Università degli Studi di Milano

Faculty of Agricultural and Food Sciences

Department of Agricultural and Environmental Sciences. Production, Landscape, Agroenergy

PhD School "Agriculture, Environment and Bioenergy"

XXXI Cycle 2015-2018

Impact of agricultural practices on biodiversity of soil invertebrates, assessment through DNA Metabarcoding approach

Dottorando: Sumer ALALI

Matricola: R11223

Relatore: Prof. Stefano BOCCHI

Correlatore: Dott. Matteo MONTAGNA

Coordinator: Prof. Daniele BASSI

Academic year 2018-2019

Index

Abstract

In spite of the increase of the organically farmed areas worldwide, it has been always doubted if the organic farming really enhances the soil biodiversity, as the main component in the agroecosystems. This doubt was a target of many studies, trying to reveal the true impact of the applied agricultural practices in the adopted farming strategies, targeting the soil invertebrates as bioindicators of the impact. Unfortunately, the doubt is still, due to the limitations in the sampling and taxonomy of the soil's invertebrates communities. In the last years, the molecular approaches represent promising methods to overcome these limitations. Thus, the DNA metabarcoding was applied targeting the COI gene in the DNA extracted from soil samples collected in different farming strategies (organic *vs* non organic), with a different cropping systems (stable meadow *vs* barley) and different levels in the field from the margin to center, this sampling was performed in three seasons (May, July and October). In addition, the soil properties (pH, texture, N%, C% and C/N ratio) were determined for the selected samples. The illumine MiSeq run was performed and the obtained reads were processed bioinformatically to get the OTU table (Operational Taxonomic Unite). This OTU table was used for the statistical and ecological analysis. Finally, the QBS, a soil quality index depends on the soil inhibiting microarthropods, was calculated by its classic method

and estimated based on the obtained molecular data, to check the correlation between the resulted values.

Results showed that the DNA metabarcoding approach represents a promising method for the assessment of soil biodiversity in the agroecosystem, but this approach is not able to detect the seasonal changes of the soil invertebrates' communities. Regarding the farming strategies, the farming management as organic or non organic (conventional) did not affect significantly the community structure of soil invertebrates and the biodiversity indices Shannon and Pielou's evenness, while the species richness was significantly lower in the conventional farm. Soil invertebrates' communities were significantly affected by the crop and the position of the field (as margin or field), and the C/N ratio. For Rotifera and Tardigrada communities' structure were affected by the farming strategy, while insects' communities were affected by the pH of the soil. The role of the margin of the field as a reservoir is increased in the cultivated fields (barley), while in the stable meadows the interactions between the margin and the center of the field are lower. Finally, the soil biological quality is decreased from the margin to the center of the field (of the same field), also decreased in the barley field comparing to the stable meadows. The obtained molecular index mQBS that is developed based on the QBS-ar is a promising approach for the soil biological quality estimation.

Introduction

Agriculture, as almost all human activities, have significant implications on wild species of flora and fauna. Especially under the general tendency of achieving a high production through adopting new techniques and strategies for farming such as the agricultural intensification, some management techniques may create a fundamental habitat changes that cause significant shifts in biodiversity (McLaughlin and Mineau 1995).

The main target of this shift is expected to be the integral component of the soil biota. The soil faunal communities are very diverse, and the most abundant mesofauna in soil include nematodes, collembolan, acari, enchytraeids, tardigrades, rotifers, proturans, as well as immature stages of many larger species of invertebrates (Hamilton *et al*. 2009). This huge diversity make it challenging to estimate the real changes in biodiversity caused by the different agricultural practices of the adopted farming strategy. The limitations in faunal biodiversity assessment are mainly associated with the low efficiency of the taxonomical approaches for faunal community analysis (André *et al*. 2002). No one method could extract all faunal groups and these approaches require an expertise for identification and quantification of the organisms (Hamilton *et al*. 2009; Coleman *et al*. 2004).

Extensive studies in the last years suggested the molecular techniques for complex community analysis as a solution to overcome the above-mentioned limitations (Elbrecht *et al*. 2017a, Hamilton *et al.* 2009). The DNA Metabarcoding approach, consisting of direct extraction of DNA from an environmental sample, is considered a powerful tool for elucidating mechanistic insights in ecological and evolutionary processes, with the ability to explore ecosystem-level processes, to analysis the species, community diversity, and dynamics (Bohmann *et al.* 2014).

The main goal of the study is the evaluation of soil invertebrates biodiversity of farms subjected to different agricultural strategies (organic *vs* non-organic or the so-called "conventional"; Knapp *et al*. 2018), using a DNA metabarcoding approach targeting a fragment of the mitochondrial metazoan gene COI.

This PhD thesis consists of the following chapters:

1- Introduction.
2- Materials and Methods.
3- Results.
4- Discussion.
5- Conclusions.
6- References.

1- Soil fauna in the agroecosystems

1-2 Soil faunal biodiversity

Soil is a dynamic and living resource represents a unique balance between physical, chemical and biological factors. This balance condition is crucial for the production of food and ecosystem functions (Doran *et al.* 1996); soils are considered one of the most diverse habitat on earth, and house a large proportion of animal species "Fauna" including mainly the invertebrates (Wardle 2002).

The soil fauna or edaphic fauna play a key role in many soil functions, such as organic matter decomposition, humus formation and nutrient element cycling; moreover, affect the porosity, aeration, infiltration and distribution of organic matter in soil horizons, modifying soil structure and improving its fertility (Menta et al. 2011). These fauna consist of a huge variety of animals such as nematodes, arthropods and earthworms (Jeffery et al. 2010). In particular, invertebrates are considered a fundamental part in determining the suitability of soils for the sustainable production of healthy crops or trees (Stork and Eggleton 1992).

The types of invertebrates that make the greatest contribution to soil quality were classified into three groups based on their size and the way they interact with their habitats (Anderson 1988):

a- **Microfauna:** these are invertebrates of less than 100 μm, mostly nematodes, which are associated with water films on the surface of soil.

b- **Mesofauna or meiofauna**: this diverse group of invertebrates are of sufficient size to overcome the surface tension of water on soil particles but are not large enough to disrupt the soil structure in their movement through soil pores, their bodies size is between 100 μm and 2 mm. This group includes acari, collembolan, enchytraeid worms, small millipedes (Diplopoda), and many small larval and adult insects.

c- **Macrofauna:** this group consists of species large enough to disrupt the soil structure by their burrowing and feeding ranging from 2 mm to 20 mm in body size. The most important taxa are Isopoda, larger Diplopoda, Isoptera, Coleoptera, Diptera, Formicidae, Annelida (earthworms) and Mollusca.

In general, the micro- and mesofaunas appear to accelerate decomposition (Castanho et al. 2012), as well as mediating mineral transport processes in the soil (Lavelle et al. 2006), in particular, arthropods are the most abundant and important functional group in soil food webs and maintainer of the soil ecosystem functionality (Goncalves and Pereira, 2012). The role of each group in enhancing the soil quality have been long studied.

For example, springtails (Collembola) decompose organic matters and increase the mineralization by feeding on fungi and scavenging (van Amelsvoort et al. 1988), exhibiting by that a strong control on soil ecosystem functions (Blair et al.1992). They also influence the microbial community, directly by feeding on fungal and bacterial biomass and indirectly by fragmenting litter in such a manner as to increase surface area for microbial colonization (Lussenhop 1992). Earthworms help to increase pore volume, field water holding capacity and infiltration rates (Lee and Foster 1991). Even though phytophagous nematodes often reduce plant primary production, soil nematodes may enhance decomposition by 16% in field soil and up to 30% in litter (Stork and Eggleton 1992). Other invertebrates as holometabolous insect groups such as Coleoptera (beetles) and Diptera (flies) are important in soils to breakdown dung, carrion and leaf litter, and therefore return nutrients to the soil; Scarabaeid beetles may be especially important in this role (Greenslade 1985; Kalisz and Stone 1984).

1-2- Effect of agricultural practices on soil properties

It is known that the agricultural practices such as tillage, fertilization and the selected cover crop are the main factors that modify the soil's physical and chemical properties (Bronick and Geoderma 2005). For example, tillage reduces soil organic matter

and nitrogen content in the upper layer (Sapkota 2012), it also alters many aspects of the soil's physical properties such as soil water, aeration, compaction, porosity, and temperature (Unger 1990; Prasad and Power 1991). Another effective practice is the addition of animal wastes, which has beneficial effects on soil pH, soil structure, resistance to erosion, soil temperature, organic matter content of soil, water infiltration and soil water retention (Barnett 1982).

For the biological properties of the soil, especially under intensification in agriculture practices in the last decades, it was increased the loss in terms of both abundance and taxonomic diversity of soil faunal communities like arthropods and earthworms (Menta et al. 2011). However, many studies have highlighted the direct effect of these practices on the biological properties of the soil fauna (Tuck et al. 2014; Gabriel et al. 2013; Weibull 2003). Regarding the tillage, for example, it was found that the no-tillage farming systems showed higher soil microbial biomass, respiration and enzyme activity, and a higher abundance and diversity of microarthropods (Tabaglio et al. 2009). The use of pesticides with no doubt has raised risks of ecosystem functions loss, for example, earthworms were found to be highly susceptible to lethal and sub lethal effects of neonicotinoids and fipronil (Pisa et al. 2015).

1-3- Sustainable farming strategies

The concerns about the negative effects of the agricultural practices make it urgent to develop more sustainable farming strategies, resulting in the IPM (Integrated Pest Management) strategies as an "approach that combines different crop protection practices with careful monitoring of pests and their natural enemies (Bajwa and Kogan 2002)". These alternative strategies aim to help making crop protection more sustainable, and are considered as the best way forward, and the EU has placed them as obligatory within its 2009 Sustainable Use Directive on pesticides 2009/128/EC (European Parliament 2010). The practices and the national thresholds are described in the national regulations like the annual "Linee guida Nazionali di produzione integrate", with more regional thresholds could be added.

Another strategy aiming the reduction of the synthetic chemicals and more sustainability of the agroecosystems were presented by the organic farming as "a potential solution for the ecosystem services loss, and to enhancing functional biodiversity to bring sustainability to production (Padel 2002; Buguna-Hoffmann 2000; Altieri 1999)". Another proposed definition according to the FAO/WHO Codex Alimentarius Commission, 1999, organic agriculture is a "holistic production management system which promotes and enhances agro-ecosystem health, including

biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. This is accomplished by using, where possible, agronomic, biological, and mechanical methods, as opposed to using synthetic materials, to fulfil any specific function within the system (FAO 1999)".

Denmark was the first country to introduce a national support programme for the organic farming in 1987, followed by Germany which have used the framework of the EU extensification programme (EC Reg. 4115/88) to introduce a support for conversion to organic farming. By 1996, all EU member states, had introduced policies to support organic farming within the agroecosystem (EC Reg. 2078/92). After that, the common framework of this programme and the regulatory base provided by EC Reg. 2092/91.

These mentioned regulations have the main objectives of: a) the sustainability of the agricultural production under a stable management system; b) obtaining a high quality products; c) producing a varieties of products which responding the consumers demands, through methods that are not harmful for the environment or the human and animals health. Thus, the basis of the organic production are depending on the sustainability and the

saving of the natural resources, while the eligibility and other conditions of the schemes in each country vary widely. However, they have to respect regulations such as EC Reg. 889/2007 and EC Reg. 217/2006, which concern about the use of the organic fertilizers, and the main regulatory EC Reg. 2092/91, till the latest one 2018/848/EC.

In Italy, respecting the European regulations of the organic farming there are many National legislations, like the legislative decree n. 220/1995, derived from the articles 8 and 9 of EC Reg. 2092/91.

In the recent years we have witnessed a continuous growth in the organically farmed areas worldwide (Willer and Lernoud 2016), But the assumption is still in doubt whether the organic farming reduce the impact on soil's biodiversity or not (Reganold and Wachter 2016; Pimentel et al. 2005)?

Many researches have investigated this doubt, comparing the soil faunal communities in organic and non-organic "named as conventional if there is a synthetic chemicals application with respect to the IPM thresholds" (e.g., Tuck et al. 2014; Mader et al. 2002; Altieri 1999). However, the results have made more uncertainties, since that the species composition of soil invertebrates was not affected by the farming system according to some studies (Blackburn and Arthur 2001). For example, in the

olive groves, both conventional and organic, no differences were recovered in Isopoda and Coleoptera species diversity (Hadjicharalampous et al. 2002), this fact could be attributed to the availability of more suitable micro-habitats, due to the presence of crop itself, the weed and stones, that offer refuges to this groups of arthropods (Hadjicharalampous et al. 2002).

Some other studies provided evidences that organically managed fields showed higher biodiversity than conventionally farmed ones in the soil invertebrate's communities, including arthropods, nematodes and earthworms (Gabriel et al. 2013). On the contrary, conventional farms showed a higher species richness and abundance in specific groups like carabids (Coleoptera) (Weibull 2003). Another example was the organic vineyards and maize which were poorest than the conventional ones in Isopoda species (Hadjicharalampous et al. 2002).

This confusion in the results, when using the soil fauna as an effect measurement of the farming strategies, could be attributed to many reasons:

- Soil faunal taxon diversity and abundance are so great, so the using of one group of the soil invertebrates is neglecting by consequence the other presented groups and ignoring the complicity of the soil faunal communities (Creer et. al. 2010; Maraun et al. 2003).

- The soil invertebrate group are dynamic and have certain responses to these agriculture practices, and may be reflecting some aspects of the soil physical and chemical properties (Bardgett and van der Putten 2014; Sylvain and Wall 2011). For example, 50% of the abundance and diversity of earthworm species were explained by the amount of phosphorus, nitrogen, and calcium in soil, soil acidity, and the diversity or mass of fungi, plant litter and roots (Mueller et al. 2016).
- Finally, the knowledge of soil animal diversity remains limited, to a great extent, because very few studies have simultaneously assessed diversity of different soil animals; this should also be due to the number and complexity of methods needed to study such cryptic organisms (Sylvain and Wall 2011).

Due to these limitations, even the more comprehensive surveys of soil organisms typically cover less than half of the taxonomic groups that represent common types of soil invertebrates (e.g. Bardgett and van der Putten, 2014; Postma-Blaauw et al. 2012; Scherber et al. 2010; van der Wal et al. 2009;).

2- Molecular tools for biodiversity assessment

Because of the huge diversity of the soil inhibiting communities of invertebrates, the effective measurement of the soil biodiversity needs a novel and effective sampling and estimation procedures. Thus, the developed molecular methods occurred in the last 10 years represent a possible candidate for such assessments, especially for 'hyperdiverse' groups, such as arthropods, nematodes and other soil invertebrates, such as the DNA metabarcoding.

The term ‘DNA barcoding’ is of recent use in the literature (Hebert et al. 2003; Floyd et al. 2002). It relies on the use of a standardized DNA region as a tag for rapid and accurate species identification (Hebert and Gregory 2005). Nevertheless, DNA barcoding is not a new concept. This term was firstly used in 1993 in a paper that did not receive very much attention from the scientific community (Arnot et. al. 1993). Furthermore, the concept of species identification using molecular tools is older still back to 1982, through the discerning the origin of fresh meats (Kang'Ethe et al. 1982).

DNA barcoding has received much attention recently, and is being further developed through many international initiatives (Valentini et al. 2009); it is believed that DNA barcoding might play an important role in biodiversity assessment, both for present and for past animal and plant communities. This technique could

be defined as: Genomic approaches to taxon diagnosis exploit diversity among DNA sequences to identify organisms (Wilson 1995); these sequences can be viewed as genetic 'barcodes' that are embedded in every cell (Hebert et al. 2003). Or as: "A novel system designed to provide rapid, accurate, and automatable species identifications by using short, standardized gene regions as internal species tags. As a consequence, it will make the Linnaean taxonomic system more accessible, with benefits to ecologists, conservationists, and the diversity of agencies charged with the control of pests, invasive species, and food safety (Hebert and Gregory 2005)".

DNA barcoding enhances biodiversity inventories by being faster and cheaper, and by overcoming the taxonomic impediment (Valentini et al. 2009; Rougerie et al. 2009; Newmaster et al. 2007). It could allow biodiversity assessment through the identification of taxa from the traces of DNA present in environmental samples such as soil or water. Same studies have demonstrated this technique as a tool for the estimation of biodiversity of environments with low accessibility like the study of the microbial biodiversity in deep sea with the possibility to use classical biodiversity indices, such as species richness, Simpson's index and Shannon's index (Herrera et al. 2007; Gomez-Alvarez et al. 2007; Margurran et al. 2004). In this aspects, the estimation of biodiversity indices can be based on operational taxonomic units

(OTU), detected using the barcoding approach (Floyd et al. 2002; Blaxter et al. 2005). This OTU is used to classify groups of closely related individuals, grouped by similarity to be equivalent to, but not necessarily, a classical Linnaean taxonomy level like species (Sneath and Sokal 1973).

One step beyond DNA barcoding is the DNA Metabarcoding (Deagle et al. 2014; Yu et al. 2012), which is a rapid method of biodiversity assessment that uses universal PCR primers to mass-amplify a taxonomically informative gene from mass collections of organisms or from environmental DNA. Briefly, a high-throughput sequencer is used usually to get the output as a long list of DNA sequences of the amplified target amplicon. The output data set needs to be reduced by using a bioinformatics processing to cluster the sequences into OTUs. Finally, a representative sequence is taken from each OTU and is assigned a taxonomy using one or more of the databases (Ji et al. 2013).

The official barcode sequences are tied to a curated specimen deposited in a museum and meet certain metadata standards, the intent being to provide auditable taxonomies, which are managed and available through the Barcode of Life Database (BOLD, http://www.barcodinglife.org)(Ratnasingham and Hebert 2007).

It is widely accepted to use the Metabarcoding for eukaryote biodiversity as a rapid, cheap and comprehensive measurement tool (yang et al. 2014).

Compared to the traditional taxonomic methods, DNA Metabarcoding identified more than twice the number of taxa than the morphology-based protocol, and yielded a higher taxonomic resolution (Elbrecht et al. 2017a).

Recent studies have highlighted that DNA Metabarcoding can achieve comparable assessment results, representing a feasible and reliable method to identify invertebrates in ecosystem's bio-assessment, and offers powerful advantage over morphological identification in providing identification for taxonomic groups that are unfeasible to identify in routine protocols (Elbrecht et al. 2017a; Valentini et al. 2009).

The targeted molecular taxonomic marker in this approach could be amplified from a directly extracted DNA from the collected samples (Soil, water, etc…) which is referred as Environmental DNA.

This environmental DNA (eDNA) is defined as: trace DNA in samples such as water, soil, or feces, it is a mixture of potentially degraded DNA from many different organisms (Bohmann et al. 2014).

For animals, the gene region proposed as a standard barcode is a 658 base pair region in the gene encoding the mitochondrial cytochrome c oxidase 1 (COI or *cox1*) (Hebert et al. 2003b). This 650 bp fragment of the mitochondrial DNA (mtDNA) has been

used successfully for species-level identification in several animal groups (Ji et al. 2013), and can serve as the core of a global bio-identification system for animals as proposed by Hebert et al. (2003). This choice was made based on reasonable and well-established reasons:

a- Mitochondrial DNA has a haploid mode of inheritance, elevated rate of molecular evolution, lacks introns and has limited recombination (Tsaousis et al. 2005).

b- INDELs (insertion/deletion events) are rare in mtDNA protein coding genes and Universal primers for the COI gene are robust (Zhang and Hewitt 1997; Folmer et al. 1994).

c- Finally, the mode of molecular evolution of COI usually facilitates species discrimination while also retaining phylogenetic information for the majority of animal taxa (Hebert et al. 2003b).

The COI is easily sequenced and provides a species-level specificity for birds (Hebert et al. 2004), mammals (Hajibabaei et al. 2007), fishes (Ward et al. 2005), and various arthropods (Hajibabaei et al. 2006). However, this approach encounters two major problems: i) the DNA degradation in archival specimens and processed biological material often prevents the recovery of PCR fragments longer than 200 bp, impeding barcode recovery for the reference database construction (Goldstein et al. 2003; Hajibabaei et al. 2006; Wandeler et al. 2007). ii) Some of the current approaches

cannot be used for comprehensive analysis of environmental samples, because the high sequence variability necessitates the use of distinct primer sets for each major taxonomic group (Meusnier et al. 2008).

2-1- Advantages *vs* Limitations of DNA Metabarcoding

In spite of the latest and rapid improvements in the use of DNA metabarcoding for the assessment of local biodiversity from soil, water and fecal samples (Valentini et al. 2009), it still has some limitations related to the targeted fragment properties and to the length of the targeted fragment, which could be summarized as the following:

- **a-** Nuclear copies of fragments of mitochondrial DNA are common and can be preferentially amplified in some circumstances (Zhang and Hewitt 1996), leading to potential identification errors.
- **b-** The heteroplasmy "the presence within a cell or organism of mitochondria with different genetic constitutions (Dictionary of Biology, Oxford University Press 2004)" can confuse the identification system (Kmiec et al. 2006).
- **c-** The targeted fragment COI (usually ~500 bp), which prevents the amplification of degraded DNA (Hebert et al. 2003b). Fortunately, many potential primers are proposed and used to overcome this limitation, targeting the degraded DNA in environmental samples where the target is DNA

from dead animals and DNA fragments sometimes are shorter than 200 bp (Taberlet et al. 2007; Hajibabaei et al. 2006; Meusnier et al. 2008; Elbrecht et al. 2017b).

d- Finally, bioinformatics involved in the procedure are complex (Zaiko et al. 2015).

Regardless the aforementioned limitations, this approach offers multiple advantages; DNA metabarcoding is generally reliable, cost-effective and easy molecular identification tool with a wide applicability across metazoan taxa (Virgilio et al. 2010).

2-2- Metabarcoding application in the agroecosystems

Metabarcoding has been widely and successfully used in recent ecological studies and has significantly affected the scale, the velocity and the precision of the outcomes from such studies (Creer et al. 2016), proofing its importance as a tool for investigating microbiology, mycology (Tedersoo et al. 2014; Caporaso et al. 2011) and metazoan (Taberlet et al. 2012; Valentini et al. 2009) communities in complex habitats, supported by the latest improvements in the High-throughput sequencing techniques (Sigut et al. 2017).

Examples of the recent successes in applying the Metabarcoding with the utility of eDNA are ranging from species detection, biodiversity assessments, population genetics, reconstruction of past flora and fauna and the detection of invasive marine species

(Elbrecht and leese 2015). These applications have covered different ecosystems and targeted many organisms within them, i.e. air pollen identification (Kraaijeveld et al. 2015), terrestrial mammals (Murray et al. 2012), birds (Andersen et al. 2012) and plants (Yoccoz et al. 2012, Jørgensen et al. 2012). Covering also the reptiles (Lacoursière-Roussel et al. 2016), amphibians (Valentini et al. 2016), and invertebrates (Gardham et al. 2014). Reaching to the marine and deep seas fish and invertebrates communities (Yamamoto et al. 2017; Hawkins et al. 2015). Revealing some interesting and specific relationships like the host/parasitoid interactions at different taxonomic levels (Sigut et al. 2017).

Nevertheless, this approach has not been implemented widely in the agroecosystems, except for a few cases of investigating in such a complex ecosystem with a direct anthropic stresses. Like the case of the honeybee monitoring for hive co-existing parasites, biological information and the product characteristics (Utzeri et al. 2018). Another research studied the agriculture effect on bat's diet of pest arthropods (Aizpurua et al. 2018). Stable meadows biodiversity responses to different levels of cattle grazing stress has been studied for different kingdoms (Plants, Bacteria, Fungi and Metazoan) (Montagnaet al. 2018). Finally, the potential biological control role of carabids in farmlands through the diet analysis (Kamenova et al. 2018).

3- Soil Health indicators

The capacity of soil to function as a vital living system in order to sustain plant and animal health and productivity is referred as the "soil health or biological quality" (Laishram et al. 2012). This could be also defined as the ability of soil to perform or function according to its potential, and changes over time due to human use and management or to natural events, that determine agricultural sustainability (Acton and Gregorich 1995). As a fundamental part of the agroecosystem, the soil properties are directly affected by the management and agricultural activities that reflects in the multiple functions of soil, and so its quality (Huang et al. 2007).

Soil quality can be assessed by using many indicators, like the chemical and physical indicators: soil's organic matter, bulk density and aggregate stability (Liebig and Doran 1999; Six et al. 2000), or simply by measuring the yield trends over time under a consistent management system, i.e. rotation, tillage type, fertilizer regime, etc…, with the decrease indicating a loss of health (Poulson and Johnston 1994). Further studies reached to evaluate soil quality through more complex indices, such as integrated soil quality indexes (SQIs)(Doran et al. 1994), multi-variable indicator kriging (MVIK)(Nazzareno and Michele 2004) and soil quality dynamics (Larson and Pierce 1994).

The traditional indices used for the soil health evaluation have used the soil properties and the productivity of the crops as indicators, ignoring the soil's living organisms as bioindicators, which reflects easily the anthropogenic disturbances (e.g., pollution, land use changes) or natural stressors (e.g., drought, late spring freeze)(Holt and Miller 2011). In particular, mesofauna represent a major component of soil biological communities and play a critical role in maintaining soil quality and many of ecosystem functions (Barrios 2007). Including decomposition, nutrient cycling, and soil formation, which facilitates water supply and regulates local erosion and climate (Lavelle et al. 2006; Barrios 2007). Such functions are key components soil health (Doran and Zeiss 2000).

The attraction towards using soil animals as bioindicators rose in the last decades, especially after the conference 'Soil Health: Managing the Biological Component of Soil Quality' held in the USA in November 1998, which concerned about the importance and utility of soil organisms as indicators of soil quality and determinants of soil health. In general, most groups of soil's invertebrates have met the five required criteria to be used as soil quality bioindicators, which are: a) the utility in defining ecosystem processes. b) The good correlation with physical, chemical, and biological properties. c) The sensitivity to management and climatic variations. d) The accessibility and

utility to agricultural specialists, producers, conservationists, and policy makers, e) they are easy and inexpensive to measure (Doran and Parkin 1996).

For example, nematodes are being used as one of the potential parameters to measure the impact of disturbances and to monitor changes in structure and functioning of the below-ground ecosystem, an applicable index were developed using the nematodes the so-called Nematode Maturity Index; which is based on the proportion of colonizers and persisting nematodes in samples (Bongers 1999).

However, arthropods are the most used organisms as soil health indicators, since they are responsible of many ecosystem functions in soils, many of arthropod's groups were proposed as bioindicators of soil quality and ecosystem health, due to their life-history characteristics, their small size, and variation of ecological preferences, relatively high fecundity, and ease of sampling (Gerlach et al. 2013). Such as Acari and Collembola, their abundances are useful for understanding how biota respond to the impacts and intensity of land-use on ecosystems (Arroyo et al. 2013; Rutgers et al. 2009). For example, studies found that Oribatid abundances were lowest in mineral soils and correlated with all soil properties except moisture content, while Collembola abundances was negatively influenced by increased moisture

levels in upland peat habitats where their abundances were lowest (George et al. 2017). In addition, greater abundances of Collembola indicated the use of organic fertilizers and high-level of agricultural management (Cluzeau et al. 2012).

3-1- QBS index "*Qualità biologica dei suoli*"

In general, soil invertebrate-based indices consider the consistency and richness of populations (van Straalen 1998; Jacomini et al. 2000), but their application is often limited by the difficulties in classification and the correct sample's collection. These crucial limitations led to the introduction of a simplified eco-morphological index that does not require the classification of organisms to species level, allows a wider application of these methodologies (Bongers 1990, 1999; Pankhurst 1997).

Based on the concept of eco-morphological index, the integrated QBS index "Qualità Biologica del Suolo" was proposed by Parisi et al (2001).

The QBS-ar index is based on the fact that the higher soil quality, the higher will be the number of microarthropod groups adapted to soil habitats (Sacchi and Testard 1971). Thus, QBS-ar is applied to soil microarthropods, through evaluating the microarthropods' level of adaptation to the soil environment life. Basically, the morphological characters that reveal adaptation to soil environments, such as: reduction or loss of pigmentation and

visual apparatus, streamlined body form, with reduced and more compact appendages (hairs, antennae, legs), reduction or loss of flying, jumping or running adaptations (Parisi 1974).

The main phases for QBS-ar application are: (1) sampling, (2) microarthropods' extraction, (3) preserving the collected specimens, (4) determination of biological forms, (5) calculation of QBS-ar index (Parisi 2001).

3-2- From traditional to Molecular approaches

Even though the QBS index depends on the concept of eco-morphological values, it still needs some knowledge of taxonomy, also there is a little margin of errors related to the samples collecting, the need of more replicates, the possible disturbance while taking and transporting the samples which could complicate the measurement or misleading the soil quality values. In addition, the general tendency nowadays is toward a smaller sample size and more rapid analysis, pushing the researches to find a molecular replacement for the traditional methods of survey and biodiversity measurements. Especially, with the proves that the results obtained using the molecular approaches are broadly similar to results obtained using a traditional, microscopy-based approach (Hamilton et al. 2009).

4- Study objectives:

Back to the controversial matter if organic and conventional farming have different impacts on the soil organisms or not, with the application of the recent advances in molecular taxonomy, benefitting of the next generation DNA sequencing technologies.

In the present study, using the DNA metabarcoding technique as a tool, targeting a fragment the metazoan molecular taxonomy marker (COI), the soil invertebrates' communities of selected farms in the Po Plain (Italy) were characterized in order to address the main following questions:

i) Is soil invertebrates' biodiversity higher in organic respect to conventional farms?
ii) Does the cropping system, as a cultivated crop (i.e., barley) or stable meadow, affect the soil invertebrate communities?
iii) Is the margin of the field a reservoir for the soil invertebrates' diversity of the cultivated area?
iv) In addition, the impact of soil properties on soil invertebrate communities were evaluated.
v) Finally, the correlation between the classic index of soil biological quality (QBS-ar) and the obtained molecular was investigated.

Materials and Methods

1- Study Area

To study the impact of the agricultural strategies on the soil invertebrates' biodiversity, fields were selected in the agricultural park of south Milan "Parco Agricolo Sud Milano". This rural area is regulated by the "Piano Territoriale di Coordinamento (PTC) del parco agricolo sud Milano D.G.R. n VII/818 del 3 agosto 2000", so all the agricultural activities are defined in the "Piano di settore agricolo Art. 19 L.R. 24/90; art. 7 N.T:A del PTC". According to that, and taking in consideration the National regulations (PAN) and the EC regulations, the agricultural activities should be organic and conventional (with integrated production and protection strategies); the fields were selected in the city of Albairate (Fig. 1, Table 1), south west Milan, according to the following criteria:

a) Representing the farming systems of the agricultural park of south Milan, which are organic and conventional
b) Similar geomorphological and microclimatic conditions: elevation, exposure, slope, soil profiles, bedrock.
c) The two farms are similar in the presence of stable meadows and barley fields.

d) The organic farm has adopted this strategy for more than 10 years.
e) The stable meadows in the two farms are about 100 years old.

The chosen farms are:

a- Organic farm (Cascina Isola Maria): representing the organic farming system, a typical grain (cereals/pulses)-livestock farming of the Pianura Padana, with a certified organic management according to the regional, national and European regulations of the organic agricultural production, which prohibits the use of the synthetic chemicals. Two fields were chosen in this farm, the first was a stable meadow for a 100 years (samples labeled as IMS), and the second was a barley field (IMB) part of a rotation.

b- Non-organic Farm (Cascina Visconta): representing the conventional farming system, a typical grain-livestock farming of the Pianura Padana, with this type of management it is allowed the use of the authorized synthetic pesticides, with the respect of the integrated production regulations under the regional, national and European legislations. In addition, two fields were chosen here, a stable meadow for 100 years (VS) and a barley field (VB).

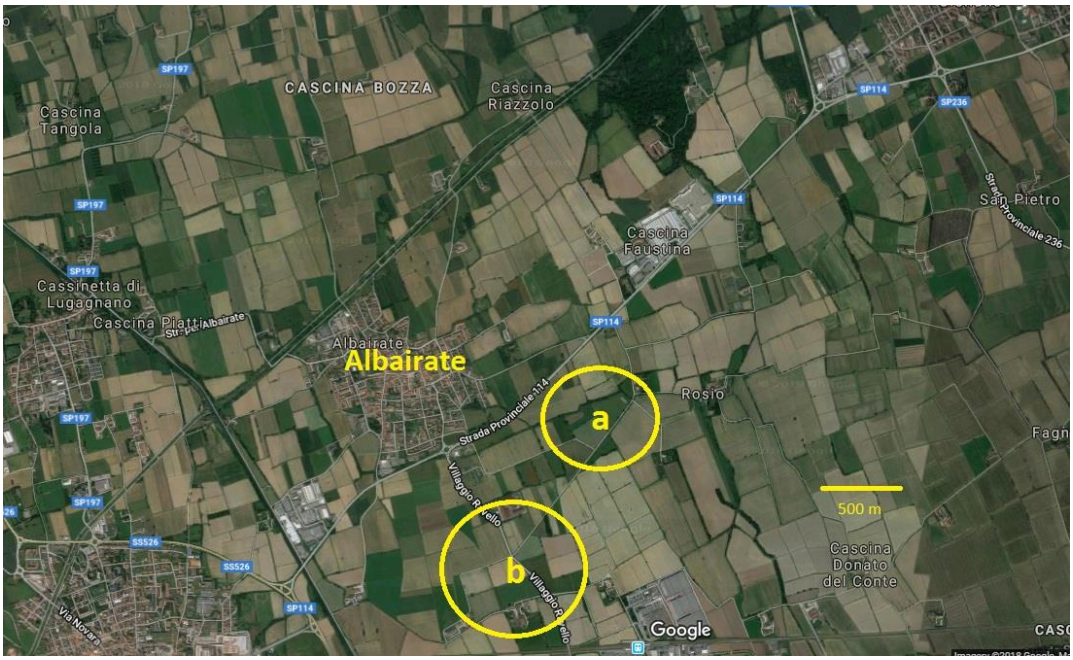

Fig. 1: study area, a) Organic Farm (Isola Maria), b) Conventional farm (Visconta).

Information crops history cultivated in each of the selected fields were reported for the last five years (Table 2), and more details for the agricultural practice for the year of sampling 2016 were collected (Table 3a, 3b), all this data were recovered from the so called: “quaderno di campagna” (farm notebook).

Table 1: The collected samples and their coordinates, showing the codes used for the samples, the farm, the crop and the position in the field:

Sample ID	Farm	Management strategy	Crop	Position (m)*	Sampling point (Latitude, longitude)
IMS0	Isola Maria	Organic	Meadow	0	45.420465° N, 8.956538° E
IMS15	Isola Maria	Organic	Meadow	15	45.420506° N, 8.956801° E
IMS30	Isola Maria	Organic	Meadow	30	45.420462° N, 8.956983° E
IMS45	Isola Maria	Organic	Meadow	45	45.420459° N, 8.957159° E
IMB0	Isola Maria	Organic	Barley	0	45.417159° N, 8.951422° E
IMB15	Isola Maria	Organic	Barley	15	45.417210° N, 8.951545° E
IMB30	Isola Maria	Organic	Barley	30	45.417302° N, 8.951720° E
IMB45	Isola Maria	Organic	Barley	45	45.417360° N, 8.951875° E
VS0	Visconta	Conventional	Meadow	0	45.407753° N, 8.945991° E
VS15	Visconta	Conventional	Meadow	15	45.407864° N, 8.945776° E
VS30	Visconta	Conventional	Meadow	30	45.407744° N, 8.945815° E
VS45	Visconta	Conventional	Meadow	45	45.407621° N, 8.945881° E
VB0	Visconta	Conventional	Barley	0	45.411183° N, 8.950575° E
VB15	Visconta	Conventional	Barley	15	45.411079° N, 8.950586° E
VB30	Visconta	Conventional	Barley	30	45.410921° N, 8.950641° E
VB45	Visconta	Conventional	Barley	45	45.410802° N, 8.950672° E

* The distance of the transect, where the sample was collected, from the margin to the center of the field, (0) is the field margin; (15) is 15 meters from the margin; (30) is 30 meters from the margin; (45) is 45 meters from the margin or the field center.

Table 2: History of the cropping system of the selected fields in the last five years.

Farm	Field	Type	Total Area (m2)	Area (m2)	2016	2015	2014	2013	2012	2011
Isola Maria	Stable Meadow	Organic	8320	520	Field edges and not cultivated area					
		Organic		7800	Meadow					
	Barley	Organic	16070	419	Field edges and not cultivated area					
		Organic		15651	Barley	Fresh Peas	Clover - forage	Corn - silage	Mixed forage	Silage Corn and waxy corn
		Organic				Sorghum for grains			Barley	
Visconta	Stable Meadow	Conventional	4570	70	Field edges and not cultivated area					
		Conventional		4500	Meadow					
	Barley	Conventional	61798	2598	Field edges and not cultivated area					
		Conventional		59200	Barley	Corn - for grains	Corn - silage	Corn - for grains	Corn - for grains	Corn - for grains
		Conventional			Mais - silage					

Table 3a: Detailed agricultural practices in the selected fields of farm Isola Maria for the sampling year.

Farm	Field	Total Area (m2)	Use	Area (m2)	altitude (m a.s.l)	Slope (%)	Exposition	Agrotechniques	Materials	Synthetic Chemicals
Isola Maria	Stable Meadow	8320	Field edges and not cultivated area	520	121.9	0.23	Est	Cut 3-4 times	Solid organic Matter (Cow manure)	NO
			Mixed Meadow	7800						
	Barley	16070	Field edges and not cultivated area	419	122.1	0.23	Est	Flooting irrigation	Solid organic Matter (Cow manure)	NO
			Barley	15651				Plowing and settlement Seeding	Liquid organic Matter (Cow's)	

Table 3b: Detailed agricultural practices in the selected fields of farm Visconta for the sampling year.

Farm	Field	Total Area (m2)	Use	Area (m2)	altitude (m a.s.l)	slope (%)	Exposition	Agrotechniques	Materials	Synthetic Chemicals
Visconta	Stable Meadow	4570	Field edges and not cultivated area	4500	121	0.37	Est	Cut 2-3 times	Solid organic Matter (Cow manure)	NO
			Mixed Meadow	70					Urea (2-3 times) Nitrites	
	Barley	61798	Field edges and not cultivated area	2598	120.7	0.21	Est			
			Barley	59200				Flooting irrigation	Solid organic Matter (Cow manure) Liquid organic Matter (Cow's) Amm. Nitrate 225 kg /ha Amm.Nitrate 225 kg /ha	NO
			Corn - silage	59200				Flooting irrigation	Solid organic Matter (Cow manure) Liquid organic Matter (Cow's) Urea 450 kg/ ha (2 times)	herbicides herbicides

After the selection of the study area, the work was planned as indicated in the detailed scheme in (Fig. 2).

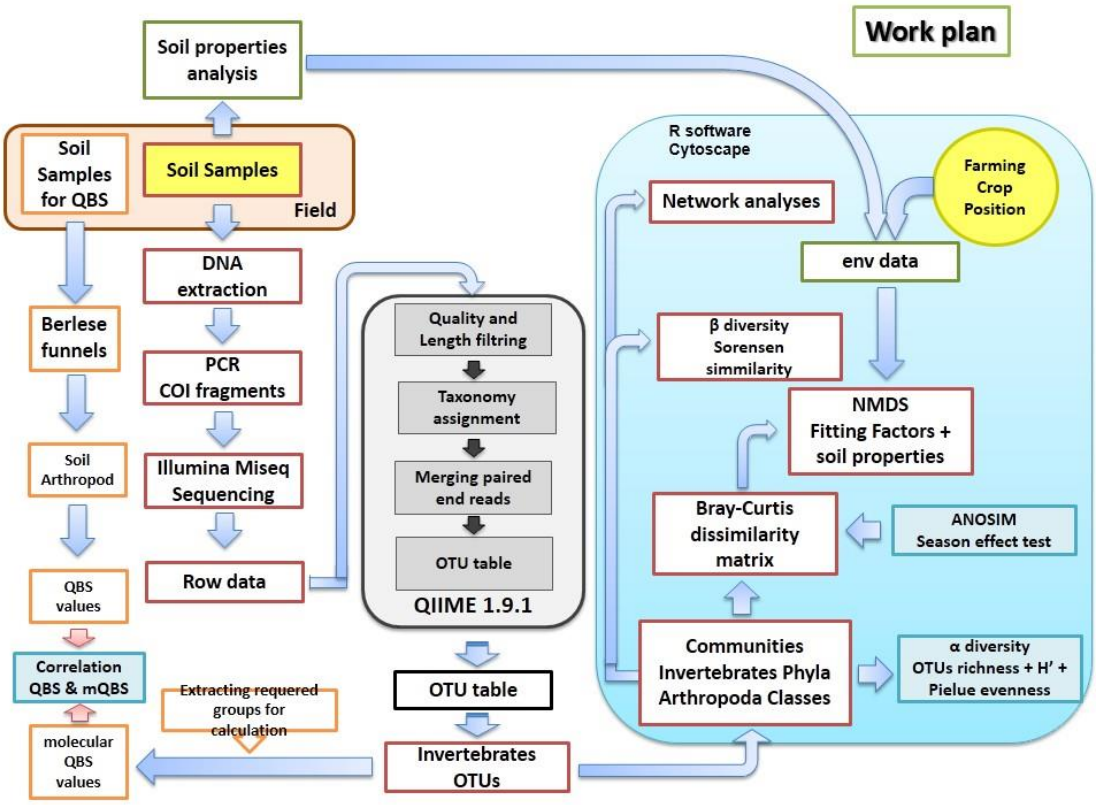

Fig. 2: The applied work steps from sampling to data analysis.

2- Soil Sampling

Soil samples of the top soil layer were taken from each field at 4 sampling sites starting from the edge of the field to the center as the following: from a starting point at the edge of the field (coordinates in the table 1), 3 points with intervals of 15 m were assigned to reach almost the field's center, with a 30 cm long soil

hole borer (Fig. 3a), 5 holes to the right and 5 holes to the left of each point were taken with intervals of 1 m between each hole, the 10 cores of soil for each level were put together in a plastic bag then closed firmly and labeled with the site and date. In (Fig. 3b) a diagram of the sampling in each field.

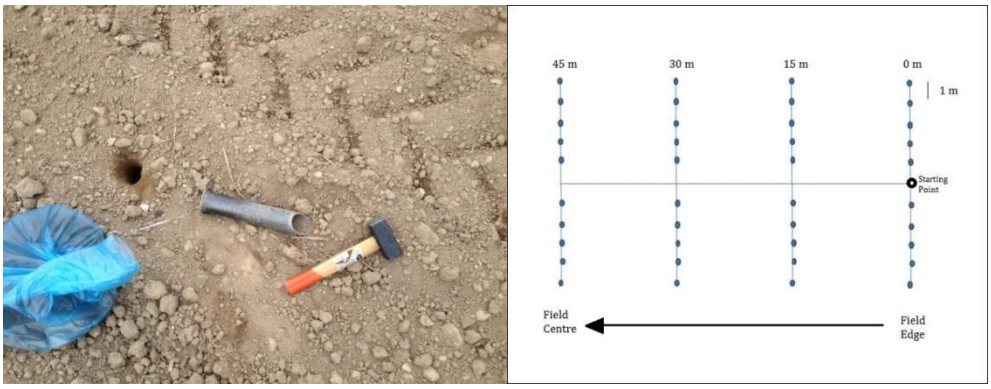

Fig. 3: a) soil sampling equipment; b) sampling diagram in each field

These steps were repeated for the four fields (IM and V), and sampling was repeated in the same assigned spots three times in 2016:

The spring period at 03/05/2016, the Summer Period at 04/07/2016 and the fall period at 28/10/2016. To cover the possible seasonal changes in soil faunal communities.

Bags of the soil samples were transferred directly from the field to a workbench in the site, sieved with 2 mm sieves to remove any existing roots and rocks, then 3 sterile 50 ml tubes were filled with a sieved soil as three replicated of each sample, labeled with details, then kept in a portable thermal box (about 4°C), then transferred to the laboratory where kept in -20 °C until DNA

extraction Fig. 4. The rest of the soil sample was transferred in labeled plastic bags to the laboratory for properties analysis.

Fig. 4: soil samples sieving and replicates preparation for the DNA extraction.

Soil borers, Sieves and cloves were surface sterilized with diluted NaOH (4%), then clean water and dried between each sample to reduce cross-contamination.

3- Soil physio-chemical analysis

These analyses were conducted in the Soil Laboratory in the Department of Agricultural and Environmental Science, according to the laboratory handbook (Metodi di analisi chimica del suolo, Capitolo 2) the soil samples Physio-chemical properties were determined as the following:

3-1- Soil Texture

Pipette method was used for the soil texture determination, this method determines the relative masses of sand, silt and clay in the soil sample (thus, the texture), and soil sample was prepared as the following:

Soil samples were air dried for about 2 weeks, then sieved with 2 mm sieves, split until having a quantity of about 500 g of dried soil. This 500 g of soil were smashed gently with a wooden roller and passed through a 2 mm sieve, this steps were repeated twice, then the samples were split to get a final sample of soil of about 250 g. sample were kept in a clean dry plastic bottles with double caps until use.

After the sample preparation, the determination of the soil texture of each sample were performed as the following:

a- About 10 g of the soil sample were put in a 200 ml plastic bottle with double caps.

b- Add 5 ml of mixture (40 g/l of $(NaPO_3)6$ + 10 g/l of $Na_2CO_3.H_2O$).

c- Add 125 ml of deionized water.

d- Close the caps well and put them in a rotary agitator for at least 16 hours (overnight) with a speed of 40 rpm.

e- In this time, glass Petri dishes (9 cm diameter) should be numbered and weighted after drying in the oven (105 °C) several times, until the weight of each dish is constant.

f- Number of 500 ml cylinders with 25 ml pipettes were cleaned with deionized water and let dry. In addition, funnels and 0.2 mm sieves were cleaned; the laboratory temperature should be 20°C all the time.

g- After the rotary agitator was finished, the sample were transferred into the cylinder through the 0.2 mm sieve by the help of plastic funnel, the bottle was washed with deionized water several times to take all the soil particles, the remaining particles which are larger than 0.2 mm were discarded.

h- Complete the volume of soil suspension in the cylinder to 500 ml with deionized water.

i- Close the top of the cylinder with a cap, put the palm of hand over the top, grasp the bottom of cylinder and invert it for about 30 seconds to re-suspend the soil particles. Place the cylinder gently on the bench and remove the cap with timing started.

j- Take the first sample by the attached pipette (10 ml) at 1:55 min, in one of the numbered and weighted dishes.

k- Put the dish in the oven at 105 °C for about 3-4 Hours.

l- 5 ml of the mixture (40 g/L of $(NaPO_3)6$ + 10 g/L of $Na_2CO_3.H_2O$) should be dried in a plate for 3-4 hours to record the weight of salts in the sample, at least two replicates.

m- The weight of the dried dish after 3-4 h in the oven was recorded, the weight number 1, which refers to the Silt and Clay content.

n- Repeat the shaking (step i) then place the cylinder gently on the bench, remove the cap with timing started.

o- Take the second sample by the attached pipette (10 ml) after 20 Hours.

p- Dry the sample in the oven at 105 °C for about 3-4 Hours.

q- Weight the dishes and record the weight number 2, which refers to the Clay content.

r- Final calculation should be done to calculate the net weight of the Silt, Clay and Sand in the sample.

s- Results of each sample were compared with the triangle of soil Classification of USDA.

3-2- pH determination

the pH meter (CRISON®) was used to determine the pH of a soil sample in water , about 15 g of the prepared soil sample were put in a plastic bottle, deionized water were added in the ratio (1:2.5 – soil : water), the bottles were sealed well and mixed for two hours in the rotary agitator. The soil pH was measured directly at the end of the shaking.

3-3- Carbon to Nitrogen Ratio (C/N)

This method is used to determine the content of the total carbon and the total nitrogen in a soil sample, the measurement depends on burning a little quantity of the soil sample to obtain two gases (N_2, CO_2), and those gases will be separated by gas-

chromatography separation, which determine their relevant presence in the sample.

It is important to detect the presence on any inorganic carbon in the sample before the analysis, this was conducted by adding a few drops of HCl to a few grams of the soil sample in a Petri dish and observe the occurrence of the reaction and forming the bubbles, in the case of the negative reaction the sample contain just organic carbon.

For the determination of the C/N ratio the element analyzer (NC thermoquest, Model NA 1500 serie 2) was used, the soil sample was prepared as the following: the previously dried and sieved soil sample was used, about 10 grams were taken, grinded well in a ceramic mortar then passed through 0.05 mm sieve, this step was repeated until all the sample passes through the sieve, the resulted fine powder like soil were mixed well and kept in a dry small plastic bottle, 40 ± 1 mg of each sample were taken in a small tin capsule and closed well, then formed to a ball shape gently with fingers. The ready capsule of each sample were kept in a labeled Eppendorf tube, three capsules of the standard "Atropine" were prepared in three quantities: 0.75 ± 0.1 mg, 1.5 ± 0.1 mg and 3 ± 0.1 mg.

The analyses of the standards and samples were done under the recommended conditions: T= 1004 – 1020 °C, Pressure = 95 KPa, He= 120 ml/min, He Ref = 60 ml/min O2= 30 – 35 ml/min.

The obtained results for each sample of carbon and nitrogen were divided to get the C/N ratio.

4- DNA extraction

Each replicate of the samples were homogenized by liquid nitrogen in mortars. After homogenization an amount of about 500 µg was taken in sterile 1.5 ml Eppendorf for the extraction; DNA was extracted separately from each replicate by using an extraction kit NucleoSpin@Soil, 50 samples (MACHEREY-NAGEL GmbH & Co), according to the included protocol, SL1 was used, and the final dilution was done by adding 40 ul of SE. The concentration of the resulted DNA was measured by NanoDrop (ND8202, Software NanoDrop 1000 3.7), 1 µl of the DNA was used, after that an equimolar pool was prepared for each sample of the three replicates. The pool of DNA was kept at (-20°C) until use.

5- PCR amplification of COI fragments

The obtained pooled DNAs were used as a template for PCRs with primers targeting the mitochondrial COI.

Three PCRs were performed by using three pairs of primers to amplify the COI fragments; the three replicates of the extracted DNA were used. Primers with the references are described in the table 4:

Table 4: the set of primers used for the amplification of the COI fragments.

PCR	Primer	Sequence (5'–3')	Fragment size (bp)	Reference
PCR1	CO1F2 F CO1R2 R	TCTACYAATCATAAAGATATTGGTAC ACTTCTGGATGACCAAAGAATCA	680	Arribas et al. 2016 Modified from Folmer et al. 1994
PCR2	mlCOIintF F jgHCO2198 R	GGWACWGGWTGAACWGTWTAYCCY CC TAIACYTCIGGRTGICCRAARAAYCA	313	Leray, 2013
PCR3	FoldF F FoldR R	TCNACNAAYCAYAARRAYATYGG TANACYTCNGGRTGNCCRAARAAYCA	658	Yu et al. 2012

For the PCR1, and PCR3, the used Taq was KAPA (KAPA® HiFi HotStart Ready Mix, KAPA Biosystems INC., MA, USA), while the Taq Quiagen (QIAGEN ® PCR Master Mix Kit, QIAGEN, Venlo Netherlands) was used for the PCR2.

The Thermo-cycle:

For the PCR1 and PCR2: 16 initial cycles: denaturation for 10s at 95°C, annealing for 30s at 62°C (−1°C per cycle) and extension for 60s at 72°C, followed by 25 cycles at 46°C annealing temperature.

For the PCR4: Amplification was accomplished by initial denaturation for 3 min at 94°C followed by 5 initial Cycles: Denaturation for 30s at 94°C, annealing for 30s at 45°C, extension for 1 min at 72°C, followed by 35 cycles: Denaturation for 30s at 94°C, annealing for 1 min at 51°C, extension at 72°C for 1 min,

finally 10 min at 72°C and 4°C storage. Success of PCR amplifications was checked on 1.5% agarose gels. A clear single band of expected length indicated the success of the PCR.

Amplicons were cleaned-up with Agencourt AMPure XP (Beckman, CA - USA) and quantified with a sizes check using Agilent DNA7500 and the DNA 12000 kit (Agilent Technologies, Woldbronn, Germany), and then an equimolar pool [75 nM] of the three PCR products was prepared for each sample.

6- Library preparation and Illumina MiSeq run

Libraries were prepared with a second stage PCR using Nextera XT Index 1 Primers (N7XX) and Nextera XT Index 2 Primers (S5XX) from the Nextera XT Index kit, following 16S Metagenomic Sequencing Library Preparation protocol (http://www.illumina.com/content/dam/illuminasupport/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide15044223-b.pdf). The libraries obtained were quantified by Real Time PCR with KAPA Library Quantification Kits (Kapa Biosystems, Inc. MA - USA) pooled at equimolar concentration [200 nM], followed by gel purification using Wizard® SV Gel and PCR Clean-Up System (Promega corporation- USA). Sequencing was performed by MiSeq sequencer (Illumina, CA – USA) using reagent kit v2 with paired-end reads of 250 bp.

7- COI database preparation

All available cytochrome oxidase I (COl) sequences from the BOLD website were retrieved (Ratnasingham and Hebert, 2013) the files from BOLD consist of excel spreadsheets containing, among other metadata, the nucleotide sequence and the taxonomic assignment of each entry. All non-COl entries were removed, as well as entries with COl sequences shorter than 400 nucleotides or containing more than two consecutive "N"s. COl sequences and their relative taxonomic assignments were recovered from the remaining entries. The taxonomy classifications were formatted to follow QIIME (Caporaso et al. 2010) requirements, i.e. each entry had its taxonomy expressed in seven taxonomic levels from Kingdom to species. The subfamily level was removed and if a given taxonomic level was missing, it was replaced by a generic placeholder (e.g., "s " for species, "g_" for genus, and “p_" for phylum).

The sequence file was then processed to remove redundant sequences. CD-HIT -EST (Fu et al. 2012; Li and Godzik 2006) was used to cluster the sequences at both 99% and 100% sequence similarity. The most abundant sequence was used as representative sequence for each cluster. Taxonomy files corresponding to both sequence files (i.e. 99% and 100% sequence similarity) were generated from the original one.

This database contained also representative sequences of Bacteria, Fungi, Archaea and Plantae kingdoms in order to check the

presence of any contamination or any other non target reads in the obtained sequences.

8- Analysis of sequence data

The paired-end reads were quality-filtered and trimmed to retain only reads longer than 200pb with a Phred score > 30. The forward reads were then clustered into OTUs (Operational Taxonomic Unites) using uclust (Edgar 2010) and the most abundant reads chosen as representative sequence. The sequence was then united with its reverse counterpart using a custom script. The united sequence was subsequently used for the taxonomic assignment with BLAST (Altschul et al. 1997), and a custom build database comprising sequences from Nematode, Acari, Collembola, Annelida, Arthropoda, Rotifera, and Tardigrada, as well as from other kingdoms (i.e., Fungi, Bacteria and Planta to check the contamination). Most of the analysis was carried out using the QIIME 1.9 (Caporaso et al. 2010) pipeline. The generated OTU table was used for the subsequent analyses.

9- Diversity and Statistical analyses

Invertebrates OTU table was used as input for the diversity analyses carried out with R packages (R Project 3.0.2; http://cran.r-project.org/); in particular the **vegan** package was used (Dixon 2003; Oksanen et al. 2017). In order to legitimate us to consider the samples collected in the three periods as biological replicates of the sample, we performed a test for the differences

among the communities by nonparametric one-way analysis of similarity (ANOSIM; Clarke 1993), based on the Bray-Curtis dissimilarity (Bray & Curtis 1957) of presence/absence OTU table (999 permutations). Since no differences were recovered among the communities collected in the three periods (Table S4), they were then considered as biological replicates of the sampling point. The community data on which the analyses were preformed after that were obtained by filtering the presence-absence OTU table adopting the following criterion: the presence of a taxon in a sample is counted when it occurred in at least two out of three replicates.

The α-diversity was calculated using as estimators the Shannon index (Shannon 1948), the Pielou's evenness (Pielou 1975), and the observed species richness. For each community, a Non-metric Multi-Dimensional Scaling (NMDS; Kruskal 1964) based on the Bray-Curtis dissimilarity of the OTU table was performed using *metaMDS* in **vegan**. The correlation between the communities and the physical and chemical parameters of the soil was investigated by fitting the previous NMDS ordination scores with the *envfit* function. The permutation of the community composition-based dissimilarity matrix allowed assessment of the significance of the fitted vectors, and the r^2 coefficient was calculated.

In order to evaluate shift of the invertebrates communities from field hedges to field center, the nestedness and turnover

component of the β-diversity were calculated using Simpson-based multiple-site dissimilarity (β_{SIM}; Baselga 2010) and nestedness-resultant multiple-site dissimilarity (β_{NES}; Baselga 2010). The overall β-diversity of the invertebrates communities, as a whole and splitted at phylum and arthropods class levels, associated with the four transects was estimated using the Sørensen-based multiple-site dissimilarity (β_{SOR}; Baselga 2010), implemented in R package betapart (Baselga & Orme 2012).

Venn diagrams, acquired with the R package **gplots**, provided a visualization of the number of taxa, establishing the core diversity for the different analyzed farming systems and fields.

10- Bipartite network analyses

Bipartite network analyses of each community under the tested farming systems and the crops were performed by calculating the matrices of similarity between samples in R and visualized in Cytoscape (Shannon et al. 2003). Nodes in the network corresponded to samples in three replicates (three sampling times) and the invertebrate's group OTUs, with links indicating the presence of an OTU in the sample.

The network parameters (connectance, nestedness, cluster coefficient, niche overlap, and robustness) were calculated for each network by using the function *networklevel* in R package **Bibartite** (Dormann et al. 2008, Dormann and Strauss 2014).

The calculated parameters for each network are described as the following:

- Connectance: Realized proportion of possible links.
- Nestedness: Nestedness temperature of the matrix (0 means cold, i.e. high nestedness, 100 means hot, i.e. chaos).
- Cluster coefficient: for the samples is simply the number of realized links divided by the number of possible links. Introduced by Watts & Strogatz (1998).
- Niche overlap: the mean similarity in interaction pattern between species of the same level, calculated by default as Horn's index ('dist="horn"').
- Robustness: robustness.HL refers to the robustness of the higher level (samples) to extinctions in the lower level (species).

11- Correlation between the QBS-ar and the molecular QBS

11-1- Sampling

At the field margin and middle zones of the sampling points selected previously, 3 samples at a transect with 2 m intervals were taken, the sample consisted of 10 × 10 cm area, and a depth up to 10 cm of soil taken with a plain spatula, samples were placed in a paper bag, labeled and transported to the laboratory.

11-2- Microarthropods' extraction

A simple Berlese–Tullgren funnel was used for extraction (15 cm diameter, 2 mm mesh, and 40 W lamp at 15 cm distance). Soil arthropods were extracted within 24 hours from sampling.

The soil core was carefully placed on the mesh above the funnel together with all the soil lost from sample during handling before inserting a bottle filled with preservative liquid (2 parts 75% ethanol and 1 part glycerol) beneath the funnel.

The extraction system were kept free from vibrations and other disturbance. The extraction duration was 5 days.

11-3- Calculation of QBS-ar index

Arthropods in the preservative liquid were identified and the QBS-ar index was calculated for each sample based on the presence of an individual of the microarthropod reported in the table of Eco-morphologic indices (EMIs) (Parisi 2001).

Whenever two eco-morphological forms were present in the same group, the final score is determined by the higher EMI. In other words, the most highly adapted microarthropods belonging to a group determine the overall EMI score for that group. The QBS-ar score of a sample was calculated by the sum of the EMIs of all collected groups in the sample.

11-4- The molecular QBS.

Appling the basics of the classic taxonomical QBS-ar to the recovered OUT table was performed, the groups of arthropods mentioned in the Eco-morphologic indices (EMIs) were extracted from the OUT table of the samples of the margin and the center of the studied fields, based on the presence/absence recorded in the OUT table, a molecular QBS value. Finally, a Pearson correlation was calculated between the QBS-ar and the molecular QBS.

Results

1- Soil samples' physio-chemical properties

A total of 48 soil samples were taken and analyzed for their physio-chemical properties, this resulted in detecting that the soil texture was loamy in most of the samples, and sandy loam in the margin zone of the barley field (Org.) and all the samples of the barley field (Conv.) (Table 5).

Soils of the organic farm were more acidic comparing to the conventional ones, since that the pH values were significantly lower ($t = -3.75$, p value = 0.001) (Table 5), also they contained a significantly higher percentage of Nitrogen (N %) ($t = 13.03$, p value = 0.000), while no significant differences were found between the two farms in terms of organic carbon content (C %) and the C/N ratio (p value > 0.05). No differences were found between the cropping systems (p value > 0.05) for all the studied parameters (pH, N%, C% and C/N ratio). Significant differences were found between the positions in the field (p value < 0.01), where the margin zone samples were significantly lower in pH values, and significantly higher in terms of N%, C% and C/N ratio.

Table 5: Samples site and farming information, and results of soil analysis for each sample

Sample ID	Texture	pH	N %	C %	C/N ratio
IMS0	Loam	4.59 ± 0.04	0.39 ± 0.01	4.55 ± 0.03	11.77 ± 0.03
ISM15	Loam	5.97 ± 0.1	0.31 ± 0.02	2.82 ± 0.02	9.11 ± 0.07
IMS30	Loam	5.96 ± 0.03	0.35 ± 0.02	3.15 ± 0.02	9 ± 0.1
IMS45	Loam	6.1 ± 0.04	0.39 ± 0.01	3.64 ± 0.01	9.25 ± 0.11
IMB0	Sandy loam	5.86 ± 0.03	0.13 ± 0.01	1.35 ± 0.02	10.02 ± 0.03
IMB15	Loam	5.87 ± 0.01	0.13 ± 0.01	1.25 ± 0.04	9.44 ± 0.13
IMB30	Loam	6.13 ± 0.02	0.13 ± 0.01	1.31 ± 0.01	9.75 ± 0.07
IMB45	Loam	5.89 ± 0.02	0.16 ± 0.02	1.58 ± 0.02	9.68 ± 0.1
VS0	Loam	6.64 ± 0.12	0.27 ± 0.01	2.67 ± 0.02	10.04 ± 0.05
VS15	Loam	6.32 ± 0.1	0.3 ± 0.02	2.7 ± 0.03	9.12 ± 0.02
VS30	Loam	6.12 ± 0.01	0.28 ± 0.01	2.61 ± 0.03	9.45 ± 0.02
VS45	Loam	6.14 ± 0.05	0.33 ± 0.01	2.96 ± 0.03	9.1 ± 0.04
VB0	Sandy loam	6.15 ± 0.04	0.24 ± 0.04	2.7 ± 0.73	11.13 ± 0.06
VB15	Sandy loam	6.45 ± 0.14	0.11 ± 0.01	1.15 ± 0.06	10.13 ± 0.01
VB30	Sandy loam	6.2 ± 0.1	0.11 ± 0	1.06 ± 0.04	9.81 ± 0.01
VB45	Sandy loam	6.21 ± 0.1	0.11 ± 0.02	0.99 ± 0.03	9.97 ± 0.01
t test	Between farms	-3.75	13.03	1.821	-0.853
Sig.		0.001	0	0.082	0.403
t test	Between crop	-1.137	-1.351	-0.68	-0.134
Sig.		0.267	0.19	0.503	0.895
F	Between positions	51.679	15.964	50.787	999.11
Sig.		0.000	0.000	0.000	0.000

2- Dataset description, soil invertebrates α-diversity

The 48 metabarcoding libraries yielded about 13M paired reads that after the filtering procedures resulted in 2,313,488 high quality reads. Reads were clustered into 18,034 OTUs (used threshold 3% of sequence identity) of which 13,551 were assigned to invertebrates (corresponding to 1,561,294 reads). The remaining OTUs were discarded as non-target groups (Table S1).

No differences, in terms of community composition, were recovered among the three sampling seasons (ANOSIM $R_{INVERTEBRATES}$ = 0.01; p-value = 0.31), this R value means the strength of the factor in its effect on the samples, thus the three temporal replicates were considered as true replications for the following analyses (Table 6).

Table 6: Results of ANOSIM analysis showing that the season has no effect on the distributions of the soil communities of Metazoan, Invertebrates and Arthropods

	Metazoan		Invertebrates		Arthropods	
	R	Significance	R	Significance	R	Significance
Season	0.011	0.298	0.012	0.309	0.001	0.417

Arthropods represent the most abundant invertebrate group in each sample as assigned OTUs (84.4 ± 1.7 %), followed by annelids (6.5 ± 1.9 %) and molluscs (about 5%) (Table 8; Fig 5). Within arthropods, the most abundant group is represented by insects (68.0 ± 2.0 %) followed by Arachnida (22.3 ± 1.4 %) and collembolans with a percentage of about 5 % (Fig.5, Table 8). Furthermore, the recovered insects OTUs were dominated by Hymenoptera, Diptera and Lepidoptera (27.33 ± 1.31%, 20.58 ± 1.2 % and 19.96 ± 1.43% respectively).

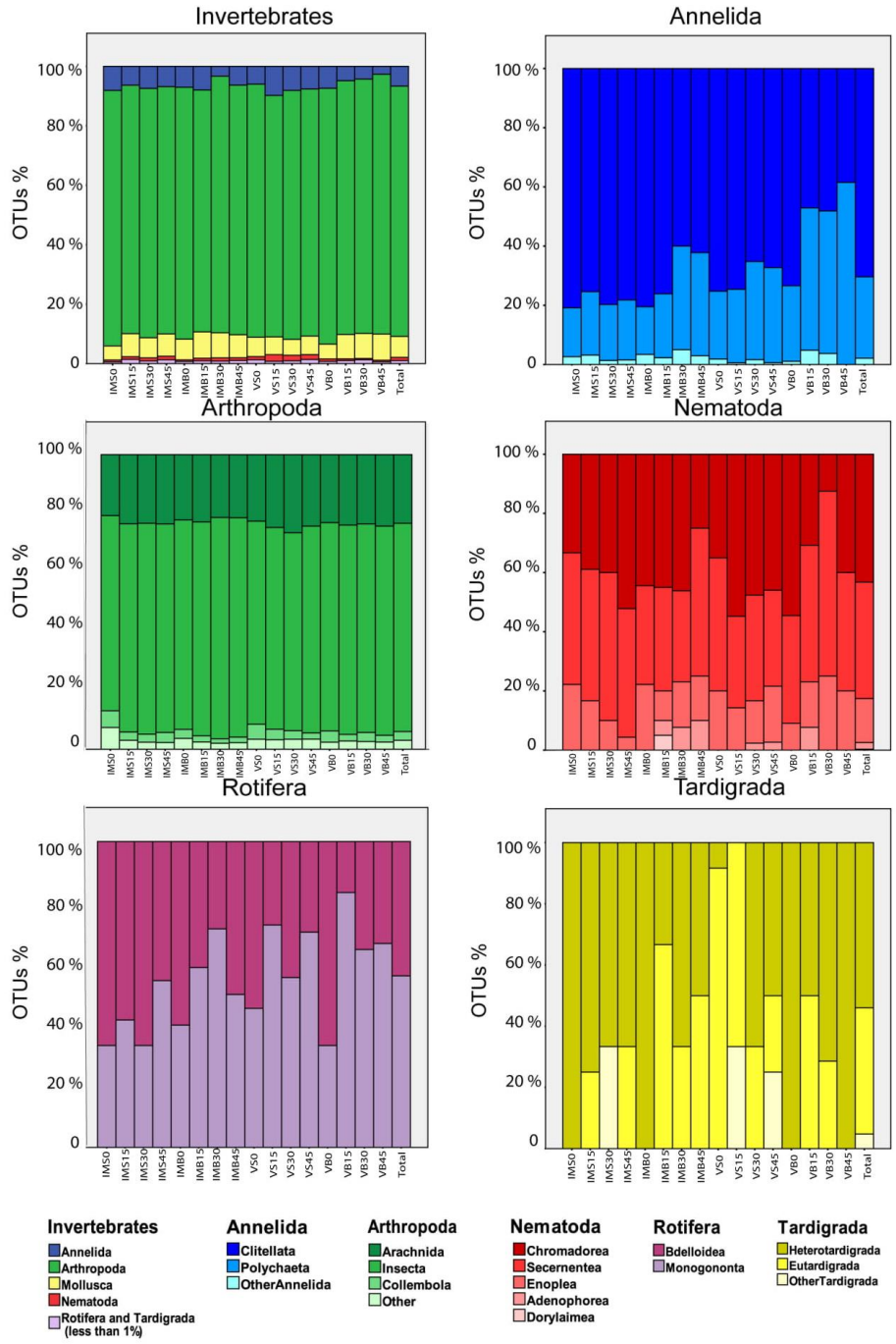

Fig. 5: Percentages of the assigned OTUs in the samples to the Invertebrates and its Phyla, and the main classes in each Phylum.

The OTU richness spans from 977 in the center of the conventional barley field (VB45) to 2270 in the center of the conventional stable meadow (VS45), while Shannon Index values ranged from 5,34 ± 0,91 in the center of the conventional barley field (VB45) to 6,83 ± 0,1 in the center of the organic stable meadow (IMB45), finally the Pielou's evenness values ranged between 0,74 ± 0,08 in the organic stable meadow (IMB15) and 0,86 ± 0,01 at the margin of the conventional barley (VB0) (Table 7). No significant differences, in terms of Shannon's index and Pielou's evenness, were observed among samples collected from organic and conventional farming system, field use and the position of the samples in the field (Anova, p-value > 0.05); while in the case of OTUs richness differences were recovered among the field use and the position in the field (Anova, p-value < 0.01) (Table 7, S2).

Sample ID	Invertebrates OTUs	H' Mean ± SE	Pielou's Mean ± SE	OTUs (% of Invertebrates OTUs)					OTUs (% of Arthropods OTUs)					
				Annelida	Mollusca	Nematoda	Rotifera	Tardigrada	Arthropoda	Insecta	Arachnida	Collembola	Diplura & Protura	Chilopoda & Diplopoda
IMS0	1412	6.24 ± 0.42	0.84 ± 0.04	115 (8.1%)	67 (4.7%)	9 (0.6%)	6 (0.4%)	2 (0.1%)	1213 (85.9%)	782 (64.5%)	244 (20.1%)	67 (5.5%)	3 (0.2%)	66 (5.4%)
IMS15	1974	6.61 ± 0.37	0.85 ± 0.02	126 (6.4%)	153 (7.8%)	18 (0.9%)	24 (1.2%)	4 (0.2%)	1649 (83.5%)	1112 (67.4%)	369 (22.4%)	44 (2.7%)	5 (0.3%)	18 (1.1%)
IMS30	1991	6.54 ± 0.26	0.84 ± 0.03	148 (7.4%)	135 (6.8%)	20 (1%)	15 (0.8%)	3 (0.2%)	1670 (83.9%)	1147 (68.7%)	373 (22.3%)	44 (2.6%)	4 (0.2%)	11 (0.7%)
IMS45	1941	6.49 ± 0.39	0.84 ± 0.04	133 (6.9%)	146 (7.5%)	23 (1.2%)	22 (1.1%)	3 (0.2%)	1614 (83.2%)	1094 (67.8%)	363 (22.5%)	53 (3.3%)	4 (0.2%)	11 (0.7%)
IMB0	1678	6.33 ± 0.44	0.82 ± 0.04	118 (7%)	117 (7%)	9 (0.5%)	10 (0.6%)	2 (0.1%)	1422 (84.7%)	988 (69.5%)	308 (21.7%)	43 (3%)	2 (0.1%)	35 (2.5%)
IMB15	2211	5.83 ± 0.69	0.74 ± 0.08	176 (8%)	196 (8.9%)	20 (0.9%)	17 (0.8%)	3 (0.1%)	1799 (81.4%)	1243 (69.1%)	391 (21.7%)	36 (2%)	2 (0.1%)	19 (1.1%)
IMB30	1202	6.27 ± 0.49	0.88 ± 0.03	40 (3.3%)	102 (8.5%)	13 (1.1%)	7 (0.6%)	3 (0.2%)	1037 (86.3%)	744 (71.7%)	211 (20.3%)	15 (1.4%)	0 (0%)	10 (1%)
IMB45	2135	6.83 ± 0.1	0.87 ± 0.01	135 (6.3%)	165 (7.7%)	20 (0.9%)	18 (0.8%)	4 (0.2%)	1793 (84%)	1281 (71.4%)	368 (20.5%)	33 (1.8%)	4 (0.2%)	18 (1%)
VS0	1814	6.55 ± 0.36	0.85 ± 0.02	109 (6%)	118 (6.5%)	20 (1.1%)	11 (0.6%)	12 (0.7%)	1544 (85.1%)	1039 (67.3%)	340 (22%)	77 (5%)	11 (0.7%)	21 (1.4%)
VS15	1850	6.33 ± 0.24	0.83 ± 0.03	181 (9.8%)	110 (5.9%)	42 (2.3%)	11 (0.6%)	3 (0.2%)	1503 (81.2%)	992 (66%)	358 (23.8%)	51 (3.4%)	4 (0.3%)	23 (1.5%)
VS30	2261	6.11 ± 0.43	0.78 ± 0.05	184 (8.1%)	121 (5.4%)	42 (1.9%)	18 (0.8%)	3 (0.1%)	1893 (83.7%)	1225 (64.7%)	484 (25.6%)	53 (2.8%)	5 (0.3%)	32 (1.7%)
VS45	2270	6.13 ± 0.54	0.78 ± 0.06	174 (7.7%)	142 (6.3%)	37 (1.6%)	27 (1.2%)	4 (0.2%)	1886 (83.1%)	1266 (67.1%)	438 (23.2%)	38 (2%)	3 (0.2%)	31 (1.6%)
VB0	1268	6.38 ± 0.17	0.86 ± 0.01	94 (7.4%)	63 (5%)	11 (0.9%)	6 (0.5%)	3 (0.2%)	1091 (86%)	740 (67.8%)	241 (22.1%)	40 (3.7%)	1 (0.1%)	13 (1.2%)
VB15	2154	6.45 ± 0.2	0.83 ± 0.02	104 (4.8%)	176 (8.2%)	13 (0.6%)	18 (0.8%)	4 (0.2%)	1839 (85.4%)	1255 (68.2%)	422 (22.9%)	40 (2.2%)	7 (0.4%)	19 (1%)
VB30	1877	6.49 ± 0.21	0.85 ± 0.02	81 (4.3%)	158 (8.4%)	8 (0.4%)	17 (0.9%)	7 (0.4%)	1606 (85.6%)	1101 (68.6%)	366 (22.8%)	48 (3%)	6 (0.4%)	18 (1.1%)
VB45	977	5.34 ± 0.91	0.77 ± 0.1	26 (2.7%)	86 (8.8%)	5 (0.5%)	3 (0.3%)	3 (0.3%)	854 (87.4%)	588 (68.9%)	201 (23.5%)	19 (2.2%)	5 (0.6%)	8 (0.9%)
			% mean ± SD	6.5±1.9	7.1±1.4	1±0.5	0.8±0.3	0.2±0.1	84.4±1.7	68±2	22.3±1.4	2.9±1.1	0.3±0.2	1.5±1.1

3- Factors affecting soil invertebrates communities

NMDS analyses were performed on OTU table of invertebrates communities as whole and its Phyla of Annelida, Mollusca, Nematoda, Rotifera & Tardigrada and Arthropoda, the Arthropoda also where analyzed as whole and its Classes of Insecta, Arachnida, Collembola, Diplura & Protura and Chilopoda & Diplopoda (due to the low number of taxa in some groups they were analyzed together). Stable solutions were achieved in two dimensions (stresses ranged from 0.07 to 0.15; Fig. S1), fitted with soil physical and chemical properties, the agricultural strategy (farming and crop) and the position of sampling.

Focusing on the agricultural practices (Table 8), No significant effect was found for the farming system as organic or conventional on the invertebrates' communities' structure; except the case of the Rotifera & Tardigrada (p-value < 0.05). The significant effects were found for the crop as barley or a stable meadow on the Invertebrates (p-value < 0.05) and its phyla of Mollusca, Nematoda and Arthropoda; as well for the Arthropoda classes of Arachnida and Insecta (p-value < 0.01). Finally, the sampling position affected the communities of Annelida, Arthropoda, and the classes of Arthropoda: Arachnida, Insecta, Collembola, Chilopoda & Diplopoda (p-value < 0.05). These patterns are highlighted by the non-overlapping standard error ellipses representing the 95% confidence area around the mean of samples for the position in the

field, it was clear the separation of the field margin in all the studied groups from the other ellipses, and the samples of the same crop were aggregated together regardless the farming system as organic or conventional in Invertebrates and its phyla of Mollusca, Nematoda and Arthropoda; as well for the Arthropoda classes of Arachnida and Insecta (Table 8, Fig. 6).

Regarding the soil properties, the invertebrates ordination was significantly correlated with the chemical properties of the soil (pH, N%, C% and C/N ratio), while the N%, C% and C/N ratio were significantly effective on Annelida, Mollusca and Nematoda, in addition to that the Rotifera & Tardigrada communities were correlated with the pH of the soil (p-value < 0.01). For the Arthropoda and its classes, the significant effect of the soil properties (pH, N%, C% and C/N ratio) was found for the Arthropoda and Insecta, Arachnida communities were correlated with N%, C%, C/N ratio and the soil texture, Collembola communities were correlated just with the C/N ratio (p-value < 0.05), while the Chilopoda & Diplopoda were significantly correlated with the soil's pH (p-value < 0.001) and the C% (p-value < 0.05). Finally, the Diplura & Protura communities were not correlated with any of the soil parameters (Table 8).

Table 8: Correlation and the corresponding significances between invertebrates' communities and the tested factors (farming, crop, position and soil physical and chemical parameters)

Factors		Invertebrate	Annelida	Mollusca	Nematoda	Rotifera & Tardigrada	Arthropoda	Arachnida	Insecta	Collembola	Chilopoda & Diplopoda	Diplura & Protura
Farming	r^2	0.029	0.088	0.151	0.062	0.247	0.038	0.042	0.028	0.021	0.171	0.067
	p value	0.664	0.316	0.079	0.394	0.018*	0.607	0.519	0.691	0.749	0.062	0.891
Field	r^2	0.272	0.160	0.289	0.344	0.116	0.275	0.274	0.292	0.078	0.050	0.067
	p value	0.016*	0.088	0.007**	0.002**	0.177	0.008**	0.008**	0.009**	0.328	0.570	0.317
Position	r^2	0.407	0.388	0.317	0.295	0.211	0.473	0.431	0.450	0.458	0.378	0.019
	p value	0.038*	0.038*	0.083	0.096	0.193	0.013*	0.031*	0.020*	0.017*	0.033*	0.898
Texture	r^2	0.115	0.068	0.128	0.113	0.079	0.108	0.202	0.127	0.066	0.029	0.030
	p value	0.179	0.369	0.133	0.181	0.303	0.186	0.024*	0.139	0.388	0.712	0.783
pH	r^2	0.391	0.344	0.218	0.064	0.738	0.421	0.166	0.419	0.155	0.837	0.213
	p value	0.048*	0.055	0.210	0.681	0.002**	0.039*	0.299	0.027*	0.339	0.001***	0.168
N_percent	r^2	0.522	0.504	0.375	0.508	0.355	0.538	0.517	0.537	0.198	0.265	0.151
	p value	0.007**	0.009**	0.043*	0.012*	0.049*	0.009**	0.011*	0.005**	0.235	0.136	0.334
C_percent	r^2	0.535	0.642	0.388	0.430	0.538	0.556	0.481	0.562	0.334	0.398	0.127
	p value	0.004**	0.001***	0.036*	0.025*	0.011*	0.006**	0.013*	0.004**	0.076	0.031*	0.366
ratio_CN	r^2	0.833	0.277	0.886	0.440	0.473	0.791	0.903	0.827	0.453	0.401	0.051
	p value	0.001***	0.129	0.001***	0.017*	0.017*	0.001***	0.001***	0.001***	0.016*	0.057	0.669
	* 0.05	** 0.01	*** 0.001									

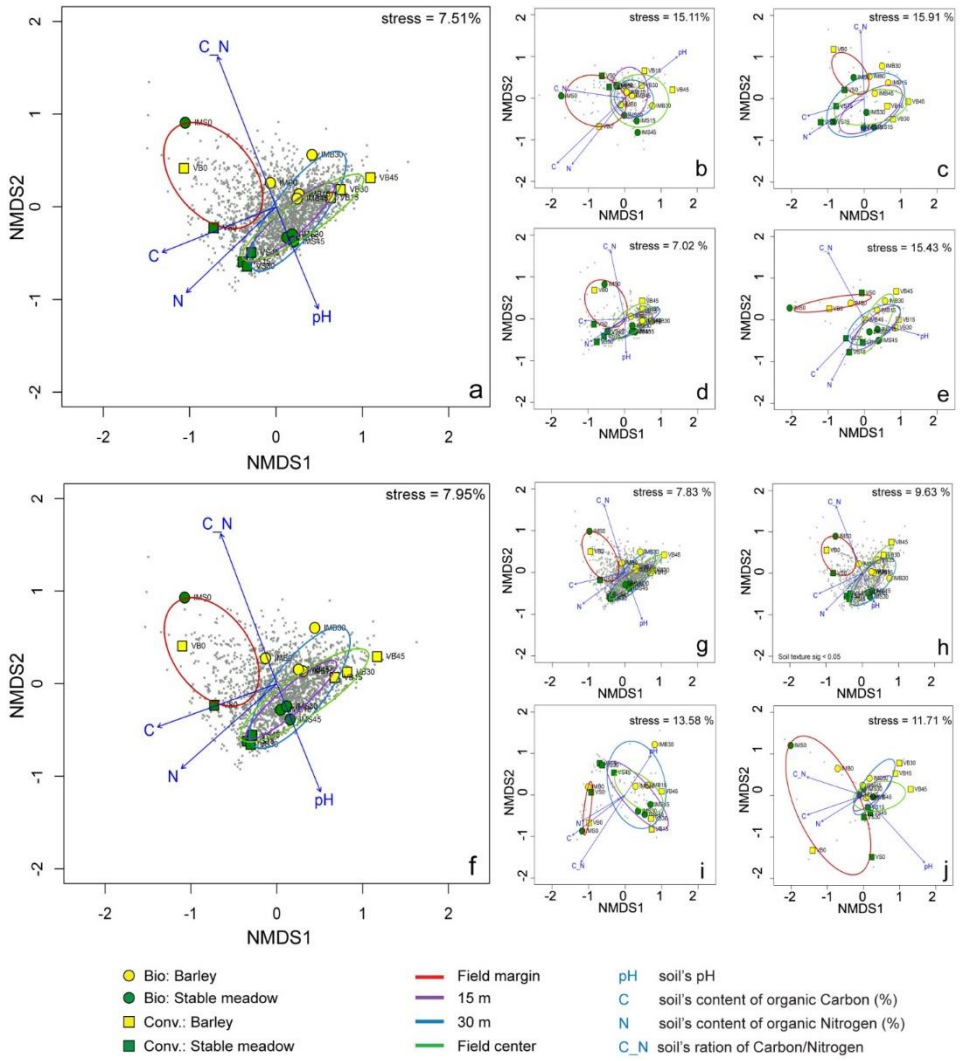

Fig. 6: Biplot of the two dimensional Nonmetric Multi-Dimensional Scaling representing correlations between the invertebrates communities and factors (farming, crop, position and soil physical and chemical parameters the soil), a) Invertebrates; b) Annelida; c) Nematoda; d) Mollusca; e) Rotifera & Tardigrada f) Arthropoda; g) Insecta; h) Arachnida; i) Collembola; j) Chilopoda & Diplopoda. Grey crosses represent the identified OTUs, the blue vectors the mean direction and strength of correlation with soil physical and chemical features, standard error ellipses representing the 95% confidence area around the mean of the levels of the position in the field.

4- Shifting of soil communities from margin to field center

Beta diversity:

A unique pattern was observed for all the studied groups under the farming and crop variables, summarized in the separation of the margin zone based on the β_{SOR} matrix of dissimilarity, while the three samples of the field levels were grouped in another cluster (Figs. 7a, 7b). The overall β-diversity (β_{SOR}) for the invertebrates communities ranged from 0.58 in the stable meadow (Conv.) to 0.69 in both Stable meadow (Org.) and Barley (Conv.). The turnover (β_{SIM}) ranged from 0.51 in barley (Org.) to a maximum of 0.66 in stable meadow (Org.). The nestedness (β_{SNE}) ranged from 0.03 in the stable meadow (Org.) to a maximum of 0.10 in both barley fields. These values of the overall β-diversity and its components were varied between the studied groups and the farming variables (Figs. 7a, 7b).

In details, the phyla of the invertebrates showed a similar behavior of the invertebrates: Annelida overall β-diversity (β_{SOR}) was higher in the barley field (Conv.) with 0.77 and the nestedness (β_{SNE}) was higher in the Barley (Org.) with a value of 0.21; Mollusca overall β-diversity (β_{SOR}) was 0.65 in the stable meadow (Org.) and decreased to 0.54 in the barley (Org.). The turnover (β_{SIM}) was low in both barley fields with a values of 0.43 and 0.40 in Conv. and Org. respectively, nestedness (β_{SNE}) values were higher in the

Barley (0.19) and lower in the stable meadow both of the conventional farming; regarding the Rotifer & Tardigrade, the overall β-diversity (β_{SOR}) was higher than 0.64 in all sites and reached a maximum of 0.75 in the stable meadow (Org.) and the barley (Conv.). The turnover (β_{SIM}) was higher in the stable meadows (0.65 in both farming systems) and lower in the barley fields, the nestedness (β_{SNE}) was higher in the barley (Conv.) with a value of 0.16.

Regarding the groups (Classes) of the Arthropoda, the Insecta and Arachnida had the same behavior of the Invertebrate and Arthropoda in general (values of overall β-diversity (β_{SOR}) of values about 0.57 to 0.70, and nestedness (β_{SNE}) less than 0.10) (Fig 7b), while a different values were estimated in the other groups; Collembola β-diversity (β_{SOR}) reached a maximum of 0.85 in the barley (Org.), the nestedness (β_{SNE}) were lower than 0.10, turnover (β_{SIM}) values were 0.80 in barley (Org.) and decreased to 0.57 in stable meadow (Conv.).

Invertebrates' communities associated with those fields under the effect of the two farming systems were found to be similar in their patterns in terms of Sørensen dissimilarity, with some exceptions when the values were estimated for smaller groups. The turnover components accounted for the majority of the overall β-diversity, while the nestedness component contributed only marginally to the overall β-diversity in all cases (higher value was 0.26). The

results indicate that the Invertebrates communities are relatively constant, with high dissimilarity between the field samples and the sample of the margin zone as visualized in the heatmaps of the dissimilarity (Fig. 7a, 7b).

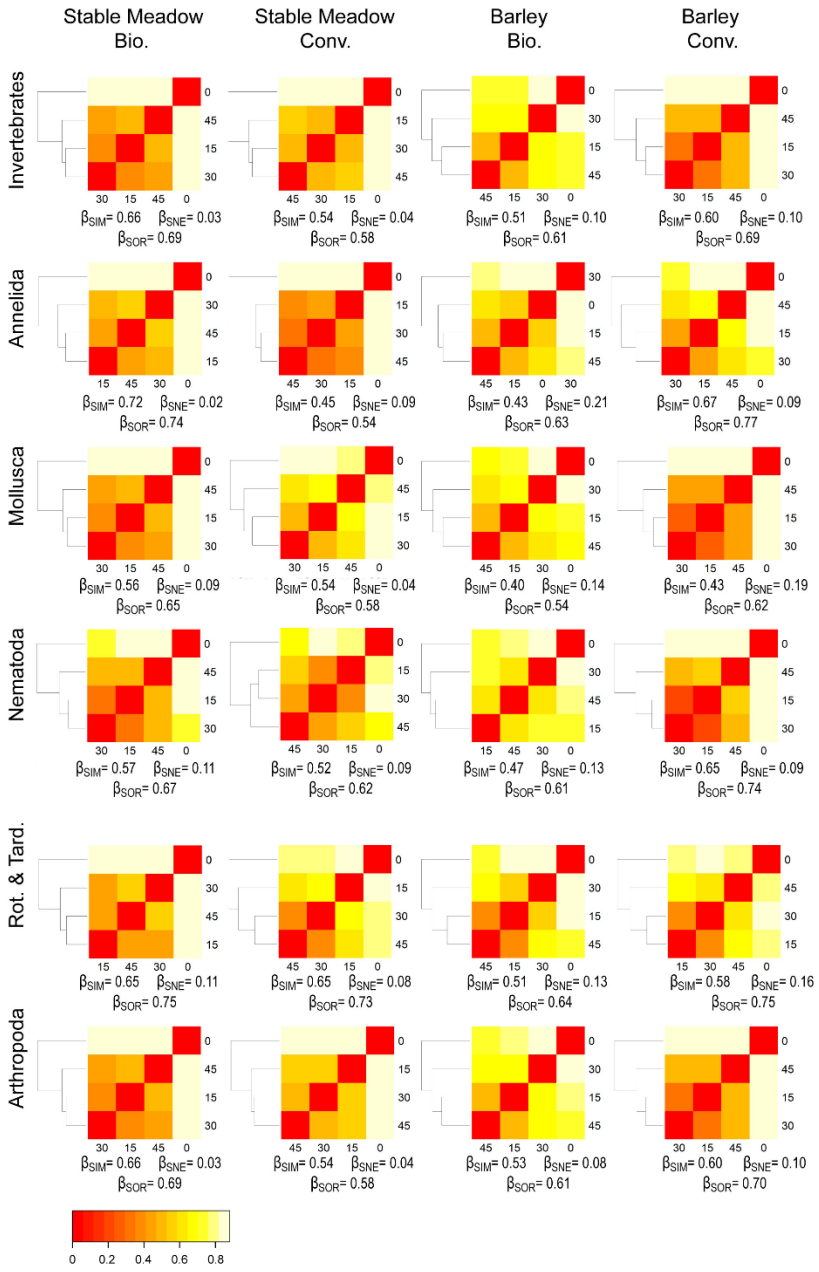

Fig. 7a: Heatmaps of the Sørensen-based multiple-site dissimilarity index inferred on Invertebrates communities; hierarchical clustering is reported on the left of each heatmap; on the bottom of each heatmap the overall β-diversity is reported as values of β_{SOR}, β_{SIM} and β_{SNE}; the legend shows the values of the Sørensen-based multiple-site dissimilarity.

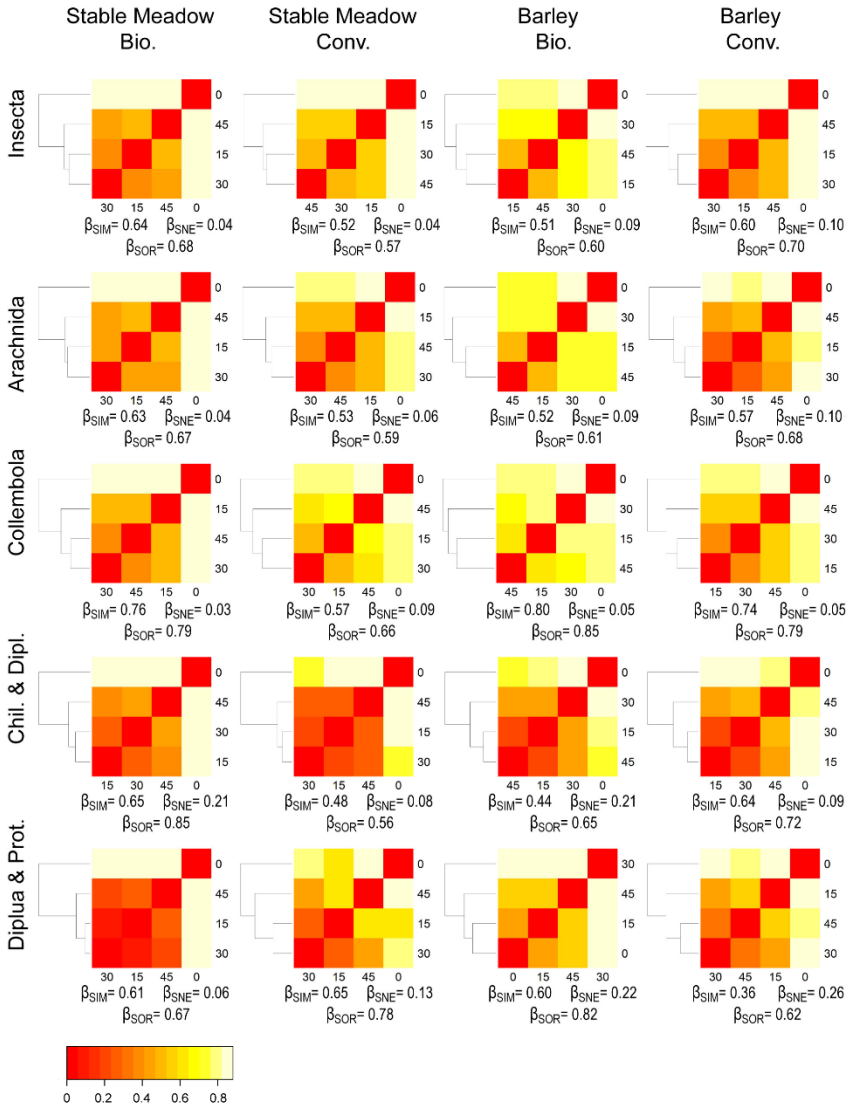

Fig. 7b: Heatmaps of the Sørensen-based multiple-site dissimilarity index inferred on Invertebrates communities; hierarchical clustering is reported on the left of each heatmap; on the bottom of each heatmap the overall β-diversity is reported as values of β_{SOR}, β_{SIM} and β_{SNE}; the legend shows the values of the Sørensen-based multiple-site dissimilarity.

5- The Core diversity of the recovered invertebrates communities

Venn diagrams were performed on Invertebrates communities to highlight the differences in the core diversity between the farming systems and the crops in each farm. The abundance of the core diversity was 720 OTUs (~9% of the total invertebrates OTUs), this core diversity was dominated by Insecta OTUs (55%) followed by Arachnida OTUs with 22% (Fig. 8, Table S3). The stable meadows were higher than barley fields in the number of unique OTUs in the two farming systems; stable meadow (Conv.) has 1258 unique OTUs and stable meadow (Org.) has 1114 OTUs comparing to the barley field (Conv.) which has 775 OTUs and the barley field (Org.) with 623 OTUs. Analyzing the composition of those unique OTUs showed that the higher percentage was the Insecta, but the barley field (Conv.) contained the highest percentage of Insecta (59%), this percentage decreased to 49% in the stable meadow (Org.). Percentage of Annelida followed an opposite behavior, being higher in the stable meadow (Org.) with 14% and decreased remarkably to 5% in the barley field (Conv.).

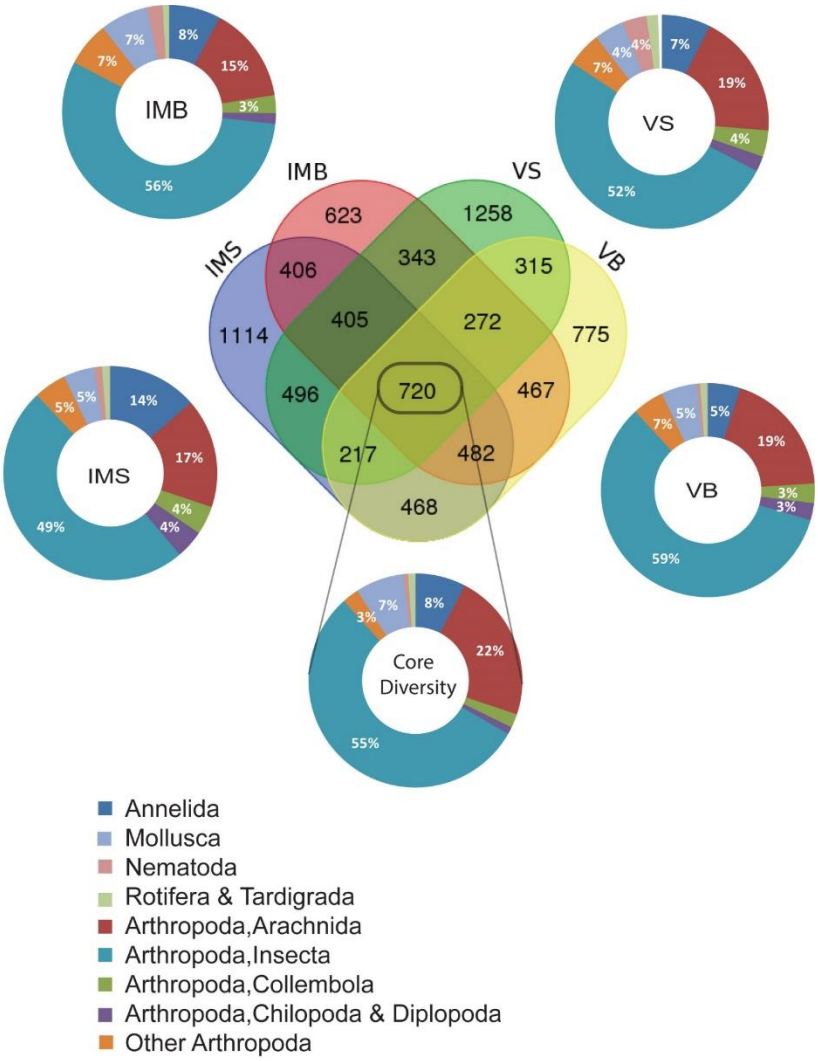

Fig. 8: Venn diagrams of invertebrates communities reporting the shared OTUs among the studied fields; the unique OTUs in the organic stable meadow (IMS), the organic barley field (IMB), the conventional stable meadow (VS) and the conventional barley field (VB); the percentage of the taxonomy assigned to this OTUs is reported as well (more details in table S3).

Since that, the margin zone of the field was separated in the NMDS analyses of the Invertebrates communities, the Venn diagrams were designed for the margin zone and the middle of the field for each of the studied groups of invertebrates separately (Fig. 9).

Results showed a commun pattern for almost all the Invertebrates groups, which could be summerized as: in the stable meadows, the highest number of unique OTUs is recorded in the center of the field in the organic farm, this value was decreased in the margin with a few number of shared OTUs between the margin and the center of the field. In the stable meadow in the conventional farm, the number of unique OTUs was higher in the center of the fied and decreased in the margin with a high number of shared OTUs. For example, the Invertebrate number of unique OTUs was 1005 in the center of the organic stable meadow, this was decreased to a 877 OTUs in the margin of the organic stable meadow, with only a 87 shared OTUs, while in the conventional stable meadow, the uniqe OTUs number decreased from 957 OTUs in the center of the field to a 688 OTUs in the margin of the field with a 456 shared OTUs (Fig. 9).

Regarding the barley field, the highest number of unique OTUs was recorded in the center of the organic field: this number was decreased in the megine with a high number of shared OTUs, while at the conventional barley field, the number of OTUs was higher at

the margin of the field and decreased in the center with a low number of shared OTUs between the margnin and the center.

For example, the highest number of unique Invertebrates OTUs was 928 in the center of the organic barley field, this value was decreased to 588 OTUs with 476 OTUs shared between them, while in the conventional barley field, the number of unique OTUs was 727 in the margin and decreased to a 412 OTUs in the center of the field with only 21 shared OTUs (Fig. 9).

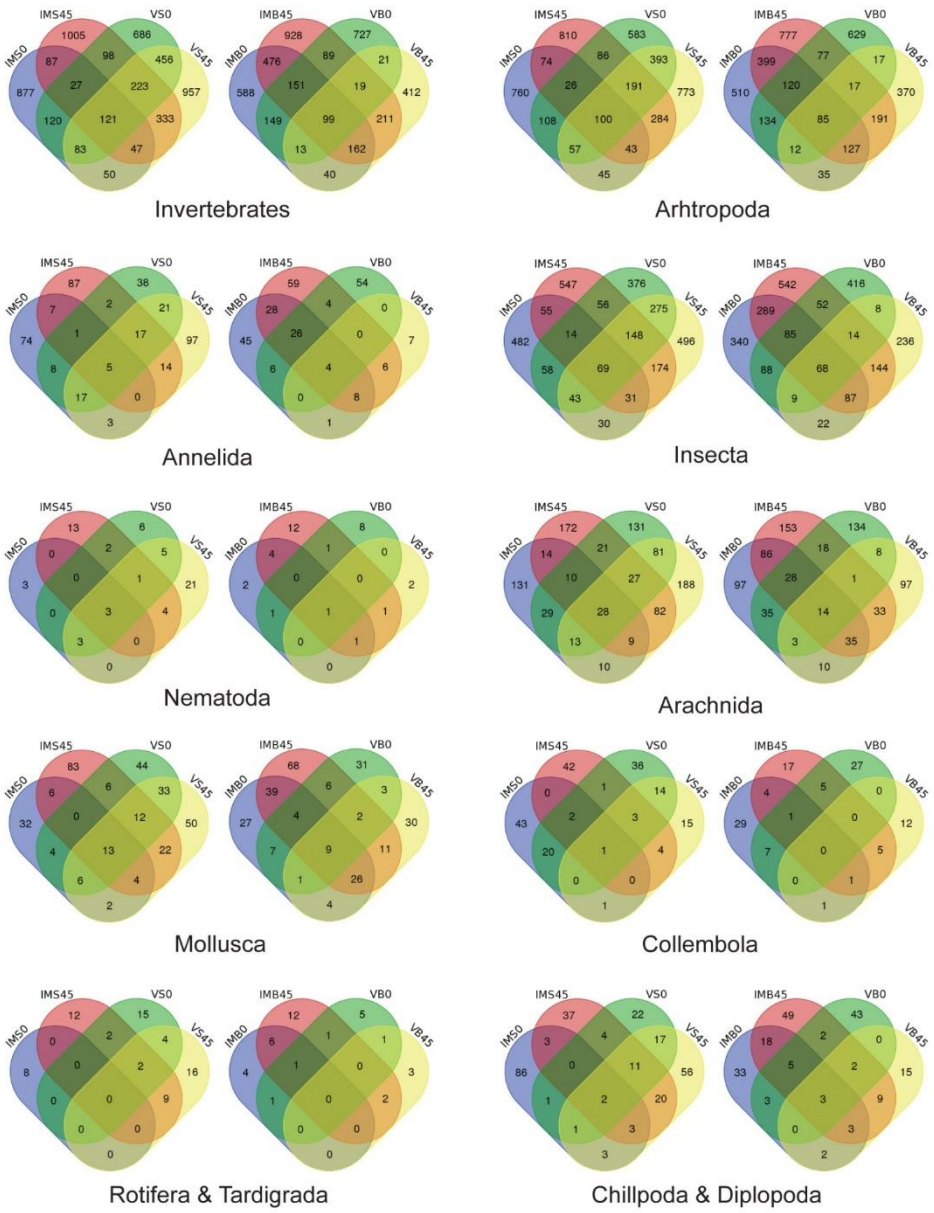

Fig. 9: Venn diagrams of invertebrates communities reporting the shared OTUs in the studied fields between the margin and the field; Organic stable meadow (IMS), the organic barley field (IMB), the conventional stable meadow (VS) and the conventional barley field (VB).

6- Visualization of the connections between the margin and field through the ecological network

The bipartite network plots confirmed the difference between the margin and the field levels in the invertebrates' communities and the partial networks of the invertebrate phyla and the Arthropoda classes (Fig.10a, 10b). in addition, some differences were observed in the effects of the management strategy.

In the case of the invertebrates as whole, the stable meadow management in the organic farm led to form a separated communities at the margin and in the center of the field with a low number of connections between the two zones, the same pattern was not found for the stable meadow of the conventional farm, since that a contrast pattern was visualized, showing more connections between the margin and the center of the field samples.

Regarding the calculated parameters of the visualized networks, results showed a high values of robustness in all the analyzed networks, ranging from 74 to 83 (Figs. 10a, 10b, S2; Table 10). The Stable meadow in the organic farming was higher in the values of nestedness (e.g. 52.16 for Invertebrates, 57.83 for Annelid), with a low percentage of shared OTUs (e.g. 10.1 for Invertebrates, 8.33 for Annelida), and moderate values of connectance (e.g. 35 for Invertebrates, 31 for Annelida). Comparing the obtained values for

the stable meadow of the conventional farming, lower values were recorded for the nestedness (e.g. 46.67 for Invertebrates, 30.13 for Annelida), with a higher percentage of shared OTUs (e.g. 27.25 for Invertebrates, 21.59 for Annelida), and higher values of connectance (e.g. 42 for Invertebrates, 47 for Annelida).

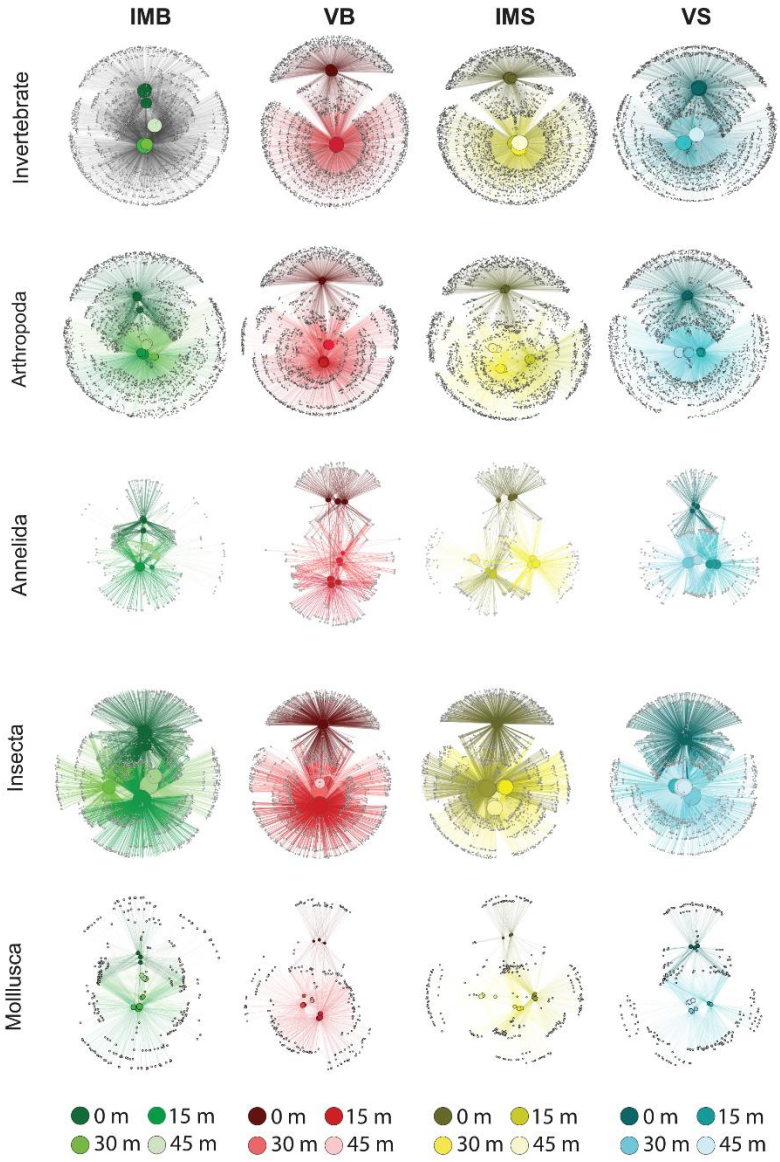

Fig. 10a: Bipartite network analysis of the soil invertebrates' communities, representing sample/OTU interactions. Sample nodes (colored circles; legend show the colors of the sampling levels in the field); OTU nodes are grey, with the edges connecting sample nodes to OTUs present in it. Each samples node size is proportional to its abundance in terms of number of OTUs; (IMS) organic stable meadow, (IMB) organic barley field, (VS) conventional stable meadow, (VB) conventional barley field.

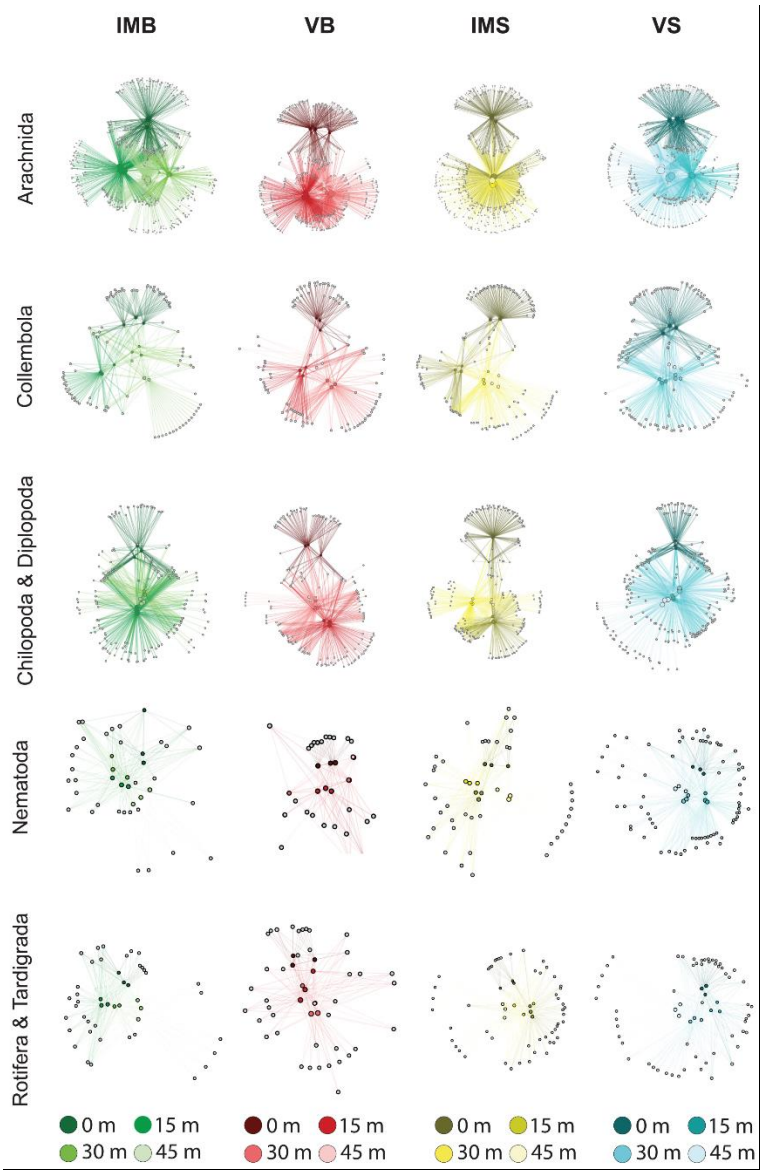

Fig. 10b: Bipartite network analysis of the soil invertebrates communities, representing sample/OTU interactions. Sample nodes (colored circles; legend show the colors of the sampling levels in the field), OTU nodes are grey, with the edges connecting sample nodes to OTUs present in it. Each samples node size is proportional to its abundance in terms of number of OTUs; (IMS) organic stable meadow, (IMB) organic barley field, (VS) conventional stable meadow, (VB) conventional barley field.

Table 10: parameters of the bipartite network, calculated for each group of the soil invertebrates and each of the studied fields

Groups	Fields	Nestedness	% Shared OTUs	Connectance	Cluster coefficient	Niche overlap	Robustness
Annelida	IMB	27.1	34.52	35	37	42	79
	IMS	57.83	8.33	31	33	36	78
	VB	37.12	13.24	29	32	28	77
	VS	30.13	21.59	47	54	57	83
Arachnida	IMB	37.69	30.62	38	38	44	80
	IMS	49.54	11.96	37	42	41	80
	VB	35.89	10.29	36	31	38	80
	VS	44.9	24.59	42	40	50	82
Arthropoda	IMB	37.65	28.97	37	38	44	79
	IMS	52.22	10.38	35	39	41	80
	VB	36.98	9.52	34	30	37	80
	VS	47.67	27.82	42	41	52	82
Chilopoda & Diplopoda	IMB	31.14	22.35	38	39	44	80
	IMS	46.6	4.74	33	34	36	78
	VB	31.19	11.04	34	26	35	79
	VS	44.56	20.57	41	41	51	81
Collembola	IMB	39.96	8	23	24	23	74
	IMS	57.41	4.73	29	30	30	77
	VB	43.92	3.85	27	28	28	76
	VS	44.67	25.6	37	38	44	81
Insecta	IMB	37.04	30.29	38	39	45	81
	IMS	51.36	10.84	36	40	41	80
	VB	37.09	9.51	34	30	38	79
	VS	46.07	30.06	42	42	54	83
Invertebrate	IMB	36.89	29.75	38	38	45	80
	IMS	52.16	10.1	35	38	41	80
	VB	36.6	9.74	34	30	37	78
	VS	46.67	27.25	42	42	53	82

Mollusca	IMB	29.27	36.09	43	40	49	83
	IMS	37.83	10.47	37	39	43	80
	VB	27.57	10.98	40	37	40	82
	VS	48.29	32.5	42	40	53	82
Nematoda	IMB	31.18	25.81	39	40	46	80
	IMS	30.93	10	36	39	42	79
	VB	38.28	4.17	36	32	43	79
	VS	39.36	18.06	38	34	44	81
Rotifera & Tardigrada	IMB	32.78	20	34	32	38	79
	IMS	40.4	0	31	34	30	77
	VB	29.93	5.13	33	29	33	80
	VS	40.37	12.5	34	30	39	79

7- **Correlation between the QBS-ar and the molecular QBS.**

The QBS-ar values were calculated and the soil quality classes were defined based on the proposed table by Parisi et al (2005). Results showed that the higher quality class were found for the margin zone of the organic stable meadow and the central zone of the conventional stable meadow, and the lowest value was found for the center of the conventional barley field (Table 11).

Table 11: Soil quality classes of the studied fields based on the QBS-ar index values

Sample ID	QBS-ar Score (Mean ± SE)	Soil quality class
IMS0	83.3 ± 10.3	4
IMS45	46 ± 6.9	3
IMB0	57.6 ± 5.1	3
IMB45	25 ± 1.9	2
VS0	74 ± 4.1	3
VS45	73.6 ± 11.3	4
VB0	81.6 ± 1.2	2
VB45	9.6 ± 0.7	0

A positive and significant correlation was found between the QBS-ar and the one calculated based on the molecular data (mQBS), with a value of r=0.536 and p-value < 0.05.

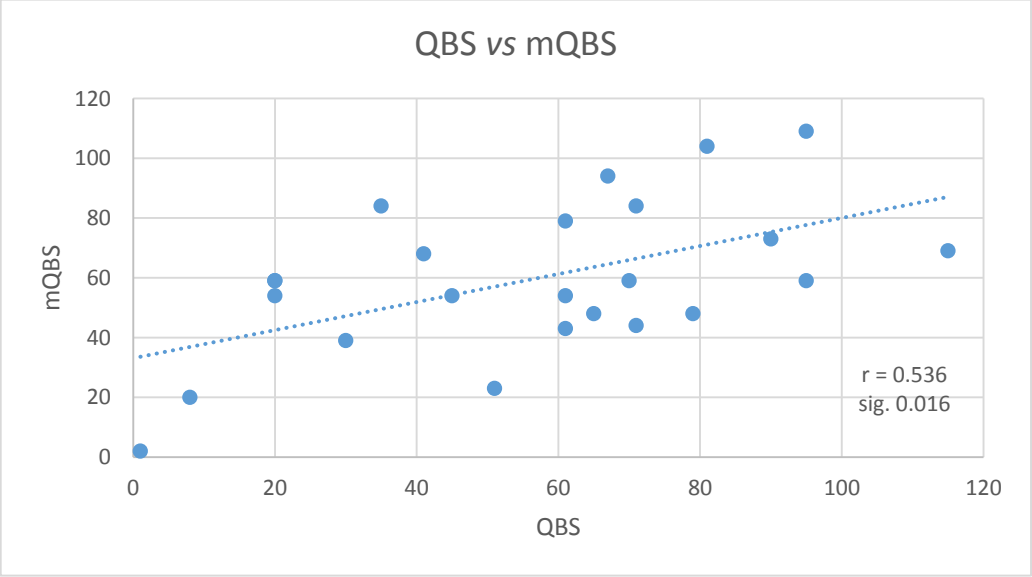

Fig. 11: scatter plot of the QBS-ar values and the molecular estimated one (mQBS).

Discussion

1- Dataset description, soil invertebrates α-diversity

The recovered soil fauna were dominated by Arthropoda in all samples of about 85% in accordance with Decaëns et al 2006, Culliney 2013, which was dominated in turn by the Insecta class, this could be expected according to Evans (2012), considering that the insects are the greatest represented group in soils in the agroecosystems. On the other hand, all the invertebrates phyla (Annelida, Arthropoda, Nematoda, Mollusca, Rotifera & Tardigrada) and the Arthropoda classes (Arachnida, Insecta, Chilopoda & Diplopoda, Collembola) were presented in all samples, with a some variation in the percentages. This presence of arthropods is essential, as they are a major component of functional biodiversity within agroecosystems, contribute to sustainability through processes including nutrient cycling and pest control (Anderson et al, 2013).

These differences in the composition were not reflected in significant differences in the biodiversity indices Shannon's and Pielou's evenness due to the farming system, crop and the position in the field. Thus, hypothesis of differences between the farming systems, cropping system and position was not approved in terms of the biodiversity indices Shannon's and Pielou's evenness.

While for the OTUs richness, even though the DNA metabarcoding is efficient for species richness survey (Creer et al 2016, Elbrecht and leese 2015), this index was not affected significantly by the farming strategy, but significant differences between the cropping systems were found. These differences could be explained by the main applied agricultural practices in the cultivated field (barley), such as tillage and irrigation, resulting in the reduction of the recovered OTUs richness comparing to the stable meadow, which was higher in the recovered OTUs richness due to the low disturbance of the soil. This effect was increased considering the position in the field, since that the center of the barley field was the lowest in OTUs richness in both farms.

2- Factors affecting soil invertebrates communities

The invertebrate' communities structure, as whole, was not significantly affected by the farming system itself as found by Hadjicharalampous et al (2002), which could be explained by the use of similar organic fertilizers (kind and quality) as found by (Hartmann et al 2014). Since that the invertebrate groups differently responded to the factors: the position in the field (Margin *vs* field levels), the crop, the soil pH and C/N ratio. These effects are related directly to the crop-growing practices, which are the key factor for the survival of soil invertebrates' species (Booij and Noorlander 1992). In particular the tillage and the added fertilizers, which are considered the main, factors of the reduction or stimulation of the taxa richness of the soil organisms (Hubbard et al. 1999; Petersen et al., 2003), this correlation

between the characteristics of the soils and the communities was confirmed by Santorufo et al (2012), and it was clear that this disturbance of the soil was more effective on the groups that have a low fecundity or long life cycles, such as Annelids, insects and Chilopods, while the effect was less on other groups such as Collembola and Nematoda (Rusek and Marshall, 2000; Behan-Pelletier, 1999). The effect of the soil acidity degree (pH) could be attributed to the bottom-up effects, since that the soil pH had a significant influence on the soil bacteria and fungi communities (Mulder et al., 2005, 2009; Fierer et al., 2009) as well as the activity of soil enzymes (Sinsabaugh et al., 2008), which could affect the soil invertebrates communities.

Once again, the hypothesis of the differences between the farming strategies in their impact on the soil invertebrate's community structure was not confirmed as found by Foissner 1992; Blackburn and Arthur 2001, while the effects of cropping system and the position in the field were confirmed, forming a distinct community separation based on this factors.

3- Shifting of soil communities from margin to field center

Beta diversity:

The separation of the margin communities for all the studied groups was confirmed by the β-diversity analysis (Sørensen dissimilarity),

highlighting the importance of the field margins for the conservation of the soil biodiversity components (Noordijk et al 2010).

This importance could be explained by the enhancing of the biodiversity margin communities of soil invertebrates, since that the turnover component of the β-diversity was less than 10% in almost all the studied groups under the different farming and grouping systems, accompanied by a high dissimilarities between the margin and the field levels, increasing by the presence of the margin the functional biodiversity by providing habitat for beneficial species (Anderson et al, 2013)

This point was better explained through the bipartite plots, which have shown the connectance and the shared OTUs between the two habitats (Margin *vs* field).

4- The Core diversity of the recovered invertebrates communities

This core diversity was dominated by Insecta OTUs (55%) followed by Arachnida OTUs with 22%. The stable meadows were higher than barley fields in the number of unique OTUs in the two farming system. This high percentage of insect is expected according to Evans (2012), but the observed point of the higher percentage in the stable meadow of Annelida in the organic farm (14% of the community) could be related to their sensibility to the practices of tillage and plowing, so the percentages were lower in the barley fields in both farming systems; interestingly, this percentage was lower also to about 7% which could

be explained by the input of the added nitrate and urea in the conventional stable meadow since this compounds have some negative effects on Clitella (Annelida) according to Armendáriz et al (2012).

5- Visualization of the connections between the margin and field through the ecological network

The obtained networks were all characterized by high robustness, which could be related to the presence of huge number of analyzed OTUs in each sample, this plots confirmed the separation of the margin communities from those of the field levels for invertebrates and the partial network of the invertebrate phyla and the Arthropoda classes. The stable meadow management in the organic farm helped to form stable separated communities at the margin and the field levels, while the stable meadow in the conventional farm formed more dynamic and connected communities between the two habitats (margin *vs* field). Showed by the parameters Nestedness and the tendency of the network for clustering, these values were higher in the stable meadows comparing to the barley fields, and in the stable meadowes they were even higher than the organic stable meadow comparing to the conventional one. This could be attributed to the stress of the additional practice applied in the conventional stable meadow, resulting in a response of the invertebrates with a movement ability, highlighting the importance of the field margins in the sustainability of the agroecosystems (Meek et al 2002).

6- **Correlation between the QBS-ar and the molecular QBS.**

The use of the soil microarthropods as soil quality indicators in agroecosystems is accepted (Stork and Eggleton, 2009), and the QBS-ar is on of the most reliable indices since it depends on the adaptation of these microarthropods to the soil habitat (Parisi et al, 2000), Confirming in the obtained results that the soil quality of the field margins and the grasslands are higher comparing with the arable fields (Menta et al 2011). On the other hand, the correlation between the classic QBS-ar and the molecular estimated one (mQBS) is expected since that Hamilton (2009) has found a similar results of correlation between the molecular survey of the soil fauna with the taxonomic identification, this results needs to be more tested for other crops since that the cropping system has affected significantly the soil's invertebrates communities. Based on this results it is also confirmed that the field margins are higher in invertebrates biodiversity and thus in soil quality.

Conclusions

- The DNA Metabarcoding approach represents a promising method for the assessment of soil biodiversity in the agroecosystem, could be a useful tool for the estimation of the direct impact of the agricultural practices.
- The DNA Metabarcoding of the soil invertebrates could not reveal the seasonal changes of the soil invertebrates' communities.
- The recovered invertebrates' communities of the soil were dominated by arthropods (80%).
- The farming management as organic or conventional did not affect significantly the community structure of soil invertebrates and the biodiversity indices Shannon and Pielou's evenness.
- The species richness was significantly lower in the barley fields and much lower under the conventional farming system.
- Soil invertebrates communities were significantly affected by the crop and the position of the field (as margin or field), and the C/N ratio.
- Rotifera and Tardigrada communities' structure were affected by the farming strategy, while insects' communities were affected by the pH of the soil.

- The role of the margin of the field as a reservoir is increased in the cultivated fields (barley), while in the stable meadows the interactions between the margin and the center of the field are lower.
- The soil biological quality is decreased from the margin to the center of the field (of the same field), also decreased in the barley field comparing to the stable meadows.
- The obtained molecular index mQBS that is developed based on the QBS-ar is a promising approach for the soil biological quality estimation.

References

1- Acton, D.F., Gregorich, L.J., 1995. The Health of our Soils: Toward Sustainable Agriculture in Canada. Agric. Agri-food Can., CDR Unit, 960 Carling Ave., Ottawa, ON K1A 0C6.

2- Aizpurua, O., Budinski, I., Georgiakakis, P., Gopalakrishnan, S., Ibañez, C., Mata, V. & Zrncic, V. 2018. Agriculture shapes the trophic niche of a bat preying on multiple pest arthropods across Europe: Evidence from DNA metabarcoding. Molecular ecology, 27(3), 815-825.

3- Altieri, M. 1999. The ecological role of biodiversity in agroecosystems. Agricult Ecosys Environ 74: 19-31. Agriculture, Ecosystems & Environment. 74. 19-31. 10.1016/S0167-8809(99)00028-6.

4- Altschul, S.F., Madden, T.L., Schaffer, A.A., et al. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res, 25, 3389-3402.

5- Andersen, Kenneth & Lise Bird, Karen & Rasmussen, Morten & Haile, James & Breuning-Madsen, Henrik & Kjaer, Kurt & Orlando, Ludovic & Thomas P Gilbert, M & Willerslev, Eske. 2011. Meta-barcoding of 'dirt' DNA from soil reflects vertebrate biodiversity. Molecular ecology. 21. 1966-79. 10.1111/j.1365-294X.2011.05261.x.

6- Anderson J.M., 1988. Invertebrate-mediated transport processes in soils. Agriculture, Ecosystems and Environment, 24, pp. 5-19.

7- Anderson, A., Carnus T., Helden A. J., Sheridan H., and Purvis G. 2013. The influence of conservation field margins in intensively managed grazing land on communities of five arthropod trophic groups. Insect Conservation and Diversity (2013) 6, 201–211 doi: 10.1111/j.1752-4598.2012.00203.x.

8- André, Henri M., Ducarme Xavier., Lebrun Philippe. 2002. Soil biodiversity: myth, reality or conning?. Oikos. 96 (1): 3 – 24. https://doi.org/10.1034/j.1600-0706.2002.11216.x.

9- Armendáriz, L., Ocón, C., & Capítulo, A. R. 2012. Potential responses of oligochaetes (Annelida, Clitellata) to global changes: Experimental fertilization in a lowland stream of Argentina (South America). *Limnologica-Ecology and Management of Inland Waters*, *42*(2), 118-126.

10- Arnot David E., Caroline Roper, Riad A.L. Bayoumi. 1993. Digital codes from hypervariable tandemly repeated DNA sequences in the Plasmodium falciparum circumsporozoite gene can genetically barcode isolates, Molecular and Biochemical Parasitology, Volume 61, Issue 1,1993, p 15-24, https://doi.org/10.1016/0166-6851(93)90154-P.

11- Arribas, P. , Andújar, C. , Hopkins, K. , Shepherd, M. , Vogler, A. P. and Yu, D. 2016. Metabarcoding and mitochondrial metagenomics of endogean arthropods to unveil the mesofauna

of the soil. Methods Ecol Evol, 7: 1071-1081. doi:10.1111/2041-210X.12557

12- Arroyo, J., Keith, A., Schmidt, O., & Bolger, T. 2013. Mite abundance and richness in an Irish survey of soil biodiversity with comments on some newly recorded species. *The Irish Naturalists' Journal, 33*(1), 19-27. Retrieved from http://www.jstor.org/stable/24394126

13- Bajwa, W. I. and M. Kogan. 2002. Compendium of IPM Definitions (CID)- What is IPM and how is it defined in the Worldwide Literature? IPPC Publication No. 998, Integrated Plant Protection Center (IPPC), Oregon State University, Corvallis, OR 97331, USA

14- Bardgett, R. D., and van der Putten, W. H. 2014. Belowground biodiversity and ecosystem functioning. *Nature* 515, 505–511. doi: 10.1038/nature13855

15- Barnett, G.M., 1982. Utilisation de, fumiers et des lisiers sur Jes cultures: Jes consequences agronomiques. In: Fumiers: Rappon du collogue sur les fumiers. Ministere de L' Agriculture, Des Pecheries et de L' Alimentation du Quebec, pp. 57-77.

16- Barrios E.. 2007. Soil biota, ecosystem services and land productivity, Ecological Economics, 64 (2):,269-285, https://doi.org/10.1016/j.ecolecon.2007.03.004.

17- Baselga, A. 2010. Partitioning the turnover and nestedness components of beta diversity. Global Ecology and Biogeography,

19(1), 134–143. https://doi.org/10.1111/j.1466-8238.2009.00490.x

18- Baselga, A., & Orme, C. 2012. Betapart: an R package for the study of beta diversity. Methods in Ecology and Evolution, 3(5), 808–812. https://doi.org/10.1111/j.2041-210X.2012.00224.x

19- Behan-Pelletier, V.M., 1999. Oribatid mite biodiversity in agroecosystems: role for bioindication. Agric. Ecosyst. Environ. 74, 411–423.

20- Blackburn, J., & Wallace, A. 2001. Comparative abundance of centipedes on organic and conventional farms, and its possible relation to declines in farmland bird populations. Basic and Applied Ecology, 2(4), 373-381.

21- Blair, J.M., Crossley, D.A., Callaham, L.C., 1992. Effects of litter quality and microarthropods on N dynamics and retention of exogenous 15N in decomposing litter. Biol. Fert. Soils 12, 241–252.

22- Blaxter, M. et al. 2005 Defining operational taxonomic units using DNA barcode data. Philos. Trans. R. Soc. Lond. B Biol. Sci. 360, 1935– 1943

23- Bohmann K, Evans A, Gilbert MTP, Carvalho GR, Creer S, Knapp M et al 2014 Environmental DNA for wildlife biology and biodiversity monitoring. Trends EcolEvol 29:358–367

24- Bongers T 1990 The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83:14–19

25- Booij, C. H. J. & J. Noorlander., 1992. Farming system and insect predators. Agri. Ecosys. & Envi., 40(1-4): 125-135.

26- Bray, J. R., & Curtis, J. T. 1957. An ordination of upland forest communities of southern Wisconsin. Ecological Monographs, 27, 325–349. https://doi.org/10.2307/1942268

27- Bronick C.J. and R. Lal, 2005. Soil structure and management: a review, Geoderma, 124 (1–2): 3-22, https://doi.org/10.1016/j.geoderma.2004.03.005.

28- Buguna-Hoffmann, L., 2000. Stimulating positive linkages between agriculture and biodiversity. Recommendations for building blocs for the European conservation agricultural action plan on biodiversity, European centre for nature conservation, (ECNC-Technical Report Series),Tilburg.

29- Caporaso J. G., Lauber C. L.,Walters W. A. et al. 2011 Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. U.S.A. 108 (Suppl 1), 4516–22.

30- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., ... Knight R. 2010 167 QIIME allows analysis of high-throughput community sequencing data. Nature Methods, 7(5),335-336. doi:IO.I038/nmeth.f.303

31- Castanho, C., Lorenzo, L., & De Oliveira, A. 2012. The importance of mesofauna and decomposition environment on leaf decomposition in three forests in southeastern Brazil. *Plant Ecology, 213*(8), 1303-1313. http://www.jstor.org/stable/23267451

32- Clarke K. R. 1993 Non-parametric multivariate analyses of changes in community structure. Austral Ecol. 18, 117–43.

33- Cluzeau, D., Guernion, M., Chaussod, R., Martin-Laurent, F., Villenave, C., Cortet, J., Ruiz- Camacho, N., Pernin, C., Mateille, T., Philippot, L., Bellido, A., Rougé, L., Arrouays, D., Bispo, A., Pérès, G., 2012. Integration of biodiversity in soil quality monitoring: baselines for microbial and soil fauna parameters for different land-use types. Eur. J. Soil Biol. 49:63–72. http://dx.doi.org/10.1016/j.ejsobi.2011.11.003 (Bioindication in Soil Ecosystems)

34- Creer S, Deiner K, Frey S, Porazinska D, Taberlet P, Thomas K, Potter C, Bik H. 2016. The ecologist's field guide to sequence-based identification of biodiversity. Methods in Ecology and Evolution 7:10081018 DOI 10.1111/2041-210X.12574.

35- Culliney, T. W. 2013. Role of Arthropods in Maintaining Soil Fertility, Agriculture 2013, 3, 629-659; doi:10.3390/agriculture3040629.

36- Decaëns, T.; Jiménez, J.J.; Gioia, C.; Measey, G.J.; Lavelle, P. The values of soil animals for conservation biology. Eur. J. Soil Biol. 2006, 42, S23–S38.

37- Dixon, P. 2003. VEGAN, a package of R functions for community ecology. Journal of Vegetation Science, 14(6), 927–930. https://doi.org/10. 1111/j.1654-1103.2003.tb02228.x

38- Doran, J.W. and Parkin, T.B. 1994. Defining and assessing soil quality. Pages 3-21, In: Doran, J.W.; Coleman, D.C;, Bezdieck, D.F; and Stewart, B.A. (Editors), Defining Soil Quality for a Sustainable Environment, SSSA Special Publication No. 35. Soil Science Society of America, Madison, WI, USA.

39- Doran, J.W. and Parkin, T.B. 1996. Quantative indicators of soil quality: a minimum data set. Pages 25-37, In: Doran, J.W. and Jones, A.J. (Editors), Methods for Assessing Soil Quality. SSSA Special Publication No. 49, Soil Science Society of America, Madison, WI, USA.

40- Doran, J.W., Zeiss, M.R., 2000. Soil health and sustainability: managing the biotic component of soil quality. Applied Soil Ecology 15, 3–11.

41- Doran, J.W.; Sarantonio, M. and Leibig, M. 1996. Soil health and sustainability. Advances in Agronomy 56: 1-54.

42- Dormann, C. F. and Strauss, R. 2014. A method for detecting modules in quantitative bipartite networks. – Methods Ecol.Evol. 5: 90 – 98.

43- Dormann, C. F. et al. 2008. Introducing the bipartite package: analysing ecological networks. – R News 8: 8 – 11.

44- Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 2010; 26: 2460–2461. pmid:20709691

45- Elbrecht V, Leese F 2015 Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass—sequence relationships with an innovative Metabarcoding protocol. Plos One, 10, e0130324

46- Elbrecht V, Peinert B, Leese F 2017b. Sorting things out: Assessing effects of unequal specimen biomass on DNA metabarcoding. Ecol Evol;7:6918–6926.

47- Elbrecht V, Vamos EE, Meissner K, Aroviita J, Leese F 2017a. Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. Methods Ecol Evol. 2017;

48- European Parliament. 2010 Pesticides: framework for Community action to achieve a sustainable use of pesticides. See http://www.europarl.europa.eu/oeil/file.jsp? id=5372322 (accessed 28 June 2010).

49- Evans, N., & Paulay, G. 2012. DNA barcoding methods for invertebrates. In *DNA Barcodes* (pp. 47-77). Humana Press, Totowa, NJ.

50- FAO 1999, Guidelines for the production, processing, labelling and marketing of organically produced foods. Joint FAO/WHO

Food Standards Program Codex Alimentarius Commission, Rome, CAC/ GL 32, 1999, p. 49.

51- Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. Ecology Letters 12, 1238e1249.

52- Floyd, R. et al. 2002 Molecular barcodes for soil nematode identification. Mol. Ecol. 11, 839–850

53- Foissner, W. 1992. Comparative studies on the soil life in ecofarmed and conventionally farmed fields and grasslands of Austria. In Biotic Diversity in Agroecosystems (pp. 207-218).

54- Folmer O., Black M., Hoeh W., Lutz R. & Vrijenhoek R. 1994 DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Marine Biol. Biotechnol. 3, 294–9.

55- Fu, L., Niu, B., Zhu, Z., Wu, S., & Li, W. 2012 CD-HIT: accelerated for clustering the next generation sequencing data. Bioinformatics, 28(23),3150-3152. doi: 10.1 093/bioinformatics/bts565.

56- Gabriel D, Sait SM, Kunin WE, e Benton TG 2013. Food production vs. biodiversity: comparing organic and conventional agriculture. Journal of Applied Ecology 50:355–364

57- Gardham S., Grant C. Hose, Sarah Stephenson, Anthony A. Chariton, Chapter Three DNA Metabarcoding Meets Experimental Ecotoxicology: Advancing Knowledge on the Ecological Effects of

Copper in Freshwater Ecosystems, Editor(s): Guy Woodward, Alex J. Dumbrell, Donald J. Baird, Mehrdad Hajibabaei, Advances in Ecological Research, Academic Press, Volume 51, 2014, 79-104, https://doi.org/10.1016/B978-0-08-099970-8.00007-5.

58- George P B.L., Aidan M. Keith, Simon Creer, Gaynor L. Barrett, Inma Lebron, Bridget A. Emmett, David A. Robinson, David L. Jones, 2017. Evaluation of mesofauna communities as soil quality indicators in a national-level monitoring programme, Soil Biology and Biochemistry, 115: 537-546. https://doi.org/10.1016/j.soilbio.2017.09.022.

59- Gerlach, J., Samways, M. & Pryke, J. J Insect Conserv 2013. Terrestrial invertebrates as bioindicators: an overview of available taxonomic groups. 17: 831. https://doi.org/10.1007/s10841-013-9565-9.

60- Goldstein PZ, Desalle R: Calibrating phylogenetic species formation in a threatened insect using DNA from historical specimens. Molecular Ecology 2003, 12(7):1993-1998.

61- Gomez-Alvarez, V. et al. 2007 Comparative bacterial diversity in recent Hawaiian volcanic deposits of different ages. FEMS Microbiol. Ecol. 60, 60–73

62- Gonçalves, J., Pereira, F., Amorim, A., & van Asch, B. 2012. New method for the simultaneous identification of cow, sheep, goat, and water buffalo in dairy products by analysis of short species-

specific mitochondrial DNA targets. Journal of Agricultural and Food Chemistry, 60, 10480–10485.

63- Greenslade, P.W.N. 1985. Pterygote insects and the soil: Their diversity, their effects on soils and the problem of species identification. Quaestiones Entomologicae 21:571–585

64- Hadjicharalampous, E., Kalburtji, K. & Mamolos. 2002. soil arthropods (coleoptera, isopoda) in organic and conventional agroecosystems. Environmental management (2002) 29: 683. Https://doi.org/10.1007/s00267-001-0056-5

65- Hajibabaei M, Singer GA, Clare EL, Hebert PDN. 2007. Design and applicability of DNA arrays and DNA barcodes in biodiversity monitoring. BMC Biol 2007, 5:24.

66- Hajibabaei M, Smith MA, Janzen DH, Rodriguez JJ, Whitfield JB, Hebert PDN. 2006 A minimalist barcode can identify a specimen whose DNA is degraded. Molecular Ecology Notes 2006, 6:959-964.

67- Hamilton H C, Strickland M S, Wickings K, et al. 2009. Surveying soil faunal communities using a direct molecular approach. Soil Biol Biochem, 2009, 41: 1311–1314

68- Hartmann M, Frey B, Mayer J, MaÈder P, Widmer F. Distinct soil microbial diversity under long-term organic and conventional farming. ISME J. 2014;1-18.

69- Hawkins, J., de Vere, N., Griffith, A., Ford, C. R., Allainguillaume, J., Hegarty, M. J., et al. 2015. Using DNA metabarcoding to identify

the floral composition of honey: A new tool for investigating honey bee foraging preferences. PLoS ONE, 10, e0134735.

70- Hebert PD, Cywinska A, Ball SL, deWaard JR.2003. Biological identifications through DNA barcodes. Proc Biol Sci 2003, 270(1512):313-321.

71- Hebert PD, Stoeckle MY, Zemlak TS, Francis CM.2004 Identification of birds through DNA barcodes. PLoS Biology 2004, 2(10):E312.

72- Hebert PDN, Ratnasingham S, deWaard JR 2003b Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc R Soc Lond B Suppl 270:96–99

73- Hebert, Paul D. N. and Gregory T. R. 2005. The Promise of DNA Barcoding for Taxonomy, Systematic Biology, Volume 54, Issue 5, 1 October 2005, Pages 852–859, https://doi.org/10.1080/10635150500354886

74- Herrera, A. et al. 2007 Species richness and phylogenetic diversity comparisons of soil microbial communities affected by nickel-mining and revegetation efforts in New Caledonia. Eur. J. Soil Biol. 43, 130– 139

75- Holt, E. A., & Miller, S. W. 2011. Bioindicators: using organisms to measure environmental impacts. Nature Education Knowledge, 3(8) https://doi.org/10.1002/ece3.3192

76- HUANG, J.; XU, Q.; SUN, Z.J.; TANG, G.L.; SU, Z.Y. 2007. Identifying earthworms through DNA barcodes. Pedobiologia, v.51, p.301-309, 2007.

77- Hubbard, V. C., Jordan, D., & Stecker, J. A. 1999. Earthworm response to rotation and tillage in a Missouri claypan soil. *Biology and Fertility of Soils*, *29*(4), 343-347.

78- Jacomini, C., Nappi, P., Sbrilli, G., Mancini, L., 2000. Indicatori ed Indici Ecotossicologici e Biologici Applicati al Suolo: Stato Dell'arte. Agenzia Nazionale per la Protezione dell'Ambiente (ANPA). RTI CTN_SSC 3/2000.

79- Jeffery S, Gardi C, Jones A, Montanarella L, Marmo L, Miko L, Ritz K, Peres G, Römbke J, van der Putten WH. 2010. European Atlas of Soil Biodiversity. European Commission, Publications Office of the European Union; 2010.

80- Ji Y, et al. 2013 Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. Ecol. Lett. **16**,1245–1257. (doi:10.1111/ele.12162)

81- Jørgensen, T., Kjær, K., Haile, J., Rasmussen, M., Boessenkoel, S., Andersen, K., Coissac, E., Taberlet, P., Brochmann, C., Orlando, L., Gilbert, M.P.T., Willerslev, E., 2012. Islands in the ice: detecting past vegetation on Greenlandic nunataks using historical records and sedimentary ancient DNA Meta-barcoding. Molecular Ecology 21, 1980e1988.

82- Kalisz, P.J., and Stone, E.L.. 1984. Soil mixing by Scarab beetles and pocket gophers in north central Florida. Soil Sci. Soc. Am. J. 48:169–172

83- Kamenova S., V. Bretagnolle, M. Plantegenest and E. Canard. 2018. DNA metabarcoding diet analysis reveals dynamic feeding behavior and biological control potential of carabid farmland communities. doi: http://dx.doi.org/10.1101/332312.

84- Kang'Ethe, E. K., Jones, S. J., & Patterson, R. L. S. 1982. Identification of the species origin of fresh meat using an enzyme-linked immunosorbent assay procedure. Meat Science, 7(3), 229-240.

85- Kmiec, et al. 2006. Heteroplasmy as a common state of mitochondrial genetic information in plants and animals Curr. Genet., 50. 2006, pp. 149-159

86- Knapp S. and M. G.A. van der Heijden. 2018. A global meta-analysis of yield stability in organic and conservation agriculture. Nature communications. DOI: 10.1038/s41467-018-05956-1.

87- Kraaijeveld, K., Weger, L. A., Ventayol García, M., Buermans, H., Frank, J., Hiemstra, P. S., et al. 2015. Efficient and sensitive identification and quantification of airborne pollen using next-generation DNA sequencing. Molecular Ecology Resources, 15, 8e16.

88- Kruskal, J. B. 1964. Nonmetric multidimensional scaling: A numerical method. Psychometrika, 29(2), 115–129. https://doi.org/10.1007/ BF02289694

89- Lacoursière-Roussel, A., Dubois, Y., Normandeau, E., & Bernatchez, L. 2016. Improving herpetological surveys in eastern North America using the environmental DNA method. Genome, 59(11), 991-1007.

90- Laishram J., Saxena k.g., Maikhuri r.k. and Rao k.s., 2012. Soil Quality and Soil Health: A Review, International Journal of Ecology and Environmental Sciences 38 (1): 19-37.

91- Larson, W. E., & Pierce, F. J. 1994. The dynamics of soil quality as a measure of sustainable management. Defining soil quality for a sustainable environment, (definingsoilqua), 37-51.

92- Lavelle P., T. Decaëns, M. Aubert, S. Barot, M. Blouin, F. Bureau, P. Margerie, P. Mora, J.-P. Rossi, 2006, Soil invertebrates and ecosystem services, European Journal of Soil Biology,42, Supplement 1, S3-S15, https://doi.org/10.1016/j.ejsobi.2006.10.002.

93- Lee KE Foster RC 1991 Soil fauna and soil structure. Soil Research 29, 745-775. https://doi.org/10.1071/SR9910745

94- Leray M, Yang JY, Meyer CP, Mills SC, Agudelo N, Ranwez V, Boehm JT, Machida RJ. 2013 A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing

coral reef fish gut contents. Front. Zool. **10**, e34. (doi:10.1186/1742-9994-10-34)

95- Li, W. & Godzik, A. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics, 22, 1658– 1659.

96- Liebig, M. A., and J. W. Doran. 1999. Impact of organic production practices on soil quality indicators. J. Environ. Qual. 28:1601-1609. doi:10.2134/jeq1999.00472425002800050026x

97- Lussenhop, J., 1992. Mechanisms of microarthropodmicrobial interactions in the soil. Adv. Ecol. Res. 23, 1–33.

98- Mader P, Fliebbach A, Dubois D, Gunst L, Fried P, Niggli U.2002. Soil fertility and biodiversity in organic farming. SCIENCE 955:1694-1697

99- Maraun, M., Martens, H., Migge, S., Theenhaus, A., Scheu, S., 2003. Adding to "the enigma of soil animal diversity": fungal feeders and saprophagous soil invertebrates prefer similar food substrates. European Journal of Soil Biology 39, 85e95.

100- Margurran, A.E. 2004. Measuring Biological Diversity. Blackwell Science

101- McLaughlin, Alison & Mineau, Pierre. 1995. The impact of agricultural practices on biodiversity. Agriculture, Ecosystems & Environment. 55. 201-212. 10.1016/0167-8809(95)00609-V.

102- Meek, B., Loxton, D., Sparks, T., Pywell, R., Pickett, H. & Nowakowski, M. (2002) The effect of arable field margin

composition on invertebrate biodiversity. Biological Conservation, 106, 259–271.

103- Menta C., A. Leoni, C. Gardi and F. D. Conti. 2011. Are grasslands important habitats for soil microarthropod conservation?. Biodivers Conserv (2011) 20:1073–1087 DOI 10.1007/s10531-011-0017-0

104- Menta C., et al. "Are grassland important habitats for soil microarthropod conservation?" Biodiversity Conservation 20.5 (2011):1073-1087.

105- Meusnier I., Singer G. A.C., Landry J. F., Hickey D. A., Hebert P.D.N., Hajibabaei M., 2008. universal DNA mini-barcode for biodiversity analysis. J BMC Genomics, 9 (1): 1471-2164. https://doi.org/10.1186/1471-2164-9-214.

106- Montagna M, Berruti A, Bianciotto V, et al. Differential biodiversity responses between kingdoms (plants, fungi, bacteria and metazoa) along an Alpine succession gradient. Mol Ecol. 2018;27:3671–3685. https://doi.org/10.1111/mec.14817

107- Mueller K E et al. 2016. Light, earthworms, and soil resources as predictors of diversity of 10 soil invertebrate groups across monocultures of 14 tree species, Soil Biology and Biochemistry, Volume 92, 2016, Pages 184-198, https://doi.org/10.1016/j.soilbio.2015.10.010.

108- Mulder, C., Den Hollander, H.A., Vonk, J.A., Rossberg, A.G., Jagers op Akkerhuis, G.A.J.M., Yeates, G.W., 2009. Soil resource supply

influences faunal size-specific distributions in natural food webs. Naturwissenschaften 96, 813e826.

109- Mulder, C., Van Wijnen, H.J., Van Wezel, A.P., 2005. Numerical abundance and biodiversity of below-ground taxocenes along a pH gradient across the Netherlands. Journal of Biogeography 32, 1775e1790.

110- Murray, D. C., Pearson, S. G., Fullagar, R., Chase, B. M., Houston, J., Atchison, J., ... & Gilbert, M. T. P. 2012. High-throughput sequencing of ancient plant and mammal DNA preserved in herbivore middens. Quaternary Science Reviews, 58, 135-145.

111- Nazzareno, D and Michele, C. 2004. Multivariate indicator Kriging approach using a GIS to classify soil degradation for Mediterranean agricultural lands. Ecology Indicators, 4: 177–187

112- Newmaster, s. G., Fazekas, a. J., Steeves, r. A. D. and Janovec, j. 2008. Testing candidate plant barcode regions in the myristicaceae. Molecular ecology resources. 8 (3): 480-490. http:https://doi.org/10.1111/j.1471-8286.2007.02002.x.

113- Noordijk, J., Musters, C. J. M., van Dijk, J., & de Snoo, G. R. 2010. Invertebrates in field margins: taxonomic group diversity and functional group abundance in relation to age. *Biodiversity and conservation*, *19*(11), 3255-3268.

114- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. 2017. VEGAN: Community ecology package. R package version 2.4-3.

115- Padel Susanne, , Nicolas H. Lampkin, , Stephan Dabbert, , Carolyn Foster, 2002, Organic farming policy in the European Union, in Darwin C. Hall, L. Joe Moffitt (ed.) Economics of pesticides, sustainable food production, and organic food markets (advances in the economics of environmental resources, Volume 4) Emerald Group Publishing Limited, pp.169 – 194

116- Parisi, V., 1974. Soil Biology and Ecology, Techniques of Researches. Boringhieri, Torino (in Italian).

117- Parisi, V., 2001. The biological soil quality, a method based on microarthropods (in Italy). Acta Naturalia de L'Ateneo Parmense 37, 97–106.

118- Parisi, V., Menta, C., Gardi, C., Jacomini, C., Mozzanica, E., 2005. Microarthropod communities as a tool to assess soil quality and biodiversity: a new approach in Italy. Agriculture, Ecosystems and Environment 105, 323–333.

119- Petersen, S.O., Henriksen, K., Mortensen, G.K., Krogh, P.H., Brandt, K.K., Sørensen, J., Madsen, T., Petersen, J., Grøn, C., 2003. Recycling of sewage sludge and household compost to arable land: fate and effects of organic contaminants, and impact on soil fertility. Soil Tillage Res. 72:139–152 Soil Agroecosystems: Impacts of Management on Soil Health and Crop Diseases. 10.1016/S0167-1987(03)00084-9.

120- Pielou, E. C. 1975. Ecological diversity. New York, NY: John Wiley & Sons.

121- Pimentel, D., Hepperly, P., Hanson, J., Douds, D., & Seidel, R. 2005. Environmental, energetic, and economic comparisons of organic and conventional farming systems. AIBS Bulletin, 55(7), 573-582.

122- Pisa, L.W., Amaral-Rogers, V., Belzunces, L.P. et al. 2015. Effects of neonicotinoids and fipronil on non-target invertebrates. Environ Sci Pollut Res (2015) 22: 68. https://doi.org/10.1007/s11356-014-3471-x

123- Postma-Blaauw, M.B., de Goede, R.G.M., Bloem, J., Faber, J.H., Brussaard, L., 2012. Agricultural intensification and de-intensification differentially affect taxonomic diversity of predatory mites, earthworms, enchytraeids, nematodes and bacteria. Applied Soil Ecology 57, 39e49.

124- Powlson, D.S. and Johnston, A.E. 1994. Long-term field experiments: their importance in understanding sustainable land use. In: Greenland, D.J. and Szabolcs, I. (eds) Soil Resilience and Sustainable Land Use. CAB International, Wallingford, UK, pp. 367-394.

125- Prasad. R. and Power, J.F., 1991. Crop residue management. Adv. Soil Sci .. 15: 205-251.

126- Ratnasingham S & Hebert PDN. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Mol Ecol Notes. 2007; 7: 355–364. pmid:18784790

127- Ratnasingham S & Hebert PDN. 2013. A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. PLoS One 2013; 8(8): e66213.

128- Reganold, J. P., & Wachter, J. M. 2016. Organic agriculture in the twenty-first century. Nature plants, 2(2), 15221.

129- Rougerie R., Decaëns T., Deharveng L., Porco D., James S.W., Chang C., Richard B., Potapov M., Suhardjono Y., and Hebert P.D.N.. 2009. DNA barcodes for soil animal taxonomy. *Pesquisa Agropecuária Brasileira*, *44*(8), 789-802.https://dx.doi.org/10.1590/S0100-204X2009000800002

130- Rusek, J., Marshall, V.G., 2000. Impacts of airborne pollutants on soil fauna. Annu. Rev. Ecol. Syst. 31, 395–423.

131- Rutgers, M., Schouten, A.J., Bloem, J., Van Eekeren, N., De Goede, R.G.M., Jagers op Akkerhuis, G.A.J.M., van derWal, A., Mulder, C., Brussaard, L., Breure, A.M., 2009. Biological measurements in a nationwide soil monitoring network. European Journal of Soil Science 60, 820e832.

132- Sacchi, C.F., Testard, P., 1971. Ecologie Animale. Doin, Paris.

133- Santorufo, L., Van Gestel, C. A., Rocco, A., & Maisto, G. 2012. Soil invertebrates as bioindicators of urban soil quality. *Environmental Pollution*, *161*, 57-63.

134- Sapkota, T.B., Mazzoncini, M., Bàrberi, P. et al. 2012. Fifteen years of no till increase soil organic matter, microbial biomass and arthropod diversity in cover crop-based arable cropping systems

Agron. Sustain. Dev. 32: 853. https://doi.org/10.1007/s13593-011-0079-0

135- Scherber, C., Eisenhauer, N., Weisser, et al. 2010. Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. Nature 468, 553e556.

136- Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13:2498–504.

137- Shannon, C. E. A. 1948. Mathematical theory of communication. Bell System Technical Journal, 27(3), 379–423, 623–656. https://doi.org/ 10.1002/j.1538-7305.1948.tb01338.x

138- Sigut M, Kostovč´ık M, Sˇigutova´ H, Hulcr J, Drozd P, Hrček J 2017 Performance of DNA metabarcoding, standard barcoding, and morphological approach in the identification of host–parasitoid interactions. PLoS ONE 12(12): e0187803. https://doi.org/10.1371/journal. pone.0187803

139- Sinsabaugh, R.L., Lauber, C.L.,Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252e1264.

140- Six, J., K. Paustian, E. T. Elliott, and C. Combrink. 2000. Soil Structure and Organic Matter I. Distribution of Aggregate-Size Classes and Aggregate-Associated Carbon. Soil Sci. Soc. Am. J. 64:681-689. doi:10.2136/sssaj2000.642681x

141- Sneath P. H. A. and Sokal R. R. 1973. Numerical taxonomy. The principles and practice of numerical classification. San Francisco, W.H. Freeman and Company. USA. p. 573.

142- Stork N E and Eggleton P 1992 Invertebrates as determinants and indicators of soil quality. Am. J. Altem. Agric. 7, 38-55.

143- Stork, N., & Eggleton, P. 1992. Invertebrates as determinants and indicators of soil quality. American Journal of Alternative Agriculture, 7(1/2), 38-47. Retrieved from http://www.jstor.org/stable/44479649

144- Sylvain, Z.A., Wall, D.H., 2011. Linking soil biodiversity and vegetation: implications for a changing planet. American Journal of Botany 98, 517e527.

145- Tabaglio, V., Gavazzi, C., Menta, C., 2009. Physico-chemical indicators andmicroarthropod communities as influenced by no-till, conventional tillage andnitrogen fertilisation after four years of continuous maize. Soil Till. Res. 105,135–242.

146- Taberlet P, Coissac E, Pompanon F, Brochmann C, Willerslev E 2012 Towards next-generation biodiversity assessment using DNA metabarcoding. Mol Ecol 21:2045–2050

147- Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., et al. 2007. Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. Nucleic Acids Research, 35, e14.

148- Tedersoo L., Bahram M., Põlme S., et al. 2014. Global diversity and geography of soil fungi. Science 346: 1256688.

149- Tsaousis, A. D., D. P. Martin, D. Ladoukakis, D. Posada and E. Zouros, 2005 Widespread recombination in published animal mtDNA sequences. Mol. Biol. Evol. 22: 925–933

150- Tuck SL, Winqvist C, Mota F, Ahnstroem J, Turnbull LA, Bengtsson J. 2014. Land-use intensity and the effects of organic farming on biodiversity: a hierarchical meta-analysis. Journal of Applied Ecology 51:746–755.

151- Unger, P.W., 1990. Conservations tillage systems. Adv. Soil Sci., 13: 27-68.

152- Utzeri V. J., A. Ribani, G. Schiavo, F. Bertolini, S. Bovo, L. Fontanesi. 2018. Application of next generation semiconductor based sequencing to detect the botanical composition of monofloral, polyfloral and honeydew honey,Food Control. 86: 342-349. https://doi.org/10.1016/j.foodcont.2017.11.033.

153- Valentini A, Taberlet P, Miaud C, Civade R, Herder J, Thomsen PF, et al. 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Mol Ecol. 2016; 25: 929–942. pmid:26479867

154- Valentini A., F. Pompanon and P. Taberlet. 2009. DNA barcoding for ecologists. Trends in Ecology and Evolution 24 (2): 110-117. doi:10.1016/j.tree.2008.09.011.

155- Van Amelsvoort. P.A.M., van Dongen,C., and van der Werff P. A., 1988. The impact of Collembola on humification and mineralization of soil organic matter. Pediobiologia. 31: 103-111.

156- van der Wal, A., Geerts, R.H.E.M., Korevaar, H., Schouten, A.J., Jagers op Akkerhuis, G.A.J.M., Rutgers, M., Mulder, C., 2009. Dissimilar response of plant and soil biota communities to long-term nutrient addition in grasslands. Biology and Fertility of Soils 45, 663e667.

157- van Straalen NM 1998 Evaluation of bioindicator systems derived from soil arthropod communities. Appl Soil Ecol 9:429–437

158- Virgilio, M., Backeljau, T., Nevado, B., & De Meyer, M. 2010. Comparative performances of DNA barcoding across insect orders. BMC bioinformatics, 11(1), 206.

159- Wandeler P, Hoeck PE, Keller LF. 2007. Back to the future: museum specimens in population genetics. Trends Ecol Evol 2007, 22(12):634-642.

160- Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PD. 2005. DNA barcoding Australia's fish species. Philos Trans R Soc Lond B Biol Sci 2005, 360(1462):1847-1857.

161- Wardle, D.A., 2002. Communities and Ecosystems: Linking the Aboveground and Belowground Components. In: Monographs in

Population Biology, vol. 34. Princeton University Press vii þ 392 pp.

162- Watts, D. J. and Strogatz, S. 1998. Collective dynamics of 'small-world' networks. Nature 393, 440–442

163- Weibull, A. C., Östman, Ö., & Granqvist, Å. 2003. Species richness in agroecosystems: the effect of landscape, habitat and farm management. Biodiversity & Conservation, 12(7), 1335-1355.

164- Willer H. and J. Lernoud. 2016. The world of organic agriculture. Statistics and emerging trends 2016. 17 edition. Research institute of organic agriculture fibl and ifoam organics international, Frick and Bonn.

165- Wilson, K. H. 1995 Molecular biology as a tool for taxonomy. Clin. Infect. Dis. 20(Suppl.), 192–208

166- Yamamoto, S., Masuda, R., Sato, Y., Sado, T., Araki, H., Kondoh, M., ... & Miya, M. 2017. Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. Scientific reports, 7, 40368.

167- Yang C, Wang X, Miller JA et al. 2014 Using metabarcoding to ask if easily collected soil and leaflitter samples can be used as a general biodiversity indicator. Ecological Indicators, 46, 379–389.

168- Yoccoz, N.G., Bråthen, K.A., Gielly, L., et al. 2012. DNA from soil mirrors plant taxonomic and growth form diversity. Mol.Ecol. 21, 3647–3655

169- Yu, D. W., Ji, Y. , Emerson, B. C., Wang, X. , Ye, C. , Yang, C. and Ding, Z. 2012. Biodiversity soup: metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. Methods in Ecology and Evolution, 3: 613-623. doi:10.1111/j.2041-210X.2012.00198.x

170- Zaiko A, Martinez JL, Ardura A, Clusa L, Borrell YJ, Samuiloviene A, et al. 2015. Detecting nuisance species using NGST: Methodology shortcomings and possible application in ballast water monitoring. Mar Environ Res. 2015; 112: 64–72. pmid:26174116

171- Zhang, D. X., & Hewitt, G. M. 1996. Nuclear integrations: challenges for mitochondrial DNA markers. Trends in ecology & evolution, 11(6), 247-251.

172- Zhang, D.-X. & Hewitt, G. M. 1997 Assessment of the universality and utility of a set of conserved mitochondrial primers in insects. Insect Mol. Biol. 6, 143–150.

Appendix

Table S1: The obtained reads assigned to the Kingdoms showing the number of reads assigned and the OTUs.

Sample ID	Sampling season	Sequences						Assigned OTUs					
		All	Animalia	Fungi	Bacteria	Others (Archea, Plantae)	No blast hit	All	Animalia	Fungi	Bacteria	Others (Archea, Plantae)	No blast hit
IMS0	May	25947	17564	7586	379	14	404	4058	2854	1017	102	9	76
IMS0	July	71455	54599	15129	818	25	884	3121	2212	783	60	5	61
IMS0	October	19422	13016	6101	209	10	86	2281	1523	683	38	7	30
IMS15	May	26813	16877	8963	490	5	478	3257	2293	762	98	2	102
IMS15	July	76796	41731	33403	1035	46	581	5771	4132	1356	152	8	123
IMS15	October	36582	21854	13539	650	24	515	3900	2820	882	95	4	99
IMS30	May	43532	26859	15344	796	35	498	3725	2666	830	119	6	104
IMS30	July	54755	39809	13540	761	32	613	4119	3025	855	124	6	109
IMS30	October	57887	34593	21906	730	6	652	4635	3379	1026	113	2	115
IMS45	May	23875	18125	5216	259	37	238	3303	2414	712	79	12	86
IMS45	July	102893	62876	36325	2966	70	656	4879	3439	1196	136	7	101
IMS45	October	42770	26728	15059	548	5	430	4084	2930	966	95	4	89
IMB0	May	28489	19396	8184	440	60	409	4710	3289	1179	122	19	101
IMB0	July	65581	46742	16134	1793	60	852	4770	3412	1085	148	15	110
IMB0	October	23611	19105	3799	375	22	310	2950	2136	627	103	7	77
IMB15	May	41686	31422	9179	739	47	299	4190	2967	1016	110	15	82
IMB15	July	108575	73519	28598	4286	523	1649	5356	3835	1189	175	19	138
IMB15	October	57493	39321	15350	1962	115	745	4661	3241	1161	138	14	107
IMB30	May	1589	1002	482	91	3	11	779	541	200	28	2	8
IMB30	July	71871	51121	17145	2469	381	755	4768	3356	1125	163	16	108
IMB30	October	35903	18594	14973	1755	157	424	4023	2635	1155	121	15	97
IMB45	May	36104	23219	10645	1434	95	711	4391	3140	969	143	15	124

IMB45	July	72414	47384	22135	1467	262	1166	5307	3720	1291	156	20	120
IMB45	October	45256	26765	16689	1239	40	523	4699	3371	1070	136	14	108
VS0	May	74101	47351	22680	1697	99	2274	5640	3922	1397	160	12	149
VS0	July	35812	24363	10168	669	10	602	3413	2321	920	82	2	88
VS0	October	69763	61452	5198	1368	16	1729	3320	2352	736	105	3	124
VS15	May	28961	17960	9975	533	8	485	3816	2682	931	98	3	102
VS15	July	47135	31620	13889	785	8	833	4409	3111	1060	120	3	115
VS15	October	25728	16012	8774	483	35	424	3118	2177	747	90	5	99
VS30	May	77544	50631	24769	1505	16	623	5214	3740	1244	127	4	99
VS30	July	52313	35423	15231	957	22	680	4320	3017	1101	105	7	90
VS30	October	50352	34604	13642	1139	11	956	4054	2850	976	117	5	106
VS45	May	67620	43863	21269	1417	10	1061	4862	3507	1114	126	4	111
VS45	July	65334	47766	14726	1963	17	862	4643	3365	1001	150	7	120
VS45	October	56209	42804	11602	1130	17	656	3533	2531	805	102	4	91
VB0	May	39931	21091	17207	708	8	917	3733	2464	1073	97	3	96
VB0	July	28409	18288	8991	539	41	550	3061	1915	940	100	5	101
VB0	October	27579	15342	11615	344	7	271	2977	1908	949	59	2	59
VB15	May	50282	34203	13468	1001	62	1548	4194	3051	960	119	11	53
VB15	July	25320	18629	5784	666	74	167	3459	2557	751	100	10	41
VB15	October	49851	33596	13970	1329	217	739	4731	3540	976	134	6	75
VB30	May	48762	32628	14057	1069	145	863	4577	3340	1026	117	15	79
VB30	July	11422	8094	2994	216	29	89	2436	1778	550	65	8	35
VB30	October	42164	25877	14159	1077	228	823	4329	3151	975	118	8	77
VB45	May	22013	19589	1878	246	17	283	2324	1750	451	65	6	52
VB45	July	2176	1462	676	25	4	9	760	509	227	15	3	6
VB45	October	70566	46220	21336	1851	153	1006	4540	3331	991	131	15	72
Total		2313488	1561294	664452	51403	3347	32992	18034	13551	3602	466	31	384

Table S2: Analysis of variance (ANOVA) of the differences between the samples in the OTUs richness, Shannon and Pielou's evenness indices.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	H	5.469	15	.365	1.268	.277
	P	.071	15	.005	1.607	.127
	OTUs	3625212.313	15	241680.821	5.383	.000
Intercept	H	1909.336	1	1909.336	6638.561	.000
	P	32.895	1	32.895	11117.562	.000
	OTUs	68394712.687	1	68394712.687	1523.386	.000
Farming	H	.346	1	.346	1.204	.281
	P	.003	1	.003	1.050	.313
	OTUs	10710.187	1	10710.187	.239	.629
Field	H	.214	1	.214	.746	.394
	P	.000	1	.000	.013	.910
	OTUs	414222.521	1	414222.521	9.226	.005
Position	H	.224	3	.075	.260	.854
	P	.009	3	.003	.980	.415
	OTUs	715269.729	3	238423.243	5.311	.004
Farming * Field	H	.006	1	.006	.020	.888
	P	.003	1	.003	1.179	.286
	OTUs	197505.021	1	197505.021	4.399	.044
Farming * Position	H	2.463	3	.821	2.855	.053
	P	.029	3	.010	3.229	.035
	OTUs	631178.563	3	210392.854	4.686	.008
Field * Position	H	.273	3	.091	.316	.814
	P	.018	3	.006	2.004	.133
	OTUs	768786.563	3	256262.188	5.708	.003
Farming * Field * Position	H	1.943	3	.648	2.251	.101
	P	.010	3	.003	1.075	.373
	OTUs	887539.729	3	295846.576	6.590	.001
Error	H	9.204	32	.288		
	P	.095	32	.003		
	OTUs	1436688.000	32	44896.500		
Total	H	1924.010	48			
	P	33.061	48			
	OTUs	73456613.000	48			
Corrected Total	H	14.673	47			
	P	.166	47			

OTUs	5061900.313	47			

Table S3: Core diversity OTUs and the unique OTUs in the studied fields.

Taxa	Core Diversity OTUs	Unique OTUs in IMS	Unique OTUs in IMB	Unique OTUs in VS	Unique OTUs in VB
Annelida,Clitellata	33	135	44	74	0
Annelida,OtherAnnelida	2	0	0	0	0
Annelida,Polychaeta	20	16	5	19	0
Arachnida,Arthropoda	0	0	0	0	148
Arthropoda,Arachnida	162	185	91	238	0
Arthropoda,Branchiopoda	0	7	3	6	0
Arthropoda,Chilopoda	1	48	6	18	0
Arthropoda,Collembola	15	48	17	50	0
Arthropoda,Diplopoda	7	0	4	11	0
Arthropoda,Diplura	0	1	0	7	0
Arthropoda,Insecta	396	548	348	650	0
Arthropoda,Malacostraca	16	36	31	36	0
Arthropoda,Maxillopoda	1	6	2	12	0
Arthropoda,Merostomata	0	3	1	0	0
Arthropoda,Ostracoda	0	0	0	8	0
Arthropoda,OtherArthropoda	0	2	5	1	0
Arthropoda,Pycnogonida	1	1	0	3	0
Bdelloidea,Rotifera	0	0	0	0	2
Branchiopoda,Arthropoda	0	0	0	0	5
Chilopoda,Arthropoda	0	0	0	0	2
Chromadorea,Nematoda	0	0	0	0	2
Clitellata,Annelida	0	0	0	0	13
Collembola,Arthropoda	0	0	0	0	23
Diplopoda,Arthropoda	0	0	0	0	18
Eutardigrada,Tardigrada	0	0	0	0	2
Gastropoda,Mollusca	0	0	0	0	42
Insecta,Arthropoda	0	0	0	0	454
Malacostraca,Arthropoda	0	0	0	0	21
Maxillopoda,Arthropoda	0	0	0	0	4
Merostomata,Arthropoda	0	0	0	0	1
Mollusca,Gastropoda	53	52	45	57	0
Monogononta,Rotifera	0	0	0	0	4
Nematoda,Adenophorea	0	0	1	0	0
Nematoda,Chromadorea	1	8	7	29	0
Nematoda,Dorylaimea	0	0	1	0	0

Nematoda,Enoplea	2	0	0	3	0
Nematoda,Secernentea	2	5	6	14	0
Ostracoda,Arthropoda	0	0	0	0	1
OtherAnnelida,Annelida	0	0	0	0	1
OtherArthropoda,Arthropoda	0	0	0	0	4
Polychaeta,Annelida	0	0	0	0	24
Protura,Arthropoda	0	0	0	0	1
Pycnogonida,Arthropoda	0	0	0	0	1
Rotifera,Bdelloidea	2	8	2	4	0
Rotifera,Monogononta	5	4	3	4	0
Secernentea,Nematoda	0	0	0	0	2
Tardigrada,Eutardigrada	0	1	1	12	0
Tardigrada,Heterotardigrada	1	0	0	2	0
Sum	720	1114	623	1258	775

Table S4: QBS-ar values vs mQBS.

Sample ID	QBS-ar	mQBS	Sample ID	QBS-ar	mQBS
IMS0	65	48	VS45	61	54
IMS0	90	73	VS45	45	54
IMS0	95	109	VS45	115	69
IMB0	61	43	IMS45	41	68
IMB0	61	79	IMS45	67	94
IMB0	51	23	IMS45	30	39
VB0	95	59	VB45	8	20
VB0	71	44	VB45	1	2
VB0	79	48	VB45	20	59
VS0	81	104	IMB45	20	54
VS0	71	84	IMB45	35	84
VS0	70	59	IMB45	20	59

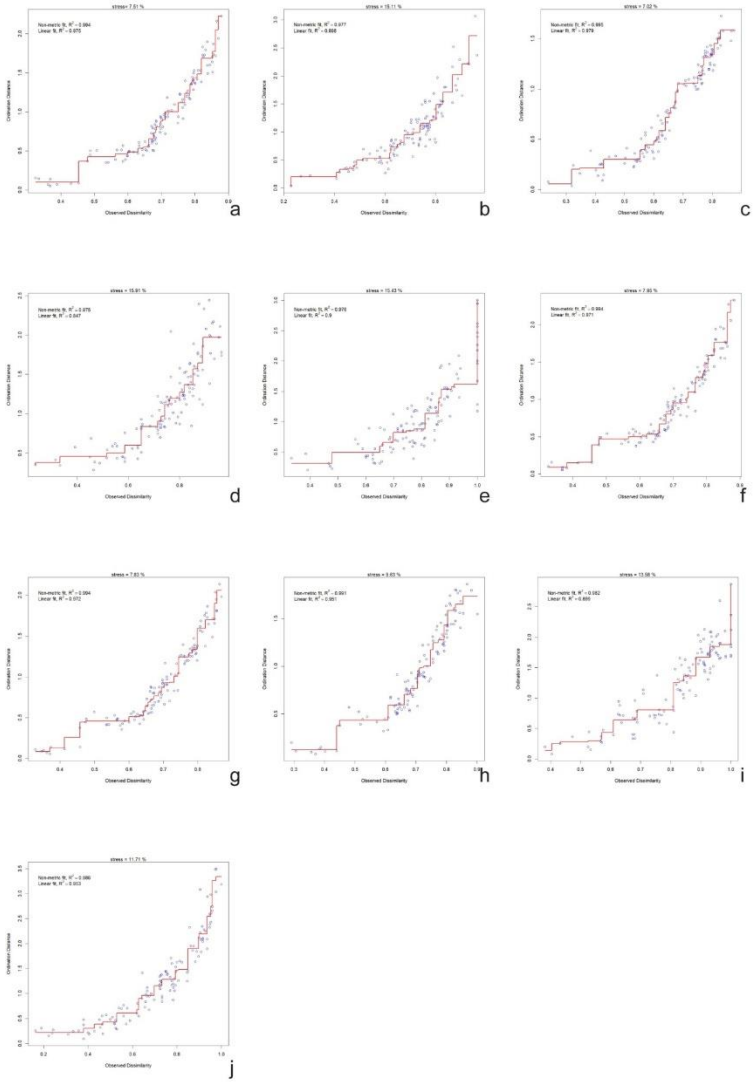

Fig. S1: Shepard diagrams for soil Invertebrates communities. Relationship between NMDS ordination distance and original observed distance. NMDS ordination was undertaken on presence-absence species matrix. (a) Invertebrates communities as whole; (b) Annelida communities; (c) Nematoda ; (d) Mollusca communities; (e) Rotifera and Tardigrada communities; (f) Arthropoda communities; (g) Insecta communities; (h) Arachnida communities; (i) Collembola communities; (j) Chilopoda and Diplopoda communities.

SOIL DNA METABARCODING: EVALUATING THE EFFICIENCY OF MULTIPLEX PRIMER SETS IN RECOVERING THE SOIL INVERTEBRATE'S COMMUNITY AS SOIL QUALITY INDICATORS

DNA METABARCODING DEL SUOLO: VALUTAZIONE DELL'EFFICIENZA DI ALCUNI SET DI PRIMER NELL'INVESTIGARE LA COMUNITÀ INVERTEBRATA DEL SUOLO COME INDICATORI DELLA QUALITÀ DEI SUOLI

Sumer Alali[1*], Paola Cremonesi[2], Bessem Chouaia[1], Valeria Mereghetti[1], Flavia Pizzi[2], Matteo Montagna[1], Stefano Bocchi[1]

[1] Università degli Studi di Milano, Dipatimento di Scienze Agraria e Ambiente-Produzione, Territorio, Agroenergia, via Celoria 2, 20133 Milano, Italy
[2] Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche, via Einstein, 26900 Lodi, Italy
[*] sumer.alali@unimi.it

Abstract
DNA Metabarcoding was used to investigate the efficiency of two sets of primers (combinations *A* and *B*) for characterizing the soil invertebrate's communities in different farming management systems. Soil samples were taken from three different sites in the South-West of Milan and DNA was extracted directly. PCR was applied by using 4 pairs of previously published primers targeting invertebrate's *cox1*, followed by Illumina Miseq sequencing. The results showed that the presence of the most popular primer pair used in barcoding studies (LCOI490-HCO2198) has affected negatively the taxonomic assignment of OTUs, since about 67.88 % of the obtained sequences where not identified. Our analysis showed that a higher percentage of Arthropoda, Annelida, Nematoda and Rotifera &Tardigrada (41.6, 5.9, 0.8 and 1% of total reads, respectively) was obtained with primer combination *B*; thus this primers set can be considered a promising method to evaluate the soil arthropods community.

Keywords: mitochondrial *cox1*, biodiversity, Illumina MiSeq.
Parole chiave: citocromo ossidasi subunità I, biodiversità, Illumina MiSeq.

Introduction:
Biodiversity assessment is the key factor in understanding the relationships between biodiversity and ecosystem functioning/services. The effects of major anthropogenic stressors on global ecosystems, including elevated CO_2, pollution, habitat loss and fragmentation, add urgency to this field, demanding an increasing focus on mechanistic and predictive studies. So far, there is a well-acknowledged biodiversity identification gap related to eukaryotic meiofaunal organisms (Creer et al., 2010). The most abundant micro- and mesofauna in soil include nematodes, microarthropods (i.e., Collembola, Acari, Insecta), Anellidae as Enchytraeids, and to a lesser extent, Tardigrades, Rotifers, and Proturans. Therefore, the accurate assessment of the taxonomic structure of these communities is both time-consuming and requires a high level of taxonomic expertise (Hamilton et al., 2009). The limitations inherent in morphology-based identification systems and the dwindling pool of taxonomists prompt the need for a new approach to taxon recognition (Hebert et al., 2003a,b).
DNA metabarcoding, a promising new technology, involves the direct extraction of DNA from soil samples, PCR amplification of the extracted DNA with specific primers, followed by libraries preparation with sample-specific tags and sequencing through Next Generation Sequencing technologies (Hamilton et al., 2009). Although presence–absence measures can provide useful indicators of biological diversity, they are often insufficient to link biological diversity to ecosystem functioning (Faust and Raes, 2012).
The Cytochrome Oxidase I (*cox1*) gene is typically used for DNA metabarcoding technique and extensive reference sequences are already available in online databases (Ratnasingham and Hebert, 2007, 2013), but, as previously evaluated, the utility of DNA metabarcoding remains limited due to severe primer bias, which prevents the detection of all taxa present in a sample (Piñol et al., 2014; Elbrecht and Leese, 2015). Therefore, the selectionof primers is the most critical component to assess macroinvertebrate bulk samples with DNA metabarcoding. Several *cox1* barcoding primers with different levels of degenerates base have been developed of which many are now used or could be suitable for

F. Ventura, G. Seddaiu, G. Cola (a cura di), Atti del XX Convegno AIAM e XLVI Convegno SIA.
Milano, 12-14 settembre 2017.
DOI 10.6092/unibo/amsacta/5692

Metabarcoding studies (e.g., Folmer et al., 1994; Hebert et al., 2004; Meusnier et al., 2008; Leray et al., 2013). One of the first primers pair designed on the 5' region of the mitochondrial *cox1* gene is the LCOI490-HCO2198 from Folmer et al. (1994), which has been successfully used in a plethora of DNA barcoding studies targeting a wide taxonomic range (e.g., Hebert et al., 2003a,b; Sheffied et al., 2009; García-Morales and Elías-Gutiérrez 2013; Magoga et al., 2016; Montagna et al., 2016). However, Metabarcoding primers typically recover approximately 80–90% or less of the taxa present in a sample (Leray et al., 2013; Brandon-Mong et al., 2015; Elbrecht and Leese, 2015). Furthermore, many primers have not been thoroughly evaluated for primer bias and the proportion of undetected taxa, making development and testing of universal primers a pressing issue.
In the present study, two combinations of primers sets targeting the mitochondrial *cox1* were tested in order to evaluate their efficiency in characterizing meso- and meio-faunal soil communities.

Material and methods:
Three sites under different farming management were chosen in the South-West area near Milan. The farms are located in the municipalities of Albairate (MI) and Cisliano (MI).
Three different types of soil samples (organic farm, conventional farm and forest), representing three main types of land-use in the study area, were collected in replicate in two farms from stable meadows and barley, during three different seasons (*i.e.*, spring, summer and autumn),.
Soil samples were homogenized in laboratory, grounded with liquid nitrogen and the DNA was extracted from each replicate by Nucleospin® soil kit (Macherey-Nagel, Düren - Germany) following the procedure described by Capra and co-workers (2016).
Fragments of the *cox1* gene ranging from ~300 to 650 bp were amplified using the primers reported in the table (Tab. 1), and the two combinations of tested primers were: *A* = primers 1+2+3+4, *B* = primers 1+3+4. Samples were randomly selected for applying the primers combination resulting in 29 samples for *A* and 26 samples for *B* combination, respectively.

Tab.1: Primers used for the amplification of COI gene.
Tab.1: Primer usati per l'amplificazione del gene COI.

PCR_ID	Primers	Sequence 5' – 3'	References
1	COIF2	TCTACYAATCATAAAGATATTGGTAC	Arribas et al, 2016
	COIR2	ACTTCTGGATGACCAAAGAATCA	
2	LCOI490	GGTCAACAAATCATAAAGATATTGG	Folmer et al, 1994
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	
3	mlCOIintf	GGWACWGGWTGAACWGTWTAYCCYCC	Leray et al, 2013; Geller et al, 2013
	JgHCO2198	TAIACYTCIGGRTGICCRAARAAYCA	
4	Foldf-foldr	GTGTATCTACGGTTGG CAATCCAGCAAGTCAGG	Yu et al. 2012

Libraries were assembled pooling PCR products, according with the primers combinations (*A* and *B*), in equimolar concentrations and sequenced on a paired 2X250 bp run on Miseq platform (Illumina, San Diego, CA, USA). Raw sequences were processed rebuilding full amplicon fragments via pair overlapping and analyzed using QIIme platform. In order to identify the obtained Operational Taxonomic Units (OTUs), a *cox1*-based reference database was built. The *cox1* dataset contains our target taxa (*i.e.*, phyla belonging to Animalia) with the inclusion of Archea, Bacteria and Fungi representatives in order to detect cross-amplifications between the used primers with non-target taxa. A descriptive statistic was performed to determine the frequencies and percentages of OTUs in samples and sites.

Results and discussions:
A total of 13,506,930 raw reads were obtained by the adopted sequencing strategy, 79.9% of the reads were lost after filtering due to their low quality resulting in a total number of 2,713,429 high-quality reads to be assigned to OTUs and analyzed (mean = 50,247 ± 3,477 sequences/sample).
The 2,713,429 reads were assigned to 194,668 OTUs, 67% of them were unspecific in the set *A* and only 21% were unspecific in the set *B* (Fig. 1, a). Regarding the OTUs assignment, ~63% of the reads obtained from the set *B* and ~22%

F. Ventura, G. Seddaiu, G. Cola (a cura di), Atti del XX Convegno AIAM e XLVI Convegno SIA.
Milano, 12-14 settembre 2017.
DOI 10.6092/unibo/amsacta/5692

of those from set *A* were assigned to the Animal kingdom. Focusing on our target groups, the invertebrates inhabiting soil (Fig. 1, b), the set *B* had a higher percentage of sequences assigned to Arthropods (41.6 %), Annelida (5.9 %), while in the case of set *A* the percentage of Arthropoda was 10.9 % and Annelida 5.4 % of the sequences.

Despite the assignment of 22.6% of sequences to non Animalia phyla (Fungi, Bacteria, etc…) in the set *B* (which was only 10.9% in the set *A*), the set *B* could recover a percentage of Arthopods four times higher than set *A* (41.6% and 10.9% in set *B* and set *A* respectively). Also higher percentages of sequences in the set *B* were assigned to Nematodes and other Metazoans like Rotifera and Tardigrades comparing with the set *A* (0.8% and 1% in set *B*; 0.01% and 0.3% in set *A*, respectively).

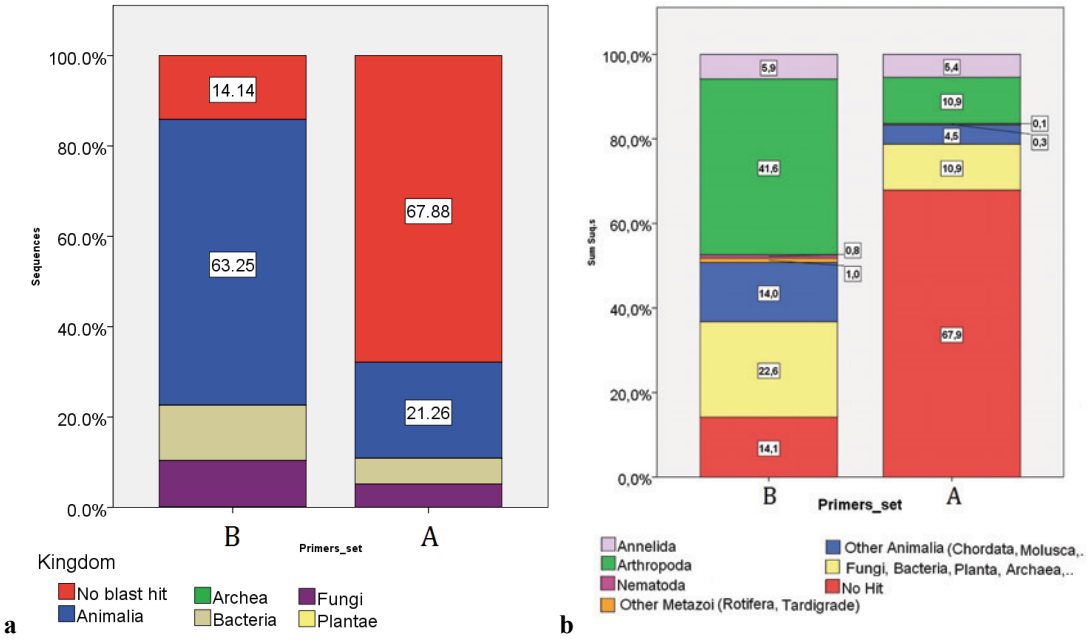

Fig.1: Percentage of the sequences assigned at the level of Kingdom (a) and at the level of Phyla for Animalia (b).
Fig.1: Percentuale delle sequenze assegnate a livello di Regno (a) e per i phyla animali (b).

Separating the obtained data according to the sampling site, the results confirmed that the primer set is a main factor affecting the recovered data, since the percentages of assigned OTUs to Arthropodes and Other groups of interest of Animalia were higher in the set *B* regardless the sites (Tab. 2). For example, the percentage of sequences assigned to Arthropodes in set *B* was four times higher than that of the set *A* in the Organic and Conventional sites.

Tab. 2: OTUs and percentages of sequences according to the primer set and the sampling sites.
Tab. 2: OTUs e percentuali di sequenze ottenute con i due set di primer nei tre siti di campionamento.

Primers_set	*A*			*B*		
Farm	Organic	Forest	Conventional	Organic	Forest	Conventional
Annelida	5,4%	7,9%	4,3%	4,0%	0,3%	7,2%
Arthropoda	9,2%	9,9%	14,5%	39,5%	3,7%	43,8%
Nematoda	0,1%	0,1%	0,1%	0,9%	0,0%	0,8%
Other Metazoi (Rotifera, Tardigrade)	0,2%	0,1%	0,4%	1,1%	0,0%	1,0%
Other Animalia (Chordata, Molusca)	4,1%	3,2%	5,9%	15,8%	2,0%	13,2%
Fungi, Bacteria, Plantae, Archaea	9,6%	15,6%	10,8%	22,5%	48,6%	22,0%
No Hit	71,4%	63,1%	64,1%	16,3%	45,3%	12,0%

Conclusions:

The major result of this preliminary study is that the set of primers, used to assembly PCRs, is a crucial factor affecting the capability to analyze efficiently the desired group of interest. The primer combination *B* could represent a promising method to evaluate the soil invertebrate's communities.

Acknowledgments:

We would like to thank the owners of the three farms in Cisliano - Albairate (MILANO) for their collaboration and facilitating the collection of soil samples.

References:

Arribas, P., Andujar, C., Hopkins, K., Shepherd, M., Vogler, A. P., & Yu, D. (2016). Metabarcoding and mitochondrial metagenomics of endogean arthropods to unveil the mesofauna of the soil. Methods in Ecology and Evolution, 7(9), 1071–1081.

Brandon-Mong, G. J., Gan, H.M., Sing, K.W., Lee, P. S., Lim, P. E., andWilson, J. J., 2015. DNA metabarcoding of insects and allies: an evaluation of primers and pipelines. Bull. Entomol. Res. 105, 717–727.

Capra, E., Giannico, R., Montagna, M., Turri, F., Cremonesi, P., Strozzi, F., Leone, P., Gandini, G., Pizzi, F. 2016. A new primer set for DNA metabarcoding of soil Metazoa. European Journal of Soil Biology, 77: 53-59. Creer, S., Fonseca, V. G., Porazinska, D. L., Giblin-Davis, R. M., Sung, W., Power, D. M., Thomas, W. K., 2010. Ultrasequencing of the meiofaunal biosphere: Practice, pitfalls and promises. Molecular Ecology, 19(SUPPL. 1), 4–20.

Elbrecht, V., and Leese, F., 2015. Can DNA-based ecosystemassessments quantify species abundance? Testing primer bias and biomass—sequence relationships with an innovative metabarcoding protocol. PLoS ONE 10:e0130324

Faust K., Raes J., 2012. Microbial interactions: from networks to models. Nat. Rev. Microbiol. 10, 538–550.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

García-Morales, A.E., Elías-Gutiérrez, M. 2013. DNA barcoding of freshwater rotifera in Mexico: evidence of cryptic speciation in common rotifers. Mol. Ecol. Resour. 13,1097-1107.

Geller J, Meyer C, Parker M, Hawk H. 2013 Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Mol. Ecol. Resour. 13, 851–861.Hamilton, H. C., Strickland, M. S., Wickings, K., Bradford, M. A., & Fierer, N., 2009. Surveying soil faunal communities using a direct molecular approach. Soil Biology and Biochemistry, 41(6), 1311–1314.

Hebert P. D. N., Cywinska, A., Ball, S. L., deWaard, J. R. 2003a. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London, Series B 270, 313–321.

Hebert, P. D. N., Ratnasingham, S., & Waard, J., 2003b. Barcoding animal life : cytochrome c oxidase subunit 1 divergences among closely related species Barcoding animal life : cytochrome c oxidase subunit 1 divergences among closely related species. Proc. R. Soc. Lond. B, 270, S96–S99.

Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H., and Hallwachs, W., 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proc. Natl. Acad. Sci. U.S.A.

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., et al., 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Front. Zool. 10:34.

Meusnier, I., Singer, G. A., Landry, J.-F., Hickey, D. A., Hebert, P. D., and Hajibabaei,M., 2008. A universal DNA mini-barcode for biodiversity analysis. BMC Genomics 9:214.

Magoga G., Sassi, D., Daccordi, M., Leonardi, C., Mirzaei, M., Regalin, R., Lozzia, G., Montagna, M. 2016. Barcoding Chrysomelidae: a resource for taxonomy and biodiversity conservation in the Mediterranean Region. Zookeys 597, 27-38.

Montagna, M., Mereghetti, V., Lencioni, V. & Rossaro, B., 2016 Integrated Taxonomy and DNA Barcoding of Alpine Midges (Diptera: Chironomidae). PLoS ONE, 11 (3), e0149673.

Piñol, J., Mir, G., Gomez-Polo, P., and Agustí N., 2014. Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative metabarcoding of arthropods. Mol. Ecol. Resour. 15, 1–12.

Ratnasingham, S., and Hebert, P., 2013. A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. PLoS ONE 8:e66213.101, 14812–14817.

Ratnasingham, S., and Hebert, P., 2007. BOLD: the Barcode of Life Data System (http://www.barcodinglife.org). Mol. Ecol. Notes 7, 355–364.

Sheffield, C. S., Hebert, P. D. N., Kevan, P. G., Packer L. 2009. DNA barcoding a regional bee (Hymenoptera: Apoidea) fauna and its potential for ecological studies. Mol. Ecol. Res. 9, 196–207.

Yu, D. W., Ji, Y., Emerson, B. C., Wang, X., Ye, C., Yang, C., & Ding, Z. (2012). Biodiversity soup: Metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. Methods in Ecology and Evolution, 3(4), 613–623.

F. Ventura, G. Seddaiu, G. Cola (a cura di), Atti del XX Convegno AIAM e XLVI Convegno SIA.
Milano, 12-14 settembre 2017.
DOI 10.6092/unibo/amsacta/5692

Session 6.
Ecology, Biodiversity and Conservation

Arthropod-Mediated Ecosystem Services in Agriculture

PO279

ASSESSING THE IMPACT OF AGRICULTURAL STRATEGIES ON SOIL ARTROPODS: A CASE STUDY USING ENVIRONMENTAL DNA

Sumer Alali, Università degli Studi di Milano, Dipatimento di Scienze Agraria e Ambiente-Produzione, Territorio, Agroenergia, Milano, Italy
Bessem Chouaia, Università degli Studi di Milano, Dipatimento di Scienze Agraria e Ambiente-Produzione, Territorio, Agroenergia, Milano, Italy
Paola Cremonesi, Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche, Lodi, Italy
Giulia Magoga, Università degli Studi di Milano, Dipatimento di Scienze Agraria e Ambiente-Produzione, Territorio, Agroenergia, Milano, Italy
Flavia Pizzi, Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche, Lodi, Italy
Stefano Bocchi, Università degli Studi di Milano, Dipartimento di Scienze Politiche e Ambientali, Milano, Italy
Matteo Montagna, Università degli Studi di Milano, Dipatimento di Scienze Agraria e Ambiente-Produzione, Territorio, Agroenergia, Milano, Italy

Elucidating how agricultural practices affect soil arthropod's communities is of relevant for both scientific and economic interests. Thus, using DNA metabarcoding approach, Arthropods communities inhabiting soil of organic and conventional farms were characterized. Soil samples were collected from organic and conventional farms, covering the margin and three levels towards the center of a stable meadow and a barley field in both farms; each sample consisted of 10 homogenized cores of soil (~560 cm^3), sampling was performed in spring, summer and autumn Soil texture, pH, N and C parameters were measured. DNA was extracted from three replicates of each soil sample. A fragment mitochondrial cox1 was amplified using three primer pairs and sequenced using Illumina Miseq. Raw sequences were processed and analyzed using Qiime to obtain Operational Taxonomic Units (OTUs) table. Approximately 75% of the obtained reads were identified as Animalia, and among these ~80% as Arthropods. α-diversity indices barley field in conventional farming were significantly lower (OTUs= 854, H'= 5.34±0.53, Pielou's evenness= 0,77±0,06) comparing with the other samples (OTUs>1100, H'>6 and Pielou's evenness>0,8). Fitting the farming system, the field, position as factors in NMDS showed that Arthropods communities were not affected by the collecting season and the farming system (organic *vs* conventional), instead crop *vs* stable meadows and the position in the field (margin *vs* middle) have a strong effect. Soil properties affected the Arthropods communities, especially the pH on Chilopoda and Diplopoda and the C/N ratio on Arachnida and Insecta. Our results pointing out that the strategy of farm management does not affect the arthropod communities of the soil as much as the soil properties itself, while the position in the filed had a major effect, highlighting the importance of green corridors for maintaining the soil biodiversity and the agroecosystem functioning.

Keywords: Soil Arthropods, DNA metabarcoding, agroecosystems, biodiversity

PO280

BUMBLEBEES DO NOT EXPAND THEIR DIET UNDER REDUCED WORKFORCE, AS REVEALED BY POLLEN DNA BARCODING

Paolo Biella, University of South Bohemia, Faculty of Science, Department of Zoology, České Budějovice, Czech Republic 2 Czech Academy of Sciences, Biology Centre, Institute of Entomology, České Budějovice, Czech Republic
Nicola Tommasi, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy
Asma Akter, University of South Bohemia, Faculty of Science, Department of Zoology, České Budějovice, Czech Republic 2 Czech Academy of Sciences, Biology Centre, Institute of Entomology, České Budějovice, Czech Republic
Jan Klecka, Czech Academy of Sciences, Biology Centre, Institute of Entomology, České Budějovice, Czech Republic
Anna Sandionigi, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy
Massimo Labra, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy
Andrea Galimberti, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy

Pollinators are affected by a high amount of stressors, as parasites, diseases, lack of food resources and some agricultural practices, including pesticides. Among pollinators, bees are particularly dependent on the flower resources, since they collect pollen and nectar for feeding the larvae. However, during their life cycle, social bees might undergo workforce losses because workers could be overpowered by environmental stressors. It might be expected that the remaining workers compensate the lack of incoming resources by individually collecting more resources per foraging trip. In this study, commercial colonies of the bumblebee *Bombus terrestris* were experimentally manipulated by removing half of the workers, in order to investigate changes in foraging strategies. Before and after the manipulation, the pollen pellets from the curbiculae were collected from the individual workers returning to the nests after their foraging trips in a flower-rich natural area in the Czech Republic. The manipulated colonies were compared with untreated ones in the same period. Meta-barcoding of the pollen's DNA was performed by targeting the ITS2 region using High throughput Sequencing (HTS). From the plant species associated to each bumblebee worker, the ecological network was derived and changes in niche breadth and network structures were tested. Overall, bumblebees were feeding on 34 plant taxa, revealed by DNA barcoding. However, only minor changes in the diet breadth of the bumblebees or in the feeding networks were found after the manipulations. At the end of the experiment, the manipulated colonies were smaller and without new queens. These results may suggest that bumblebees lack of plasticity in individual foraging, because they do not expand their foraging niche when less resources arrive in the nest due to workforce losses, with implications for population size.

Keywords: Bumblebees; *Hymenoptera Apidae*; Network Analysis; Foraging; DNA barcoding of diet; Feeding; Beneficial insects; Social insects; Pollinators

PO281

VALORISATION OF CHICKEN MANURE USING INSECTS: *HERMETIA ILLUCENS* IN THE VALORIBIO PROJECT

Sara Bortolini, BIOGEST-SITEIA, University of Modena and Reggio Emilia, Italy
Laura Macavei, BIOGEST-SITEIA, University of Modena and Reggio Emilia, Italy
Jasmine Hadj Saadoun, Dept. of Life Sciences, University of Modena and Reggio Emilia, Italy
Giorgia Foca, Department of Life Sciences, University of Modena and Reggio Emilia, Italy
Alessandro Ulrici, Department of Life Sciences, University of Modena and Reggio Emilia, Italy
Fabrizio Bernini, Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Italy
Daniele Malferrari, Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Italy
Lara Maistrello, Department of Life Sciences, University of Modena and Reggio Emilia, Italy