such as arable lands, vineyards and olive groves. We also examined different agricultural management practices, comparing conventional and organic methods, and analysing protected and unprotected sites at a local scale in the Marche region.

Nonetheless, the index was used alongside with other soil quality indicators that focus on specific soil arthropod groups and other soil organisms. The results obtained using the QBS-ar index are generally aligned with those from other indices. This outcome confirmed that microarthropods are particularly sensitive bioindicators. Nonetheless, employing multiple indicators could provide a more comprehensive understanding. We recommend using a combined approach for a fuller picture and a complete soil quality assessment. In conclusion, we believe that this study has enhanced our understanding of the QBS-ar index, providing valuable insights that can inform both theoretical discussions regarding its use and standardization, as well as its practical applications for various purposes.

Looking to the future, we hope that the index will be adopted more widely across the country for soil quality mapping. We also aspire for its global use to increase, allowing other nations to implement it effectively, as it is occurring in Italy. Furthermore, we hope that microarthropods will be recognized as significant indicators within the new soil monitoring law, and that they, along with the QBS-ar index, will be integrated into decision-making processes.

In final, it is hoped that the determination of food quality from soil health will continue to be deepened, with a holistic view always being employed. The use of more indicators will be

attempted, with a view to understanding which of them has a stronger link and can potentially be more significant for its prediction.

10 LIST OF PUBLICATIONS

Peer-reviewed journals publications:

- Perinelli, D.R.*; Coletta, M.*; Sabbatini, B.; D'Alessandro, A.; Fabiani, F.; Passacantando, A.; Bonacucina, G.; La Terza, A. A.A.A. GoodWinesWANTED: Blockchain,Non-Destructive Ultrasonic Techniques and Soil Health Assessment forWine Traceability. *Sensors* 2025, 25, 3567. <https://doi.org/10.3390/s25113567>
- Aguiar J.F., Liberal I.M., Muñoz M.L., Cantos-Villar E., Puertas M.B., Archidona-Yuste A., Casimiro-Soriguer A., Coletta M., D'Alessandro A., Gonz  les-Robles A., Gramaje D., Renard D., Roy M., La Terza A., Tarifa R., Vacher C., Veen C., Ochoa-Hueso R. (2025) Development of whole-soil microbial inoculants based on solid-phase fermentation for the regeneration of the functioning of vineyard soils. *Rhizosphere*, 101039 <https://doi.org/10.1016/j.rhisph.2025.101039>
- Coletta, M., Monticelli, M., D'Alessandro, A., Gentili, C., Torresi, A., Waris, N., La Terza, A. (2025) Managing soil to support soil biodiversity in protected areas agroecosystems. A comparison between arable lands, olive groves, and vineyards in the Conero Park (Italy). *Environmental Monitoring and Assessment*, 197:200 <https://doi.org/10.1007/s10661-025-13658-7>
- D'Alessandro, A.* , Coletta, M.* , Torresi, A., Dell'Ambrogio, G., Renaud, M., Ferrari, B. J., & La Terza, A. (2024). Evaluation of the Impact of Plant Protection Products (PPPs) on Non-Target Soil Organisms in the Olive Orchard: Drone (Aerial) Spraying vs. Tractor (Ground) Spraying. *Sustainability*, 16(24), 11302. <https://doi.org/10.3390/su162411302>
- Angeloni S., Caprioli G., Cespi M., Acquaticci L., Mustafa A.M., Santanatoglia A., D'Alessandro A., Coletta M., Waris N., La Terza., A. (2024) An Innovative Formulation to Improve Spent Coffee Characteristics as Soil Fertilizer: Nutrient, Chemical Characterization and Effects on Plant Germination. *Biocatalysis and Agricultural Biotechnology* Volume 61 103394 <https://doi.org/10.1016/j.bcab.2024.103394>

Submitted munuscripts:

- Coletta M., Torresi A., D'Alessandro A., Monticelli M., Bharti D., Kumar S., Chelli S., La Terza A. Which environmental factors have the greatest influence on the QBS-ar index? Insights from a three-year monitoring in arable lands within the Sentina Reserve (Italy). Manuscript submitted to *Applied Soil Ecology*. <http://dx.doi.org/10.2139/ssrn.5160488>

Manuscripts in preparation:

- Coletta M., Scheu S., Chen T.W., La Terza A. Mini-review of microarthropod bioindicators for measuring soil health using the Soil Biological Quality Indices: QBS-ar and QBS-c. Manuscript in preparation. To be submitted to *Soil Organisms*.
- D'Alessandro A., Coletta M., Sagratini G., Ricci M., La Terza A., Effects of compost made from compostable bioplastic packaging added to organic waste on wheat health and rhizosphere microbiome: A field experiment. Manuscript in preparation. To be submitted to: *Journal of Hazardous Materials*.
- Waris, N., Coletta, M., Acquaticci, L.,Caprioli, G., Cespi, M., Vittadini, E. Silkina, A., Halim, R., La Terza, A. Formulating, characterizing, and assessing the phytotoxic effects of two microalgae-based fertilizers using *Nannochloropsis* sp. and two organic wastes: Brewer's spent grain and Spent coffee grounds. Manuscript in preparation. To be submitted to: *Applied Phycology*.
- Ma, Q., Brooke, F.J., Briantais, D., Serlini, N., Shivakumar, S., Costantin, C.G., Rahimi, M., Emran, M., Curran, W., Waris, N., Coletta, M., La Terza, A., Silkina, A., Halim, R. The enhancement of

Nannochloropsis cultivation by strategizing the microalgal nutritional supply with brewery wastewater: from bench to pilot scale. Manuscript in preparation. To be submitted to: Bioresource Technology.

- Coletta M., D'Alessandro A., Bhuiyan J.A., Suo X., Vittadini E., La Terza A. Soil and legumes of the Sibillini for high quality pasta. Manuscript in preparation.
- Coletta M., Scheu S., Chen T.W., La Terza A. Morphological traits of Collembola within the Sentina Reserve. Manuscript in preparation.

11 ORAL PRESENTATIONS AT NATIONAL AND INTERNATIONAL CONFERENCES

- Coletta M., Torresi A., D'Alessandro A., Monticelli M., La Terza A. 8th workshop of the Working Group on QBS-ar, Role of soil arthropods in assessing soil health. Genova, 25 November 2024.
- Coletta M., Monticelli M., Gentili C., D'Alessandro A., Torresi A., Waris N., La Terza A. The arthropod-based Biological Soil Quality index (QBS-ar): Three-year application in the Sentina Reserve (Italy). 83rd Congress of the Italian Zoological Union and the 34th Congress of the Italian Society of Protistology. Pisa, September 11 - 14 2024.
- Coletta M., La Terza A., Soil and legumes of the Sibillini for a high-quality pasta. KreativeEU, Blended intensive programme. The role of stakeholders in the construction of the gastronomic science curriculum: a case study. University of Camerino, 1 - 5 July 2024.
- Coletta M., Monticelli M., D'Alessandro A., Gentili C., Waris N., La Terza A. Application of the arthropod-based Soil Biological Quality Index (QBS-ar) in the Conero Regional Park: A comparison between three organic farming systems. Centennial Celebration and Congress of the International Union of Soil Sciences Florence – Italy. May 19 - 21, 2024.
- Coletta M., D'Alessandro A., La Terza A., Biodiversità del suolo e Blockchain: sinergie per la produzione di vini di qualità. Applicazioni nel progetto B.I.O.C.E.R.T.O. Tipicità (Fermo) 9 March 2024.
- Coletta M., D'Alessandro A., Sagratini G., La Terza A. The ABRIOPACK Project: Effect of Compost Obtained by Adding Compostable Plastic Packaging to Organic Waste on Wheat Health and Rhizosphere Microbiome. Poster accepted for oral presentation at Ecomondo, Rimini. 7-10 November 2023.
- Coletta M., Waris N., D'Alessandro A., Cespi M., Caprioli G., La Terza A., Spent coffee grounds and algae biomass residue as fertilizer: sustainable solutions for crop and soil health. Algaebrew Mid-term Consortium meeting, Camerino. 26-27 September 2023.
- Coletta M., Gentili C., Monticelli M., D'Alessandro A., La Terza A., Seminario qualità delle acque, dei suoli e biodiversità. La biodiversità delle nostre aziende agricole e lo stato di salute dei nostri suoli. Attività di monitoraggio della biologia dei suoli in Aziende Agricole del Parco del Conero mediante l'applicazione dell'indice "Qualità Biologica Suoli - artropodi (QBS-ar)" Fondazione Ferretti, Castelfidardo (AN). 1 Settembre 2023.
- Coletta M., D'Alessandro A., Vittadini E., La Terza A. Influence of Organic and Conventional Management Systems on Soil Microarthropods in Protected and Non-Protected Areas, PhD Workshop Life and Health Sciences, University of Camerino. 25 November 2022.

12 POSTER PRESENTED AT NATIONAL AND INTERNATIONAL CONFERENCES

- Coletta M., D'Alessandro A., Torresi A., Dell'Ambrogio G., Renaud M., Ferrari B. J., & La Terza A. Is drone spraying of PPPs in olive orchards an environmentally friendly alternative to conventional ground spraying that can improve olive protection without harming soil biodiversity? 1st Mediterranean Agroecology Congress (AEMED). Agrigento, Sicily, Italy. June 9-12, 2025.
- Ghosh A., Bharti D., Coletta M., La Terza A., Pathania P.C., Kumar S. Can soil ciliate protists be harmful to crops? International Symposium on Ciliate Biology & Interdisciplinary Research. Acharya Narendra Dev College. New Delhi, India. February 2025
- Monticelli M., Coletta M., Torresi A., Salvatori L., Marconi M., La Terza A. Soil Biological Quality Index based on earthworms (QBS-e) in strip cropping versus pure stands: preliminary results of the OrtoBioStrip Project. Centennial Celebration and Congress of the International Union of Soil Sciences. Florence, Italy. May 19 - 21, 2024
- Waris N., Coletta M., Monticelli M., Caprioli G., Cespi M., Vittadini E., La Terza A. Development of fertiliser from brewery waste: Hints from the AlgaeBrew Project. Centennial Celebration and Congress of the International Union of Soil Sciences Florence – Italy. May 19 - 21, 2024
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13.2 CHAPTER 2: MANAGING SOIL TO SUPPORT SOIL BIODIVERSITY IN PROTECTED AREAS AGROECOSYSTEMS: A COMPARISON BETWEEN ARABLE LANDS, OLIVE GROVES, AND VINEYARDS IN THE CONERO PARK (ITALY)

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13.3 CHAPTER 3: HOW DO SOIL CHARACTERISTICS, AGRICULTURAL PRACTICES, WEATHER AND LANDSCAPE AFFECT SOIL MICROARTHROPODS IN THE AGROECOSYSTEM? INSIGHTS FROM THREE YEARS OF MONITORING IN ARABLE LANDS WITHIN THE SENTINA RESERVE (ITALY)

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13.4 CHAPTER 4 AND FINAL SECTIONS (FINAL DISCUSSION, CONCLUSIONS, AND FUTURE PERSPECTIVES)

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14 SUPPLEMENTARY MATERIAL

14.1 CHAPTER 2: MANAGING SOIL TO SUPPORT SOIL BIODIVERSITY IN PROTECTED AREAS AGROECOSYSTEMS: A COMPARISON BETWEEN ARABLE LANDS, OLIVE GROVES, AND VINEYARDS IN THE CONERO PARK (ITALY)

Arable lands in the Conero Regional Park (Italy)

June 2022

All arable lands were cultivated with durum wheat (*Triticum durum*). On the four sites, the agricultural practices used in the previous three years were almost identical. None of the plots were irrigated and no pesticides or fertilisers were used. All farms had been managed organically for more than six years and all of them used combined sowing. There were few differences in the soil management before sowing, which took place in November 2021 in all cases, and are reported as follows.

BIA	NIC	POL	PET
			
Harrowing at a shallow depth (15-20 cm) was applied by using a disc harrow, followed by weed harrowing.	A lighter subsoiling (20-25 cm) was applied, followed by harrowing with a spring-tooth harrow.	Ploughing (30 cm) was applied, followed by harrowing.	Subsoiling (30-40 cm) was used, to break up the compacted zone and create larger pores to increase rooting and infiltration, and grubbing.

Shredding was carried out on all sites as part of the normal maintenance of the olive grove. In FAT, LUC and SEL, PPPs were used to control olive fly.

SEL	FAT	LUC	PIA
			
It was the oldest olive orchard and the only one organised in terraces.	Manual hoeing was carried out at the base of the trees and a mixed crop (field beans and sulla) was sown between the trees as green manure.	A huge variety of crops were sown between the trees as green manure.	It was the only farm where PPPs were not used and fertilisers were used, probably because of the young age of the olive trees.

Fertilising, shredding, and pruning took place in all vineyards during the year. The use of PPPs was mentioned in all farms MAR, GUA, ZAZ, and MOD, in the latter site it was more intense.

MAR	GUA	MOD	ZAZ
			
Organic fertilization is applied every year in January. It has recently been converted to organic (2019).	Field beans were used as green manure, alternatively planted, and ridged. Pelleted manure was applied in November, while Copper and Sulphur in April-July	The inter-row and the under-row were mown, and mechanical suckering was carried out. It has the lower percentage of vegetation cover and has only recently been converted to organic (2019).	In the under-row the brush cutter was used, and hoeing was carried out. Organic fertilization was applied in March.

Tab. S1. The table shows the photos and the field management information for each site and agroecosystem. In-depth interviews with field owners were conducted, the following information were recorded: use of herbicides, pesticides, and fertilisers, crop rotation, irrigation, ploughing/harrowing/subsoiling depth (measured in cm), grubbing, shredding, pruning, hoeing, and sowing.

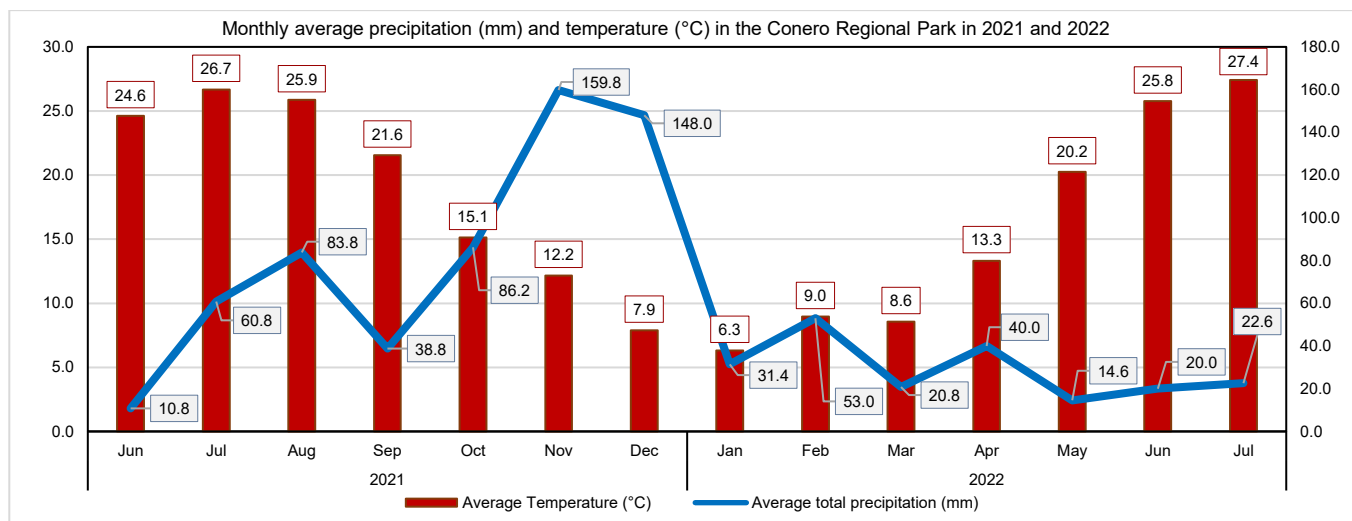


Fig. S1. The table shows the monthly average precipitation (mm) and temperature (°C) in the Conero Regional Park in 2021 and 2022. The red columns show the average temperature (°C), while the blue line shows the trend of the average total precipitation (mm). Both parameters were registered by the Camerano weather station.

Agroecosystem	Average Acari density (ind/m ²)	Average Acari density (ind/m ³)	Average Oribatid mites density (ind/m ²)	Average Oribatid mites density (ind/m ³)	% of Oribatid mites out of total mites
Arable lands	49847	433667	19636	170833	39.4 %
Olie groves	37404	325417	6006	52250	16.1 %
Vineyards	33305	289750	7816	68000	23.5 %

Tab. S2. The table shows the overall Acari and Oribatid mites density data (ind/m² and ind/m³), and the percentage of oribatid mites out of total mites for each agroecosystem.

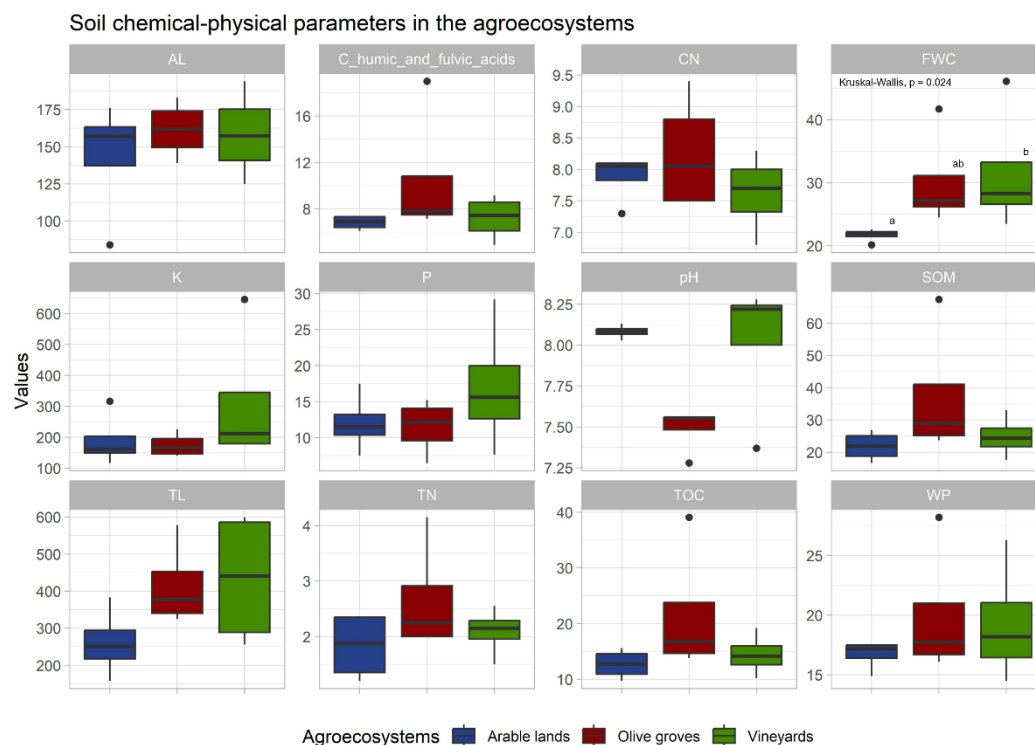


Fig. S2. Boxplots show the distribution of the values of the soil chemical-physical parameters in the three different agroecosystems. The median (horizontal line), and outliers (isolated black dots) are reported. The field water capacity (FWC) was the only parameter that showed a significant difference. For this parameter, lowercase letters represent significant differences between the average values (across

management systems) by Post-hoc Dunn tests at p -value < 0.05 . No indication is given for the other parameters because no significant differences were found. Total organic carbon, TOC; soil organic matter, SOM; total nitrogen, TN; assimilable phosphorus, P; exchangeable potassium, K; wilting point WP; total limestone, TL; active limestone, AL; carbon/nitrogen ratio, C/N ratio; carbon of humic and fulvic acids; pH.

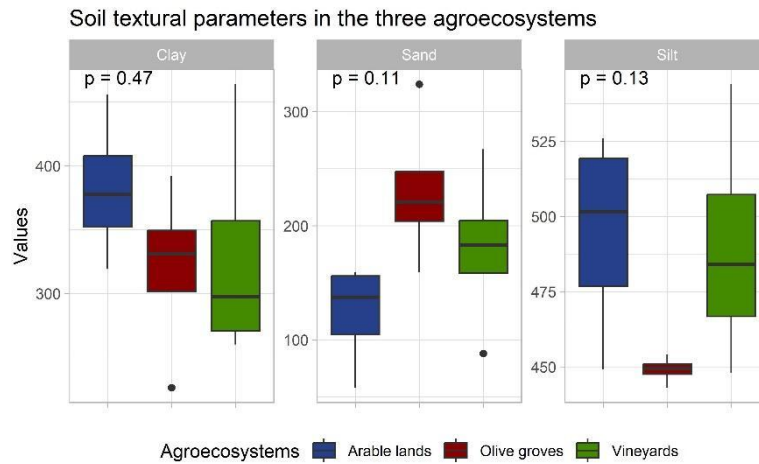


Fig. S3. Boxplots show the distribution of the soil textural parameters in the three different agroecosystems. The median (horizontal line), and outliers (isolated black dots) are reported. The content of sand, silt, and clay particles (g/kg) are reported. Arable lands and vineyards have a silty clay loam texture, while olive groves have a clay loam texture. ANOVA test at p -value < 0.05 was used.

14.2 CHAPTER 3: HOW DO SOIL CHARACTERISTICS, AGRICULTURAL PRACTICES, WEATHER AND LANDSCAPE AFFECT SOIL MICROARTHROPODS IN THE AGROECOSYSTEM? INSIGHTS FROM THREE YEARS OF MONITORING IN ARABLE LANDS WITHIN THE SENTINA RESERVE (ITALY)

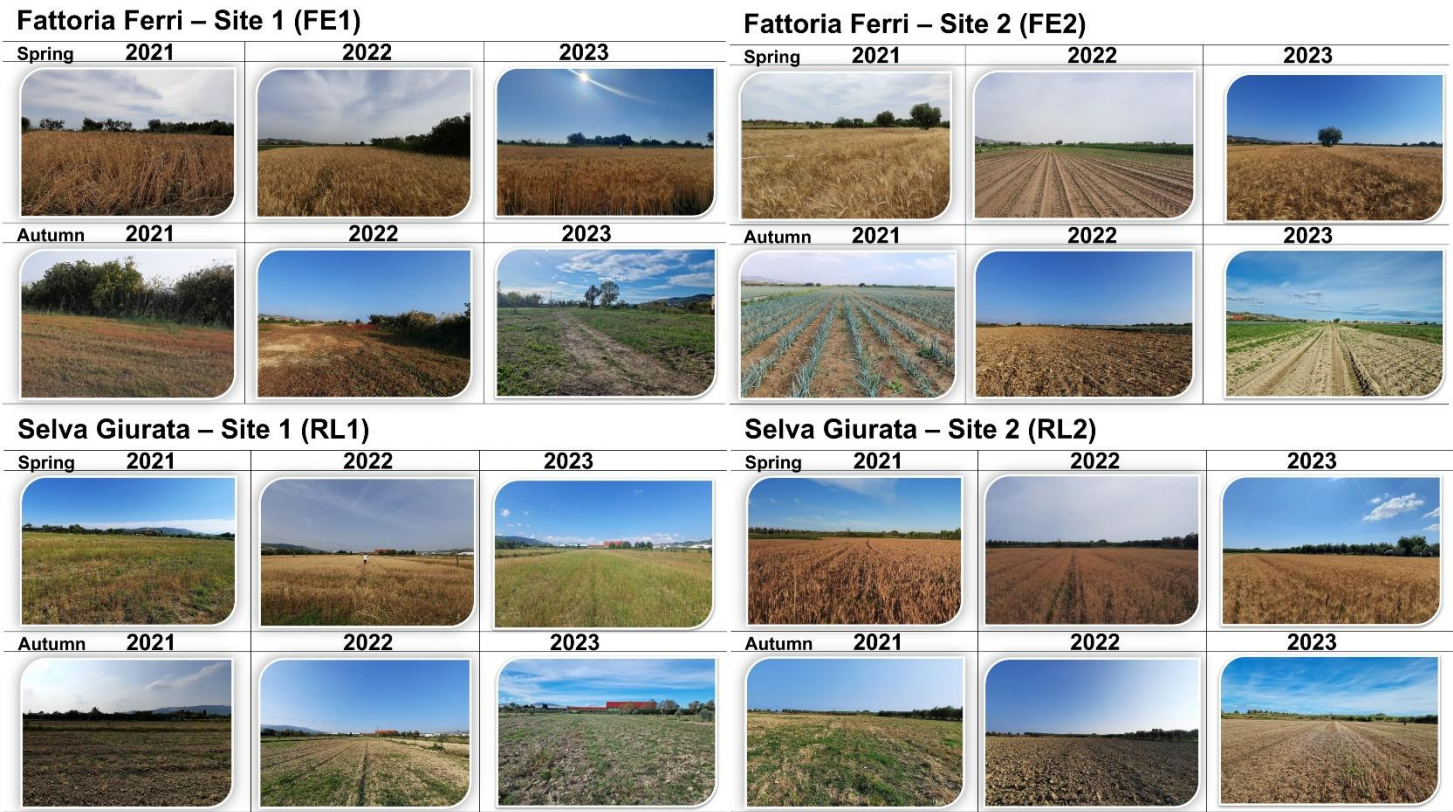


Fig. S1 - Photos of the investigated sites (FE1, FE2, RL1, RL2) taken during the spring and autumn samplings in 2021, 2022 and 2023.

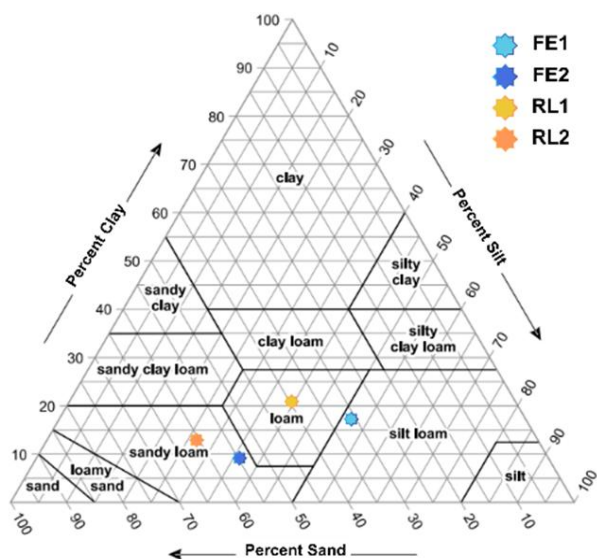


Fig. S2 - Representation of the USDA soil textural triangle showing the percentages of clay, silt, and sand particles of the four soil samples collected in the four investigated sites (FE1, FE2, RL1, RL2).

Weather	Landscape*	Agricultural practices**	Soil chemical properties	Soil textural properties	Soil environmental factors
Temperature (°C)	Artificial surfaces (%)	Tillage for seed bed preparation	Total organic carbon (g/kg)	Clay (g/kg)	Soil moisture (%)
Relative humidity (%)	Agricultural areas (%)	Crop rotation	Organic matter (g/kg)	Silt (g/kg)	Soil temperature (°C)
Precipitation (mm)	Forest and semi natural areas (%)	Fertilizer and PPPs use	pH	Sand (g/kg)	
	Wetlands and water bodies (%)	Harvesting	C/N ratio	Coarse sand (g/kg)	
		Irrigation	Total nitrogen (g/kg)	Fine sand (g/kg)	
		Sowing	Available phosphorus (mg/kg)	Very fine sand (g/kg)	
		False sowing	Exchangeable potassium (mg/kg)		
		Shredding and burial of crop residues	Cation exchange capacity (meq/100g)		
			Electrical conductivity (dS/m)		

Tab. S1 - The table shows the five macrocategories in which data referring to different variables were grouped into. The influence of each set of variables on the QBS-ar index was then assessed by using multiple linear regression (MLR). When the number of variables to be tested was significant in relation to the number of observations, the selection of variables was performed using a stepwise regression by backward elimination based on AIC.

* To improve the reliability of the analysis, the 20 different land-use and land-cover types (20) found in the landscape analysis, were grouped into four macrocategories based on the first level of the CORINE land cover classification (CLC). The percentage of each land-use and land-cover type within each macrocategory, was summed to determine the overall percentage for that macrocategory, which was then used in the analysis. The following macrocategories were used:

- **ARTIFICIAL SURFACES:** paved roads, unpaved roads, scattered buildings, continuous buildings, railway.

- **AGRICULTURAL AREAS:** arable land, gardens and parks, grassland, olive groves, vineyards.

- **FOREST AND SEMI NATURAL AREAS:** salt marsh vegetation, halophytes, hedges and shrubs, reedbed, riparian woodland, psammophilous vegetation, shoreline.

- **WETLANDS AND WATER BODIES:** watercourse, pond and small lakes, sea.

** Numerical values were assigned to the different agronomic practices based on their overall environmental impact, particularly regarding soil health. For evaluating soil tillage for seedbed preparation, four categories were defined: no tillage (fallow); shallow tillage (harrowing) (< 15 cm); medium tillage (35 cm) + post-tillage harrowing (< 15 cm); deep tillage (50 cm) + post-tillage harrowing (< 15 cm). To evaluate the effect of crop rotation, the presence or absence of crops, and the specific type of crop grown during soil sampling was considered. The crops included: durum wheat, soft wheat, leek, maize, horseradish, and field beans. Each crop was ranked based on its potential positive or negative effect on soil health, taking into account whether it tended to impoverish or benefit the soil. To all other agricultural practices applied (fertilizer and PPPs use, irrigation, sowing, false sowing, harvesting, shredding and burial), was assigned a numerical value depending on the soil disturbance they generated. Below are the numerical values given to the different variables included in the category "Agricultural practices":

Tillage for seedbed preparation:

- no tillage/fallow = 100
- shallow tillage (harrowing) = 75
- medium tillage (35 cm) + post-tillage harrowing = 25
- deep tillage (50cm) + post-tillage harrowing = 0

Crop rotation:

- horseradish and field beans = 100
- absence of crop/fallow = 85
- leek = 70
- corn = 55
- durum wheat = 40
- soft wheat = 30

Fertilizer and PPPs use

- NO = 100
- fertilization = 40
- herbicide use + 1 fertilization =25
- herbicide use + 2 fertilization =15
- herbicide use + 3 fertilization =10

Harvesting

- NO=100
- corn harvesting = 35
- wheat harvesting = 45
- leek harvesting = 55

Irrigation

- NO = 100
- flood irrigation =70
- irrigation by sprinklers =75

Sowing

- NO=100
- YES =80

False sowing

- NO = 100
- YES = 75

Shredding and burial of crop residues

- NO=100
- green manuring =95
- shredding and burial =80

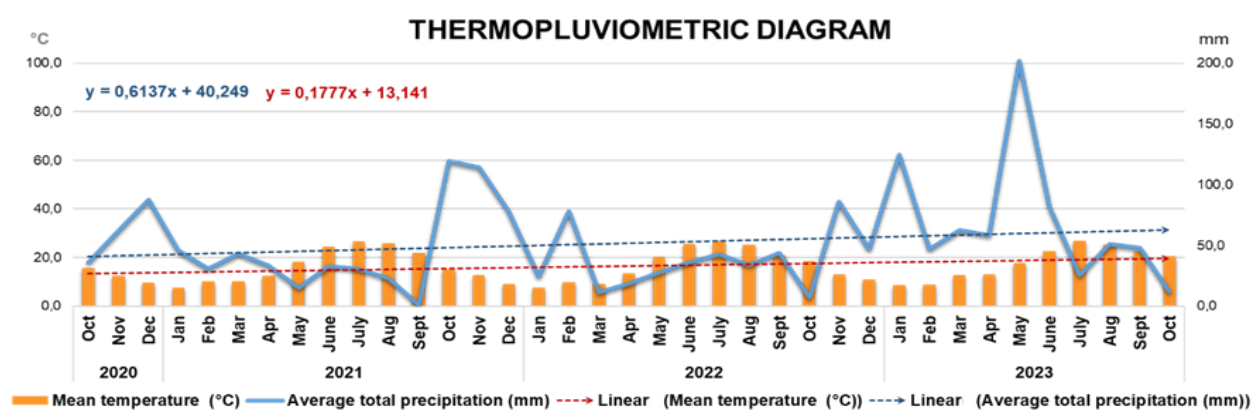


Fig. S3 - Thermopluviometric diagram of the study area from October 2020 to October 2023. Orange columns refer to the average temperature (°C), while the blue line represents the trend of the average total precipitation (mm).

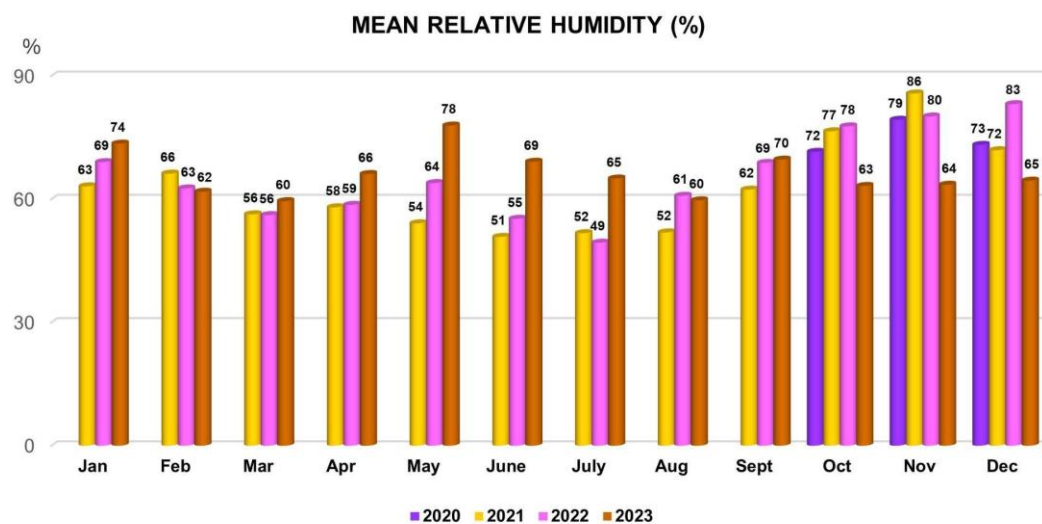


Fig. S4 - Graph of the mean relative humidity (%) within the Sentina Reserve over the period considered in the study.

15 ACKNOWLEDGEMENTS
