Università di Napoli Federico II

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Phd in Improvement and Management of Agro-forestry Resources

Soil fauna contribution to the soil system: multiscale approaches to address a complex interaction

Ph.D. Thesis

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Part of the objectives and results of this P.h.D Thesis were obtained by research work conducted in collaboration of CNR ISAFoM of Ercolano, and in particular by collaboration with Dr. Giacomo Mele (my co-tutor) and Dr.Laura Gargiulo.

The other part of results, were obtained by research work conducted in collaboration of Wageningen University (Wageningen, the Nederland) where I spent part of my PhD period. In the Department of Soil Quality, by collaboration with Prof. Jan Willem van Groenigen and Dr. Ingrid Lubbers.

Table of contents

Abstract:

The soil is one of the fundamental environmental compartments for supporting life on earth as it performs many ecosystem functions and services. The soil represents one of the most important reservoirs of biodiversity and soil fauna plays an essential role in several soil ecosystem functions. The aims of this work was to evaluate the interactions between soil fauna and its habitatin in relation to different land uses and at different scales. Such an aim has been achieved by performing a series of experiments carried out in the laboratory and in the field by means a multidisciplinary approach.

The first experiment has been directed to verify in which way and which amount the "different land use" were able to affect the soil quality. This has been determined by means of the Qbs-index (Qbs-ar), which uses the edaphic adaptation of the species, thus being also easier to use compared to other indices present in the literature. It was calculated in twelve different sites of the Telesina Valley (Benevento, Italy) correspond to four different land uses. The obtained results have shown that the Qbs index, independently on the different land uses, has provided a good biological soil quality in almost all studied sites of the Telesina Valley. In particular statistical analysis has not provided significant differences in taxa abundance between the different land uses. The Qbs-index showed the real ecological condition of the soil environment. This index, easy to use than other biological indices and considered extremely reliable, has shown the potential to be utilized for obtaining a complete soil mapping of the study sites on the biological soil quality and as an indicator of a possible soil stress state. Although this biological indicator is widely known and used, has shown limitations (i) to give information only on a single aspect of the soil quality that is the soil biodiversity and also (ii) to be not very sensitive in differentiating between different land uses. Scilicet Qbs doesn’t evaluate another important aspect, regarding the role of the soil fauna in the soil quality system, namely its contribution to soil structure formation.

This latter aspect was addressed in the second experimental phase in which it was evaluated the relationship between soil fauna and soil structure and the effect of different land uses. In four sites of the Telesina Valley corresponding each to a different land use, soil samples were collected combining the analysis about variability and abundance of soil meso and macrofauna found, with the quantification of the soil pore size distribution on undisturbed sample replicates, by means of 3D image analysis technique on the pore system. The results were very interesting because they showed a correlation between the heterogeneity of the soil structure, namely the multimodality of the soil pore size distribution and the soil fauna abundance. Despite this positive results, the used approach showed clear difficulties in the identification of the specific relationships between each soil fauna species and the produced soil pores. Thus, the analysis of natural soil samples does not allow the univocal recognition of the soil fauna species, which produced a specific soil pore formation.

To overcome the latter limitation in the successive experimental phase has been investigated the cause-effect relationships among different species of soil fauna and the soil pore system. It was done by developing an experimental design that uses repacked soil mesocosms in which many different taxa of soil fauna were inoculated, in order to identify their different biological signature. After the burrows activity of the fauna, the samples were impregnated with epoxy resin and underwent to x-ray medical CT to obtain 2D and 3D images. Then the pore size distribution for each study samples was determined, to quantify the contribution of each species. The different inoculation techniques (in lab or in the field) tested in this experiment, have been found appropriate for the identification and the quantification of the contribution of different taxonomic groups of meso and marcofauna to soil pore system formation. The preparation of traps into the field, has provided to be the most successful experimental setup. Results described in this experimental phase, proved that the identification of different contributions to the soil pore system formation has the potential to be employed for the identification and quantification of different biological activities in

natural conditions. Moreover, unlike the current literature, which is focused on the study of earthworms as "excellence ecosystem engineers", in this work has been evaluated the contribution of other soil fauna taxa in order to obtain a more complete outline for a more proper consideration of the fauna for soil quality improvement. In addition, published works on the relationship between soil fauna and soil structure are often more descriptive, they do not provide data directly related to the soil functions. The characterization of the soil structure by means of the pore size distribution based on the use of mathematical morphology algorithms has the potential to quantify the impact of the biological activity on many soil functional aspects (e.g. transport of fluids and solutes, creating new habitats). The results obtained by this new approach have the advantage they could be directly implemented in physically based models that simulate flow processes in soils.

Finally, in order to investigate about the contribute and the effect of soil fauna in the development of the main greenhouse gases (CO_2 and N_2O) I have participated to an experiment conducted by the University of Wageningen (The Netherland) where I spent my abroad period for PhD thesis. A laboratory test with inoculum of different soil fauna species was conducted. The N_2O and CO_2 fluxes were measured by means of gas monitor, Innova 1312 photo-acoustic infrared multi-gas analyser in a static closed chamber. At the end of fluxes measurement, each microcosm was used for fauna extractions and for soil analysis (e.g. DOC, pH). Excluding some evident artefacts in the results, outcomes of the experiment allowed to state that an increase in the soil respiration and in the fluxes of N_2O has been essentially provided by the presence of the earthworms, with different trends and timing for CO_2 and N_2O respect to the duration of the experiment. Fluxes of N_2O seemed also to be increased in the case of presence of only Potworms in the microcosms. Every experimental phase confirming which the interaction between soil fauna and its habitat are many and difficult to linked each other. Underlining how much the fauna is closely interrelated to its habitat and, how every modify on its habitat affect the biodiversity. Soil fauna and soil structure are interrelated each other, increasing one of this increases the other and viceversa. A good soil

management bring to higher soils biological quality (with higher richness species and greater abundance) consequently improving the soil porosity and soil structure.

Chapter 1

Introduction

1-1 Importance of soils

Soil is the basal layer of terrestrial ecosystems, rich of organisms and extremely varied, both from the taxonomic and numerical point of view. A single teaspoon of soil garden may include thousands species, millions individuals and hundreds meters of fungal networks. Scientists have estimated that about a quarter of current species on Earth live in soils. Moreover, only a small part of these species most of which are soil microorganisms - has been identified (Turbé et al., 2010). We know that there are many different soil types. Each one derived from different parent material. Many variables affect soil types and functions, between them climate, slope gradient, aspect, different vegetation and biodiversity, all closely correlated each other. The soil is closely related to all environmental compartments, into an integrated system, in which any modification to each compartment affect all system. Soil provides food, biomass and raw materials, serving as a substrate for human activities, it is a fundamental element of landscape and cultural heritage and plays a key role both as habitat and gene pool (CE 2006b). Soils, with their different typology and variety, underlie sustenance of the primary production (plant, fungi, microorganisms) and the survival of natural habitats. Soil microorganisms contribute to organic matter decomposition, recycling nutrient and carbon sequestration and storage. Together with soil macro-fauna, such as earthworms, they develop soil structure, making it more permeable for water and air (CE 2012 Biodiversity Impact). Furthermore, the soil is also the main planet terrestrial carbon deposit. Kyoto protocol (1997) underlines both, the importance to preserve soils and the need of correcting land management in order to ameliorate soils carbon sequestration. Many often all described above soil functions are taken for granted and their products have been always considered available and abundant, however, has assumed today, considerable importance. Soil degradation can be a slow process and rarely it shows dramatic

immediate effects (e.g. landslides) and it can be hard to raise public awareness about the importance of the sustainable use of soils (CE 2012a). At the same time, soil biodiversity decrease has been identified as another of major threats; then soil biodiversity it is an issue that we have to deal in the coming years (McBratney et al., 2014) and that it should be included in future conservation strategies.

Still today there are not present suitable actions to protect soil biodiversity, due to the complexity to address this issue and to the lack of data on the distribution of soil organisms at suitable soil management scale (e.g. 1:50,000 or more detailed) and for large areas. Orgiazzi et al (2016) have developed thematic maps of potential risks for soil biodiversity and preliminary guidelines to protect them (Fig 1).

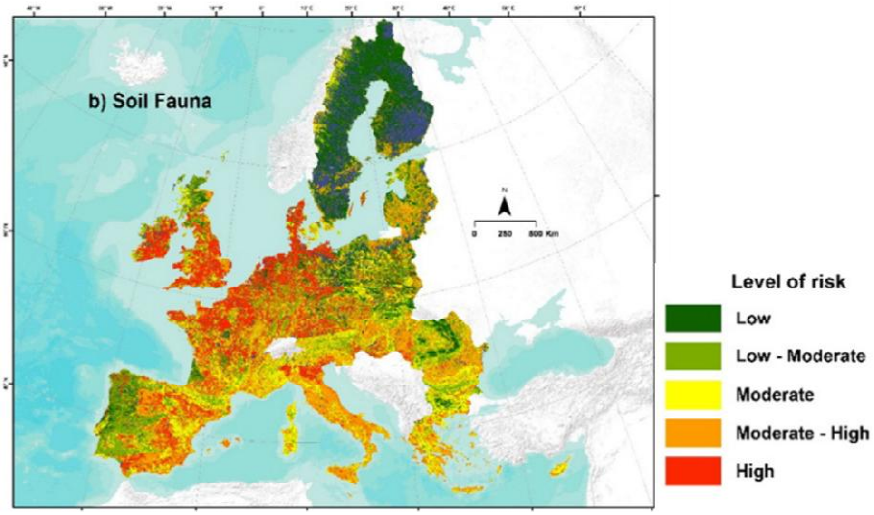


Fig 1. Maps of distribution of the potential threats to soil fauna (spatial resolution 500 m)(Orgiazzi et al. 2016).

A list of 13 potential threats to soil biodiversity was proposed to experts of different knowledge to understand their impact on the major components of soil biodiversity: soil microorganisms, soil fauna, and biological functions, in order to obtain knowledge-based rankings of threats.

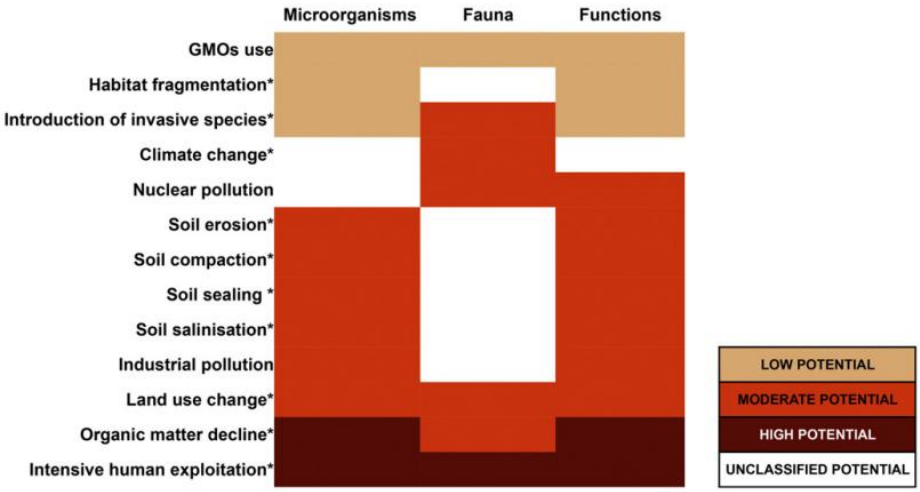


Fig 2. Classification of potential threats to soil biodiversity. The table shows the potential (from low to high) assigned to each of the 13 possible threats for the three components of soil biodiversity. The threats with significant difference in scores given to each category of soil biodiversity are indicated with * (Kruskal –Wallis test, pb 0.05),(Orgiazzi et al. 2016).

The fauna category, in particular, have a different trend than the others. The climate change, the decrease of organic matter and land use determine a moderate risks for the fauna. While the intensive human exploitation determine a high risk. This was also observed in our work. It was observed that these variables are able to strongly determine the decrease of the populations that live under the ground. To protect soils and to improve the land management, should be considered both the abiotic (decrease of soil erosion and of soil sealing) and the biotic factors (protection of soil-fauna living).

The importance of soil fauna

The less-charismatic soil organisms receive less scientific attention than the high-profile above-ground animals. “Soil biota” is an expression with a similar meaning to soil biodiversity, but is more specific and refers to the complete community within a given soil system. For example, it is possible to say that the soil biota in a grassland soil is generally more different than that in an arable system, or that grassland soils generally have higher levels of soil biodiversity than the soil in arable systems, with the same meaning is in both instances.

The biodiversity plays a key role into the soil formation processing (pedogenesis) into the ecological sequence, in the decomposition and transformation of the organic matter, carbon cycles, nitrogen, phosphorus, sulphur and water. In particular, the soil fauna plays an important role in the soil cycle, in its development and in its regeneration both for ecological and economic role. Soil fauna releases available elements for plants and other organisms (micronutrients), for the water control system, mitigating chemical and biological contamination and the genetic heritage preservation. Soil fauna can be divided in (Bullini et al., 1998):

- Hyperedaphon, who lives in low vegetation, but sometimes in edaphic environment;

- Piedaphon, who lives on the soil surface (generally in the litter);

- Hemiedaphon, who lives at medium depth, till to the soil surface;

- Euedaphon, who lives in the deep soil layers.

The natural history of soil biota allows to know the ecological conditions of soil and their quality, indeed many species have been employed as useful bio-indicators of soil quality. The soil mesofauna species are particularly indicated for the soil quality evaluation (Gupta and Yeates, 1997). In fact other soil fauna groups show more problems, such as the incomplete description of many taxa, the insufficient mapping of abundance or spatial distribution of the different species, the complex interrelationship between the different soil species and their functions within soil as well as their relationship with different environmental compartments or variables. Then it is not surprising the increasing use of microarthropod in the environmental monitoring. For instance the "International Standardization Organization" (ISO) is already using the mesofauna species as a technique of soil evaluation. (ISO 11266:1994; ISO 11267:1999; ISO11268-1:1993; ISO11268-2:1998; ISO 16387:2004). Some techniques, mainly used by entomologists, to study soil biota include the use of pitfall traps for macrofauna species who live in the soil surface (APAT, 2004), the different species of soil fauna and their different functions (spatio-temporal scales).

It's well know how the soil fauna contribute to modify the soil structure creating new habitats. Three different groups are typically involved in forming and modifying soil structure and improving the soil organic matter contributes to soil aeration, to absorb water and retain nutrients and, finally, to improve the soil structure. The different functional groups of soil fauna are called: Chemical Engineers, Biological Regulators and Ecosystem Engineers. Most of the species in soil are microorganisms, such as bacteria, fungi and protozoans, which are “Chemical Engineers”, responsable of decomposition of plant organic matter into nutrients readily available for plants, animals and humans. Soil also include many different species of invertebrates, such as Potworms, Springtails, Nematodes and Mites, which act as predators of plants, other invertebrates or microorganisms, by regulating their dynamics in space and time. These so-called "Biological Regulators" are relatively unknown to a wider audience, contrary to the larger invertebrates, such as Insects, Earthworms, Ants and Termites, ground Beetles, which show fantastic adaptations to living in a dark belowground world. The "Ecosystem Engineers" are Earthworms, Ants, Termites and some small mammals.

The ecosystem engineers have deeply influenced our assessment of the role of organisms in ecosystem functioning (Jones et al. 1994). Some organisms are no longer considered to play a role only as elements of a food web, but they are studied from the viewpoint of being responsible for altering ecosystem dynamics through the modification, maintenance and/or creation of habitats for other organisms in the ecosystem. Ecosystem engineers directly or indirectly modulate the availability of resources for other species, changing the physical state of biotic or abiotic materials (Jones et al., 1994, 1997). They are primarily physical engineers, increasing the proportion of stable aggregates in soil and thus stable inter aggregate porosity, they create biogenic structures, which can be galleries, chambers, casts. These structures are the components of stable macroaggregate structures that determine soil hydraulic properties and resistance to erosion (Blanchart et al., 1999; Chauvel et al., 1999). The main ecosystem engineers are earthworms thanks to their feeding and burrowing activities (Jones et al., 1994; Jouquet et al. 2006). Earthworms create macro-pores

through their burrowing activities and ingest soil particles and organic matter, mixing these two fractions together and expulsing them as casts at soil surface or in depth (Turbè A. 2010).

Lavelle (2002) deduces that in soil system the relative importance of regulation imposed by ecosystem engineering is likely to be greater than regulation by trophic relationships because of the specific ecological constraints observed in this environment when compared to above-ground conditions. Any kind of ecosystem engineers has the potential to enhance ecosystem function in soil, probably more than in any other ecological medium. One feature common to all these organisms is the disproportionate magnitude of their effects in terms of their biomasses and the way that their activity modulates soil resource accessibility for other soil organisms (Jouquet et al. 2006). Another important factor to consider is their influence on soil spatial and temporal heterogeneity. They affect soil processes and soil heterogeneity at different scales, they ranging from soil aggregation, the storage of soil organic matter to vegetation patterns and landscapes (Jones et al., 1997; Wilby et al., 2001). It is also important to evaluate the interaction of this soil ecosystem engineers, with other different species equally able to burrow into the soil and able to modify its structure.

However, the three different functional groups act mainly over distinct spatio-temporal scales, which provide a clear framework for management options. This is because the different organisms size, determines their different spatial aggregation patterns and dispersal distances, as well as their lifetimes, with smaller organisms acting at smaller spatio-temporal scales than the larger ones. Thus, chemical engineers are typically influenced by local scale factors, ranging from micrometres to metres and short-term processes, ranging from seconds to minutes. Biological regulators and soil ecosystem engineers, on the other hand, are influenced essentially by factors acting at intermediate spatio-temporal scales, ranging from a few to several hundreds of metres and from days to years. This provides land managers with two distinct management options for soil biodiversity: direct actions on the functional group concerned, or indirect actions at greater spatio-temporal scales than

that of the functional group concerned. (European Commission - DG ENV, Soil biodiversity: functions, threats and tools for policy makers, 2010).

Regulation of carbon and nitrous flux and climate control

Carbon dioxide (CO_2) and Nitrous oxide (N_2O) plays an important role in the current debate about climate change. They are the most dangerous greenhouses gases. Soil is estimated to contain about 2,500 billion tonnes of carbon to one meter depth. The soil organic carbon pool is the second largest carbon pool on the planet and is formed directly by soil biota or by the organic matter (e.g. litter, aboveground residues) that accumulates due to the activity of soil biota. Soil organisms increase the soil organic carbon pool through the decomposition of biomass, while their respiration releases carbon dioxide (CO_2) to the atmosphere. Carbon can also be released to the atmosphere as methane, a much more dangerous greenhouse gas than CO_2, when soils are flooded or clogged with water.

In addition, part of the carbon may leak from soils to other parts of the landscape or to other pools, such as the aquatic pool. Peatlands and grasslands are among the best carbon storage systems in Europe, while land-use change, through the conversion of grasslands to agricultural lands, is responsible for the largest carbon losses from soils. The loss of soil biodiversity, can reduce the soils ability to regulate the atmosphere composition, as well as the role of soils in counteracting global warming. (Commission - DG ENV, Soil biodiversity: functions, threats and tools for policy makers, 2010). Moreover soil biodiversity can also have indirect effects on the soil functions, turned this as a carbon sink or source. A complete understanding of the carbon cycle is fundamental for increasing the understanding of the links of carbon between the soil and the atmosphere and how this may be controlled or utilised for climate change mitigation.

Regarding Nitrous oxide (N_2O) soil emissions, they contribute significantly to global warming. While these gases represent much smaller fluxes than CO_2 gas, they are much more potent than carbon dioxide as a greenhouse gas (21 times and 310 times, respectively).This process, together with the greenhouse gases released by human activity, contributes to global warming. (European

Commission - DG ENV, Soil biodiversity: functions, threats and tools for policy makers (2010). N_2O is currently the most important anthropogenic ozone–depleting compound and will probably remain so during this century (Ravishankar et al., 2009).

Mitigation of N_2O emissions is severely hampered by a lack of understanding of its main controls. Fluxes can only partly be predicted from soil abiotic factors and microbial analyses and a possible role for soil fauna has until now largely been overlooked (I. Kuiper et all., 2013). There is sufficient evidence that soil fauna has significant effects on all of the pools and fluxes in C and N cycles, and soil fauna mineralize more N than microbes in some habitats.

Soil zoologists have long appreciated that soil fauna play key roles in regulating soil N cycling (e.g., Anderson et al. 1984, 1985, Coleman 1994), yet these roles have not been integrated into biogeochemical models (Seastedt 2000), although some of them are acknowledged (Schimel and Bennett 2004). Soil fauna can either suppress, delay, increase or accelerate soil CO_2 and N_2O emissions depending on the group through their effects on the processes of decomposition, nitrification and denitrification (Frouz et al., 2007; Kuiper et al., 2013; Wu et al., 2013). Soil fauna affects all of the pools within the soil N cycle through their effects on microbial biomass, inorganic N pools, supply of dissolved organic matter (DOM), and mass loss of organic matter.

In particular, soil invertebrate fauna can significantly affect N_2O emission increasing soil pore connectivity by means of their burrowing activity and intensifying the trophic interactions. The knowledge is ever more full of study about the relationship of soil fauna and N_2O emission, but these emissions have never been studied to the extent that we can quantitatively predict their impact on greenhouse gas emissions.

Determination of environmental stress state in different land uses

Many are the variables that influence the relationship between soil biodiversity and environmental stress state and disturbance. The relationships between soil biodiversity and ecosystem functioning are not simple (Chapin et al., 2000; Brussaard et al., 2004).

Considerable attention is receiving the insurance hypothesis (Loreau, Yachi, 1999) which suggests that ‘‘high’’ biodiversity confers an insurance against ecosystem malfunctioning under stress or disturbance. In fact soil biodiversity may well be related to efficient use of natural resources, such as water and nutrients. This holds promise for relieving pressure from agriculture on natural areas in agricultural landscapes, and even for providing habitats for species with conservation value from ‘‘natural’’ areas.

Another important variable is the managing of soil biodiversity. Whereas aboveground biodiversity is widely managed by choosing livestock and livestock breeds, crops and crop varieties, rotations, crop sequences and non-productive elements in agricultural landscapes, in most cases soil biodiversity can only be managed indirectly and the options for such management are less evident (Brussaard et al 2007).

The aim of the thesis:

Despite the importance of soil biodiversity, still today there are not present suitable actions to protect them. The current knowledge about quantitative evaluation and of the importance of the soil biota for soil quality are limited. Alike, the influence of the functioning of soil biota on the soil (e.g. porosity or gas fluxes). Many often, the studies are confined to evaluate only particular species (e.g. earthworms or microbial community). It's therefore necessary to give more relevance to all species of soil fauna by considering them with a greater connection (on a global scale) aiming at the ultimate objective of restoring and enhance the biodiversity of soil ecosystems in conjunction with the human management activities that may currently threaten them.

In such a framework, the aim of this thesis is to study soil fauna and its habitat, underlining critically its complex interactions at different scales by means of a multi-phase experimental approach, based on both field and laboratory experiments. Namely, the study of the relationship of the soil fauna with the ecological soil quality and the different land uses has been carried out by monitoring the abundance of the species in specific sites of an important agricultural district. Then, in order to investigate how the soil fauna affects the soil structure (porosity) and in which way the different species are able to improve the soil porosity, resin impregnation of undisturbed soil samples and soil pore imaging (by medical CAT) has been performed for sites where soil quality had already been investigated in the previous experimental phase. Moreover, through the study of the current knowledge in the literature, has been developed a further experimental phase aiming at individuating the porosity determined exclusively by the soil fauna. This has been possible through the construction of repacked soil mesocosms in the lab, from which 2D and 3D images has been determined and analysed. Finally in the last experimental phase has been investigated the relationship between soil fauna and soil fluxes. Particular attention has been given to the study of the CO_2 and N_2O fluxes and how the soil fauna can influence these. This was evaluated means by the accumulation of these gas into repacked soil mesocosm in which were inoculated different types

of soil fauna (individually or mixing each other) and analyzing them means by an Infrared gas analyzer (multigas monitor).

Chapter 2 Evaluation of soil fauna contribution to soil quality at landscape scale (Telesina Valley case study)

2-1 Introduction

The soil quality is determined by the ability of a specific soil to preserve the air and water quality, to sustain the animals and plants productivity, and to support the human life (Doran and Parkin 1996; Karlen et al. 2003). The soil quality can be estimated through the analysis of some chemical-physical properties or using biological indicators (Doranand Zeiss, 2000; Karlen et al., 2001).

The most important factors affecting the soil quality, such as the pH, organic matter, porosity, mineral composition, weaving, structure, microbial population and its biomass even the extracellular enzymes pool, are subjected to numerous interactions through biochemical processes which are very difficult to evaluate (Dylis, 1964; Angermeier and Karr, 1994; Dale and Beyeler, 2001). Standard approaches to soil quality evaluation are based on the use of physical, chemical and biological indicators or index. The choice largely depends on the scale and purpose of the estimate (Parisi, 2005).

The most commonly used chemical indicator is the quantification of organic matter (Liebig and Doran, 1999; Bowman et al., 2000; Brejda et al., 2000; Kettler et al., 2000; Gilley et al., 2001; Li et al., 2001;). The stability of the aggregates and the bulk density are the most important physical indicators (Liebig and Doran, 1999; Kettler et al.,2000; Gilley et al., 2001; Li et al., 2001). About 250 are the environmental indicators required by the Organization for Economic Cooperation and Development (OECD, 1999, 2000); but the indexes related to biological soil aspects are very few (CEC, 2000; Buchs, 2003). Hereafter we focus on soil quality indicators strongly focused on soil fauna. This approach is based on the followings issues.

Soil fauna is very abundant, its role in soil formation and transformation is well recognized, and most of soil fauna has its life cycle which result exceedingly dependent on its environment. Then it is possible to use the soil fauna in a soil sample in terms of bioindicator and several species have already been recognized as useful biological indicators of soil quality.

From the biological point of view, it is necessary to utilize a reliable and easy index to be able to identify an environmental stress state. Due to considerable difficulties related to taxonomic determination, often the systematic identification behaves as a limiting factor into biological monitoring systems, in which the ecological aspect prevails with respect to the taxonomic side.

New methods of soil quality evaluation have been proposed based on soil fauna; some are based on the analysis of microarthropods, (Bardgett e Cook, 1998; Büchs et al., 2003; Parisi 2001; Parisi et al., 2005; Blocksom e Johnson, 2009) while others focus on a single taxon (Graham et al., 2009).

In order to take care of all issues risen above, in this chapter the soil quality was determined using the widely applied “Biological Soil Quality” (QBS) index (Parisi 2001). The QBS index is based on the following points: the higher soil quality, more higher will be the number of soil fauna groups better adapted to soil habitats. The QBS separate the soil fauna according to the "biological forms" approach (Sacchi and Testard, 1971), in order to evaluate the level of adaptation to the life in the soil environment (Parisi, 1974). The "biological forms" are composed of different species of soil fauna with a different morphological modifications which allow them to be adapt to their hosting environment, independently from their life cycle (Parisi 2001) and taxonomy. The adaptation produce different convergence phenomena at morphological level. For example, in the soil fauna who lives in the deep soil the common characters are: a small size, depigmentation, anophthalmia (reduction of visual organ), reduction of jumping organs (appendages).

Soil fauna is particularly sensitive to soil degradation and to the disturbances caused, for example, by different land uses, by the type of agricultural cultivation or by the trampling. The important

step, therefore, it is to consider a set of characteristics, easily readable, which allows to evaluate the level of adaptation to the underground life. In some groups, the morphological adaptations change into the different species, depending on the layer in which they live: euedaphic forms (their life cycle develops all into the soil), edafoxene forms (they developed only a bit part of their life cycle into the soil), epigean forms (they live above the surface), hypogean form (they live into the soil) and species related to the litter (grass), (Angelini, 2002). In other taxa, instead, all species possess a complete adaptation to the underground life, and they can be considered a single biological form. The construction of "adaptation zone" independently of their taxonomy is definitely a big advantage since it allows to overcome the problems related to the determination of the species. This also permit independently of the biological age of their life cycle, for some kind of larvae, to assign different values rather the same of the adults. For each group, is attributed a value which can change from 1 for the species low or nothing adapting to the edaphic life to a maximum of 20 for the species that have the maximum edaphic life adaptation. This value is called EMI (Eco-Morphological Index). This value then makes it possible to characterize the various systematic groups, in terms of their confinement in the soil. The sum of the EMI values of the various group is a measure of the degree of the community's overall convergence to edaphic life. The introduction of a simplified EMI index that does not require the classification of organisms by species level allows a broader application of these methodologies (V. Parisi, C. Menta, 2008). For some taxa this variable can change within the different systematic units (Table1).

Tab 1. ([a] Some taxonomic groups get only a single EMI value, while others include a range. The former groups reach values that are considered the maximum representative scores given to the edaphic adaptation levels for those taxa. In the latter case, it was not considered correct to attribute a single value of EMI, due to the variety of characters present within the group.)

Eco-morphologic indices (EMIs) of edaphic microarthropod groups[a]

Group	EMI score
Protura	20
Diplura	20
Collembola	1–20
Microcoryphia	10
Zygentomata	10
Dermaptera	1
Orthoptera	1–20
Embioptera	10
Blattaria	5
Psocoptera	1
Hemiptera	1–10
Thysanoptera	1
Coleoptera	1–20
Hymenoptera	1–5
Diptera (larvae)	10
Other holometabolous insects (larvae)	10
Other holometabolous insects (adults)	1
Acari	20
Araneae	1–5
Opiliones	10
Palpigradi	20
Pseudoscorpiones	20
Isopoda	10
Chilopoda	10–20
Diplopoda	10–20
Pauropoda	20
Symphyla	20

For the assignment of quality classes some specific groups, considered excellent biomarkers, play key role, since their presence it is generally linked to a soil with a lot of organic matter and potentially with good quality. Some of this are Protura, Coleoptera and a specific species of Collembola (Onichiuridi). The assessment of soil quality is determined by all of these variables, together with other environmental variables reported below.

2-2 Materials and methods

The sampling sites: pedo-climatic conditions and land uses

The samplings necessary for the realization of the present work were conducted in the "Telesina Valley" site in southern Italy (Fig.1). The area is of about 20 000 ha; it is close to the city of Benevento and encompasses 13 municipalities.

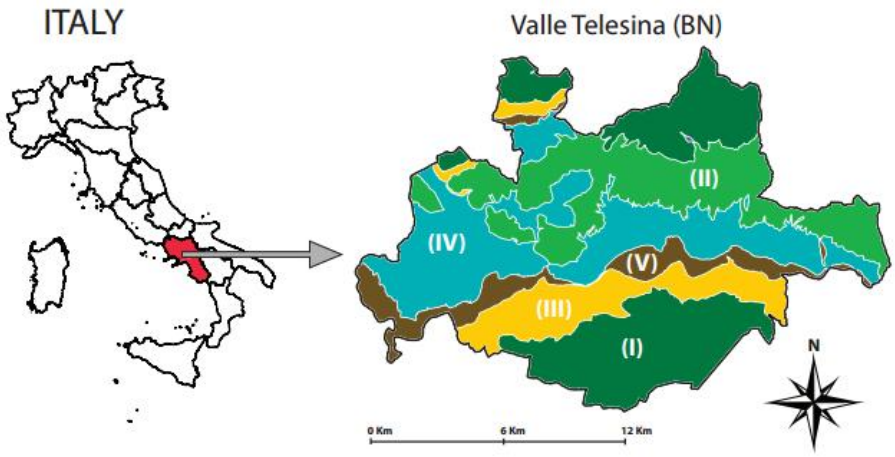


Fig.1: Map of Telesina Valley and its main landscape system: (I) Mountains, (II) Pediment plain, (IV) Ancient fluvial terraces, (V) Alluvian plain.

It is a very complex landscape with a high soil and climate spatial variability. Telesina Valley has a composite geomorphology and an east–west elongated graben where the Calore River lies.

Five different landscape systems are present (Fig. 2): (i) limestone mountains, with volcanic ash deposits at the surface; (ii) hills, comprised of marl arenaceous flysch; (iii) pediment plain, comprised of colluvium material from the slope fan of the limestone reliefs; (iv) ancient alluvial terraces; and (v) the actual alluvial plain. Such complexity is showed in the 60 soil typological units, aggregated into 47 soil mapping units.

The study area is traditionally suited to the production of high-quality wine (Bonfante et al., 2011) and olive oil in the hilly areas, while beech and chestnut forests are present in the mountain system, where there is a natural park. It is also important to emphasize the fact that, over the last decade,

Telesina Valley has experienced a large amount of soil consumption as a result of land use change due to new urbanization (Fig 3). These changes in land use have caused conflicting interests between agriculture, forestry, and urbanization and ideas of how the land should be used.

The study area includes 12 sites located within the Telesina Valley (Fig 2).

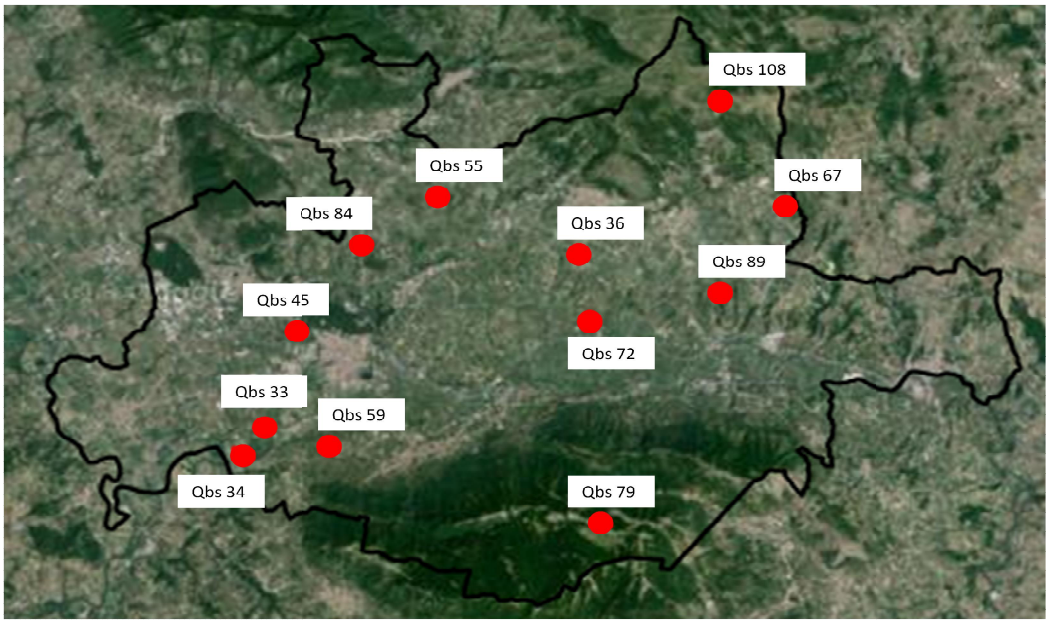


Fig 2. : 12 sites located in the Telesina Valley, where the QBS-ar index was performed.

Samples were collected in those 12 sites which correspond different land use (Fig 3). It's possible to summarize these in the table 1.

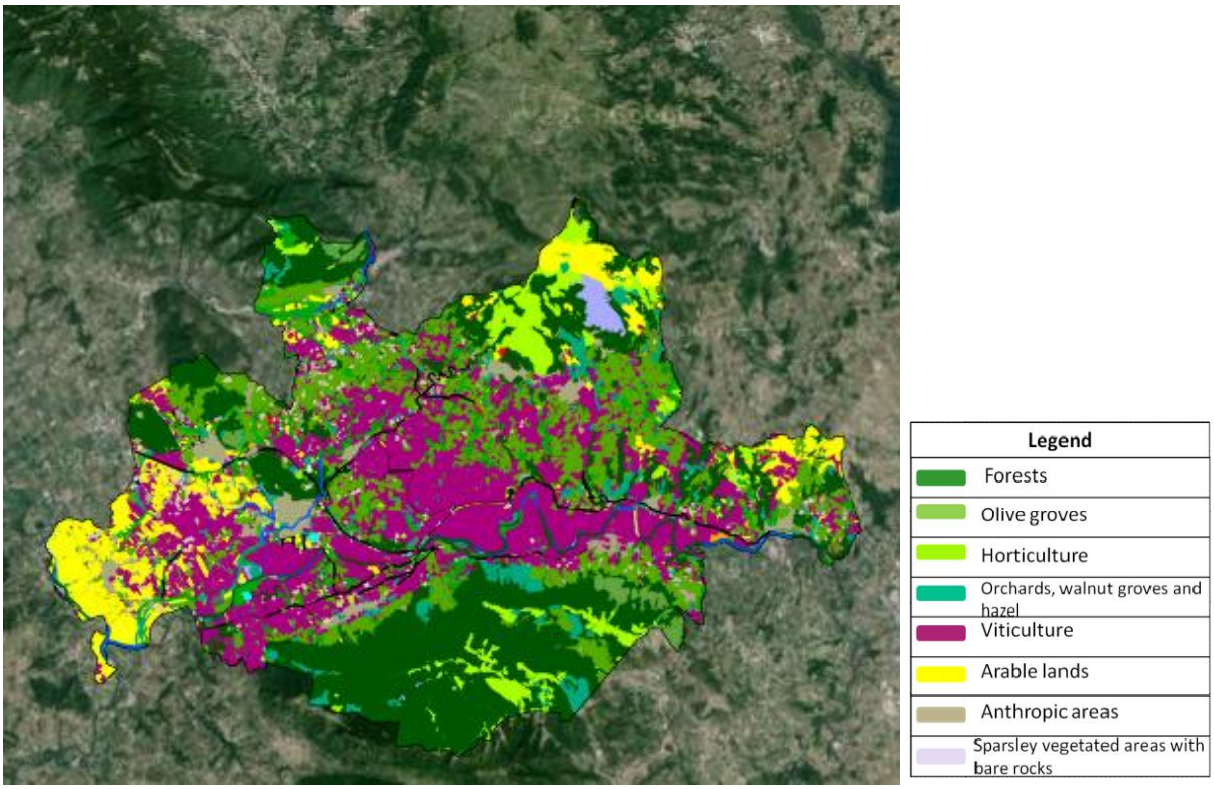


Fig 3. : Map about different land use (2011) in Telesina Valley.

Tab1: Sampling sites with geomorphological, climatic and land use information

Municipality	Elevation (av.)	Slope (av.)	Annual Temperature	Annual Rainfall	Soil Use	**Qbs-ar Value**
Telese terme (BN)	41 (ms.L.m)	0%	15.06 °C	1480 mm	Viticulture	**106**
San Lorenzello(BN)	191 (ms.L.m)	23%	14.08 °C	1000 mm	Olive groves	**185**
Vitulano (BN)	1160 (ms.L.m)	30%	15.05°C	1570 mm	Forests	**109**
Solopaca (BN)	78 (ms.L.m)	5%	16.01 °C	1480 mm	Viticulture	**166**
Telese terme (BN)	70 (ms.L.m)	1%	15.07 °C	1430 mm	Viticulture	**125**
Guardia sanframondi (BN)	224 (ms.L.m)	10%	15.01 °C	1440 mm	Viticulture	**145**
San Salvatore telesino (BN)	54 (ms.L.m)	1%	15.07 °C	1250 mm	Arable	**115**
San Lupo (BN)	352 (ms.L.m)	12%	15 °C	1420 mm	Olive groves	**126**
Guardia sanframondi (BN)	85 (ms.L.m)	5%	14.08 °C	1650 mm	Viticulture	**93**
Castelvenere (BN)	165 (ms.L.m)	9%	14.08 °C	950 mm	Forests	**173**
S. Lorenzo maggiore (BN)	190 (ms.L.m)	11%	15.01 °C	1500 mm	Olive groves	**119**
S. Lupo (BN)	777 (ms.L.m)	251%	14.08 °C	1340 mm	Arable	**129**

Use of Qbs-ar index to identify soil quality

Particular attention has been paid in collecting samples as the heterogeneity of soil matrix makes the sampling phase really sensitive to obtain representative data. Whatever soil sampling will always have an uncertain part because the environmental conditions are very variables even within

proportionate to the soil sample water content. It was possible to analyze the sample, to identify the different species, in other days closing the container, by adding preservative liquid and parafilm around the container. The soil fauna was separated from the soil by means of the use of a supersaturated solution of NaCl and leaving deposit sediment for ten minutes. The organisms went up on the surface, in one or more steps. Thereafter they were filtered leaving the soil on the bottom of the flask. Always within of the 50 MM mesh, they were accurately washed from the salt excess with water, and then poured into a watch-glass with an alcohol solution (75%). All actions were carried out with care, to avoid leaving the organisms attached on the container walls, in the funnel, in the filter or the flask.

Soil fauna identification and classification

The samples extracted have been observed by means a dissecting stereomicroscope, at least 40x magnification, with optics light bulb. The systematic species identification presents considerable difficulties however to get the Qbs-ar calculation it is not necessary to go to a detailed classification level. Using the simplified “dichotomous keys” the species present have been split based on the different "Taxa" (arriving sometime at the species level of detail) of the various edaphic groups and they have been assigned a value of the EMI-index (Eco morphological index) as shown previously (Tab 1). Therefore it is possible to proceed with Qbs-ar index calculation.

Summarizing, the Qbs-ar values were obtained through these phases: collection of soil sample, microarthropod extraction, preservation of the samples collected , the determination of different biological form, and the total calculation of Qbs-ar index. The Qbs-ar, for each site, is the sum of

the maximal value within of the three repetition, in which have been found the most "biological form" (with EMI values maximum). Moreover for the calculation of the soil quality class, one must consider the presence of specific specie as a Protura species and Coleoptera species. They are a crucial species for determining the quality classes.

Statistical analysis on taxa abundance

The total number of individuals of each systematic group has been average across the three replicates of each sample site and the standard error has been calculated. Then, after performing the Sahpiro-Wilk's test to check the normality of the distribution of the taxa abundance data, the Levene's test for homoscedasticity has been carried out in order to check the possibility to perform ANOVA with reference to the land uses. When homoscedasticity was not verified, the Welch's test was performed instead of ANOVA, in order to check dependency of taxa abundance on land uses.

2-3 Results and discussion

The first results have been directed to verify in which way and which amount the "different land use" were able to affect the soil quality. This has been determine by means of Qbs-index (Qbs-ar), which uses the edaphic adaptation of the species, thus being also easier to use compared to other indices present in the literature. The Qbs index was determined for the different taxa found in the sample which are able to live under the soil, as well as by means of the presence or the absence of the possible edaphic groups found (the edaphic groups are the ones with maximum EMI value). Means by Qbs-index it is possible to establish the soil quality class for each site. These range from 0 (bad quality) to 7 (excellent quality), (Parisi 2001). Results of Qbs index and soil quality classes for each sample site are reported in the table 2 together with the different land use of each site. Figures 4,5,6 and 7 show the abundance of each taxa found in each site grouped by the land uses Forest, Olive groves, Arable and Viticulture, respectively. Finally the abundance of the Acari species is reported in and Table 2.

Comparing the two sites of "Forests" (Fig. 4) can be noted that the site Qbs-84 (oak forest) has an higher Qbs-ar value, amounting to 173, than that of the other site. The presence of five eudaphic groups including Acari, Pseudoscorpionida, Symphila, Diplura and Microcoryphia is resulted, they are crucial species for the determination of soil quality classes and, together with the Coleoptera species, are other key taxa for the soil quality class identification. The soil quality class is equal to 6, a very high value considering that the maximum soil quality class it is 7. For the site Qbs-79 (we find a Qbs value of 109, despite the presence of three eudaphic groups and the presence of Protura and Coleoptera species, the total "soil quality class" is equal to 5.

Tab 2: For each site show the different land use, the final value of calculation of Qbs-ar index and the soil quality class that have been assigned (Parisi 2001).

***The total Qbs-ar value was calculated within three repetition.**

Site	Different land use	Qbs-ar * Value of Qbs total	Quality classes From 0 to 7
Qbs-ar 33	Viticulture	**125**	**5**
Qbs-ar 34	Viticulture	**106**	**5**
Qbs-ar 36	Viticulture	**145**	**6**
Qbs-ar 59	Viticulture	**166**	**5**
Qbs-ar 72	Viticulture	**93**	**3**
Qbs-ar 55	Olive groves	**185**	**6**
Qbs-ar 67	Olive groves	**126**	**6**
Qbs-ar 89	Olive groves	**119**	**6**
Qbs-ar 79	Forests	**109**	**5**
Qbs-ar 84	Forests	**173**	**6**
Qbs-ar 108	Arable	**129**	**6**
Qbs-ar 45	Arable	**115**	**6**

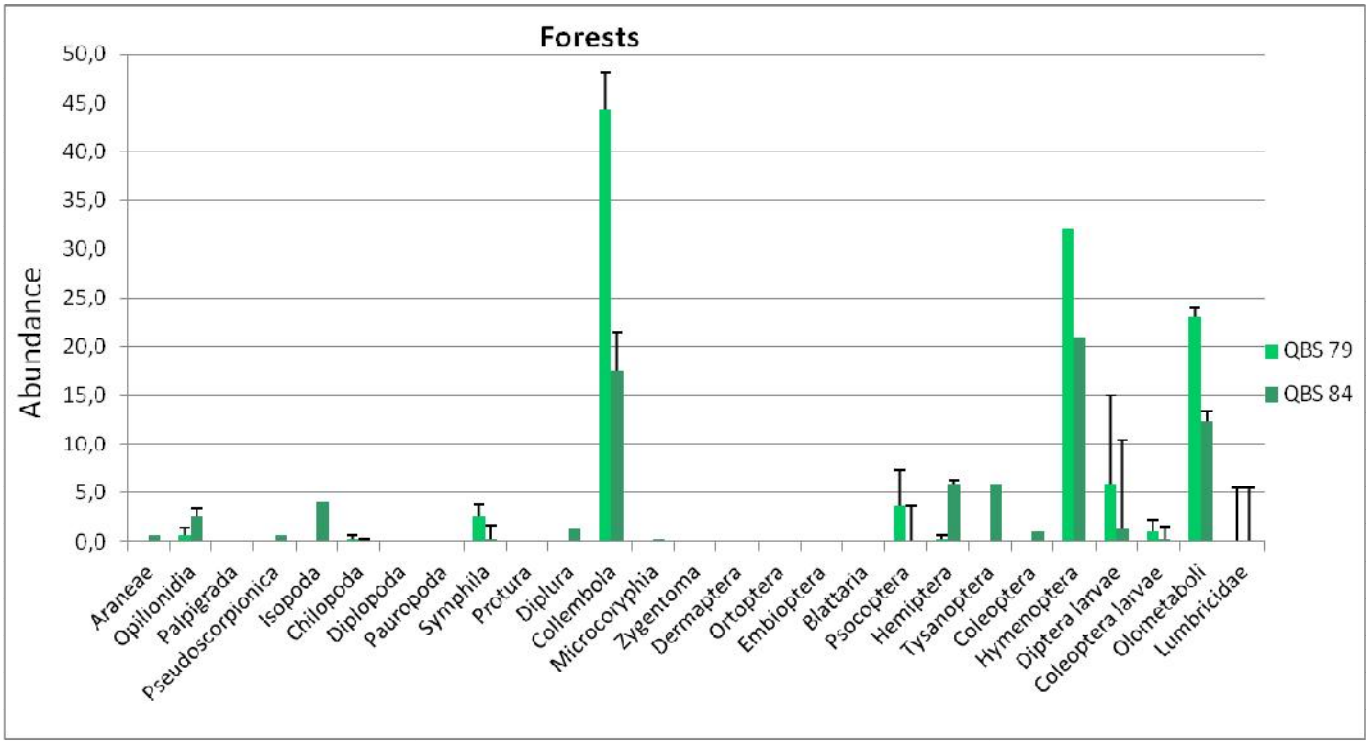


Fig 4: Mean value and standard error of the abundance of the different species found for each site.

For all sites belonging to the "olive groves" we notice the same soil quality class equal to 6. However in detail we find very different situations. For the site Qbs-55, a total value of 185 (amount of all taxa found into the sample) with the presence of seven eudaphic groups is found. For the site Qbs-67 the total value is equal to 126 with three eudaphic groups and Coleoptera species. The value of Qbs index for the last sample (Qbs-89) is 119, with only three eudaphic groups but with the presence of Coleoptera and Protura species that are able to improve the soil quality class.

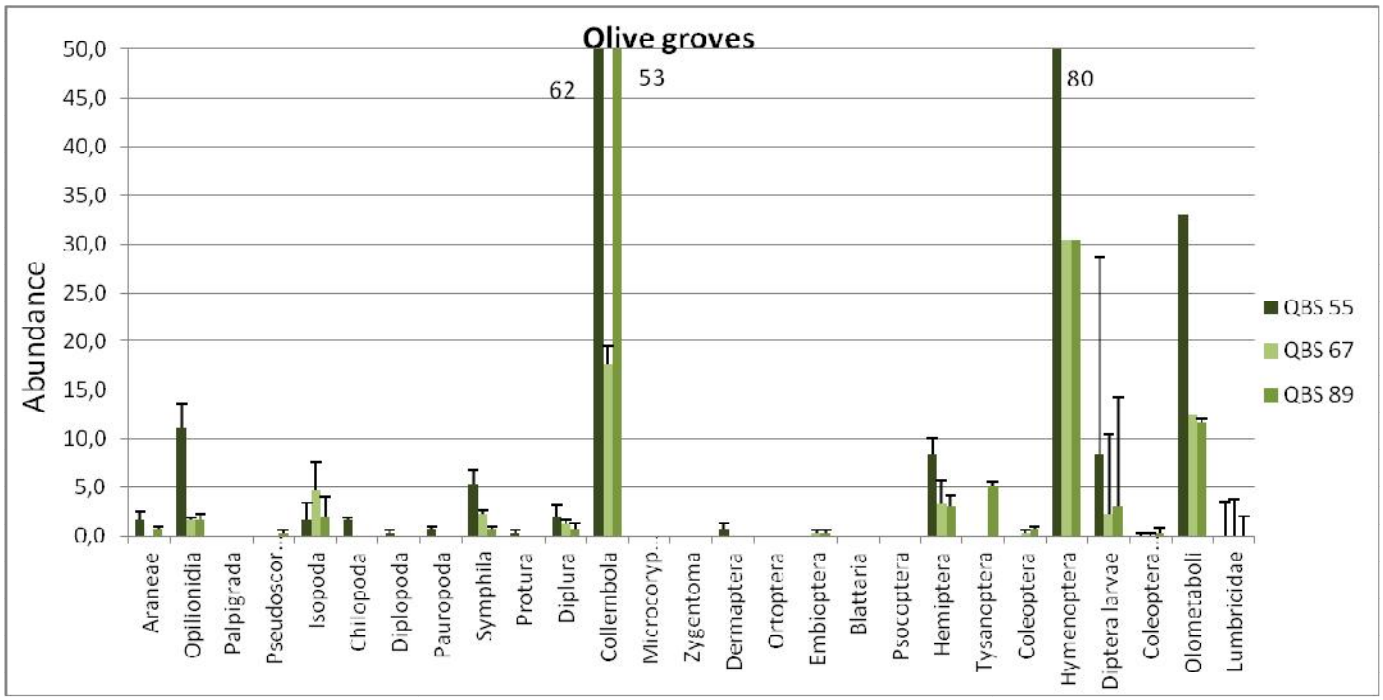


Fig 5: Mean value and standard error of the abundance of the different species found for each site.

For the arable sites the soil quality class is 6 for both, the same for the three eudaphic groups found but with different species. In the Qbs-45 (cropping system) the total value is 115 and Acari, Protura, Collembola and Coleoptera species were found. For the site Qbs-108 (not-irrigated arable) the total Qbs is 129, the species are Acari, Pauropoda, Symphila and Coleoptera.

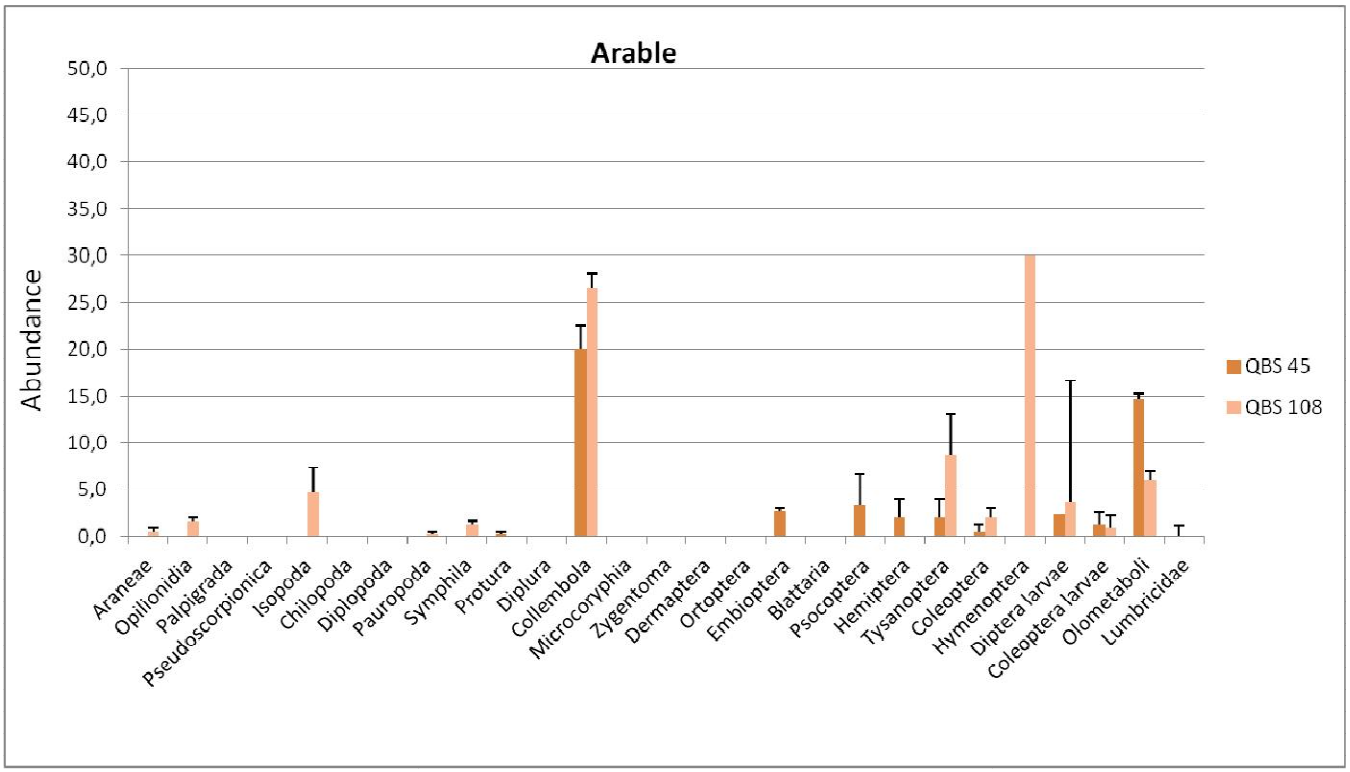


Fig 6: Mean value and standard error of the abundance of the different species found for each site.

The sites in which the land use is the viticulture show different soil quality classes. The site Qbs-33 shows a Qbs-index of 125, with four eudaphic groups composed by Acari, Pseudoscorpionida, Symphila and Diplura species, reaching the fifth soil quality class.

The Qbs-34 site, shows the same class (the fifth) of the previous site, with a total value of Qbs of 106, but contains three eudaphic groups with Acari, Chilopoda and Collembola species as well as Coleoptera species. The Qbs-36 site has the highest quality class equal to 6. Here the Qbs-index is 145, with four eudaphic groups and Acari, Pseudoscorpionida, Symphila, Diplura and Coleoptera species. Although the Qbs-59 site shows a higher total value of Qbs-index of 166, this presents only three eudaphic groups consequently a lower soil quality class, equal to 5. The other site (Qbs-72) for the "viticulture" land use is of class 3, the lowest class in these five sites. Here the Qbs value is 93, with the two eudaphic groups of Acari and Diplura species and the Coleoptera specie.

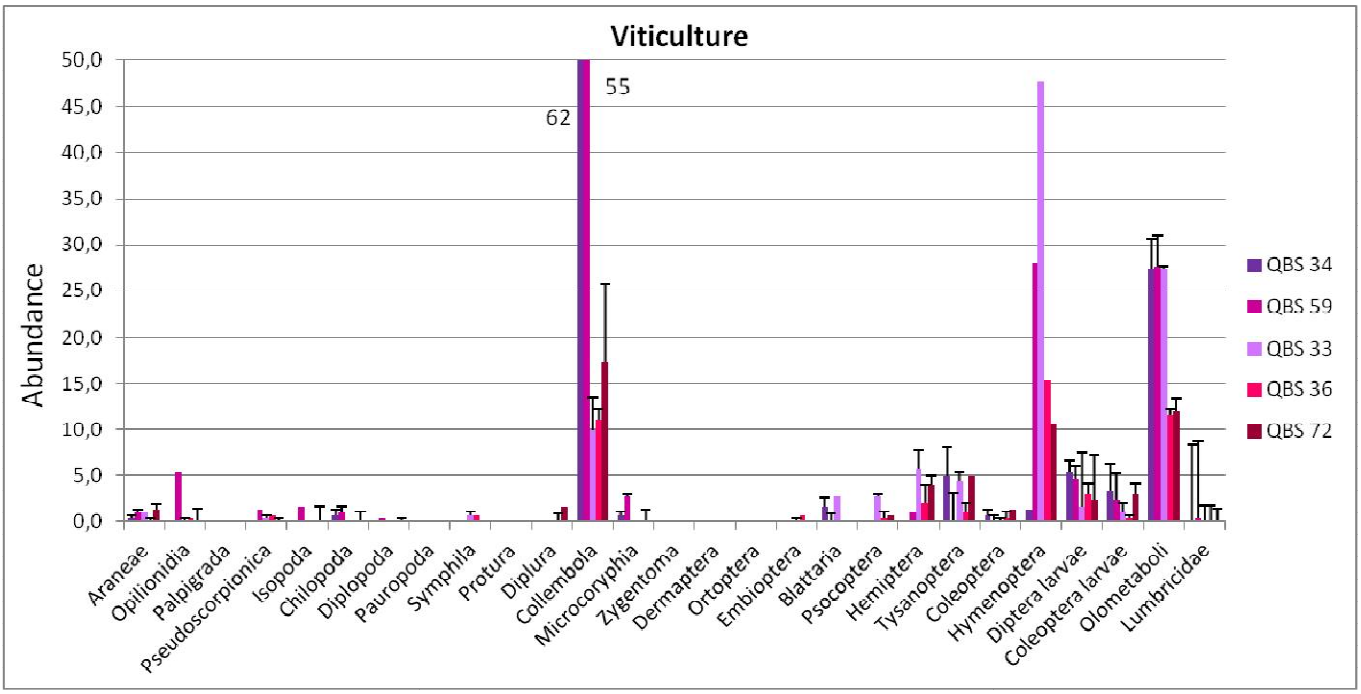


Fig 7: Mean value and standard error of the abundance of the different species found for each site.

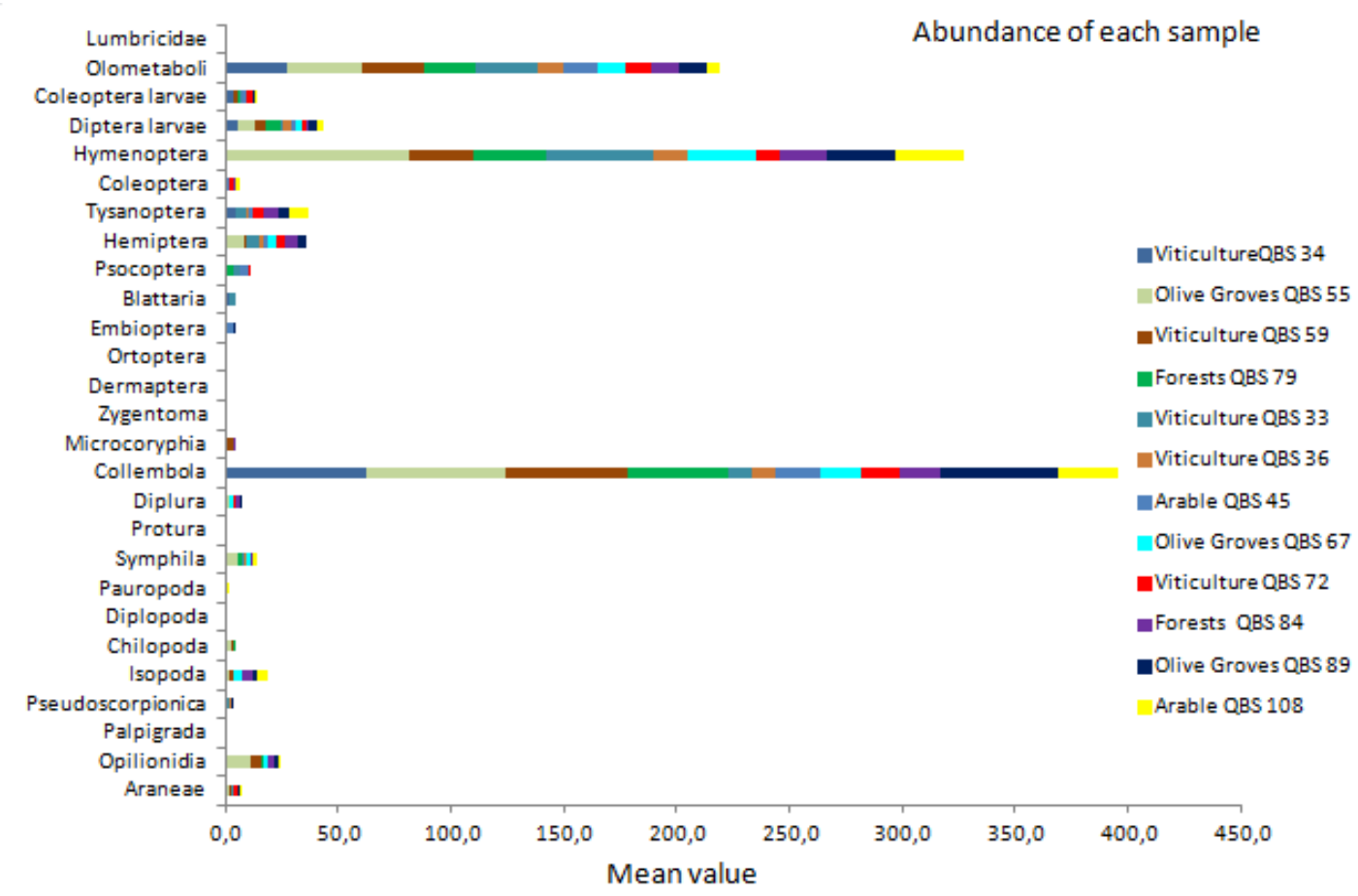


Fig 8 : Mean value of the abundance of the different species found for all sites evaluated.

Tab 3: The mean values for mites species, for all study sites.

	Mean value for the Acari species											
	QBS 34	QBS 55	QBS 59	QBS 79	QBS 33	QBS 36	QBS 45	QBS 67	QBS 72	QBS 84	QBS 89	QBS 108
Acari species	480,3	696,7	645,7	371,3	230,0	171,7	443,0	225,0	135,7	118,0	121,0	155,7

* The mean values for mites species are reported separately from the histogram as they present a very high abundance. They graphically flatten the other species , consequently are graphically difficult to showing.

In table 4 are reported the p-values of the ANOVA or, alternatively, Welch's test in order to check dependency of taxa abundance on land uses (supplementary statistical material is reported in "additional material" at the end of the thesis). P-values are in all cases greater than the significance level of the tests, thus abundance of all taxa has not resulted dependent on the land uses.

Tab 4: p-values from ANOVA(*)/Welch's test to verify dependency of taxa abundance on land uses.

Significance level α = 0,05. p-value > α: no significant difference among the mean number of taxa individuals for the different land uses p-value < α: the mean number of taxa individuals is significantly different for, at least, one land use.

Taxa	P-value *
Araneae	0,8 *
Opilionida	0,46 *
Pseudoscorpionida	0,56 *
Isopoda	0,32
Chilopoda	0,76
Diplopoda	0,78 *
Pauropoda	0,48
Symphila	0,17
Protura	0,39
Diplura	0,23*
Collembola	0,77
Microcoryphia	0,65 *
Dermaptera	0,44
Embioptera	0,25
Blattaria	0,46
Psocottera	0,51
Hemiptera	0,47 *
Tysanoptera	0,67 *
Coleoptera	0,34*
Hymenoptera	0,34*
Diptera (larvae)	0,88 *
Coleoptera (larvae)	0,11
Olometaboli	0,59 *
Acari	0,96 *

In particular, for the taxa to which positivity of Levene's test (homoscedasticity) has made applicable the ANOVA (highlight with (*) symbol in table 4), has been possible to state also that taxa abundance has greater variability between the sampling sites for a given land use than between the land uses. Such results are synoptically shown in figure 5.

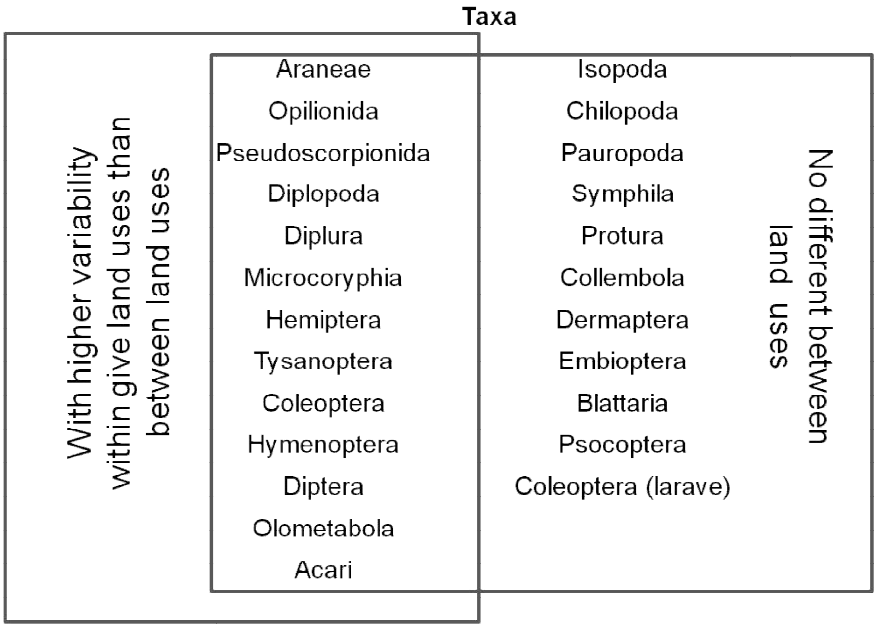


Fig 9 : Synoptic view of the results of ANOVA/Welch's test of taxa abundance with respect to land uses.

Overall considered

In all the studied sites we found lots of edaphic species, which indicate a good soil condition as they are excellently adapted to the soil life. We also found an high variability of the species. The same situation resulted from the statistical analysis on taxa abundance, by which not have been found substantial differences between the different soil land uses. Probably it was determined by an elevated basic soil quality level, naturally present in the sampled sites. Only in the site Qbs-72 (Viticulture), has been observed a low soil quality class (equal to 3). This could be, probably, determined from the impact of chemical treatments or from the intensive tillage which this has been subjected. For all the other sites, the soil quality class goes from a value of 5 to 6. This has probably due to particularly favourable condition (e.g humidity, vegetation cover (litter) or temperature) which favour a high presence of adapted species.

2-4 Conclusion

The Qbs-index showed the real ecological condition of the soil environment considered. The potentiality of this index, easy to use than other biological indices but extremely reliable, can be utilized to obtain a complete soil mapping of the study sites on the biological soil quality and as an indicator of a possible soil stress state.

The obtained results have shown that the Qbs index, independently on the different land uses, has shown a good soil quality in almost all studied sites of Telesina Valley. Statistical analysis hasn't provided significant differences in taxa abundance between the different land uses. This may states that the method has show to be not very sensitive between the studied sites not strongly contrasting sites. Although this biological indicator is widely known and used, it gives information only on a single aspect of the soil quality, namely the soil biodiversity. The Qbs doesn't evaluate others important aspect, as the contribute of soil macrofauna, the functional role of soil fauna in determining soil quality, or its contribution to soil structure formation.

Chapter 3. Soil fauna and soil structure: a study at farm scale for four different land uses in Telesina Valley

3-1 Introduction

In this chapter, the contribution of soil fauna to soil system was furtherly characterized by combining the quantification of meso- and macrofauna species with the analysis of the soil structure. Soil structure[1] is a critical physical property that affects soil ability in maintaining agricultural productivity (Hillel, 1980) as well as local and global environmental qualities (Bronick et al., 2005). Soil functions are very much affected by a key feature of the soil structure, namely the size distribution of the pore system (Bouma, 1990; Dexter et al., 2009). Pore size distribution (PoSD) strongly affects water flow processes (Coppola et al., 2009) and therefore the content and distribution of both gases and water in soils (Dexter et al., 2009; Horn et al., 1994) which, in turn, determine the species and distribution of chemical compounds (e.g., Kuka et al., 2007) as well as soil organisms.

The changes of soil structure are the result of the actions and interactions of numerous physical, chemical and biological factors with intricate feedback mechanisms (Six et al., 2004) making difficult to understand the specific effects of each single factor. In particular, as described in the Chapter 1, some soil macrofauna species, called the “Ecosystem Engineers”, such as earthworms, ants, etc., contribute to the development of soil structure making soil more permeable for water and air (CE 2012 Biodiversity Impact). They are able to modify or create new habitats for smaller soil organisms and to regulate the resource availability for other soil species, then affecting also their abundance.

[1] Soil structure may be defined either as "the shape, size and spatial arrangement of different singles soil particles and clusters of particles (aggregates)" or as "the combination of different types of pores with solid particles (aggregates)" (Pagliai 2002).

Moreover, among the causes of soil structure modification the land management is of great importance (Pituello et al., 2016; Munkholm et al., 2016; Zhang et al., 2015; Bronick et al., 2005; Pagliai et al., 2004) affect both the soil pore system and also the soil fauna abundance and species richness (Doran et al., 2000; Bedano et al., 2006; Baker, 1998).

Most of the life within the soil is restricted to the three dimensional pore space that forms its habitat. Therefore to move about through the pore network, the microorganisms must be able to squeeze themselves through the gaps which are present there. The highly dynamism of the soil system, this involve that the pore network is constantly changing owing to shrinking and swelling upon wetting and drying, as well as freezing and thawing of the soil. This means that pores once unconnected may become connected after a shrink-swell phenomena and viceversa. Another effect on the soil structure is that organisms can help in stabilising aggregates within the pore system.. This can be done through the excretion of compounds which function to stick aggregates together, or by physically binding soil aggregates together or linking between them. These effect can have beneficial impacts for example to reduce soil erosion. Larger organisms, such as earthworms, are capable of moving around soil particles, creating their own pore spaces through a process called bioturbation. These pores are called “biopores”, these are generally relatively large compared to other soil pores and so create zones of preferential flow for the water, and they reduce water run-off after rainfall. Many biopores also are created by other organisms and by plant roots which have sufficient penetrating power to force aggregates (Krogh, 2010).

In this framework, the aim of the work presented in this chapter was to investigate the relationship among the soil fauna abundance and the soil pore system in four sites of the Telesina Valley characterized by different land uses and pedo-climatic conditions. The soil structure was investigated by means of medical X-ray tomography and 3D pore image analysis.

3-2 Materials and methods

The four sampling sites: pedo-climatic conditions and land uses

The four chosen sampling sites are located in Telesina Valley and are characterized by different land uses (Fig 1.) and different pedo-climatic conditions (see paragraph 2-2, Fig.1). Three of these have already been studied in the experimental phase previously described in the Chapter 2. The fourth site (VT 77) was located in Solopaca municipality (BN), in a mountain area.

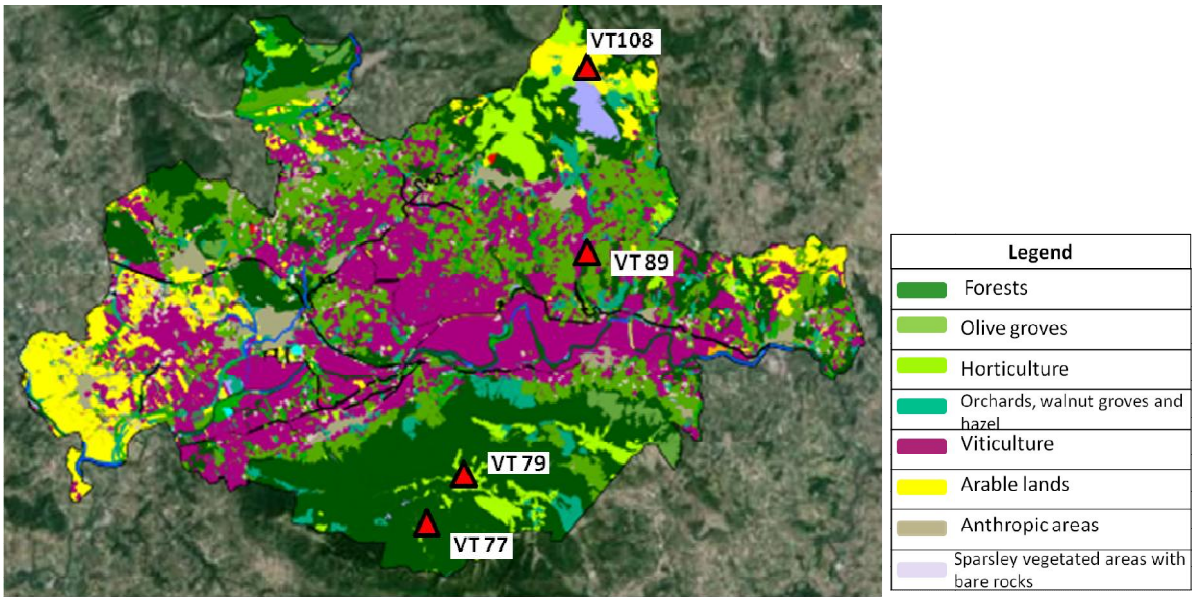


Fig 1: Map of Telesina Valley. Localization of the sample points and different "land use" : (VT 108) Not-irrigated arable / forage alternated, (VT89) Olive groves, (VT 79) Beech forest, (VT 77) Pasture.

All details about the studied sites have been summarized in Table 1.

Table 1: Summarized information about the four sampling sites.

Site	VT-79	VT-89	VT-108	VT-77
Municipality	Vitulano (BN)	S. Lorenzo maggiore (BN)	S. Lupo (BN)	Solopaca
Coordinates of the center of the area	41°10'28''N 14°36'19''E	41°14'0''N 14°38'11''E	41°16'48''E 14°38'7''E	41°10'45''N 14°10'45''E
Elevation (average)	1160 (ms.L.m)	190 (ms.L.m)	777 (ms.L.m)	1200 (ms.L.m)
Slope (average)	30%	11%	25%	11%
Aspect (average)	E (84)	O (2858)	S-O (212)	S-W (227)
Annual Rainfall	15.05°C	15.01 °C	14.08 °C	15.8 °c
Annual Temperature	1570 mm	1500 mm	1340 mm	1640 mm
Soil Use and Cover 2011	Beech Forest	Olive groves	Not irrigated arable	Field

Soil fauna characterization

After removing of litter cover, the undisturbed soil sampling for soil macrofauna characterization was performed collecting a soil sample of 10cm x 10cm with depth of 10 cm. For the soil mesofauna in each sampling sites a cylindrical soil sample of 5 cm diameter and 30 cm heigth was collected and then disassembled in three parts 10 cm high (volume of 196,25 cc), in order to separately identify and quantify the soil mesofauna for three different depths (0-10cm, 10-20cm, 20-30cm).

The undisturbed soil samples were transported in laboratory protected from thermal shock, within of 48 hours. We used a Berlese–Tullgren funnel for extraction of soil mesofauna with the same procedure described in detail in the chapter 2. An accurate systematic species identification for the macro- and mesofauna was performend by means of the use of simplified "dichotomous keys".

3D soil image analysis

From all the four sampling sites, undisturbed soil samples have been collected from 2 soil depths (0-12 cm and 12-24 cm) using PVC cylinders of 10 cm diameter and 12 cm height (volume of 942 cc). The soil cores were carefully transported in laboratory and they were impregnated with epoxy resin and diluent (Mele et al., 1999) in order to stabilize the soil structure. This resin did not change volume and tolerated up to 10% of residual water in the samples during the polymerization process. The samples were saturated from the bottom with this low-viscosity mixture by using a moderate vacuum, which provided optimal resin penetration into the pore networks. After resin polymerization, each cylindrical soil block was given four longitudinal cuts in order to obtain a regular parallelepiped inscribed in the cylinder. Although the X-ray tomography do not require the resin impregnation of the soil samples, we used this procedure to stabilize the soil structure before the transport in the medical centre where samples were scanned.

The soil blocks were imaged using the medical X-ray CT Discovery CT750 HD (General Electric) with source power set at 120 kV and current of 10 mA. The images were acquired at a resolution of 200 μm. The 3D image analysis was done in collaboration with the CNR ISAFoM of Ercolano. At the beginning of the CT-image processing the 16-bit images (DICOM format) were transformed in 8-bit images to save memory and be able to handle images with most classical software packages. This reduction in image depth was done using the software RADIANT by setting the minimum and maximum grey level values that will be kept and transformed into the final 8-bit image.

The image processing was performed in order to obtain 3D reconstructions of the internal structure of the soil blocks. Images were pre-processed and segmented through a technique of supervised 'thresholding', using CTAn software (Bruker) in order to obtain binary images, where the two separate solid and pore phases were in black and white, respectively.

Pore size distribution was determined using the own-developed software Conmorph, through the iterative application of the "opening" algorithm, which classifies the porous phase according to the spacing from the walls (Gargiulo et al, 2015).

3-3 Results and discussion

Soil fauna characterization

The results of the abundance of soil mesofauna are reported in figure 2. The higher richness species was found in the the sample VT 79 (Beech forest). Acari Oribatidae, Acari Mesostigmata and Diptera Culicidae were present in all the sampling sites. Coleoptera larvae and Psocoptera were present in all sampling sites except VT 89, Hymenoptera; Collembola and Acari Prostigmata were present in all sampling sites except VT 77, Acari Eremaidae were present in all sampling sites except VT 108. Araneae, Pseudoscorpione, Chilopoda, Coleoptera Staphilinidae, Isopoda, Diptera larvae and Protura were present only in VT 79. Tysanoptera and Hemiptera Ciminidae were present only in VT 77.

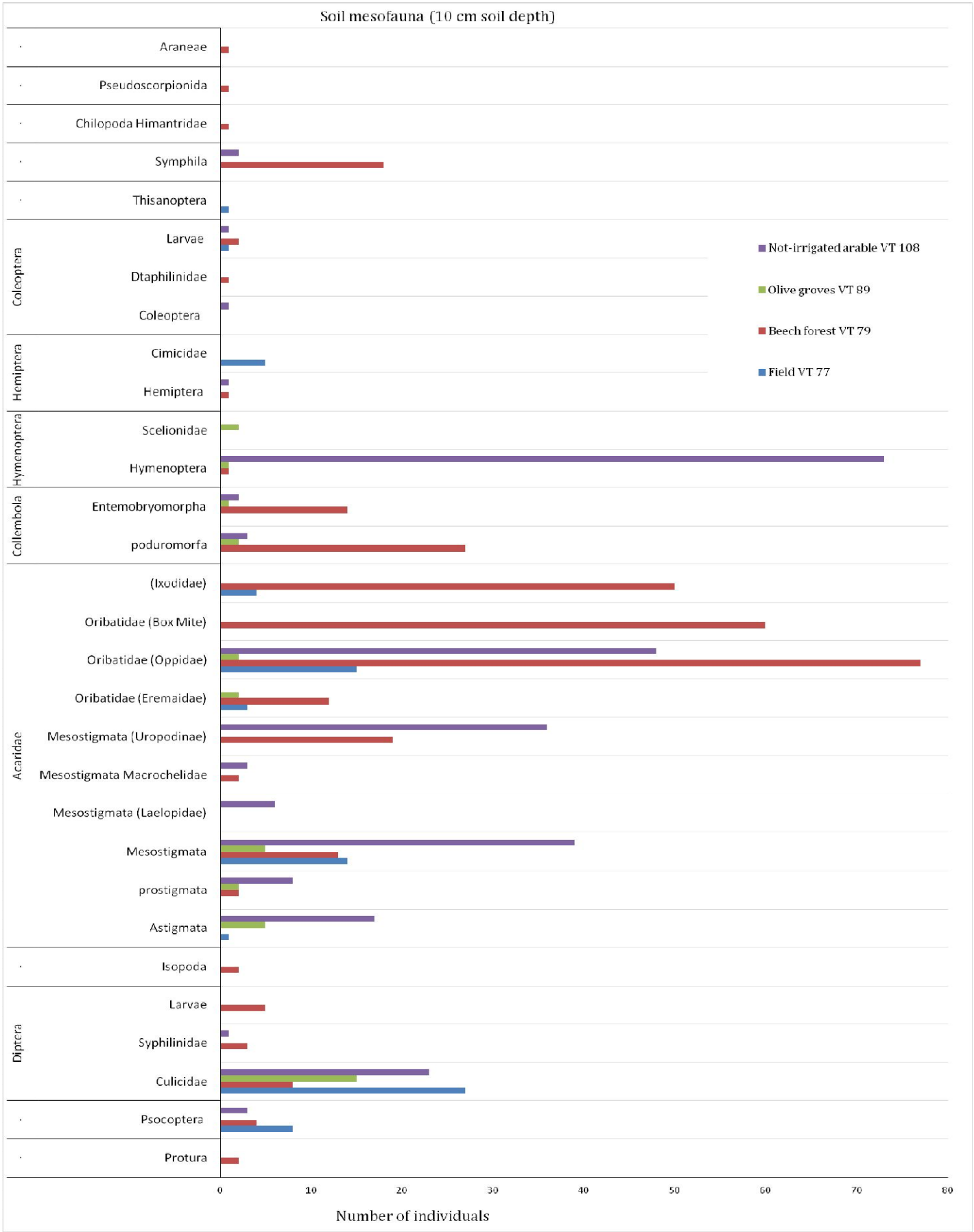


Fig 2: Abundance (number of individuals) of the mesofauna taxa for each sampling site in the first 10cm soil depth.

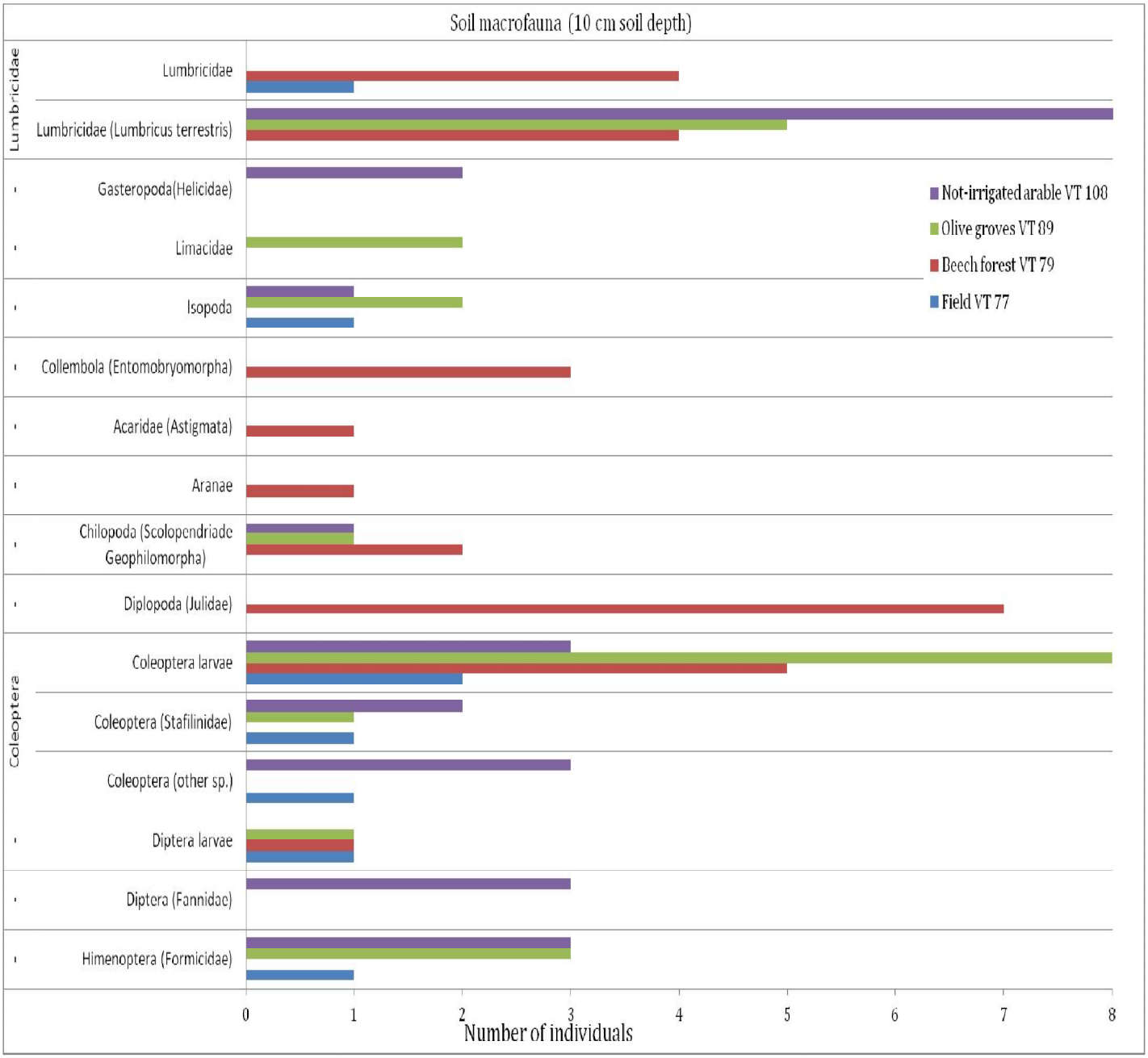


Fig 3: Abundance (number of individuals) of the macrofauna taxa for each sampling site in the first 10cm soil depth. For counting purpose larvae of a given taxa have been considered as a separate taxa.

In figure 3 are reported the results of abundance of the macrofauna taxa found in the four sampling sites. It can be noted that Coleoptera larvae and Lumbricidae resulted the taxa with overall highest abundance. Coleoptera larvae were also present in all the sampling sites, Lumbricidae and Chilopoda were present in all the sampling sites except VT 77, while Diptera larvae and Isopoda along with Himenoptera and Coleoptera were present in all sampling sites except in VT 108 and

VT79, respectively. Gasteropoda were present in VT77 and VT 108 and Arionidea were present in VT89 and VT108. Lumbricidae, Diplopoda, Mites, Aranae and Collembola were present only in VT79.

In table 2 and 3 are reported the variability and abundance of soil macrofauna and mesofauna collected in the studied sites. It can be seen that VT79 and VT108 show a much higher abundance of both macrofauna and mesofauna, and higher variability of the mesofauna. Considering that the investigated sites were sampled in the same sites described in chapter 2 and observed the same soil fauna abundance, here it is required a comparative evaluation. We must stress that the two experimental settings were very differented in terms of methods and repetitions. It is easy to understand that data between the two chapters differ. For instance, the volume of the collected samples were different (Was shown a volume of 196,25 cc compared with previous phase in which the volume was of 1000 cc).

Table 2. Variability (number of taxa) of soil fauna founded in the studied sites.

Variability	Macrofauna	Mesofauna
Field VT77	7	6
Beech forest VT79	9	15
Olive groves VT89	8	5
Not-irrigated arable VT108	9	9

Table 3. Abundance (number of individuals) of all the taxa of soil fauna founded in the studied sites.

Abundance	Macrofauna	Mesofauna
Field VT77	8	79
Beech forest VT79	28	326
Olive groves VT89	23	37
Not-irrigated arable VT108	37	267

3D soil pore image analysis

The pore size distributions of upper 0-12cm soil samples from each sampling site are shown in figure 4. The site VT 108 (not-irrigate arable) shows a pore size distribution ranging from 0.4 to 11.6mm pore size, with a well expressed multimodality. For the sample of this site the total macroporosity amounted to 28,1%, the highest value considering the four sampling sites. A multimodal pore size distribution ranging from 0.4 to 9.6 mm resulted also for the site VT 79 (Beech forest) with three peaks at 2.4, 5.2 and 9.2mm pore sizes, here the total macroporosity resulting of 23,3%. Site VT 89 (Olive groves) and site VT77 (Field) conversely show a much simpler pore size distribution with similar shape. Both pore size distributions show the dominant, highest peak at 2 mm pore size. It can be observed that the pore size distribution of the site VT 89 ranges from 0.4 and 8mm pore sizes, with a low peak at 7.6mm pore size. The site VT77 showed a pore size distribution ranging from 0.4 to 10.4mm, with some low peaks in the pore size range from 7.2 and 10.4mm (Fig 4).

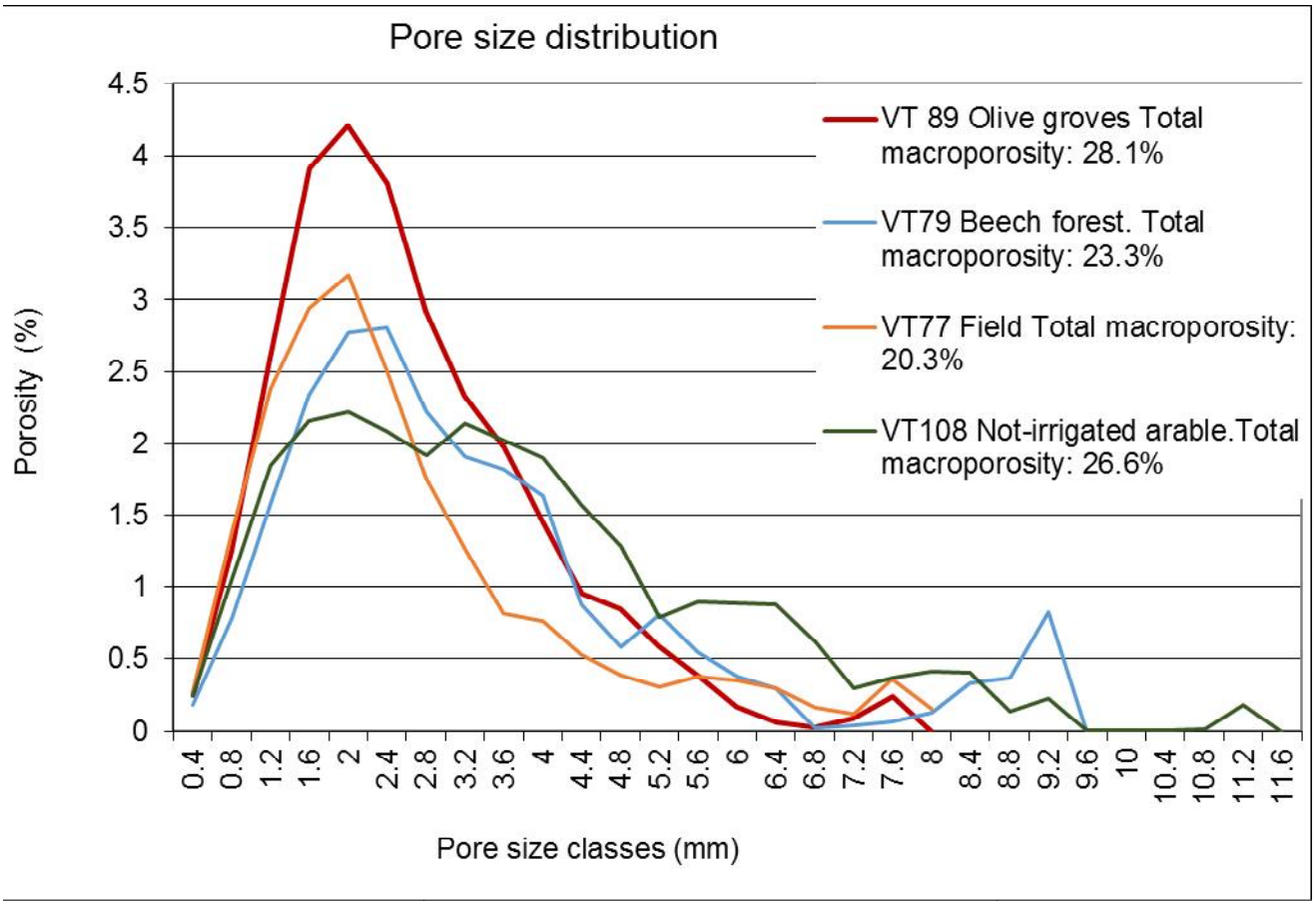


Fig 4 : Pore size distributions of the soil samples from the four studied sites (0-12 cm soil depth).

The well expressed multimodality of pore size distribution of the VT 108 sample clearly indicates coexistence of pores of different origin and size (Fig. 5). A rather similar multimodal shape has been found also for the pore size distribution of the VT 79 soil sample. The evident peak of this latter at 9.2 mm pore size corresponds to a single large cavity found in the soil sample (Fig. 6). Conversely, VT 89 and VT 77 show a simpler very similar almost unimodal shape of the pore size distributions, although VT 89 exhibits a quite higher total macroporosity (Fig. 4). This is due to the presence of one "kind" of pores although those of VT 77 are much narrower, as can be seen also from the figure 8. Results overall show that in natural environments, with less anthropic impact such as VT 79 and VT 108, we observed the presence of different types of macropores possibly of different biological origin. This was also found during the analysis of soil fauna. Actually, these two site are those with greater abundance of species. Conversely, in most anthropic environments such

as VT 77 and VT 89 the pore system resulted simpler with a low total porosity value in the case of VT 77. This is probably due to the soil compaction caused by anthropic action since the site in close to a pic-nic area having a large walkway. Similarly, for the site VT 89 it can be noticed an evident human pounding.

Interestingly, comparing the pore size distribution results with the summarized results of soil macro- and mesofauna (see tables 2 and 3) it can be noted that the complexity of the soil pore system is well correlated with abundance of both mesofauna and macrofauna and with the variability of the mesrofauna collected in the studied sites. Indeed, in agreement with our hypothesis, also in the x ray-CAT images it is conceivable that the high porosity of samples VT 108 and VT 79 is determined by the high amount of soil fauna observed. Therefore the soil fauna prove to be a fundamental factor to maintain a good soil areation, hence a good levelof soil quality. It's important to preserve this resource for a good managemento of soil.

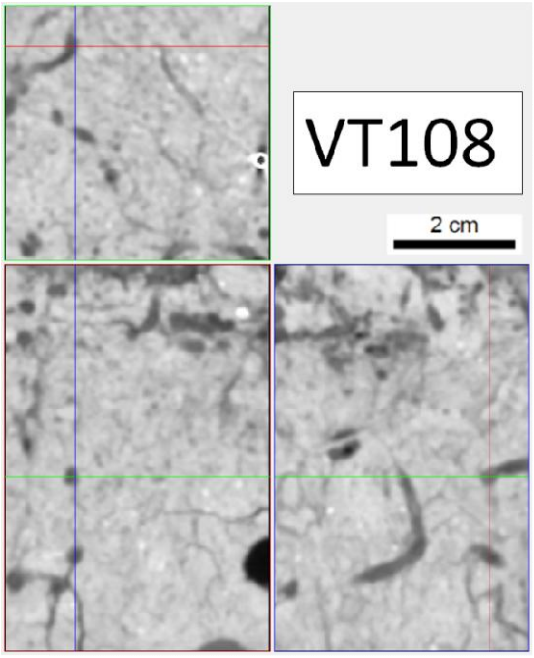


Fig 5: Sections of the VT 108 soil sample obtained from X-ray CT imaging

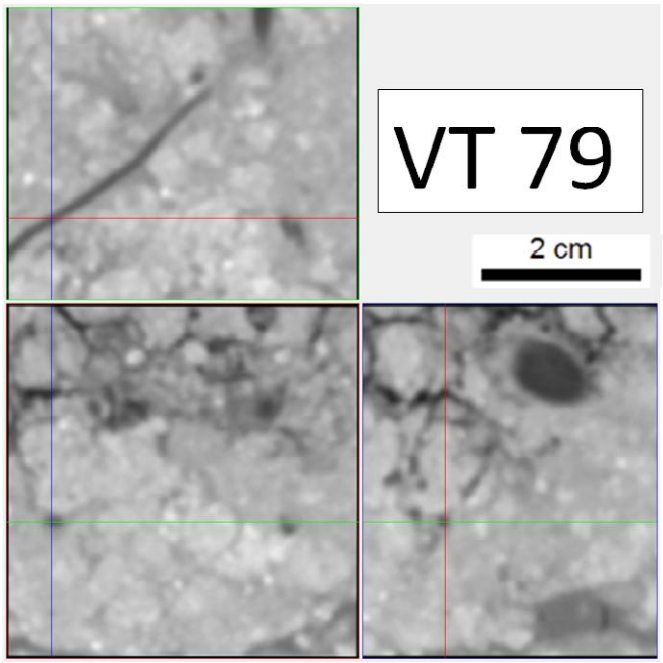


Fig. 6: Sections of the VT 79 soil sample obtained from X-ray CT imaging

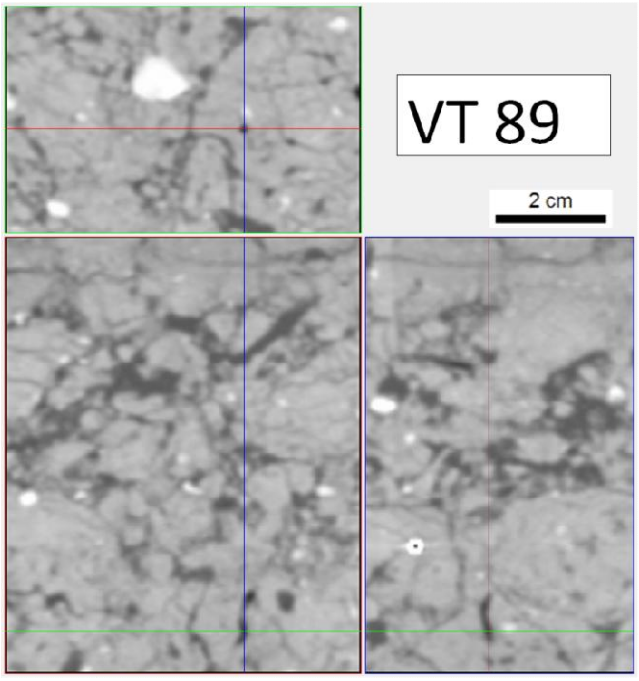


Fig. 7: Sections of the VT 89 soil sample obtained from X-ray CT imaging

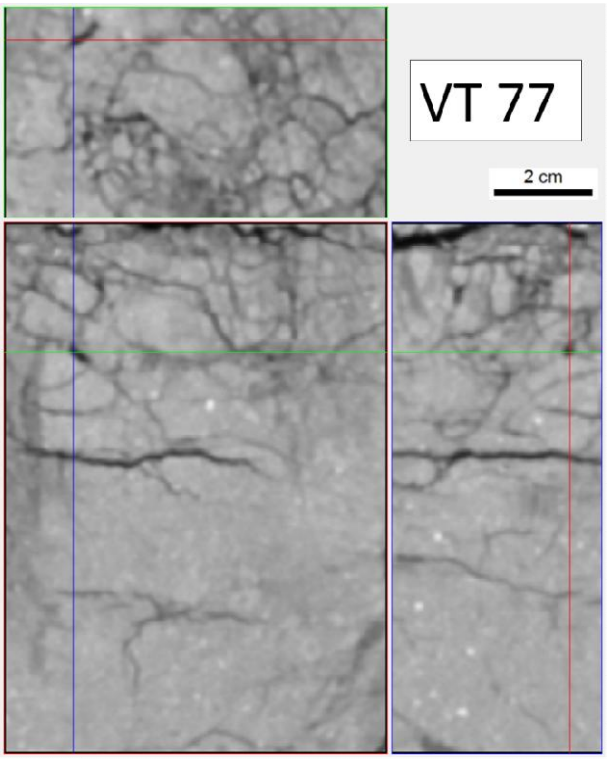


Fig. 8 : Sections of the VT 77 soil sample obtained from X-ray CT imaging

3-4 Conclusions

The aim of this study was to evaluate the relationships between macrofauna and mesofauna soil structure and different land uses. This has been verified combining the soil meso and macrofauna abundance for each sampling site with the quantification of the soil pore size distribution by means of the 3D soil image analysis technique. The results were very interesting because they showed a correlation between the heterogeneity of the soil structure, namely the multimodality of the soil pore size distribution and the soil fauna abundance. Despite these positive results it was difficult to produce further advancements because the employed approach is very much aggregated; then it was not possible to identify specific relationships between each soil fauna species and the outcoming produced soil pores. Thus, the analysis of natural soil samples did not allow the univocal recognition of the soil fauna species, which produced a specific soil pore formation neither which species prevail in the modification of the soil structure.

Chapter 4 Evaluation of soil fauna contribution at the soil core scale: a morphological approach (microtomography and micromorphology)

4-1 Introduction

Considering the results obtained from chapter 2 and 3, it is evident that despite the large bulk of research work devoted to describe and quantify soil fauna and also to develop biodiversity idexes there are still many problem in the full understanding of the role of soil fauna in determining soil quality. Most of all, results are very aggregated and empiric; this hinder a proper understanding about the role of soil fauna towards soil quality.

This chapter – rooted on previous research work – aimed to pursue a complete different approach to address soil fauna quality relationship. The basic idea is to simplify the large complexity embedded in working in natural soil where many different soil fauna and many different soil pores are already present and operating since unkown period of time. In such framework here it was developed an experimental approach based on the use of repacked soil mesocosms - combined with specific pedofauna inoculation - prepared in order to identify and quantify the specific contribution of different taxa of macrofauna to soil structure changes. As accurately described in the Introduction section of the thesis, soil fauna contributes to the soil system functioning by means of its direct influence on soil structure. Changes in habitat structure due to soil fauna activities can influence resource availability, species' abundances, and community composition of soil microorganisms.
Since the beginning of the 90s, X-ray tomography has been increasingly applied in soil biology to obtain precise and non-destructive analysis mostly of the macroporosity resulting from earthworm activity (Bastardie et al., 2005, Pierret et al., 2002, Daniel et al., 1997).

In particular most of authors used repacked soil cores with introduced earthworms (Capowiez et al., 2011, Bastardie et al., 2003, Capowiez et al., 2001, Langmaack et al., 1999, Jégou et al., 1998, Joschko et al., 1991). Indeed , the analysis of natural earthworm burrow systems is difficult as the actual composition (species, density and age structure) of the earthworm community that built the burrow system is ignored. In particular Capowiez et al. (2001, 2006, 2011) focused on the quantitative characterization of the morphology of the 3D burrow systems produced by different species of earthworms using the tools of 3D image analysis technique. The use of mesocosm experiment was used also to study the effect of the presence of earthworm burrows on the movement of arthropods (Cameron et al., 2013).

However also other macrofauna species differently contribute to the modification of soil pore system, and then to the soil functioning, by means of their burrows and bioturbation activity (Badorreck et al., 2012; Brown et al., 2010; Holden et al., 2009).

Therefore, the aim of the experimental test described in this chapter was to separately evaluate how further soil fauna species (such as Isopoda, Embioptera or Coleoptera larvae), in addition to the earthworms, are able to burrow and create new porosity in the soil. This experiment was performed combining an experiment with mesocosms in laboratory and field conditions with the 3D soil image analysis. After an incubation period in field or in laboratory, mesocosms were subjected to medical X-ray tomography. The resulting images were processed in order to obtain three-dimensional reconstructions and quantitative morphological analysis of the identified biopores.

4-2 Materials and methods

Soil fauna collection

The first step was to catch the "burrowing species" that interest us. The soil fauna collection was carried out on the slope of the Vesuvio volcano at Ercolano (South Italy, 40.8380948 N, 14.3641541 E, 120 m a.s.l.) in three different locations utilizing modified pitfall traps with sugary solution in order to attract the fauna inside (Cini et al 2012, Peter J. Landolt et al 2012, Peter J. Landolt et al 2011). The three different locations were: adjacent to a heap of compost, below a tree pine with an abundant vegetal cover, and under a hazel tree with presence of very abundant litter, therefore abundant moisture. For the construction of the modified pitfall traps have been used plastic containers superficially open, filled to half of sugary solution. A plastic funnel was placed on the top of the container, for direct the fauna inside both to prevent the escape. Between the funnel and the container was placed a gauze, to collect the fauna live (Fig 1).

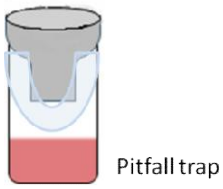


Fig 1: Pitfall trap with gauze to capture the fauna alive.

After the embedding of the pitfall traps in the field (on soil surface level) every 3-4 days the soil fauna was collected live from traps and used for the inoculum in the mesocosms.

Inoculum tests

For the inoculum test 5 different taxa were used, both adult than larvae individuals, such as Earthworms, Embioptera, Isopoda, Coleoptera (larvae), Diplopoda, etc. Before the inoculum tests the body size of the soil fauna individuals was measured (see Table 1).

In our “short-term” mesocosm experiment three different types of mesocosms were assembled, one for the lab, the other two for the field. These have been developed based on the study of different existing techniques (Berlese 1905, Tullgren 1918, Parisi 2001, Cameron et al 2013).

Each mesocosm was an open-ended system composed of two or three PVC cylinders according to the figure 2. The diameter of each cylinder, ranged between 4 and 7 cm while height between 6 and 10 cm. (Fig 2). All the mesocosms were composed of three different parts (tubes): “Source tube”, “Test tube” and “Destination tube”. These were mounted differently in the laboratory and in the field in order to avoid that the soil slipping into the test tube.

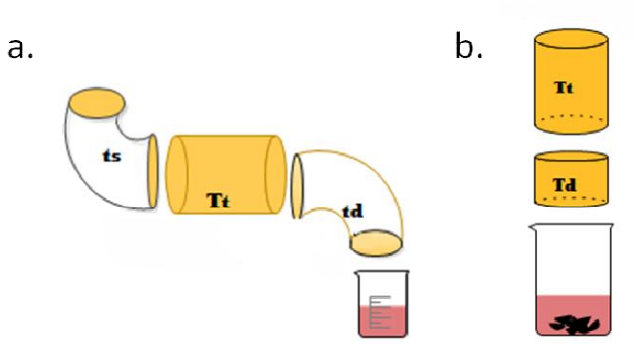


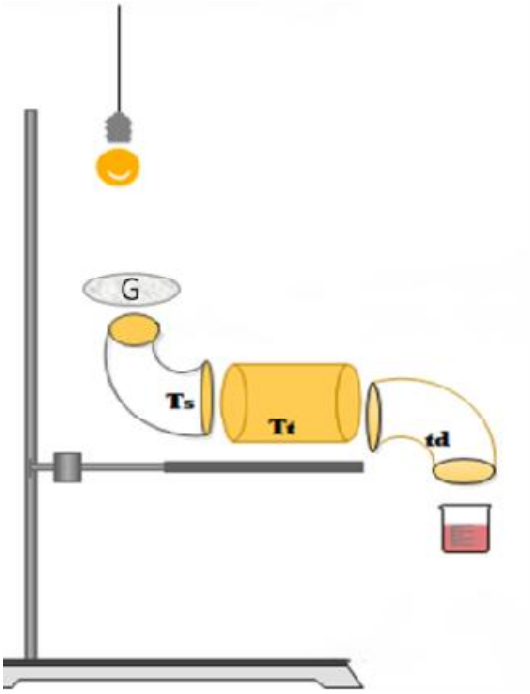
Fig 2: a) Horizontal system in the lab. b) Vertical system in the field**.**

The systems were made of:

- The "Source tube"(Ts), in which the soil fauna was mixed with soil,
- The "Test tube" (Tt), in which the soil fauna was inoculated in repacked soil mesocosm in order to obtain its specific biological signature in the soil pore system. The “Test Tubes” were prepared with 2 or 5 mm sieved soil. Before the inoculum they have been undergone to one wetting and drying cycle, in order to stabilize the soil in the cylinder.
- The "Destination tube" (Td) in which was possible to check the fauna after the two weeks of incubation. The ‘destination tube’ was connected to a container with an attractive sugary solution in order to lure the fauna inside the system.

Laboratory setup

The first innovative experimental setup is shown in Figure 3. The experimental test took place in a climatic chamber in order to fixed some climatic variables. In the lab the mesocosms were positioned horizontally in order to prevent soil slipping. Above the system an incandescence light bulb was positionated (from 25 to 40 Watt). The purpose was to dry the soil on the top in order to induce the soil fauna to enter inside the Test tube to leave their biological signature. The "Test tube" was coated with aluminium paper and with a sponge, which was frequently added with water, in order to prevent the soil from heating through the walls.

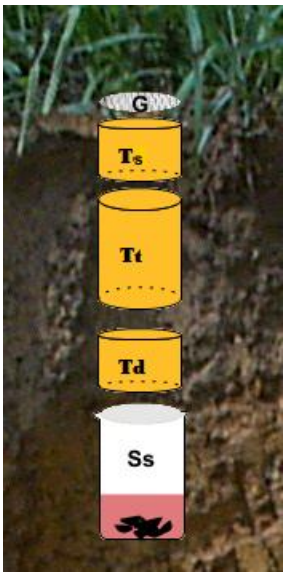


*** Legend:** (G) the gauze,(Ts) Test Source, (Tt) Test Tube, (Td) Destination tube, connected to it, the container with sugary solution.

Fig 3: Experimental setup for inoculum in the lab.

1nd field setup Inoculum in field

The second system is shown in figure 4. The fauna was mixed with soil and inoculated in the "Source Tube", covered with a 0.25 mm nylon gauze in order to allow the entry of air and light and prevents the escape of the soil fauna. In this case all tubes were mounted in vertical. The whole system was embedded in soil in different locations in the field. Also between the "destination tube" and the container with the sugary solution a nylon gauze was put in order to avoid the fall of the soil in the solution.

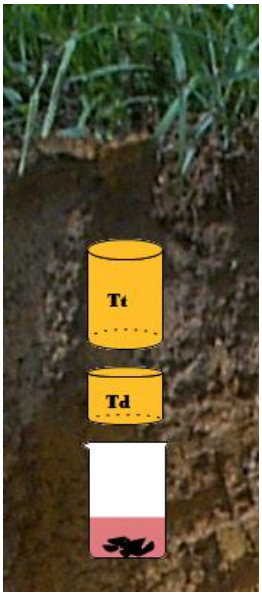


*** Legend:** (G) the gauze,(Ts) Test Source, (Tt) Test Tube, (Td) Destination tube, connected to it, the container with sugary solution.

Fig 4: Inoculum in the field.

2nd field setup (Trap-wise system)

The third was the trap-wise system shown in figure 5. The 'Test tube' was prepared using sieved soil mixed with sugary solution, without inoculation of fauna. The system was embedded in soil till a depth of 10cm in order to function as a trap in which soil fauna can enter and burrow inside of it.



*** Legend:** (G) the gauze,(Ts) Test Source, (Tt) Test Tube, (Td) Destination tube, connected to it, the container with sugary solution.

Fig 5: The Trap-wise experimental setup in the field.

After 15 days of activity, the mesocosms have been collected and disassembled, in order to verify the presence of soil fauna in the "destination tubes" of the different types of mesocosms.

3D image analysis

For the mesocosms in which we found soil fauna in the "Destination tubes", the "Test tubes" were resin impregnated and then were imaged using the medical X-ray CAT at a resolution of 200 μm as described in the Chapter 3, both the image processing was performed with the same procedure described previously in the Chapter 3.

Based on their size and shape the biopores were identified and separated from the porous phase of soil matrix using a supervised procedure. The bio-pore imaging process is exemplified in figure 6.

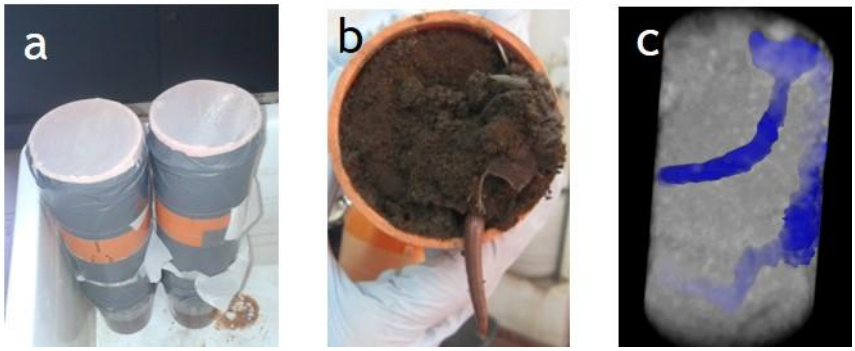


Fig 6. Bio pore imaging steps: Mesocosms for the inoculum in field (a), "Test tube" after the incubation (b), 3D imaging of biopores in the "Test tube" by X-ray tomography (c).

Pore size distribution of the bio-pores was determined using the own-developed software Conmorph, through the iterative application of the "opening" algorithm, which classifies the porous phase according to the spacing from the walls (Gargiulo et al, 2015).

The 3D-volumic information on the biopores was then simplified into 3D skeletons by determining all the ultimate eroded points (i.e. centroids) of the bio-pore objects in the 2D images constituting the reconstructed soil core volume (Capowiez *et al.*, 2011). For this purpose the "Image J" open source Java platform was used (v.1.49).

This means that a burrow corresponds to a set of connected segments joined by junction points. The burrow system is then the set of burrows found in a core.

- In order to improve the morphological characterization of the bio-pore networks, the following parameters have been computed for each burrow system:
- *Number of burrows*: counting of burrows.
- *Volume:* Total volume of the burrow system.
- *Length*: sum of the lengths of all the segments of all the burrows.
- *Longest shortest path length*: average of the burrow longest shortest path lengths weighted for the burrow longest shortest path lengths.
- *Mean diameter*: mean value of the pore size distribution calculated from the whole burrow system.
- *Tortuosity of longest shortest paths:* Average longest shortest path tortuosity across the entire burrow system weighted for the longest shortest path lengths.
- *Tortuosity:* Average burrow segment tortuosity across the entire burrow system weighted for the burrow segment lengths.
- *Vertical deviation:* Average burrow segment vertical deviation across the entire burrow system weighted for the burrow segment lengths.
- *Rate of branching*: ratio between the total number of the burrow segment junctions and the burrow system length.
- *Junction rank:* mean value of the junction rank distribution calculated from the whole burrow system.
- *Individual burrowing ratio*: Ratio between the volume of the burrow system and the total volume of the pedofauna individuals.

Except for *Volume, Mean diameter* and *Individual burrowing ratio,* all the above parameter are based on topological measurement made on the *"skeleton"* of the bio-pore network.

4-5 Results and discussion

The comparison of the presences of soil fauna in the "destination tubes" for the three different experimental setups after the inoculum tests, allowed observing that in the Laboratory setup only earthworms were found. Conversely, all the inoculated taxa, including earthworms, were found in the mesocosms of the two field experimental setups.

The qualitative observation of the images obtained from CT scans shows, for all taxa, a high bioturbation activity on surface and a specific impact on soil pore system under the surface (see figures 1a, 2a, 3a, 4a and 5a).

Were reported the quantitative results obtained from the image analysis of the bio-pores produced by the soil fauna inoculated in the mesocosms or found in the traps. Specifically, in this thesis were reported, as example, the results obtained for a "Lumbricus Terrestris" for the inoculum in the lab setup, three different taxa inoculated in the inoculums in the field (Diplopoda, Embioptea and Isopoda species), lastly for the trap-wise setup, found two different taxa; Earthworm and Coleoptera larvae. In table 1 are shown the data regarding the body size of the used taxa individuals and their "*individual burrowing ratio*", which quantifies the relative (to the body volume) contribution of each taxa to the porosity production.

Tab 1: Soil fauna body size vs burrowing ratio (for the Isopoda are reported the mean value)

	Embioptera	Isopoda	Diplopoda	Lumbricus	Coleoptera larvae	Lombricus (Trapp)
Diameter (mm)	2.6	3.6	3.5	4.8	2.5	2.8
Length (mm)	15.6	8.3	43	80	44	50
Volume (mm^3)	82.8	47.1	413.5	1446.9	215.9	307.7
Individual burrowing ratio	152.4	9.5	18.6	5.30	5.7	10.0

Embioptera has shown an individual burrowing ratio of one order of magnitude higher than the other taxa. Comparing Lumbricus results it can be noted that individual burrowing activity seems to be inversely correlated to the individual size.

Burrows description and pore size distributions

The 3D reconstruction of the internal structure of the "Test tube" inoculated with one individual of earthworm (*Lumbricus terrestris*) allowed to observe the typical large vertical burrows produced by this specie (Fig 7 a). The biopore size distribution showed that the inoculated individual contributed to the pore production in the 1.2-7.2 mm pore size range. The biopore size distribution resulted multimodal with a maximum modal value at 4.8 mm, according to the individual body size (Fig7b and table 1).

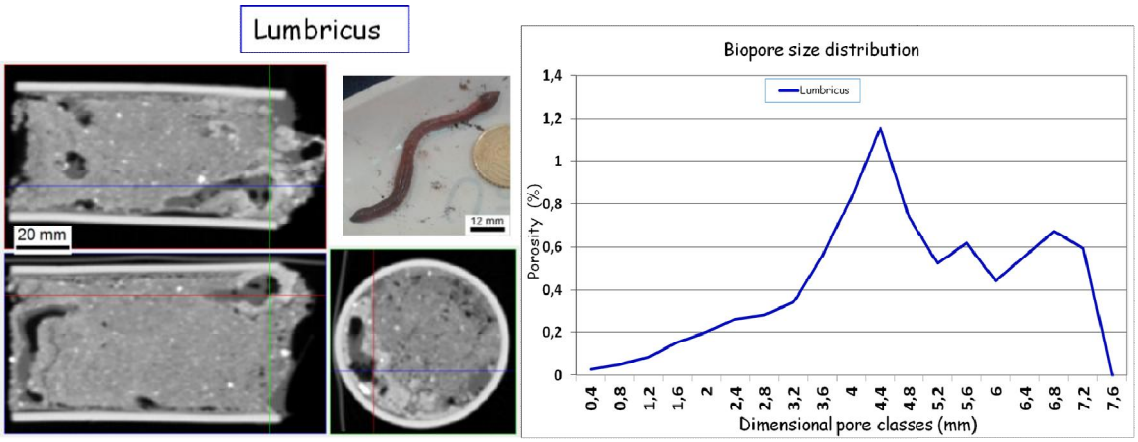


Fig 7: a) medical X-ray CAT image. b) Biopore size distribution.

Regarding the "1st field setup" we choose to show the results of three taxa which produced biopores different in shape and size. In particular the medical X-ray CT images allowed to observe that the Isopoda (16 individuals inoculated) produced biopores which appear like a small vacuoles or narrow burrows (Fig. 8a). These types of bio-pores agree with the porosity mostly distributed in the smaller pore size classes (Fig 8b). The biopore size distribution resulted unimodal, with a shift toward smaller pore size classes respect to the earthworm.

The porosity produced by the isopoda individuals showed the 1.2-3.6mm pore size range with a peak around 1.2mm (Fig 8 a).

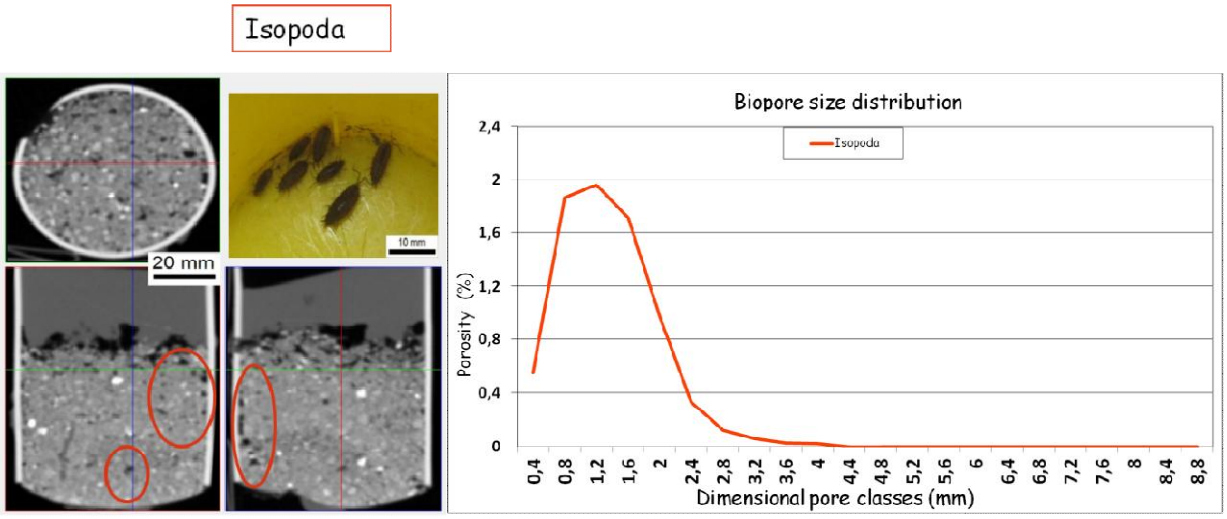


Fig 8: a) medical X-ray CAT image. b) Biopore size distribution.

Another taxa utilized for "1st field setup" was Embioptera (one individual inoculated). This taxa produced a multimodal biopore size distribution, ranging from 1.2mm to 8.4mm pore size classes, with the highest modal value of 2.4mm. This value corresponds to the diameter of the body of the inoculated individual (Fig 9a). Moreover the large pore size range is well correlated to the very high value of the individual burrowing ratio (Table 1). As it was possible to observe from the medical X ray images of the produced biopores, Embioptera created also large pore chambers which correspond to the lower modal value in the biopore size distribution (Fig 9b).

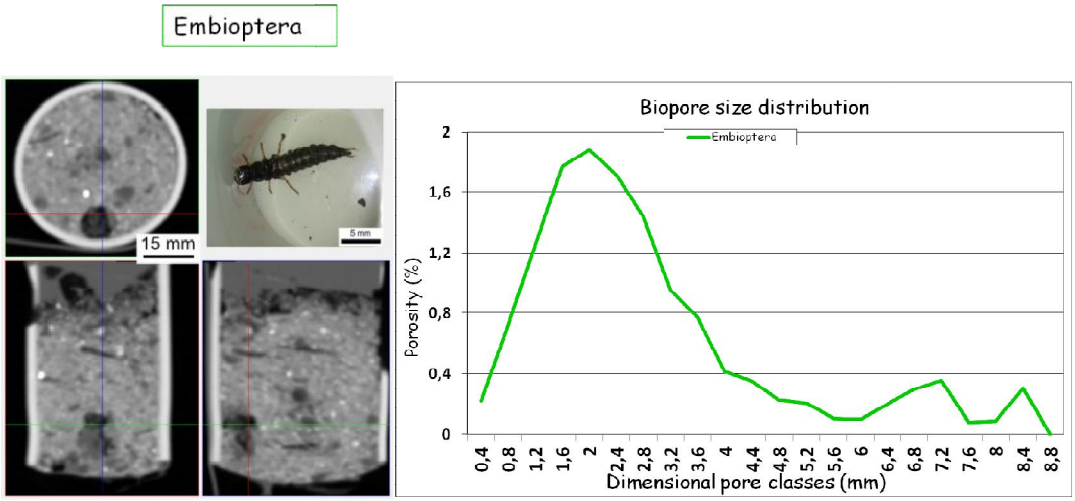


Fig 9: a) medical X-ray CAT image. b) Biopore size distribution.

The last example for the "inoculum in the field" is the Diplopoda (one inoculated individual). The biopore size distribution resulted unimodal ranging from 1.2 and 4.8mm pore size classes, with a maximum at 2.4 mm. The the medical X ray images showed concentric chambers of size corresponding to its body size (Fig. 4a).

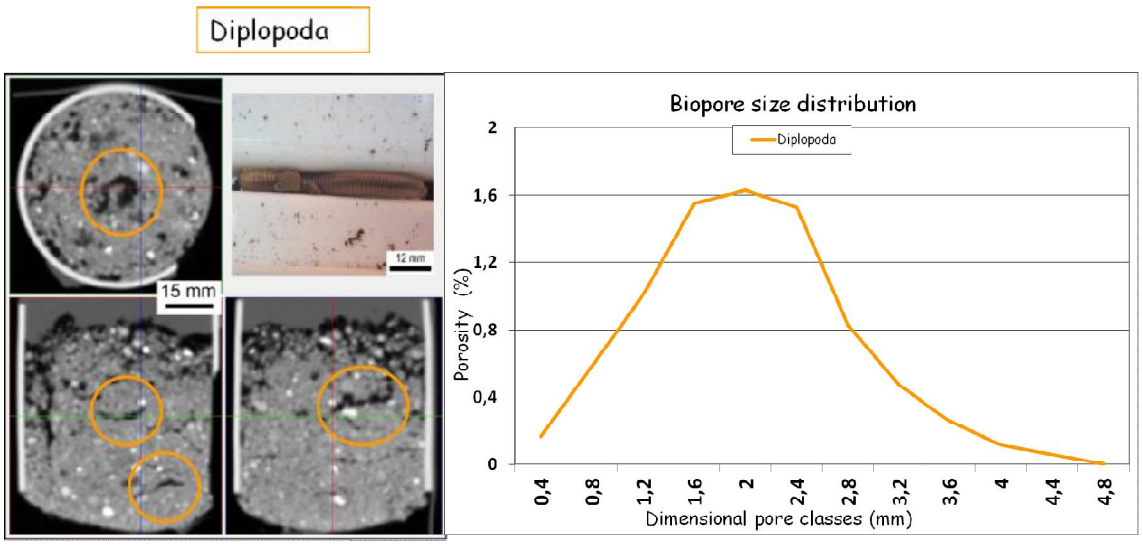


Fig 10: a) medical X-ray CAT image. b) Biopore size distribution.

Regarding the “trap-wise" setup we reported here an example of a trap in which we found two different taxa: an Earthworm and a Coleoptera larva (Elateridae) . The individuals of these two taxa were very similar in shape and size (Tab 1), but it can be noted that the two taxa produced different biopore size distributions, which cover different pore size classes. The earthworm produced a biopore size distribution which ranged from 1.2mm to 4.8mm with a modal value at 3.6mmm. The Coleoptera larva produced an unimodal biopore size distribution with a peak at 2.4mm pore size class. In both cases the modal values correspond to the diameter of the founded individuals.

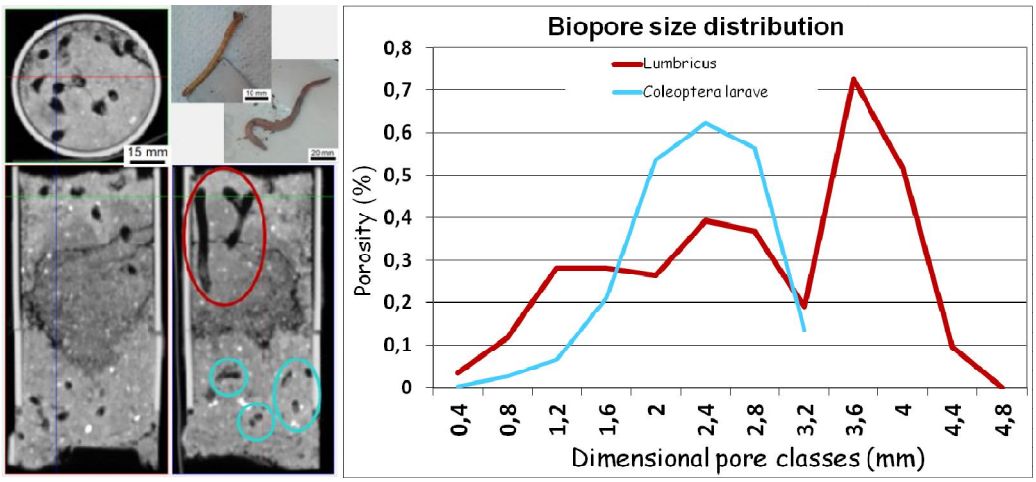


Fig 11:a) medical X-ray CAT image. b) Biopore size distribution.

In figure 12 are reported all the obtained biopore size distributions of all the tested taxa, in order to emphasize that the presence of different soil fauna groups, as it happens in the natural conditions, can produce complex pore size distribution increasing the multimodality, thus contributing to a greater heterogeneity of the structure and, therefore, to an high physical soil fertility.

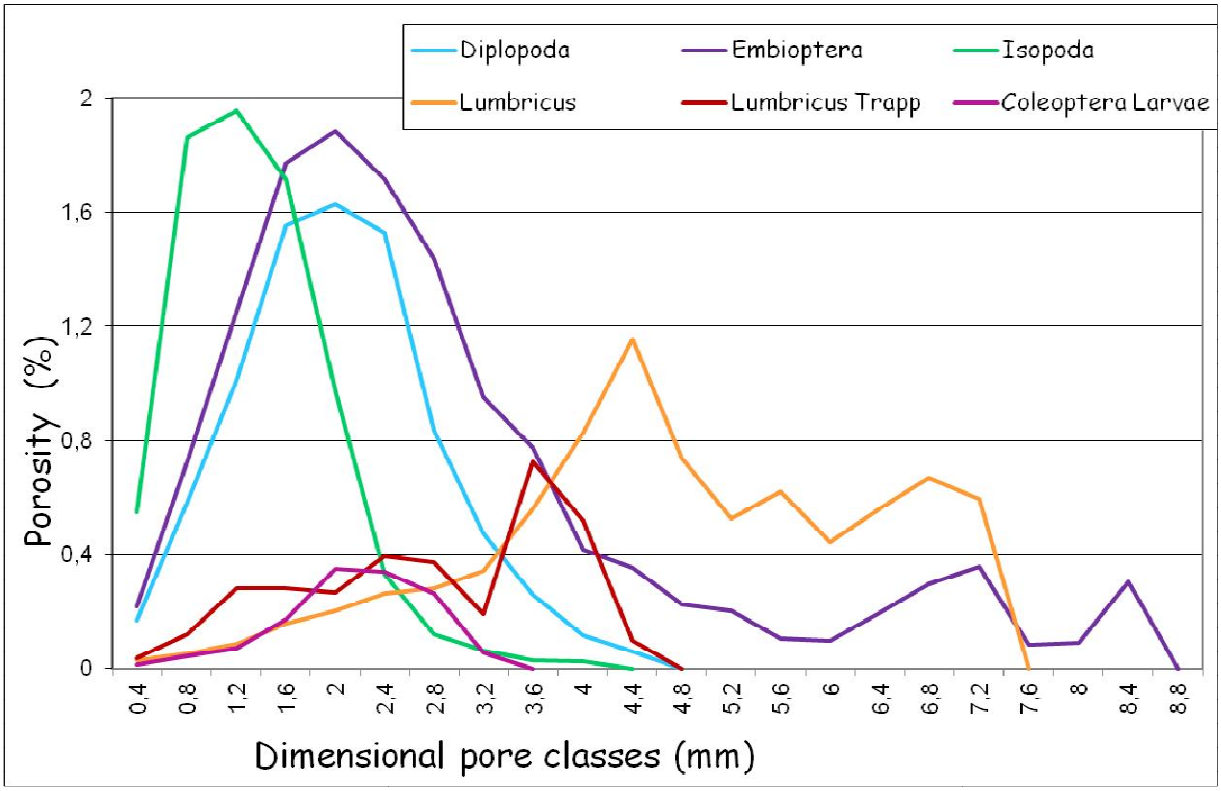


Fig 12: Biopore size distribution of all taxa studied in this experimental phase.

Burrow system topological analysis

In table 2 are reported the results of the topological parameters calculated from the skeleton of the biopores produced by one individual of Earthworm and on of Coleoptera larvae found in the single core of the "trap-wise" setup. The comparison between the results shown in the table 2 and the 3D reconstruction of the biopores in visualised in figure 7 allows to quantitatively observe the difference between the burrow systems produced by these two taxa.

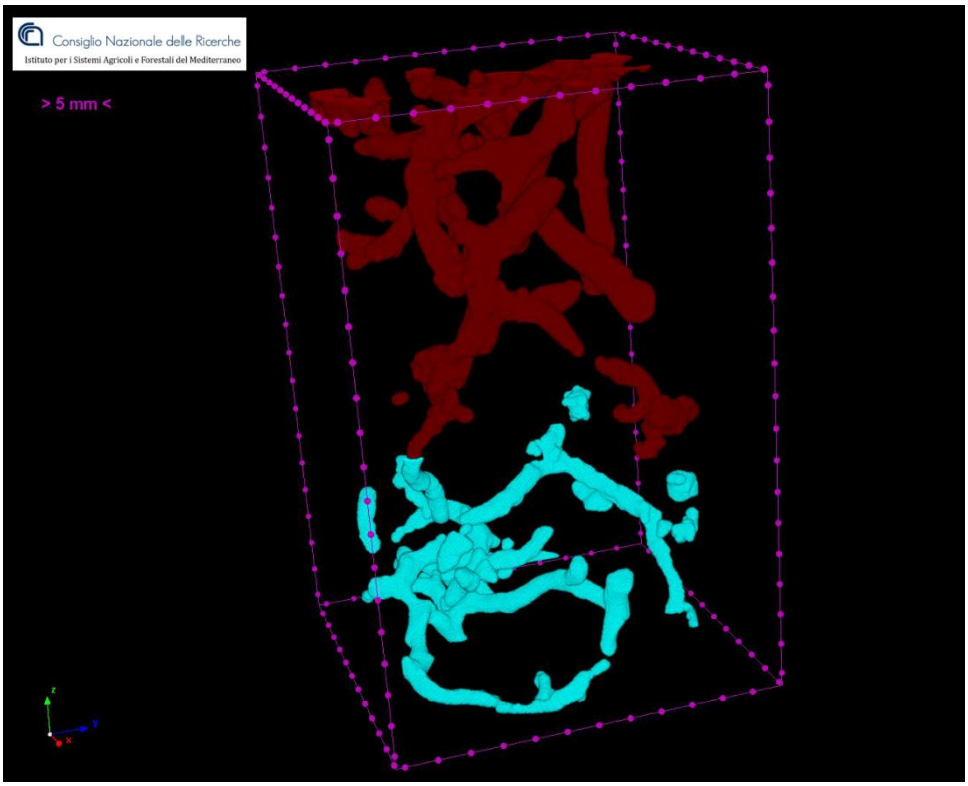


Fig13: 3D reconstruction of the biopores produced by earthworm (in red) and by Coleoptera larvae (in blue) in the "Test tube" of a trap.

As observed also from the biopore size distributions, volume, length and mean diameter of the biopores resulted higher for the earthworm. Moreover the *Tortuosity* value resulted higher for the earthworm, conversely the *Tortuosity of longest shortest paths r*esulted higher for the Coleoptera larvae. This indicated more tortuous continuous paths of the Coleoptera larvae than Earthworm Regarding the orientation of the burrow system, the vertical deviation resulted higher for the Coleptera larvae burrow system than that of the Earthworm. This means that the earthworm

produced a burrow system mainly vertical, while the biopores of the Coleptera larvae were more horizontally oriented. The burrow system of the earthworm showed the higher value of *rate of branching* while the *junction rank* resulted of 3 for the burrow systems of both the taxa. The higher value of the individual burrowing ratio obtained for the earthworm finally indicated the higher burrowing activity of the Earthworm respect to the Coleptera larva.

Tab 2. Morphological and topological parameters calculated for the skeleton of the biopores produced by two taxa found in the trap test.

	Earthworm	*Coleoptera larva*
Number of burrows	13	13
Volume (mm^3)	3071	1223
Length (mm)	547	474
Mean diameter (mm)	2.79	2.16
Tortuosity	1.26	1.21
Tortuosity of longest shortest paths	3.39	5.08
Vertical deviation (°)	30.7	61.8
Rate of branching (number/cm)	1.14	0.63
Junction rank	3.08	3.03
Individual burrowing ratio	10.0	5.70

4.6 Conclusions

The different inoculation techniques (in lab or in the field) tested in this preliminary work, have been found appropriate for the identification and the quantification of the contribution of different taxonomic groups of macrofauna to soil pore system formation. The preparation of field traps, in particular, has provided to be the most successful experimental setup. Results described in this chapter show that the identification of different contributions to the soil pore system formation has the potential to be employed for the identification and quantification of different biological activities in natural conditions. Unlike the current literature, which is focused on the study of earthworms as "excellence ecosystem engineers", in this work we wanted to evaluate the contribution of other soil fauna taxa in order to obtain a more complete outline for a more proper consideration of the fauna for soil quality improvement. This viewpoint offers many interesting challenges, in order to extend in the future the definition about the "soil ecosystem engineers". Moreover, published works on the relationship between soil fauna and soil structure are often more descriptive, they do not provide data directly related to the soil functions. The characterization of the soil structure by means of the quantitative pore size distribution based on the use of mathematical morphology algorithms has the potential to quantify the impact of the biological activity on soil properties (e.g. transport of fluids and solutes, soil fauna habitats). The results obtained by this new approach, favor the direct implementation in physically based models that simulate flow processes in soils. Then these powerful results may enable in the near future to evaluate and quantify the contribution of soil fauna towards soil functions. Indeed, this can be partially done – by modelling approaches - based of the thorough knowledge of the soil porous phase developed by soil fauna.

Despite these evidences it is also clear that it would be desirable to combine the undertaken approach with direct measurements of soil functions; this would able to provide a great insight into functional soil properties.

Chapter 5 Evaluation of soil fauna contribution at the soil core scale: a functional approach monitoring emission of greenhouse gases.

5-1 The soil fauna functions in the interactions with green house gases (CO_2 and N_2O):

Soil fauna contributes to soil system functioning also by means of its effect on C and N cycles. In particular, soil fauna affects CO_2 and N_2O emissions from soils. The last experimental phase of this thesis focused on monitoring of the main greenhouse gases CO_2 and N_2O by means of an experimental laboratory test with inoculum of different soil fauna species.

The only faunal group for which a considerable body of literature on their effects on N2O emissions exists are earthworms (Kuiper et al., 2013). They can affect N_2O emissions through altering soil structure (Drake H. et al, 2007; 2006) and incorporating plant residues into the soil (Paul B. 2012, Lubbers I. 2011). Numerous studies have confirmed that earthworms affect soil CO_2 and N_2O emissions (Rizhiya et al., 2007; Chapuis-Lardy et al., 2010; Giannopoulos et al., 2010; Lubbers et al., 2013; Frouz et al., 2014), through their direct and indirect impacts on the soil environment, the quality of resources and microbial processes (Drake and Horn, 2006; Speratti and Whalen, 2008; Nebert et al., 2011; Lubbers et al., 2013).

For other soil fauna, studies on their impact on the N cycle focus on N mineralization rates rather than on N_2O emissions (Verhoef & Brussaard, 1990; de Ruiter, 1993; Bardgett & Chan, 1999; Cole et al., 2004; Lenoir et al., 2007; Osler & Sommerkorn, 2007; Kaneda & Kaneko, 2011). A small number of studies have also shown effects of mesofauna, such as Collembola and Acari, on soil CO_2 emissions (Fox et al., 2006; Wickings and Grandy, 2011), but very few studies measured the effect of Isopoda, Collembola, Enchytraeidae or Acari species on N2O emissions (Thakur et al 2014, Kuiper et al 2013).

Little information is available on the interactive effects among soil faunal groups on soil CO2 and N2O emissions (Collison et al., 2013; Kuiper et al., 2013; Thakur et al., 2014; Wu et al., 2015).

In this experimental phase we studied the contribution of both a single species and combinations of different soil fauna groups on CO_2 and N_2O emission. We investigated the functioning of fauna respiration, and we hypothesized that as soil fauna increases, simultaneously, the N_2O emission decreases. The soil fauna species are able to facilitating a complete denitrification (e.g. by stimulating the microbial activity, or creating more microbial reaction sites). To test this hypothesis, we established the invertebrate food webs in soil microcosms with different levels of functional diversity. All the experiment reported below has been conducted in the Department of soil quality which retains all IPR issues. Below it is reported the results produced by my personal research laboratory activities performed from May to August 2015 and with special reference to measurements of fluxes of green house gases as CO_2 and N_2O.

5.2 The experimental set-up:

The experimental set-up consists of 23 treatments on soil microcosms, in which there are two controls and 21 samples in which were inoculated eight different species of soil invertebrate in order to investigate about their contribute of N_2O and CO_2 emissions. The two control were made one of only soil, the other of soil with about a 1 cm high surface layer of hay. The block of 23 treatments has 4 repetitions, totalling 5 block, therefore 115 mesocosm units. Microcosm will be incubated with living soil fauna in a randomised block design under climate controlled conditions. Soil fauna comprises different species of collembolans, earthworms, enchytraeids and mites (two species of each taxon). The fauna species originate from the field and from lab cultures of Wageningen University and from other collaborators in Amsterdam. The fauna was inoculated individually or mixed to each other in different combinations. The selection for treatments of species mixtures applied the Heemsbergen's method (Heemsbergen D.A. et al. 2004). Treatments included an increasing number of invertebrate species: 0, 1, 2, 4 and 8 species per microcosm; no

invertebrates, 8 different single species, 4 different mixtures of 2 species and 4 different mixtures of 4 species covering the whole range of more or less similar species, and the mixture of all species. Single-species treatments of all 8 species were included to quantify their per-capita effects on N_2O fluxes, C decomposition and N mineralisation. In table 1 are reported all the combinations of taxa used which determine the 21 treatments with inovulum of fauna.

The fluxes were measured from five replicates from respective blocks, by means of static closed chamber technique. N_2O and CO_2 fluxes were measured with an Innova 1312 photo-acoustic infrared multi-gas analyser (LumaSense Technologies A/S, Ballerup, Denmark), (Kool D.M.et al, 2006, Velthof G.L. et al, 2002).

As described in Pore J.R. 2016, at the end of fluxes measurement, each microcosm was cut in half, one of this part was used for fauna extractions and the other part was used for soil analysis. After mixing of the second one, a subsample was dried at 40 ºC for 48 h and analysed for dissolved organic carbon (DOC) and pH in a 0.01 M of $CaCl_2$ extraction (Houba et al., 2000). Another subsample was used to determine microbial biomass nitrogen (MBN), following the chloroform fumigation and extraction technique (Brookes et al., 1985). Subsequently, total dissolved N (Nts), ammonia (NH_4^+), nitrate and nitrite (NO_3^- NO_2^-) concentrations were measured, colorimetrically, in a K_2SO_4 extract. To calculate the dissolved organic nitrogen (DON) content, NH_4^+ and (NO_3^- NO_2^-) were subtracted from Nts (the data are not shown).

In this experimental phase, compared the mentioned article (Pore J.R. 2016), were investigated more species (Tab 1). The amount, of all species, utilized during the experimental phase, and the fauna combination that was utilized for the inoculum in each microcosm are shown in a Table 2.

The soil fauna was extracted using different extraction techniques for the mites and the enchytraeids. Enchytraeids were extracted with a Baermann funnel, a wet extraction with temperature increasing from 20ºC to 45 ºC within 3 h (Petersen and Luxton, 1982). Both mite

species were extracted with a Berlese funnel (Tullgren funnel), with a gradual temperature increase from 20 ºC to 45 ºC in 5 days (Petersen and Luxton, 1982).

Tab 1: Combination of all species of soil fauna utilized in the experimental phase.

	Single species	*2 species combination (same taxa)*	*2 species combination (different taxa)*	*4 species combination (same taxa)*		*4 species combination (different taxa)*	*8 species combination*
Spiecies	1	2		4			8
Taxa	1	1	2	2		4	4
Species combination	Pw1	Pw1	Ew1	Mi2		Pw1	Pw1
	Pw2	Pw2	Pw2	Mi1		Sp2	Pw2
	Mi1	Mi1	Sp2	Ew1		Ew1	Mi1
	Mi2	Mi2	Pw1	Ew2		Mi1	Mi2
	Sp1	Sp1	Sp1	Sp1		Ew2	Sp1
	Sp2	Sp2	Mi1	Sp2		Mi2	Sp2
	Ew1	Ew1	Ew2	Pw1		Sp1	Ew1
	Ew2	Ew2	Mi2	Pw2		Pw2	Ew2

Tab 2: Amount, of all species, of soil fauna inoculated in the experimental phase.

Taxon	*Code*		*Species*	*Treatments with the species*	*single species*	*2 species combination (same taxa)*	*2 species combination (different taxa)*	*4 species combination(same taxa)*	*4 species combination (different taxa)*	*8 species combination*	*Replicates (+ 2 controls)*	Total of individuals
Enchytraeids	Pw1	Potworm 1	E. albidus	6	50	25	25	13	13	8	8	1072
Enchytraeids	Pw2	Potworm 2	E. crypticus	6	50	25	25	13	13	8	8	1072
Mites	Mi1	Mite 1	R. robini	6	400	200	200	100	100	50	8	8400
Mites	Mi2	Mite 2	O. nitens	6	400	200	200	100	100	50	8	8400
Collembolans	Sp1	Springtail 1	S. curviseta	6	260	130	130	65	65	35	8	5480
Collembolans	Sp2	Springtail 2	F. candida	6	260	130	130	65	65	35	8	5480
Annelida	Ew1	Earthworm 1	A. caliginosa	6	4	2	2	1	1	1	8	88
Annelida	Ew2	Earthworm 2	L. rubellus	6	2	1	1	1	1	1	8	56

The soil samples used for measuring N_2O and CO_2 emission were placed with the cylindrical containers in polyvinylchloride (PVC) which could be closed with a septum-equipped cap for flux measurements. Before the flux measurements, for N_2O and CO_2, the cylinders were closed for a

period of 40 minutes, measured using a photo-acoustic infra-red gas analyzer (Innova1312), every six samples was performed the background measurement. The gas analyzer was equipped with filters to minimize interference by CO_2 (a soda-lime scrubbing filter) and N_2O concentrations were corrected for measured CO_2 concentrations and water vapor (Velthof et al., 2002). (All supplementary material is shown at the end of the thesis).

At the end of the fluxes measurements, the microcosms were decomposed. As mentioned before, the microcosm was divided in half to proceed with the others chemical analysis. In order to get an impression of the carbon (C) budgets and of the transport, into the soil solution, the dissolved organic carbon (DOC) was determined, means by extraction with a 0.01M $CaCl_2$-solution.

The soil sample dried at 40°C and extracted at 20°C in a 1:10 (w/v) ratio with 0.01 mol/l $CaCl_2$ solution of 20°C. After reaching equilibrium, which is achieved in less than 2 hours shaking, pH can be measured in the settling solution and after centrifugation, the DOC can be measured with ICP-OES. For the extraction of sampling a series consists of 30 samples, which includes 2 blanks and 2 internal reference samples (nr ISE-949 and ISE-989).

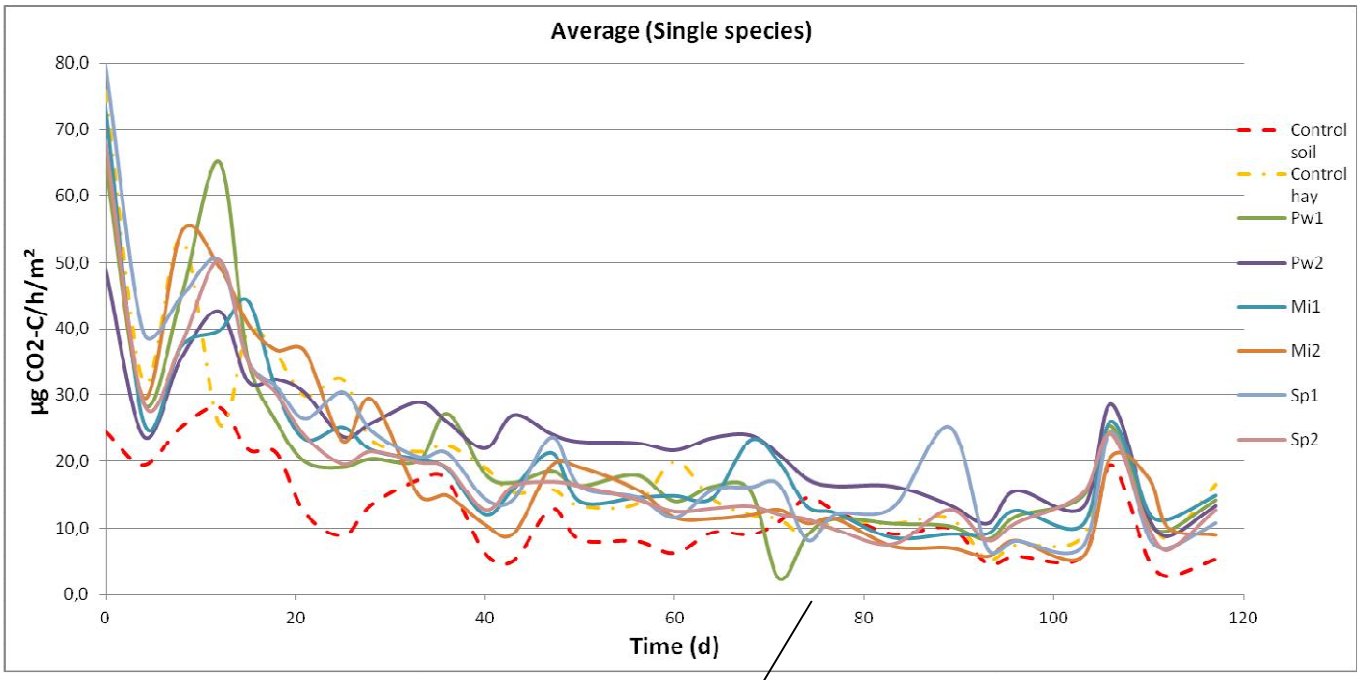
After being weighed, the samples (3,00 ± 0,03gr) were transferred to a plastic centrifuge bottle of 50 ml, added 30,0 ml calcium chloride solution and shake 2 hours in the shaking machine, in the temperature controlled room. The samples were decanted for 30 minutes then, after centrifugation, were put about 20 ml of extract supernatants into the syringe and filtrate with a 0.45 µm filter.

For measuring of pH, ± 5 ml of supernatants were transferred in a test tube then, after all extracts were sampled, the pH was measured the same day. (All supplementary material is shown at the end of the thesis).

5.3 Considerations and evaluations:

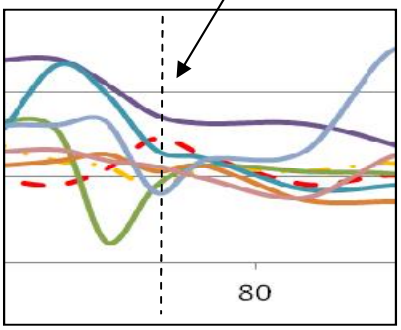
In figure 1 are reported the results of the mean values of CO2 fluxes measured day by day for all treatments consisting of single species except the earthworms. Mean values of CO2 fluxes of the single earthworms are reported in figure 2. In figure 3 and 4 are reported the CO2 fluxes results for the combinations of the couples of species of the same taxon except the earthworms and of the couple of earthworms, respectively. In figure 5 are reported the results of the mean values of CO2 fluxes measured for the four combinations of couples of species of different taxa. In figure 6 are the CO2 fluxes results of the two combination of the four species of same taxon, in figure 7 those of the

two combinations of the four species of different taxa. Finally, in figure 8 are the CO2 fluxes results of the combination of all the eight species together.



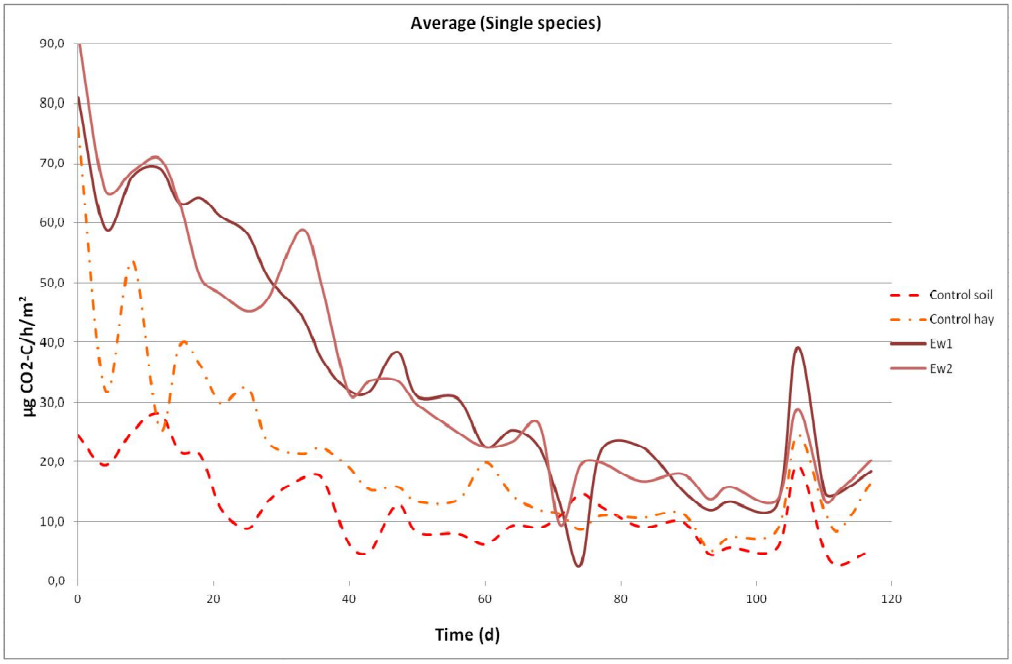
*** The codification of all the species utilized, is shown in table 2.**

Fig 1: Fluxes of CO_2 of all the single species utilized, excluding the two species of earthworms, reported in figure 2.



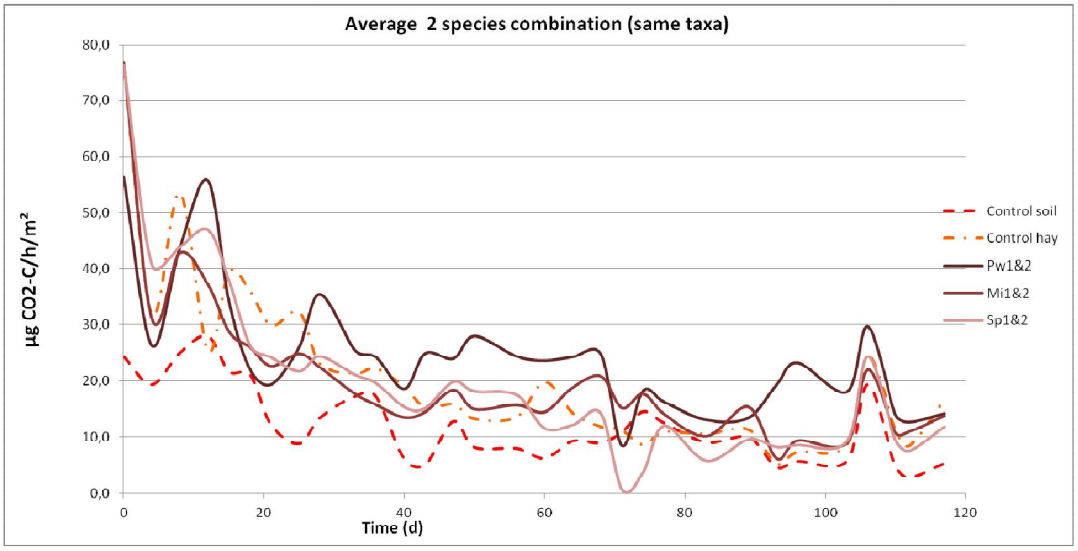
Apart from some artefacts that are evident in the results, it can be noted an overall decrease, for all treatments, of the CO2 fluxes during the experiment with a minimum reached around the 80th day. Except for the treatments of the specie combinations where earthworms are present, actually the fluxes are very similar to those of the control mesocosm with hay. Thus, only the presence of earthworms seem to provide an increase in soil respiration with respect to the control soil with a surface layer of hay. This appears particularly evident when observing the results of treatments where only earthworms are present.

Regarding the above mentioned artefacts in the results, one of these is a strong decrease of CO2 fluxes in the first ten days, likewise any singles species in its microcosm had undergo a stress due to lack of oxygen. Another one, before the 20th day, in which most of the species presented the same short term trend. The same happens between the 100th day and the 120th, probably determined by an excessive addition of water. As seen in the enlarged image in figure 1, before the 80th day it's possible to show another decreasing, excluding for the "Potworm 1" (E. Albidus specie), with a similar short term trend. The same situation is valid, for earthworm species shown in figure 2 and in the case of the figure 3.



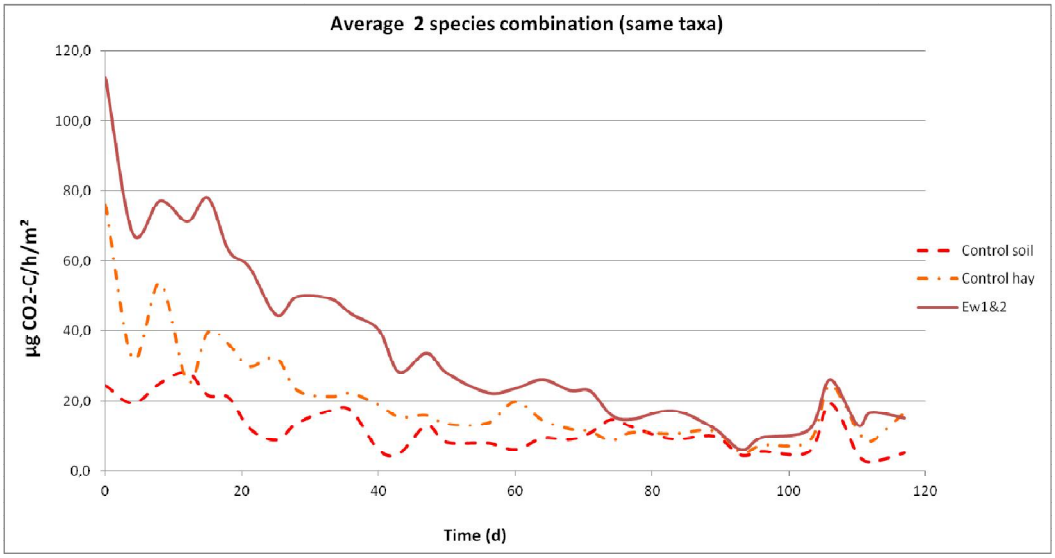
*** The codification of the species utilized, is shown in table 2.**

Fig 2: Fluxes of CO_2 of the two single species of earthworms.



*** The codification of the species utilized, is shown in table 2.**

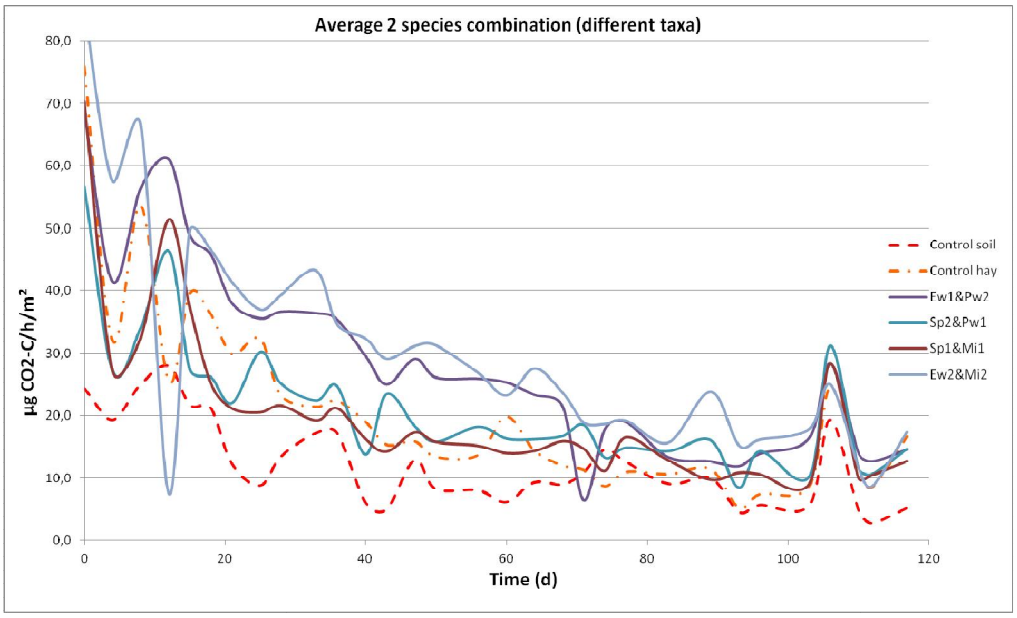
Fig 3: Fluxes of CO_2, here reported below the fluxes of the same taxa, inoculating 2 species of the same taxa in each microcosm.



*** The codification of the species utilized, is shown in table 2.**

Fig 4: Fluxes of CO_2, here shown only the inoculum of two different species of earthworm together.

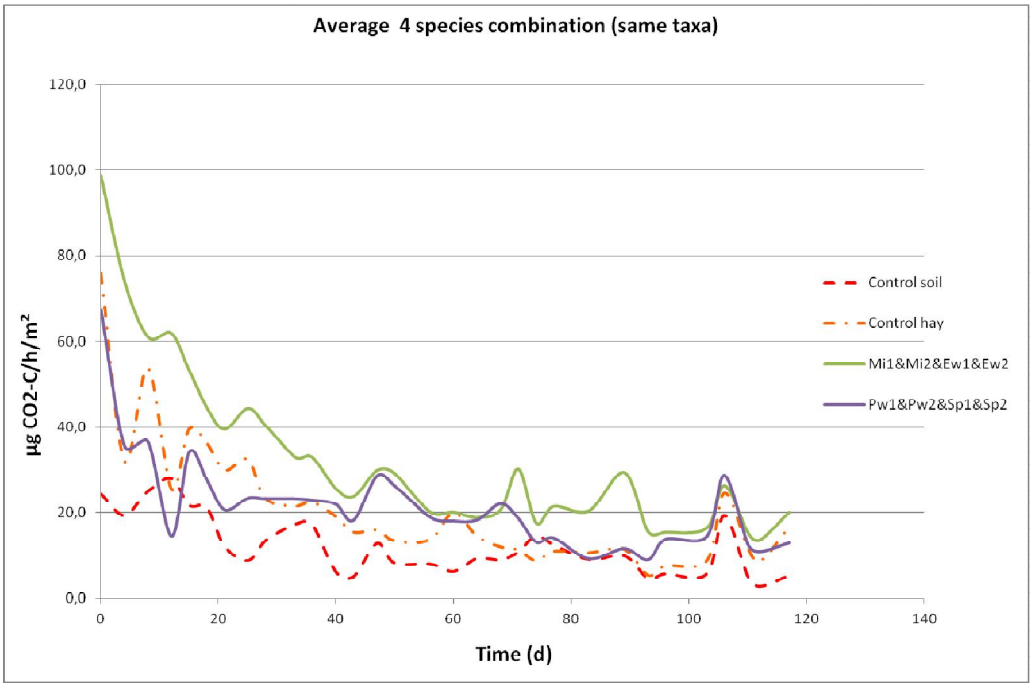
Moreover, in figure 4 it is possible to show a sharp decrease around the 15th day, for the sample in which there are 1 individuals of L. Rubellus and 200 individuals of mites, O.Niteus. Likewise they had gone to lack of oxygen.



*** The codification of the species utilized, is shown in table 2.**

Fig 5: CO_2 fluxes of 2different specie combination (of different taxa).

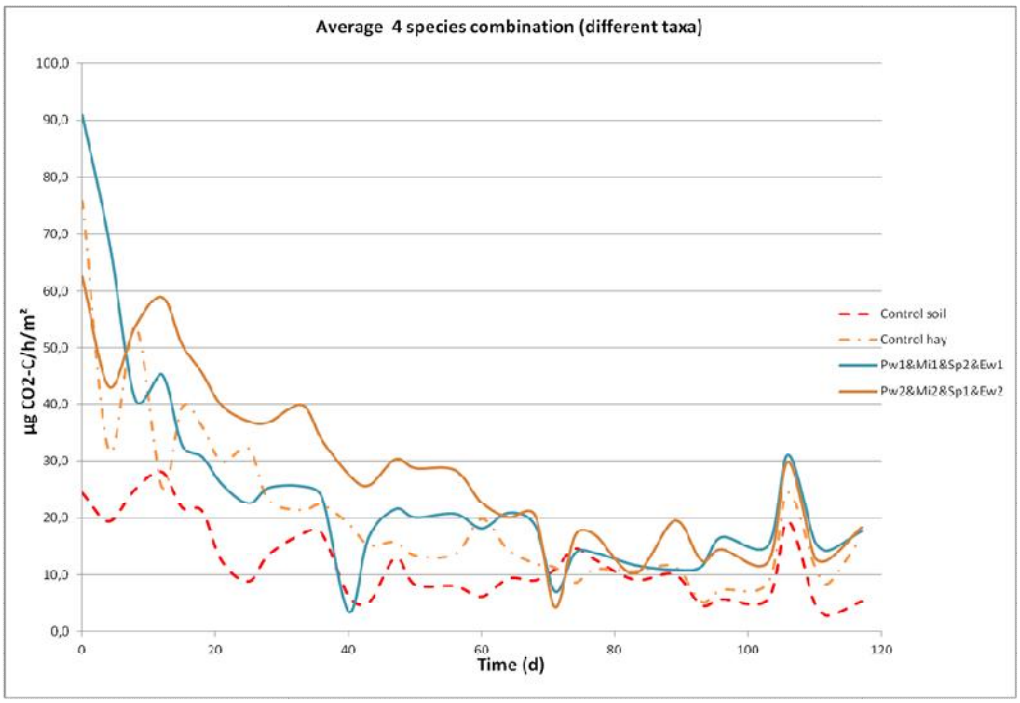
Also in the case in figure 5 and 6 there is a decrease around 15th day for the sample in wich are present two different species of Potworm, 13 individual of E.Albidus and 13 for E. Crypticus tugether with two species of Springtail, 65 individual of S.Cruiseta and the same for F. Candida. Comparing with the previous graphic in which were inoculated the single species, here there isn't the same decreasing trend.



*** The codification of the species utilized, is shown in table 2.**

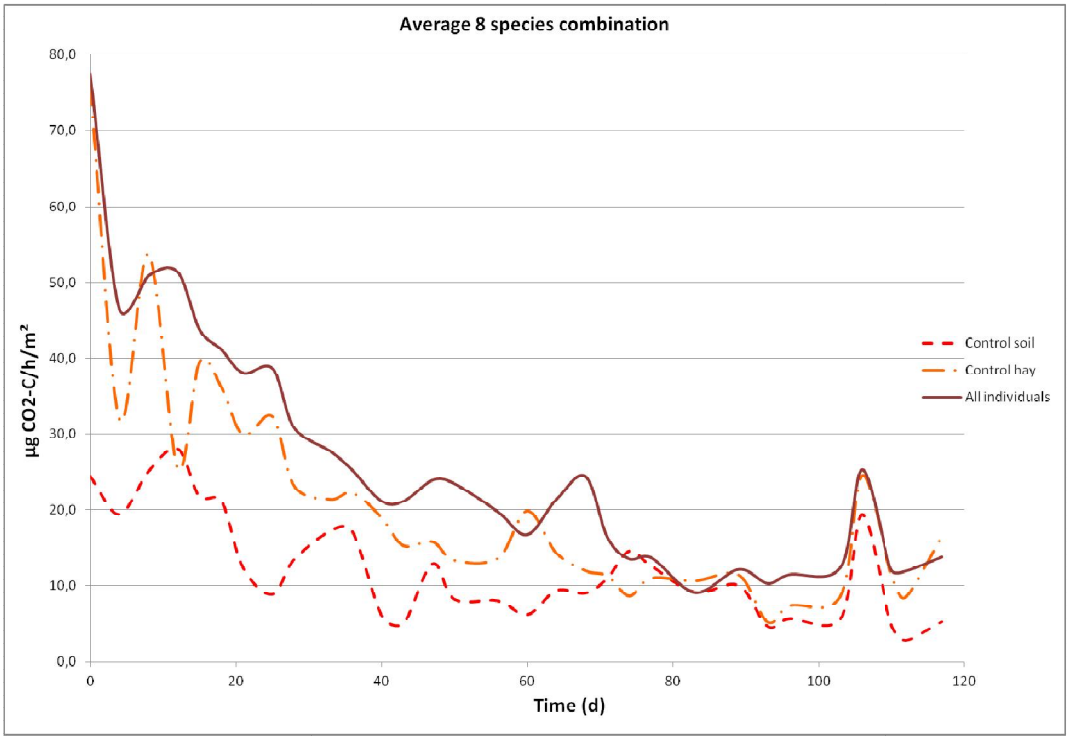
Fig 6: CO_2 fluxes of 4 species combination, utilized 2 different taxa.

The two trends in figure 7 are similar from the 15th day downwards, except for the 40th day in which the first sample has a very strong decrease.



*** The codification of the species utilized, is shown in table 2.**

Fig 7: CO_2 fluxes of 4 species combination, utilized 4 different taxa.

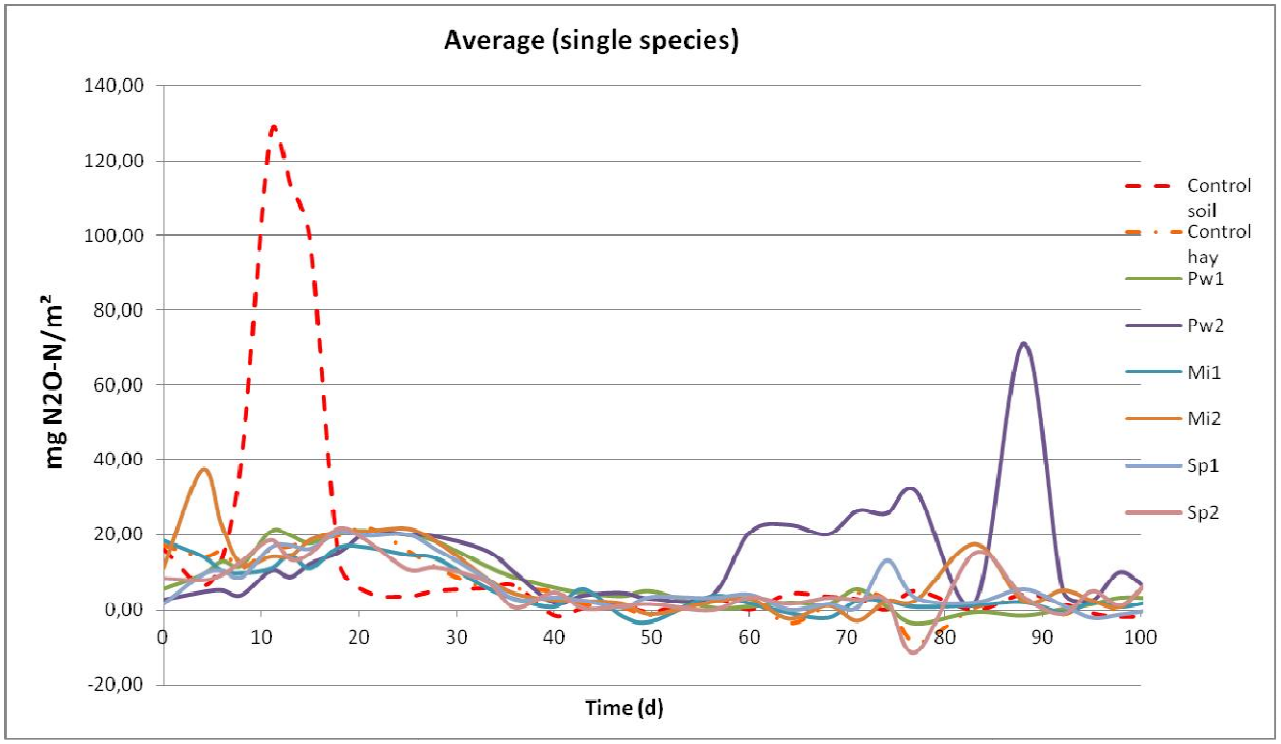


*** The codification of the species utilized, is shown in table 2.**

Fig 8: CO_2 fluxes of 8 species combination, all taxa utilized in the experimental phase.

In figures from 9 to 16 are shown the analogous results shown in figures from 1 to 8, only with reference to N_2O fluxes.

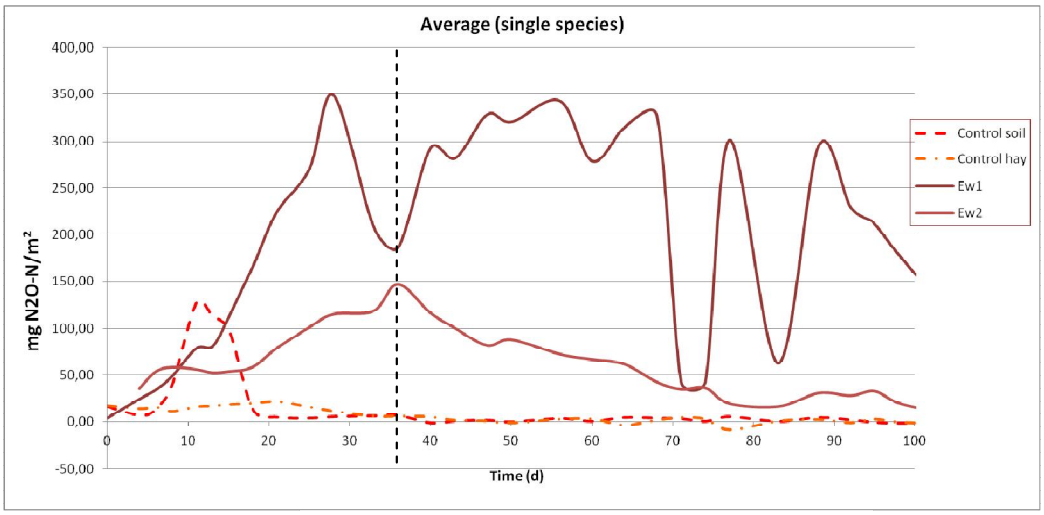
Regarding to the measurements of N_2O fluxes, similarly to the CO_2 fluxes, seem to be present complex interactions and artefacts. The most evident artefact being that of the maximum peak present at the 10th day for the control soil. Neglecting the evident artefacs in the trends, the results show, overall, an increase in the N_2O fluxes with respect to both the control microcosms, only for the treatments where the earthworms are present. Such an increase starting gradually from the beginning of the experiment and then regarding, more or less, the whole duration. Also in the treatment consisting of only the Potworms (E. Cypticus, with 50 individuals inoculating, *Pw2* case) has been registered a probably actual increase in N_2O fluxes about from the 60th to 90th day of the experiment (see figure 9).



*** The codification of the species utilized, is shown in table 2.**

Fig 9: Fluxes of N_2O of all the single species utilized, excluding the two species of earthworms, reported below.

Including, however, the contribution of the short term interactions/artefacts, some consideration can be done. The comparison of the results of the two earthworm species in figure 10, shows that the Calliginosa specie presents the highest values overall, with greater variations. This presents a maximum peack for N_2O flux at the 28° day, in addition are two evidence decrease on the trend, at the day 71 ° and 83 °. The Rubellus specie presented a rather regular trend, with a maximum peak at the 36° day. The same but opposite situation shown for the Calliginosa species in which was present an high decrease. At the 36° day the Rubellus species presented an increase in N_2O flux.

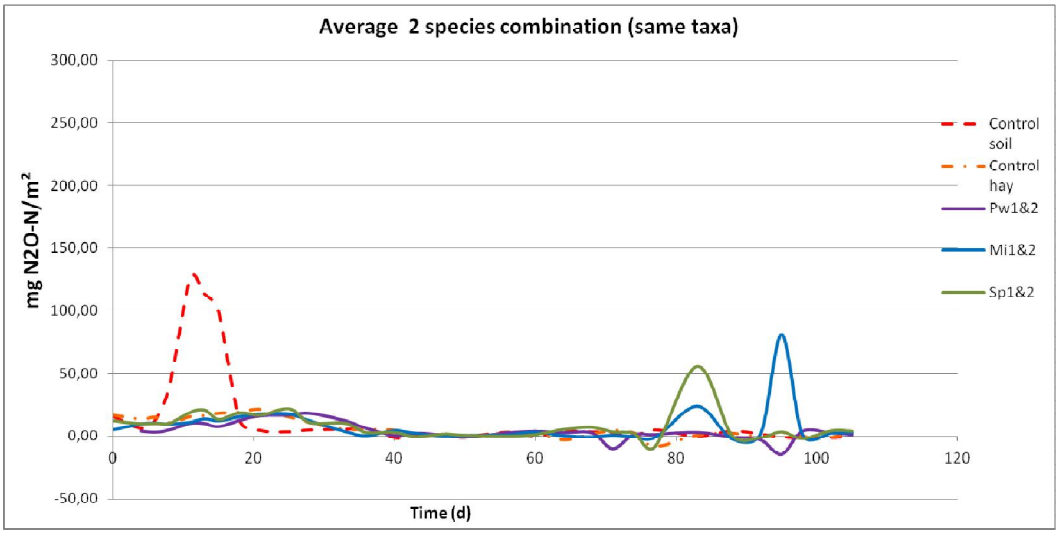


*** The codification of the species utilized, is shown in table 2.**

Fig 10: Fluxes of N_2O of two single species of earthworms.

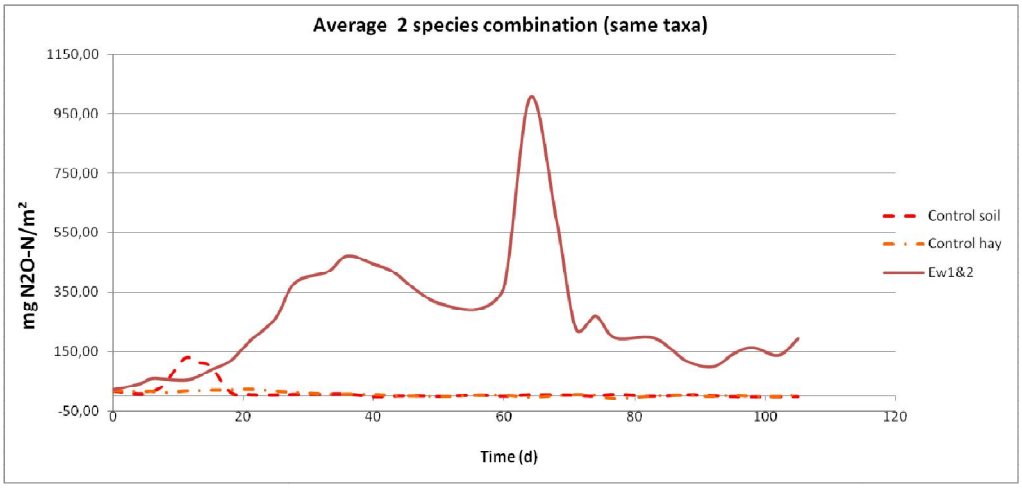
When were inoculated 2 species for each microcosm (utilized the same taxa, see figure 11) results show, comparing the previous case (single specie inoculated for each microcosm) the treatment "Pw1 Pw2" s doesn't show high values for N_2O measurements (25 individuals for each species).

For the treatment " Mites 1 and 2" two peaks at 95° and 83° day are present, together with the other treatment "Springtail 1 and 2". Finally for the 2 earthworms species inoculated together, the peak at the 64° day equal to 1006 (mg N_2O-N/m²).



*** The codification of the species utilized, is shown in table 2.**

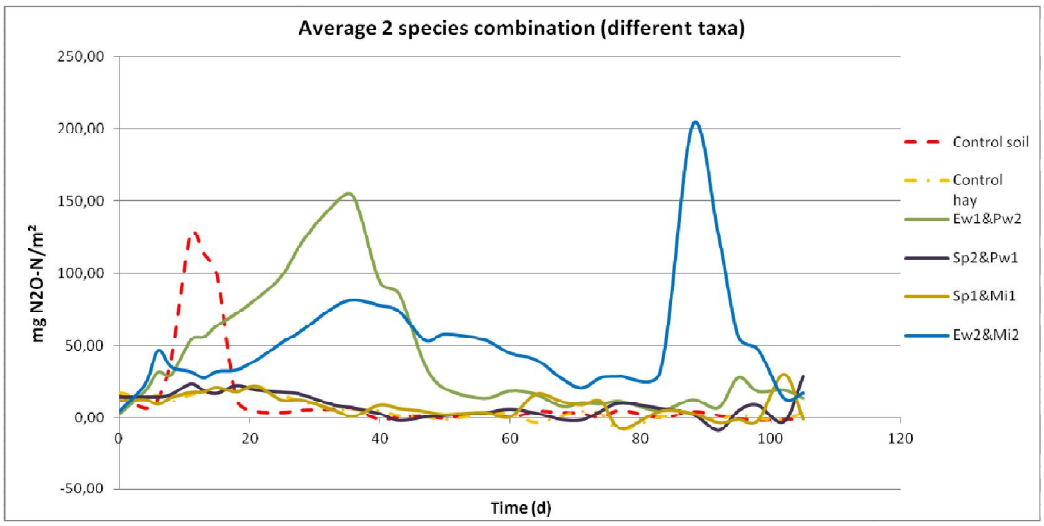
Fig 11: Fluxes of N_2O, here reported below the fluxes of the same taxa, inoculating 2 species of the same taxa in each microcosm.



*** The codification of the species utilized, is shown in table 2.**

Fig 12: Fluxes of N_2O, here shown only the inoculum of two different species of earthworm together.

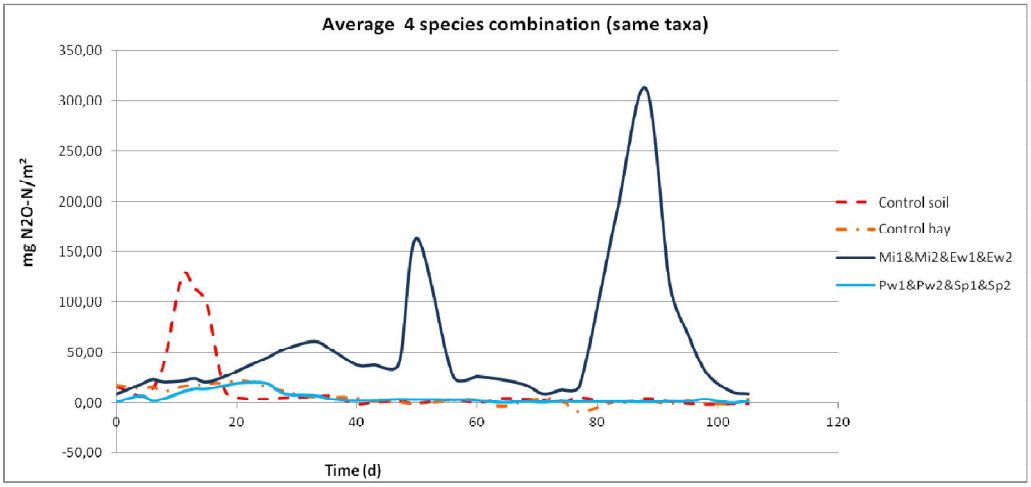
In the four treatments each with 2 species of different taxa inside inoculated (see figure 13), two samples with a high value in the N_2O fluxes can be noted. Namely the sample " Ew1- Pw2" and "Ew2-Mi2" present both two peak at the 6° and at 36° day. The same for the day 88th but for "Ew1-Pw2" the increase was very high.



*** The codification of the species utilized, is shown in table 2.**

Fig 13: N_2O fluxes of 2 different specie combination (of different taxa)

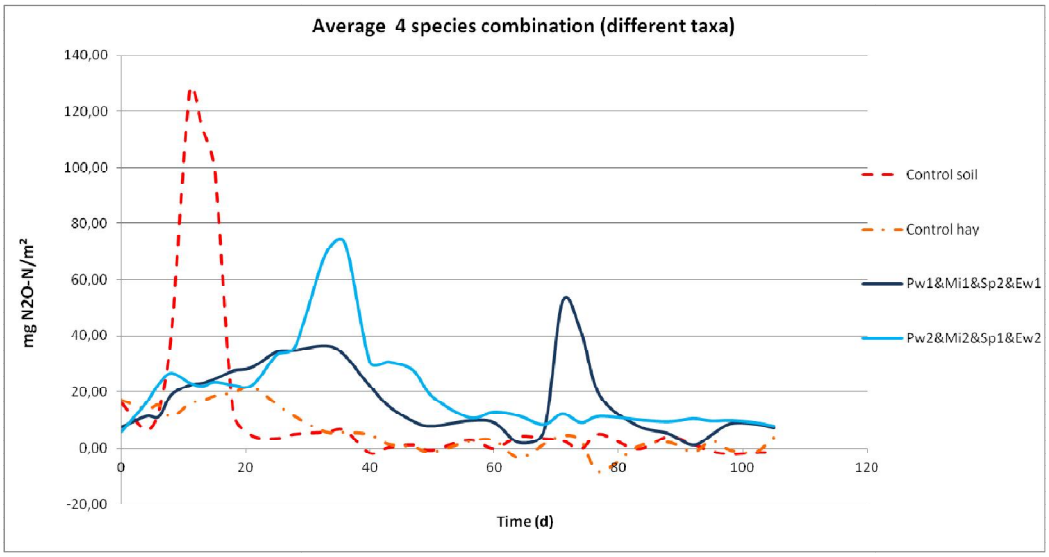
As stated previously, the samples with higher values were those with the two species of earthworm inside. The treatment " Mi1-Mi2-Ew1-Ew2" (fig. 14) contains one specie of Rubellus, one of Calliginosa, 100 individuals of Mites (R. Robini) and 100 of the other species of Mites (O. Nitens). A maximum peak at 88° day is shown and another one at 50° day and the last at 33° day.



*** The codification of the species utilized, is shown in table 2.**

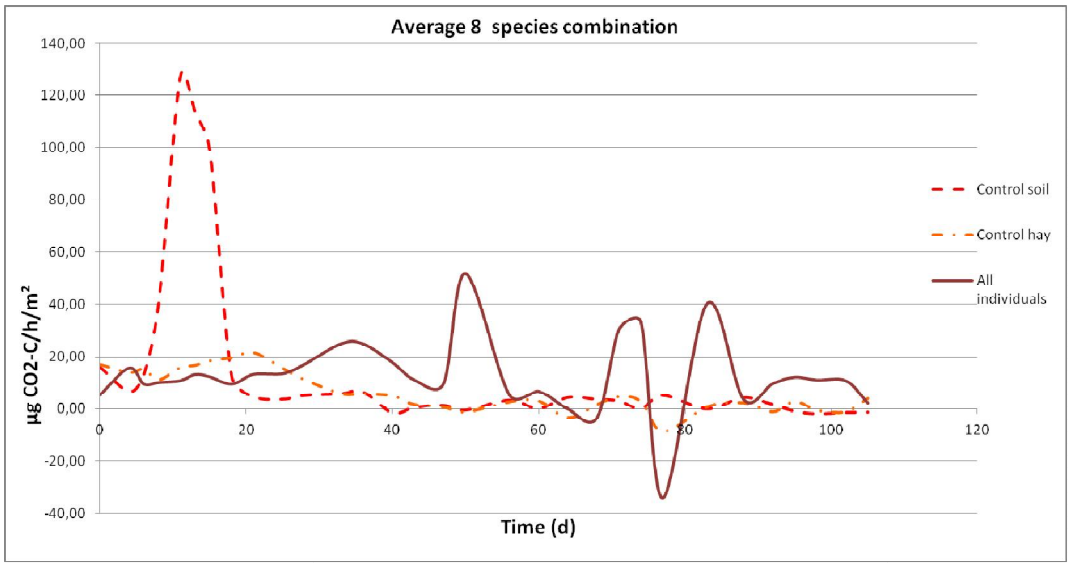
Fig 14: N_2O fluxes of 4 species combination, utilized 2 different taxa (then 4 different species).

For the case of four species of different taxa inoculated in each microcosm (figure 15) the treatment "Pw1-Mi1-Sp2-Ew1" (in which are present the Calliginosa specie) a maximum peack equal to 52,25 (mg N2O-N/m²) is present. For the other treatment (Pw2-Mi2-Sp1-Ew2) a maximum peak of 73,09 at the 36° day is shown.



*** The codification of the species utilized, is shown in table 2.**

Fig 15: N_2O fluxes of 4 species combination, utilized 4 different taxa.



*** The codification of the species utilized, is shown in table 2.**

Fig 16: N_2O fluxes of 8 species combination, all taxa utilized in the experimental phase.

Considering all 8 species together (figure 16), three peak in which the fluxes increase can be found, namely at 50°, 74° and 83° day, and an evident decrease at 77° day equal to -33,82 (mg N_2O-N/m²) is shown.

The other measurements of the experiment (Total dissolved N (Nts), ammonia (NH_4^+), nitrate and nitrite (NO_3^- NO_2^-) concentrations, colorimetrically measured in a K2SO4 extract, the dissolved organic nitrogen (DON) content, NH4+ and (NO_3^- NO_2^-) were subtracted from Nts), together with the dissolved Organic carbon (DOC) have not been reported here, as they have been made by the Group of Wageningen University. Here are reported in figure 17 only the pH results of the mesocosms of each treatment at the end of the experiment, averaged across the replicates. Not significant differences in the overall average value of 5.78 can be noted except that the minimum and maximum value of 5.45 and 6.03 were obtained for Ew2 and Ew1 treatments, respectively.

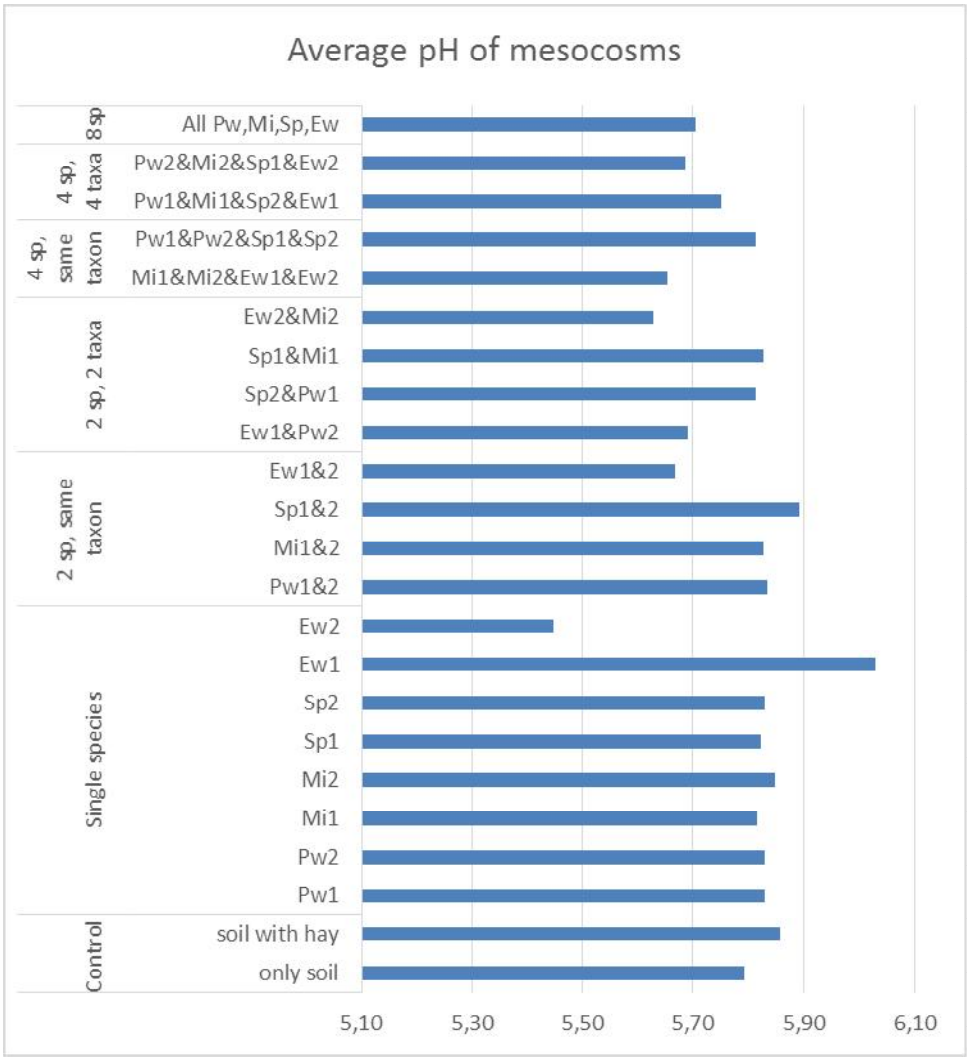


Fig 17: average pH values of mesocosms for each treatment at the end of the experiment.

5.4 Conclusion remarks:

Excluding some experimental artefacts, outcomes of the experiment described in this chapter allow to state that an increase in the soil respiration and in the fluxes of N_2O has been essentially provided by the presence of the earthworms, with different trends and timing for CO_2 and N_2O respect to the duration of the experiment. Fluxes of N_2O seemed also to be increased in the case of presence of only Potworms in the mesocosms.

However, the complex trends found as output of this experiment show the huge difficulty to control all the variables involved in complex processes involving living organisms like the soil respiration and the soil emission of N_2O. In other words it seems very difficult, although there have been many efforts, to fix the many control conditions into each microcosm. Therefore, a further in depth investigation is necessary also by analysing the other data produced in this experiment and not available for this thesis. Moreover, such a large and complex experimental phase should probably need more time to analyze and investigate all the interactions among the species.

General Conclusion:

The work carried out in this thesis, thanks to its innovative multi-step experimental approach, has allowed addressing, with a more comprehensive view, the complex relationships between soil fauna and its habitat in relation with either, different land uses and different scales. In fact, information provided with this thesis, spans from the study of the contribution of the soil fauna in defining the ecological soil quality at landscape scale, to the investigation on the relationships between soil fauna and soil structure evaluated at farm scale, till the identification of the contribution of the single taxa to the soil pore system. Moreover, the interactions between soil fauna and soil at global scale have also been addressed thanks to an experiment on the role of soil fauna in regulating emission from soil of greenhouses gases.

After the literature review of the first chapter, which led to formalise the aims of this thesis, the field experiment described in the second chapter proved that the widely used Qbs-index (QBS-ar) is very suitable to determine the real ecological condition of the soil environment considered. However it has the limitation to give information only to a specific aspect of the soil quality, namely the soil biodiversity. In particular, for the Telesina Valley a general good soil quality was found, and there wasn't significant difference in taxa abundance for different land uses.

The experiment reported in the third chapter was partially conducted in the field to collect the soil taxa and partially in the lab to quantify the soil structure organisation, and allowed to point out the correlation between the heterogeneity of the soil structure and the soil fauna abundance. Despite this results, the used approach showed difficulties in the identification of the specific relationships between each soil fauna species and the produced soil pores. However in the Telesina Valley has been found that a higher multimodality of the soil pore size distribution was correlated to the less anthropic land uses. Such finding was in agreement with the high risk of potential threats for soil fauna in human intensively exploited areas described in very recent literature.

The experiment described in the fourth chapter was conducted on repacked soil mesocosms inoculated with different taxa of soil fauna and then incubated in the field. It allowed to define new protocols for both, soil fauna lab inoculation of taxa different from earthworms, and 3D bio-pore image analysis aiming at geometrically quantify the burrow activity of those taxa. In particular, outcomes of such experiment seem to open new perspectives for a more proper and complete evaluation of other soil taxa as "soil ecosystem engineers" for soil quality improvement.

The fully lab experiment described in the last chapter, although well-conceived and conducted, made evident the huge difficulty to control all the variables that play a role in the complex processes involving living organisms like the soil respiration and the soil emission of N_2O. However, excluding some evident artefacts in the results, outcomes of the described experiment allowed to state that an increase in the soil respiration and in the fluxes of N_2O was essentially provided by the presence of the earthworms, with different trends and variable timing respect to the duration of the experiment for CO_2 and N_2O. Fluxes of N_2O seemed also to be increased in the case of presence of only Potworms in the mesocosms. Overall considered, the results obtained with this experiment can be seen as a very useful premise for a very important future research aiming at understanding the complex interactions between different combination of soil fauna species and the soil greenhouse gases emission.

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Additional material:

Additional material concerning the chapter II:

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Araneae	0,0	1,7	0,3	0,0
	0,7	0,0	1,0	0,7
		0,7	1,0	
			0,0	
			1,3	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	0,7	0,3	0,2	0,2	0,4	-5,2	5,9
Olive groves	3,0	2,3	0,8	0,7	1,4	0,4	-0,8	2,3
Viticulture	5,0	3,7	0,7	0,3	1,2	0,3	0,0	1,5
Arable	2,0	0,7	0,3	0,2	0,2	0,4	-5,2	5,9
Sources	***SS***	***df***	***MS***	***F***	***P value***	***F crit***	***RMSSE***	***Omega Sq***
Between Groups	0,5	3,0	0,2	0,4	0,8	4,1	0,4	-0,2
Within Groups	3,1	8,0	0,4					
Total	3,52	11	0,32					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
W	1,0	0,9	Alpha	0,1	type	p-value
p-value	0,8	0,5	F-stat	0,4	means	0,64156
alpha	0,1	0,1	df1	3,0	medians	0,905549
normal	yes	yes	df2	3,0	trimmed	0,64156
			p-value	0,8		
			sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Opilionida	0,7	11,0	0,0	1,7
	2,7	1,7	0,0	0,0
		1,7	0,3	
			5,3	
			0,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	3,3	1,7	2,0	2,0	2,3	-27,4	30,7
Olive groves	3,0	14,3	4,8	29,0	58,1	1,9	-3,3	12,8
Viticulture	5,0	5,7	1,1	5,5	22,1	1,4	-2,9	5,1
Arable	2,0	1,7	0,8	1,4	1,4	2,3	-28,2	29,9

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	29,76	3,00	9,92	0,95	0,46	4,07	0,56	-0,01
Within Groups	83,60	8,00	10,45					
Total	113,36	11,00	10,31					

Shapiro-Wilk Test	*Olive*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			Alpha	0,1	type	p-value
W	0,8	0,6	F-stat	0,4	means	0,069044
p-value	0,0	0,0	df1	3,0	medians	0,806691
alpha	0,1	0,1	df2	3,4	trimmed	0,069044
normal	no	no	p-value	0,8		
			sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Pseudoscorpionida	0,0	0,0	0,3	0,0
	0,7	0,0	0,0	0,0
		0,3	0,7	
			1,3	
			0,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	0,7	0,3	0,2	0,2	0,3	-3,6	4,3
Olive groves	3,0	0,3	0,1	0,0	0,1	0,3	-1,0	1,2
Viticulture	5,0	2,3	0,5	0,3	1,2	0,2	-0,1	1,0
Arable	2,0	0,0	0,0	0,0	0,0	0,3	-3,9	3,9

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	0,42	3,00	0,14	0,73	0,56	4,07	0,48	-0,07
Within Groups	1,54	8,00	0,19					
Total	1,96	11,00	0,18					

	Olive	*Viticulture*	*Welch's Test*		*Levene's Tests*	
Shapiro-Wilk Test			Alpha	0,05	type	p-value
W	0,8	0,9	F-stat	1,2	means	0,133498
p-value	0,0	0,3	df1	3,0	medians	0,323288
alpha	0,1	0,1	df2	2,9	trimmed	0,133498
normal	no	yes	p-value	0,5		
			sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Isopoda	0,0	1,7	0,0	4,7
	4,0	4,7	0,0	0,0
		2,0	0,0	
			1,7	
			0,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	4,0	2,0	8,0	8,0	1,3	-14,4	18,4
Olive groves	3,0	8,3	2,8	2,7	5,4	1,1	-1,7	7,3
Viticulture	5,0	1,7	0,3	0,6	2,2	0,8	-1,9	2,6
Arable	2,0	4,7	2,3	10,9	10,9	1,3	-14,0	18,7

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	13,56	3,00	4,52	1,36	0,32	4,07	0,59	0,08
Within Groups	26,52	8,00	3,31					
Total	40,07	11,00	3,64					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			Alpha	0,05	type	p-value
W	0,8	0,6	F-stat	1,42	means	0,003179
p-value	0,2	0,0	df1	3,00	medians	0,085976
alpha	0,1	0,1	df2	2,15	trimmed	0,003179
normal	yes	no	p-value	0,4		
			sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Chilopoda	0,3	1,7	0,0	0,0
	0,0	0,0	0,7	0,0
		0,0	0,0	
			1,0	
			0,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	0,3	0,2	0,1	0,1	0,4	-5,1	5,5
Olive groves	3,0	1,7	0,6	0,9	1,9	0,3	-0,9	2,0
Viticulture	5,0	1,7	0,3	0,2	0,9	0,3	-0,4	1,1
Arable	2,0	0,0	0,0	0,0	0,0	0,4	-5,3	5,3

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	0,42	3,00	0,14	0,40	0,76	4,07	0,40	-0,18
Within Groups	2,80	8,00	0,35					
Total	3,21	11,00	0,29					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			Alpha	0,05	type	p-value
W	0,8	0,8	F-stat	1,02	means	0,012667
p-value	0,0	0,0	df1	3,00	medians	0,75372
alpha	0,1	0,1	df2	2,86	trimmed	0,012667
normal	no	no	p-value	0,53		
			sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Diplopoda	0,0	0,3	0,0	0,0
	0,0	0,0	0,0	0,0
		0,0	0,0	
			0,3	
			0,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	0,0	0,0	0,0	0,0	0,1	-1,3	1,3
Olive groves	3,0	0,3	0,1	0,0	0,1	0,1	-0,2	0,5
Viticulture	5,0	0,3	0,1	0,0	0,1	0,1	-0,1	0,2
Arable	2,0	0,0	0,0	0,0	0,0	0,1	-1,3	1,3

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	0,02	3,00	0,01	0,36	0,78	4,07	0,38	-0,19
Within Groups	0,16	8,00	0,02					
Total	0,19	11,00	0,02					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			Alpha	0,05	type	p-value
W	0,8	0,6	F-stat	0,50	type	p-value
p-value	0,0	0,0	df1	3,00	means	0,104726
alpha	0,1	0,1	df2	3,98	medians	0,781256
normal	no	no	p-value	0,71	trimmed	0,104726
			sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Pauropoda	0,0	0,7	0,0	0,3
	0,0	0,0	0,0	0,0
		0,0	0,0	
			0,0	
			0,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	0,0	0,0	0,0	0,0	0,1	-1,9	1,9
Olive groves	3,0	0,7	0,2	0,1	0,3	0,1	-0,3	0,7
Viticulture	5,0	0,0	0,0	0,0	0,0	0,1	-0,3	0,3
Arable	2,0	0,3	0,2	0,1	0,1	0,1	-1,7	2,1

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	0,12	3,00	0,04	0,91	0,48	4,07	0,55	-0,02
Within Groups	0,35	8,00	0,04					
Total	0,472	11,000	0,043					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
W	0,8	0,7	Alpha	0,05	type	p-value
p-value	0,0	0,0	F-stat	0,43	means	0,001018
alpha	0,1	0,1	df1	3,00	medians	0,409891
normal	no	no	df2	2,30	trimmed	0,001018
			p-value	0,76		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Symphila	2,7	5,3	0,7	1,3
	0,3	2,3	0,0	0,0
		0,7	0,7	
			0,0	
			0,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	3,0	1,5	2,7	2,7	1,0	-10,9	13,9
Olive groves	3,0	8,3	2,8	5,6	11,2	0,8	-0,7	6,2
Viticulture	5,0	1,3	0,3	0,1	0,5	0,6	-1,5	2,0
Arable	2,0	1,3	0,7	0,9	0,9	1,0	-11,8	13,1

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	12,56	3,00	4,19	2,18	0,17	4,07	0,80	0,23
Within Groups	15,33	8,00	1,92					
Total	27,89	11,00	2,54					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	0,9	type	p-value
W	1,0	0,7	df1	3,0	means	0,048645
p-value	0,7	0,0	df2	2,1	medians	0,222331
alpha	0,1	0,1	p-value	0,6	trimmed	0,048645
normal	yes	no	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Protura	0,0	0,3	0,0	0,0
	0,0	0,0	0,0	0,3
		0,0	0,0	
			0,0	
			0,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	0,0	0,0	0,0	0,0	0,1	-1,1	1,1
Olive groves	3,0	0,3	0,1	0,0	0,1	0,1	-0,2	0,4
Viticulture	5,0	0,0	0,0	0,0	0,0	0,1	-0,2	0,2
Arable	2,0	0,3	0,2	0,1	0,1	0,1	-1,0	1,3

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	0,06	3,00	0,02	1,14	0,39	4,07	0,65	0,03
Within Groups	0,13	8,00	0,02					
Total	0,185	11,000	0,017					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	0,5	type	p-value
W	0,8	0,6	df1	3,0	means	0,000275
p-value	0,0	0,0	df2	2,2	medians	0,19251
alpha	0,1	0,1	p-value	0,7	trimmed	0,000275
normal	no	no	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Diplura	0,0	2,0	0,0	0,0
	1,3	1,3	0,0	0,0
		0,7	0,0	
			0,0	
			1,7	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	1,3	0,7	0,9	0,9	0,5	-5,7	7,0
Olive groves	3,0	4,0	1,3	0,4	0,9	0,4	-0,4	3,1
Viticulture	5,0	1,7	0,3	0,6	2,2	0,3	-0,5	1,2
Arable	2,0	0,0	0,0	0,0	0,0	0,5	-6,4	6,4

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	2,69	3,00	0,90	1,80	0,23	4,07	0,81	0,17
Within Groups	4,00	8,00	0,50					
Total	6,69	11,00	0,61					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	0,5	type	p-value
W	1,0	0,6			means	0,340306
p-value	1,0	0,0			medians	0,692628
alpha	0,1	0,1			trimmed	0,340306
normal	yes	no				
			sig			

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Collembola	44,3	61,7	10,0	26,7
	17,7	17,7	62,3	20,0
		52,7	11,0	
			54,7	
			17,3	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	62,0	31,0	355,6	355,6	15,9	-170,6	232,6
Olive groves	3,0	132,0	44,0	540,3	1080,7	13,0	-11,7	99,7
Viticulture	5,0	155,3	31,1	642,4	2569,6	10,0	3,2	58,9
Arable	2,0	46,7	23,3	22,2	22,2	15,9	-178,3	224,9

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	576,58	3,00	192,19	0,38	0,77	4,07	0,38	-0,18
Within Groups	4028,09	8,00	503,51					
Total	4604,67	11,00	418,61					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	0,6	type	p-value
W	0,9	0,8	df1	3,0	means	0,042749
p-value	0,4	0,1	df2	3,1	medians	0,747019
alpha	0,1	0,1	p-value	0,6	trimmed	0,042749
normal	yes	yes	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Microcoryphia	0,0	0,0	0,0	0,0
	0,3	0,0	0,7	0,0
		0,0	0,0	
			2,7	
			0,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	0,3	0,2	0,1	0,1	0,6	-7,2	7,5
Olive groves	3,0	0,0	0,0	0,0	0,0	0,5	-2,0	2,0
Viticulture	5,0	3,3	0,7	1,3	5,3	0,4	-0,4	1,7
Arable	2,0	0,0	0,0	0,0	0,0	0,6	-7,4	7,4

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	1,16	3,00	0,39	0,57	0,65	4,07	0,38	-0,12
Within Groups	5,39	8,00	0,67					
Total	6,546	11,000	0,595					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	0,6	type	p-value
W	0,8	0,7	df1	3,0	means	0,176814
p-value	0,0	0,0	df2	2,8	medians	0,645235
alpha	0,1	0,1	p-value	0,7	trimmed	0,176814
normal	no	no	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Dermaptera	0,0	0,7	0,0	0,0
	0,0	0,0	0,0	0,0
		0,0	0,0	
			0,0	
			0,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	0,0	0,0	0,0	0,0	0,1	-1,7	1,7
Olive groves	3,0	0,7	0,2	0,1	0,3	0,1	-0,3	0,7
Viticulture	5,0	0,0	0,0	0,0	0,0	0,1	-0,2	0,2
Arable	2,0	0,0	0,0	0,0	0,0	0,1	-1,7	1,7

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	0,11	3,00	0,04	1,00	0,44	4,07	0,58	0,00
Within Groups	0,30	8,00	0,04					
Total	0,407	11,000	0,037					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	0,2	type	p-value
W	0,8	0,7	df1	3,0	means	0,000966
p-value	0,0	0,0	df2	2,6	medians	0,441256
alpha	0,1	0,1	p-value	0,9	trimmed	0,000966
normal	no	no	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Embioptera	0,0	0,0	0,0	0,0
	0,0	0,3	0,0	2,7
		0,3	0,7	
			0,0	
			0,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	0,0	0,0	0,0	0,0	0,5	-6,3	6,3
Olive groves	3,0	0,7	0,2	0,0	0,1	0,4	-1,5	2,0
Viticulture	5,0	0,7	0,1	0,1	0,4	0,3	-0,7	1,0
Arable	2,0	2,7	1,3	3,6	3,6	0,5	-5,0	7,7

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	2,46	3,00	0,82	1,65	0,25	4,07	0,87	0,14
Within Groups	3,99	8,00	0,50					
Total	6,44	11,00	0,59					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	1,4	type	p-value
W	0,8	0,6	df1	3,0	means	2,02E-05
p-value	0,0	0,0	df2	2,9	medians	0,000985
alpha	0,1	0,1	p-value	0,4	trimmed	2,02E-05
normal	no	no	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Blattaria	0,0	0,0	2,7	0,0
	0,0	0,0	1,7	0,0
		0,0	0,0	
			0,0	
			0,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	0,0	0,0	0,0	0,0	0,6	-7,9	7,9
Olive groves	3,0	0,0	0,0	0,0	0,0	0,5	-2,2	2,2
Viticulture	5,0	4,3	0,9	1,5	6,1	0,4	-0,2	2,0
Arable	2,0	0,0	0,0	0,0	0,0	0,6	-7,9	7,9

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	2,19	3,00	0,73	0,95	0,46	4,07	0,49	-0,01
Within Groups	6,13	8,00	0,77					
Total	8,32	11,00	0,76					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	0,6	type	p-value
W	0,8	0,8	df1	3,0	means	0,002772
p-value	0,0	0,0	df2	3,3	medians	0,460193
alpha	0,1	0,1	p-value	0,7	trimmed	0,002772
normal	no	no	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Psocoptera	3,7	0,0	2,7	0,0
	0,0	0,0	0,0	3,3
		0,0	0,3	
			0,0	
			0,7	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	3,7	1,8	6,7	6,7	1,0	-11,4	15,0
Olive groves	3,0	0,0	0,0	0,0	0,0	0,8	-3,6	3,6
Viticulture	5,0	3,7	0,7	1,2	5,0	0,7	-1,1	2,6
Arable	2,0	3,3	1,7	5,6	5,6	1,0	-11,5	14,9

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	5,48	3,00	1,83	0,85	0,51	4,07	0,58	-0,04
Within Groups	17,26	8,00	2,16					
Total	22,74	11,00	2,07					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	0,9	type	p-value
W	0,8	0,8	df1	3,0	means	0,011254
p-value	0,0	0,0	df2	2,2	medians	0,046395
alpha	0,1	0,1	p-value	0,6	trimmed	0,011254
normal	no	no	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Hemiptera	0,3	8,3	5,7	0,0
	6,0	3,3	0,0	2,0
		3,0	2,0	
			1,0	
			4,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	6,3	3,2	16,1	16,1	1,9	-20,8	27,1
Olive groves	3,0	14,7	4,9	8,9	17,9	1,5	-1,7	11,5
Viticulture	5,0	12,7	2,5	5,3	21,0	1,2	-0,8	5,8
Arable	2,0	2,0	1,0	2,0	2,0	1,9	-23,0	25,0

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	19,84	3,00	6,61	0,93	0,47	4,07	0,60	-0,02
Within Groups	56,93	8,00	7,12					
Total	76,77	11,00	6,98					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	0,9	type	p-value
W	0,8	1,0	df1	3,0	means	0,255143
p-value	0,1	0,8	df2	2,9	medians	0,758354
alpha	0,1	0,1	p-value	0,6	trimmed	0,255143
normal	yes	yes	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Tysanoptera	0,0	0,0	4,3	8,7
	6,0	0,0	5,0	2,0
		5,0	1,0	
			0,0	
			5,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	6,0	3,0	18,0	18,0	2,2	-25,3	31,3
Olive groves	3,0	5,0	1,7	8,3	16,7	1,8	-6,2	9,5
Viticulture	5,0	15,3	3,1	5,7	22,8	1,4	-0,9	7,0
Arable	2,0	10,7	5,3	22,2	22,2	2,2	-23,0	33,7

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	16,16	3,00	5,39	0,54	0,67	4,07	0,48	-0,13
Within Groups	79,64	8,00	9,96					
Total	95,81	11,00	8,71					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	0,9	type	p-value
W	0,8	0,8	df1	3,0	means	0,14881
p-value	0,0	0,1	df2	2,4	medians	0,712034
alpha	0,1	0,1	p-value	0,9	trimmed	0,14881
normal	no	yes	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Coleoptera	0,0	0,0	0,0	2,0
	1,0	0,3	0,7	0,7
		0,7	0,3	
			0,0	
			1,3	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	1,0	0,5	0,5	0,5	0,4	-4,9	5,9
Olive groves	3,0	1,0	0,3	0,1	0,2	0,3	-1,2	1,8
Viticulture	5,0	2,3	0,5	0,3	1,2	0,3	-0,3	1,2
Arable	2,0	2,7	1,3	0,9	0,9	0,4	-4,0	6,7

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	1,39	3,00	0,46	1,30	0,34	4,07	0,76	0,07
Within Groups	2,86	8,00	0,36					
Total	4,25	11,00	0,39					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	0,5	type	p-value
W	1,0	0,9	df1	3,0	means	0,255469
p-value	1,0	0,3	df2	2,5	medians	0,398908
alpha	0,1	0,1	p-value	0,7	trimmed	0,255469
normal	yes	yes	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Hymenoptera	32,0	80,7	47,7	30,0
	21,0	30,3	1,3	0,0
		30,3	15,3	
			28,0	
			10,7	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	53,0	26,5	60,5	60,5	14,8	-161,0	214,0
Olive groves	3,0	141,3	47,1	844,5	1689,0	12,0	-4,7	99,0
Viticulture	5,0	103,0	20,6	321,2	1285,0	9,3	-5,3	46,5
Arable	2,0	30,0	15,0	450,0	450,0	14,8	-172,5	202,5

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	1705,74	3,00	568,58	1,31	0,34	4,07	0,67	0,07
Within Groups	3484,44	8,00	435,56					
Total	5190,19	11,00	471,84					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	0,6	type	p-value
W	0,8	1,0	df1	3,0	means	0,23
p-value	0,0	0,8	df2	3,1	medians	0,90
alpha	0,1	0,1	p-value	0,7	trimmed	0,23
normal	no	yes	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Diptera Larvae	6,0	8,3	1,7	3,7
	1,3	2,3	5,3	2,3
		3,0	3,0	
			4,7	
			2,3	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	7,3	3,7	10,9	10,9	1,6	-17,2	24,5
Olive groves	3,0	13,7	4,6	10,8	21,6	1,3	-1,2	10,3
Viticulture	5,0	17,0	3,4	2,4	9,6	1,0	0,5	6,3
Arable	2,0	6,0	3,0	0,9	0,9	1,6	-17,8	23,8

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	3,61	3,00	1,20	0,22	0,88	4,07	0,28	-0,24
Within Groups	43,05	8,00	5,38					
Total	46,67	11,00	4,24					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	0,2	type	p-value
W	0,8	0,9	df1	3,0	means	0,057
p-value	0,2	0,6	df2	2,9	medians	0,676
alpha	0,1	0,1	p-value	0,9	trimmed	0,057
normal	yes	yes	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Coleoptera larvae	1,0	0,0	1,0	1,0
	0,3	0,0	3,3	1,3
		0,3	0,3	
			2,3	
			3,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	1,3	0,7	0,2	0,2	0,7	-7,7	9,1
Olive groves	3,0	0,3	0,1	0,0	0,1	0,5	-2,2	2,4
Viticulture	5,0	10,0	2,0	1,7	6,7	0,4	0,8	3,2
Arable	2,0	2,3	1,2	0,1	0,1	0,7	-7,2	9,6

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	7,31	3,00	2,44	2,78	0,11	4,07	0,86	0,31
Within Groups	7,02	8,00	0,88					
Total	14,33	11,00	1,30					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	8,1	type	p-value
W	0,8	0,9	df1	3,0	means	0,019
p-value	0,0	0,5	df2	3,0	medians	0,155
alpha	0,1	0,1	p-value	0,1	trimmed	0,019
normal	no	yes	sig	no		

Land Uses (average)				
Species	Forest	Olive groves	Viticulture	Arable
Olometabola	23,0	33,0	27,3	6,0
	12,3	12,3	27,3	14,7
		11,7	11,7	
			27,7	
			12,0	

Description	ANOVA Single factor (α =0,05)							
Groups	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Forest	2,0	35,3	17,7	56,9	56,9	6,5	-65,2	100,6
Olive groves	3,0	57,0	19,0	147,1	294,2	5,3	-3,9	41,9
Viticulture	5,0	106,0	21,2	73,1	292,6	4,1	9,7	32,7
Arable	2,0	20,7	10,3	37,6	37,6	6,5	-72,6	93,2

Sources	*SS*	*df*	*MS*	*F*	*P value*	*F crit*	*RMSSE*	*Omega Sq*
Between Groups	171,23	3,00	57,08	0,67	0,59	4,07	0,51	-0,09
Within Groups	681,24	8,00	85,16					
Total	852,47	11,00	77,50					

Shapiro-Wilk Test	*Olive groves*	*Viticulture*	*Welch's Test*		*Levene's Tests*	
			F-stat	8,1	type	p-value
W	0,8	0,7	df1	3,0	means	0,162985
p-value	0,1	0,0	df2	3,1	medians	0,984148
alpha	0,1	0,1	p-value	0,5	trimmed	0,162985
normal	yes	no	sig	no		

Additional material concerning the chapter IV:

	Cumulative average of N2O fluxes mg N2O-N/m²																					
Time (d)	Control soil	Control hay	Pw1	Pw2	M1	M2	Sp1	Sp2	Ew1	Ew2	Pw1&2	M1&2	Sp1&2	Ew1&2	Ew1&Pw2	Sp2&Pw1	Sp1&M1	Ew2&M2	M1&M2& Ew1&Ew2	Pw1&Pw2& p1&Sp2	Pw1&M1& Sp2&Ew1	Pw2&M2& Sp1&Ew2	All individuals
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0,15	0,33	0,22	0,12	0,34	0,89	0,23	0,19	0,60	0,87	0,10	0,24	0,24	0,93	0,44	0,34	0,29	0,54	0,42	0,17	0,27	0,39	0,38
6	0,48	0,71	0,53	0,25	0,59	1,45	0,48	0,42	1,45	2,17	0,19	0,50	0,50	2,32	1,20	0,68	0,52	1,67	0,97	0,23	0,55	0,94	0,61
8	1,93	1,13	0,97	0,40	0,96	1,92	0,81	0,89	3,25	4,32	0,38	0,87	0,89	4,35	2,29	1,27	1,02	2,94	1,71	0,39	1,25	1,93	0,99
11	5,05	1,55	1,50	0,66	1,25	2,32	1,23	1,37	5,24	5,83	0,65	1,17	1,39	5,73	3,66	1,87	1,47	3,80	2,28	0,66	1,83	2,55	1,29
13	7,90	1,99	2,01	0,89	1,63	2,72	1,67	1,72	7,31	7,23	0,92	1,54	1,92	7,37	5,09	2,36	1,92	4,54	2,90	1,00	2,43	3,14	1,64
15	11,7	2,7	2,7	1,3	2,1	3,4	2,3	2,3	11,5	9,3	1,2	2,0	2,5	10,7	7,5	3,0	2,7	5,8	3,7	1,5	3,4	4,1	2,1
18	12,5	3,5	3,5	1,9	2,7	4,3	3,1	3,2	18,0	11,8	1,7	2,7	3,2	15,3	10,4	3,9	3,4	7,2	4,7	2,2	4,5	5,0	2,5
21	13,2	4,7	4,7	3,0	3,6	5,5	4,2	4,1	29,4	16,0	2,6	3,6	4,2	24,7	14,6	4,9	4,6	9,3	6,5	3,2	6,0	6,3	3,3
25	14,0	5,5	5,6	3,9	4,3	6,5	5,1	4,7	40,6	20,4	3,3	4,4	5,1	35,3	18,9	5,8	5,3	11,6	8,4	4,1	7,6	7,8	3,9
28	14,8	6,3	6,9	5,2	5,3	7,8	6,2	5,6	62,9	28,0	4,5	5,4	6,0	59,5	26,8	7,0	6,2	15,6	11,9	4,7	9,9	10,2	5,1
33	15,9	6,9	7,7	6,0	5,8	8,6	6,9	6,2	74,2	34,0	5,3	5,8	6,7	78,2	33,7	7,7	6,7	19,3	14,8	5,3	11,9	13,3	6,3
36	16,7	7,4	8,4	6,7	6,1	9,1	7,3	6,4	85,8	42,2	5,8	6,0	7,1	103,6	42,3	8,3	7,0	23,9	17,8	5,6	13,9	17,3	7,7
40	17,5	8,0	9,0	7,1	6,5	9,6	7,7	6,9	100,5	48,5	6,1	6,5	7,6	124,6	47,7	8,8	7,6	27,8	20,0	6,0	15,3	19,3	8,7
43	18,1	8,3	9,6	7,6	7,0	10,1	8,1	7,2	117,6	55,0	6,4	6,8	7,8	149,1	53,5	9,0	8,2	32,3	22,4	6,3	16,6	21,4	9,6
47	19,1	8,8	10,1	8,1	7,2	10,6	8,5	7,6	135,0	60,6	6,8	7,1	8,3	168,8	57,4	9,5	8,8	35,8	24,9	6,8	17,7	23,4	10,4
50	19,7	9,0	10,9	8,6	7,3	10,9	9,1	8,0	163,0	69,1	6,9	7,4	8,6	197,3	60,9	9,9	9,2	41,2	37,5	7,2	19,0	25,6	14,5
56	21,3	9,8	11,7	9,4	8,0	11,8	9,9	8,5	191,2	77,6	7,6	8,0	9,2	225,5	65,9	10,8	10,0	46,7	41,4	7,9	20,8	28,0	15,8
60	22,3	10,4	12,3	10,8	8,4	12,5	10,6	9,1	213,7	84,5	8,1	8,6	9,7	253,9	70,0	11,6	10,5	51,1	44,6	8,4	22,2	29,9	16,9
64	23,6	10,7	12,9	12,4	8,8	13,0	11,1	9,6	239,1	91,5	8,7	9,0	10,5	314,4	74,1	12,3	11,8	55,5	47,9	8,8	23,4	31,9	17,7
68	24,8	11,3	13,6	13,8	9,1	13,7	11,7	10,2	262,4	97,4	9,2	9,4	11,2	351,0	77,9	12,9	12,8	59,1	50,8	9,3	24,8	33,8	18,4
71	25,8	11,9	14,3	15,2	9,6	14,1	12,1	10,7	273,3	102,2	9,2	9,7	11,8	371,8	81,1	13,3	13,5	61,9	52,9	9,6	27,5	35,4	20,2
74	26,8	12,4	14,8	16,7	10,0	14,7	13,0	11,1	284,7	107,2	9,5	10,1	12,3	394,8	84,4	13,9	14,4	65,1	55,3	10,1	30,0	37,0	22,1
77	28,1	12,2	15,1	19,5	10,4	15,4	13,7	10,7	316,7	112,5	10,0	10,3	12,1	423,1	88,2	15,1	14,4	69,5	58,4	10,5	32,4	39,2	20,4
83	30,1	13,1	16,2	21,1	11,2	17,6	14,8	12,4	343,3	121,6	10,9	12,5	16,3	465,2	94,9	16,6	15,6	76,4	73,6	11,4	35,3	42,6	24,3
88	32,1	14,0	17,1	25,8	12,0	18,7	16,0	13,3	378,0	130,4	11,5	13,2	17,3	498,8	101,2	17,7	16,7	90,7	93,0	12,1	37,6	45,6	26,0
92	33,7	14,7	17,9	27,2	12,5	19,8	16,8	13,9	404,4	137,7	12,0	13,8	18,1	526,2	106,3	18,2	17,4	99,5	101,7	12,8	39,5	48,2	27,5
95	34,9	15,3	18,6	28,2	13,1	20,6	17,3	14,6	426,6	143,9	11,9	17,3	18,9	550,3	111,1	19,1	18,0	105,1	107,8	13,3	41,1	50,2	29,0
98	36,0	15,8	19,4	29,7	13,6	21,4	17,9	15,2	450,6	150,1	12,5	17,9	19,5	577,9	116,0	20,2	18,5	111,2	113,1	13,9	43,0	52,5	30,6
102	37,7	16,5	20,4	31,3	14,3	22,7	18,8	16,3	477,4	157,7	13,2	18,9	20,6	610,6	122,2	21,0	20,4	117,0	118,9	14,6	45,4	55,4	32,4
105	39,0	17,3	21,1	32,4	14,9	23,4	19,4	16,9	502,6	164,4	13,8	19,7	21,5	641,8	127,3	23,1	21,1	122,0	123,6	15,2	47,3	57,7	33,7

Average of CO2 fluxes (µg CO2-C/h/m²)																							
Time (day)	Control soil	Control hay	Pw1	Pw2	Mi1	Mi2	Sp1	Sp2	Ew1	Ew2	Pw1&2	Mi1&2	Sp1&2	Ew1&2	Ew1& Pw2	Sp2& Pw1	Sp1& Mi1	Ew2& Mi2	Mi1&Mi 2&Ew1& Ew2	Pw1&Pw 2&Sp1& Sp2	Pw1&Mi 1&Sp2& Ew1	Pw2&Mi 2&Sp1& Ew2	All individua ls
0	24,4	75,9	64,8	49,1	73,3	67,7	79,8	68,3	81,0	91,4	56,2	76,9	76,4	112,2	68,4	56,7	70,3	85,1	98,6	67,5	90,9	62,4	77,4
4	19,4	32,0	28,7	23,7	25,9	29,6	39,5	28,8	59,1	65,6	26,3	31,1	40,8	67,7	41,5	27,3	27,2	57,5	74,2	35,5	69,0	43,1	46,5
8	25,1	53,7	45,0	35,6	37,3	54,5	44,9	37,8	67,7	68,6	43,7	42,8	43,9	77,1	56,4	34,0	32,4	66,6	61,0	36,5	40,9	53,8	50,9
12	28,0	25,5	65,0	42,6	39,7	49,3	50,4	50,4	69,1	70,8	55,6	37,3	47,0	71,1	61,1	46,3	51,4	7,5	61,8	14,5	45,3	58,9	51,4
15	21,7	39,5	35,4	32,0	44,2	40,7	35,2	35,1	63,3	63,1	34,2	28,7	38,0	77,9	48,9	27,4	37,3	49,6	53,2	34,0	32,9	50,5	43,8
18	21,2	36,1	25,9	32,3	30,7	36,7	31,2	30,2	64,2	51,0	22,0	26,0	26,4	63,1	45,8	26,3	25,2	46,5	44,5	27,7	30,8	45,5	41,2
21	12,3	29,9	19,9	30,3	23,3	36,5	26,4	23,8	61,1	48,2	19,3	22,7	24,2	58,2	38,0	22,0	21,1	41,3	39,5	20,6	26,0	39,7	38,0
25	8,9	32,3	19,1	23,7	25,1	23,0	30,5	19,6	58,1	45,3	26,0	24,9	21,7	44,5	35,5	30,2	20,5	36,9	44,3	23,3	22,7	37,0	38,7
28	13,5	23,2	20,4	25,6	21,6	29,3	24,6	21,4	50,8	47,5	35,4	22,4	24,4	49,6	36,6	25,2	21,6	39,3	40,4	23,2	25,4	36,8	30,9
33	17,2	21,4	20,1	28,8	20,2	14,9	20,6	19,8	44,5	58,9	25,5	17,6	21,1	49,1	36,4	22,4	19,2	43,2	33,0	23,2	25,7	39,8	27,8
36	17,2	22,3	27,2	25,9	18,8	14,9	21,2	19,0	37,0	49,1	24,2	15,7	19,6	44,9	35,4	24,7	21,1	34,6	32,8	22,9	23,7	33,7	25,3
40	6,2	19,0	18,1	21,9	12,0	10,5	14,5	12,8	31,9	31,3	18,5	13,5	15,3	40,3	29,6	13,8	16,2	32,3	25,6	21,9	3,5	27,6	21,2
43	5,0	15,3	16,9	26,9	15,9	9,2	14,4	16,3	31,8	33,7	24,9	14,5	15,1	28,2	25,0	23,5	14,2	29,1	23,7	18,2	16,7	25,7	21,1
47	12,9	15,8	18,5	24,0	21,2	19,3	23,6	16,9	38,4	33,7	24,0	18,3	19,8	33,5	29,1	18,3	17,3	31,1	29,8	28,6	21,5	30,2	24,0
50	8,2	13,4	16,4	22,9	14,0	19,0	16,3	16,3	30,9	29,7	28,0	14,9	18,1	27,9	26,2	15,8	15,8	31,2	29,1	26,3	20,1	28,7	23,5
56	8,0	13,7	17,9	22,7	14,6	15,8	14,7	14,3	30,6	25,0	24,4	15,7	17,4	22,4	26,0	18,2	15,2	26,8	20,1	18,9	20,8	28,3	19,8
60	6,2	19,8	14,1	21,6	14,9	11,6	11,6	12,5	22,5	22,5	23,6	14,4	11,6	23,6	25,4	16,3	14,0	23,2	20,0	18,0	18,2	22,6	16,8
64	9,4	14,4	16,3	23,5	14,5	11,4	15,8	13,0	25,4	23,4	24,3	18,8	12,2	26,1	23,3	16,2	14,3	27,5	18,7	18,3	20,9	20,1	21,4
68	9,0	12,0	15,6	23,8	23,1	11,9	16,2	13,3	22,3	26,2	24,8	20,8	14,2	23,0	21,4	16,7	15,9	23,7	20,6	22,0	18,7	20,4	24,5
71	11,0	11,3	2,4	20,9	19,8	12,7	16,6	12,0	12,9	9,5	8,4	15,1	0,7	22,8	6,5	18,5	14,7	18,8	30,1	18,6	7,2	4,3	16,4
74	14,6	8,7	9,0	17,3	13,2	10,7	8,2	11,3	2,8	19,3	18,1	17,6	3,6	15,9	17,8	13,3	11,2	18,6	17,5	13,2	13,9	16,9	13,6
77	12,6	11,0	11,5	16,3	12,3	11,3	12,1	9,8	21,7	19,9	16,2	14,1	12,0	14,6	18,9	14,9	16,5	19,2	21,3	14,1	13,9	17,0	13,8
83	9,1	10,6	10,7	16,3	8,5	7,2	13,2	7,6	22,5	16,8	13,1	10,1	5,7	17,1	13,3	14,3	12,8	15,6	20,3	9,3	11,7	10,2	9,2
89	10,0	11,4	10,3	13,5	9,1	7,0	25,1	12,8	15,3	18,0	13,4	15,3	9,6	12,1	12,6	16,1	9,8	23,8	29,3	11,7	10,9	19,5	12,2
93	4,6	5,2	8,3	10,7	9,2	5,7	6,9	8,1	12,0	13,8	19,2	6,1	8,2	6,1	11,8	8,5	10,9	15,2	15,6	9,0	11,5	12,4	10,3
96	5,7	7,4	11,8	15,6	12,7	8,1	8,1	10,7	13,4	15,8	23,2	9,3	8,6	9,5	13,9	14,3	10,6	16,1	15,3	13,9	16,7	14,5	11,5
103	5,7	8,7	14,8	13,1	10,5	5,6	7,3	15,2	13,0	13,7	18,0	8,9	9,2	11,9	16,3	10,3	9,1	17,8	16,3	14,5	15,2	12,2	12,5
106	19,4	24,6	25,3	28,5	25,9	20,7	24,4	24,1	39,2	28,8	29,6	22,0	24,4	26,0	28,4	31,2	28,3	25,0	26,3	28,8	31,1	29,8	25,4
110	5,0	11,6	10,8	11,4	12,2	17,4	8,5	9,8	14,8	13,5	14,1	10,8	9,6	13,1	14,1	11,7	10,1	11,8	15,2	12,6	16,1	13,1	12,2
112	2,8	8,5	9,7	8,7	11,3	10,0	7,1	6,7	14,7	15,1	12,7	10,9	7,5	16,7	12,6	10,7	10,5	8,6	13,6	11,0	14,3	12,5	11,9
117	5,3	16,6	14,3	13,3	14,9	8,9	10,8	12,7	18,4	20,2	14,2	13,8	11,8	15,0	14,5	14,7	12,7	17,3	20,0	13,0	17,7	18,3	13,9