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SOIL INDICATORS SET UP TO ASSESS SUSTAINABLE MANAGEMENT IN VARIOUS AGRICULTURAL AND FOREST ECOSYSTEMS

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This thesis is dedicated to my father

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Contents

List of abbreviations

Acid pho.= acid phosphatase

Pho./Corg.=acid phosphatase specific activity

AMF= arbuscular mycorrhizal fungi

Aryl= arylsulphatase

Aryl./Corg= arylsuphatase specific activity

BR = soil basal respiration

CA=conservation agriculture

CBD= The Convention on Biological Diversity

CC =cover crop

CEC= cation exchange capacity

Chit=chitinase

Chit./Corg= chitinase specific activity

CLPP= community level physiological profile

Cmic= microbial carbon

CONV= conventional management

Corg= total organic carbon

CT= conventional tillage

DFA = discriminant function analysis

EA= enzyme activities

Extr-C= extractable carbon

Extr-N= extractable nitrogen

FA= fatty acids

FAO = The Food and Agriculture Organization

H'= Shannon Index (microbial functional diversity)

LM = living mulch

LTE= long term experiment

MDS= minimum dataset

MEE = Multi-Environment-Experiment

MR= Microresp®

MUF= methylumbelliferone

Nmic= microbial biomass nitrogen

$N\text{-}NH_4$= nitrogen as ammonium form

$N\text{-}NO_3$ = nitrogen as nitric form

ORG= organic management

Pho= acid phosphatase

Pho./Corg=acid phosphatase specific activity

PLFA= phospholipid-derived fatty acids

qCO2 = metabolic quotient

QRM=quantile regression model

RT= reduced tillage

SC=subsidiary crop

SEI= synthetic Enzyme Index

SEIc= synthetic Enzyme Index involved in C-cycle

SOCO= Sustainable agriculture and soil conservation project

SOM=soil organic matter

SQ= soil quality

TN= total nitrogen

T-RFLP= terminal restriction fragment length polymorphism

UNEP=United Nations Environment Programme

WHC= water holding capacity

I. INTRODUCTION

1. Sustainable management and soil quality

The topic of sustainability vastly concerns the management of natural resources including soil, which is considered a non-renewable resource and therefore should be a cause of intense deliberation. The definition of The Food and Agriculture Organization for sustainability is: "ensuring human rights and well-being without depleting or diminishing the capacity of the Earth's ecosystems to support life, or at the expense of others' well-being". The World Soil Charter states that soil management is sustainable "*if the supporting, provisioning, regulating, and cultural services provided by soil are maintained or enhanced without significantly impairing either the soil functions that enable those services or biodiversity. The balance between the supporting and provisioning services for plant production and the regulating services the soil provides for water quality and availability and for atmospheric greenhouse gas composition is a particular concern".*

Every year there are major consequences of bad soil management seen as: loss of soil organic matter and nutrients, compaction, salinization, contamination with heavy metals, soil sealing etc., all these can be prevented by sustainable and economically feasible management techniques (Gil and Gil, 2011). Soil management practices can be considered sustainable when they can maintain the provisions of ecosystem services such as provision of clean water, hydrologic and nutrient cycling, habitats for microorganisms and mesofauna, carbon sequestration and climate regulation over long periods of time. (Kassam et al., 2013)

There is a large amount of scientific literature that resumes the best practices of maintaining a good soil quality (SQ), hence a soil that can perform at good parameters its functions. This complexity makes the evaluation of SQ much more challenging than that of water or air quality (Carter et al., 1997). In the last decades attention was given to the activity and diversity of the soil microbial community and it was assessed that a large biomass and high biodiversity in soil may link to the degree of SQ (Carter et al., 1997).

Smyth and Dumanski (1995) stated that sustainable soil management combines technologies, policies and activities aimed at integrating socio-economic principles with environmental concerns so as to simultaneously:

(i) maintain or enhance production and services;

(ii) reduce the level of production risk;

(iii) protect the potential of natural resources and prevent degradation of soil and water quality;

(iv) be economically viable and socially acceptable.

Furthermore sustainability is in a constant state of change, perpetually requiring proper methods and new indicators to describe it. Moreover, soil resilience is the capacity to recover functional and structural integrity and it has to be taken into consideration when evaluating managed ecosystems, as any form of management can disturb the original equilibrium of the native ecosystem (Carter et al., 1997).

Soil sustainable management is linked to the characterization of soil quality, hence changes in soil properties over time will help to define effective management strategies (Gil and Gil, 2011). The evaluation of soil quality as a response to human and natural impacts allows the sustainability of the soil to be comprehensively characterized (Tóth et al., 2007). According to Zornoza et al., 2015, suitable management practices are essential to preserve soil functions and thus promote SQ.

2. Soil quality to provide ecosystem services

One of the most popular definitions of SQ was given by Doran and Parkin (1994) stating that it is the "capacity of soil to function within ecosystem and land use boundaries, to sustain productivity maintain environmental quality and promote plant and animal health". There are other several definitions of SQ such as: "the soil's capacity to store and recycle water, nutrient and energy" (Anderson and Gregorich, 1984); "the ability of soil to perform or function to its potential, and changes over time due to human use and management or to unusual events" (Mausbach and Tugel, 1995) etc. The concept of SQ includes assessments of soil properties and processes as they relate to the ability of soil to function effectively as a component of a healthy ecosystem. It includes measures of a soil's ability to produce plant biomass, maintain animal health and production, recycle nutrients, store carbon, partition rainfall, buffer anthropogenic acidity, remediate added animal and human wastes and regulate energy transformations (Schoenholtz et al., 2000).

Also the term soil health can be found whenever focusing on a living, dynamic system whose functions are mediated by a diversity of living organisms that require management and conservation (Doran and Zeiss, 2000). Assessment of SQ is essential to describe, evaluate and monitor changes in all types of soils, independently of the purpose. Therefore soil sustainability and the effects of management should be determined by measuring soil properties and processes directly (Burger, 1996;Seybold et al., 1998).

According to Franzluebbers and Haney (2006), SQ in relation to management practices can: (1) deteriorate rapidly with poor management (2) stabilize with time under adequate management, but undergo minor variations due to weather (3) improve with time using the best-available, adaptive techniques that restore key soil functions.

Ecosystem services can be defined as the conditions and processes through which natural ecosystems, and the species that make them up, sustain and fulfil human life and are linked to their main functions (Daily, 1997). The Millennium Ecosystem Assessment (2005) groups ecosystem services in four categories these can be attributed to soil as following: supporting services (nutrient cycling, soil formation, primary production, habitat, biodiversity); provisioning services (food for humans, fresh water, wood, fiber and fuel); regulating services (regulation of climate, floods, diseases and water purification) and cultural services (aesthetic, spiritual, educational and recreational). Furthermore, the ability of soil to provide ecosystem services depends on its properties and their interactions that are mostly influenced by land use and management (Adhikari and Hartemink, 2016). In order to describe soil ecosystem services Adhikari and Hartemink (2016) created a diagram representing the main soil properties that influence soil functions in relation and finally the ecosystem services provided for the human well-being (Figure 1.1).

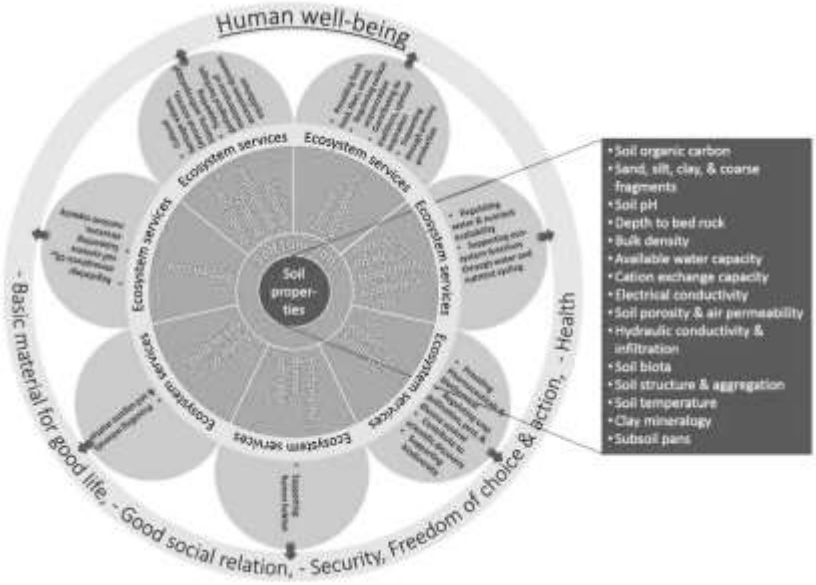

Fig 1.1. Links between key soil properties to ecosystem services through soil functions for the well-being of humans (Source: Adhikari and Hartemink, 2016).

Lal (2014), highlights that importance of the "adoption of restorative land use and recommended management practices are important to strengthening numerous ecosystem services such as: improving water quality and renewability, increasing below and above-ground biodiversity, enhancing soil resilience to climate change and extreme events, and mitigating climate change by sequestering C in soil…"

3. Soil in agriculture and forest ecosystem

Soils of natural and managed ecosystems are a critical and dynamic regulatory system that generates a multitude of functions, also known as soil functions (Blum, 2005). Soil systems in general can perform several such functions often simultaneously (de la Rosa and Sobral, 2008). FAO enumerates the following basic soil functions in global ecosystems:

1. Biomass production, including in agriculture and forestry;

2. Storing, filtering and transforming nutrients, substances and water;

3. Biodiversity pool such as habitats, species and genes;

4. Physical and cultural environment for humans and human activities;

5. Source of raw materials;

6. Acting as carbon pool;

7. Archive of geological and archaeological heritage.

3.1 The role and functions of soil in agroecosystems and in forest ecosystems

Special importance is given to soil in ecosystems due to their role in providing various functions. For example the majority of studies on soil in agroecosystems are concentrated on the soil's fertility, hence the role of soils in providing support to plant growth by provisioning water and soil nutrients in adequate amounts. Apart from the role in plant growth, in forest ecosystems soil also regulates nutrient uptake, decomposition and water availability. In the last decades, the role of forest soils as a C-sink gained a lot of attention. Forests and forest soils store 650 billion tons of carbon or nearly one third of the total in

terrestrial ecosystems (FAO, 2015). Moreover, soil plays a vital role in the development of forest and the forests play a vital role in the development of soils (pedogenesis).

Agricultural and forest ecosystems are on different poles when it comes to soil properties and this is due mainly to the difference in disturbance intensity and the quality and quantity of the soil organic matter (SOM) which in forest soils is usually higher than in agricultural ones. Arable lands are highly disturbed over a short period of time, with a high rate of oxidation of the more labile soil organic carbon fractions which then release carbon to the atmospheres CO_2 (Chan et al., 2002). On the other hand forest sites are left relatively undisturbed for the growth period of the trees ranging from 15 to 100 years (Creamer et al., 2015a). These differences in disturbance influence all soil processes and also the general soil study approaches. An important component in describing soil quality has to do with the carbon cycling and storage which has vast implications concerning the biological stability of the two ecosystems. Not only does the microbial biomass size and activity differ but also the interconnections between its populations due to the degree of land use intensity and the total organic carbon content of the soils (Creamer et al , 2015). Agricultural soils are poor from the point of view of microorganism's interconnections and food-web relationships compared to forest soils that can be categorized as stable systems with a very well developed food web.

In agroecosystems human organization has determined new structures and functions at different hierarchical levels (field, farm and landscape). The ecological succession steps are replaced by crop sequence patterns and crop spatial arrangements (monoculture, polyculture) while crop management (cultivation, fertilization, irrigation, pest and disease control) largely affects the land cover conditions and use of land resources (Caporali, 2008). In agroecosystems, soils are influenced directly by human interventions and their characteristics and properties depend on agricultural organization and practices. The sustainability in agroecosystems can be promoted by responsible actions in favour of a systemic approach to soil as the centre of all ecological processes. (Figure 1.2). Soil represents the meeting point of inputs and outputs being considered an open system due to its capability to exchange energy with the surrounding environments (Caporali, 2008).

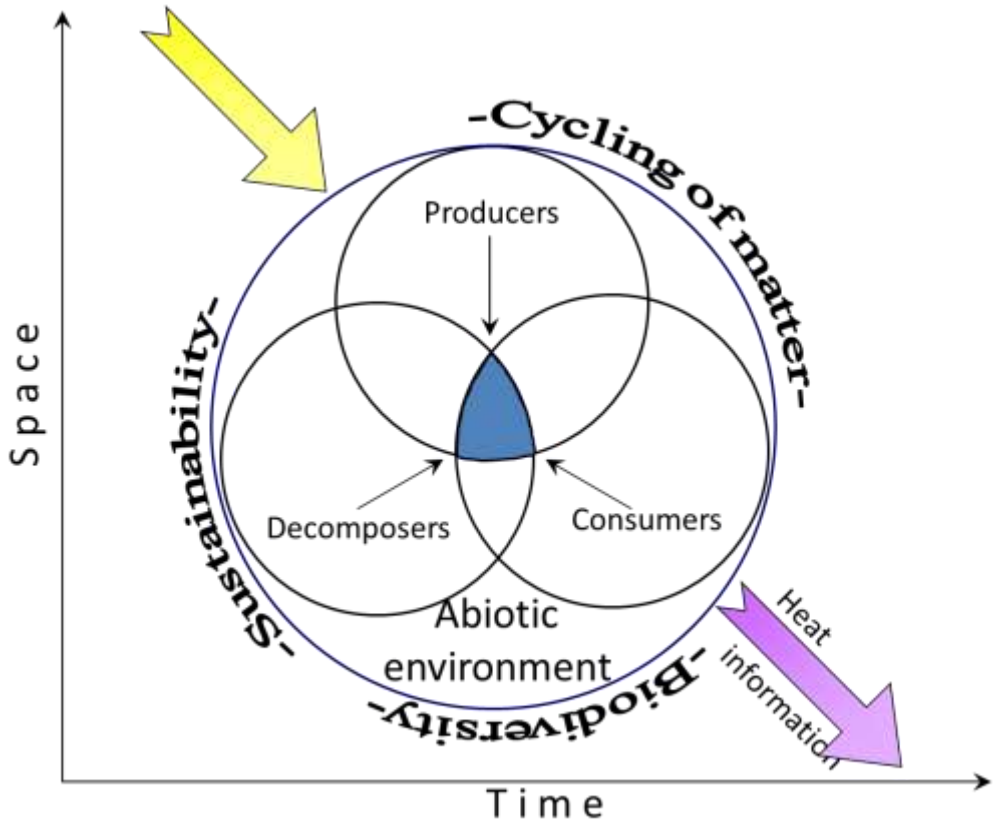

Figure 1.2 Soil as the centre of all ecological processes (source: Caporali, 2008)

In agriculture, SQ is usually connected to the fertility and the ability of soil to produce a high yield while issues of biodiversity, environmental quality or social value are often secondary to productivity (Schoenholtz et al., 2000). Stockdale et al. (2013) resumes in figure 1.3 the most widespread aspects of soil fertility resulted from the plant-soil interaction. Plant nutrient dynamics are influenced by the physical, chemical and biological soil properties. These properties can change the amount and the availability of nutrients to crop plants and influence soil productivity. After the green revolution, the use of soil fertilizers, tillage, drainage and lime addition were considered management practices that increase the soil productivity but the damages brought on by them were seen soon after when soil started to be depleted of organic matter and lost its tilth. As the awareness of the negative impact of intensive management in agriculture grew, the concept of sustainable agriculture was widely debated and soils started to occupy a central role especially for the new agricultural policies.

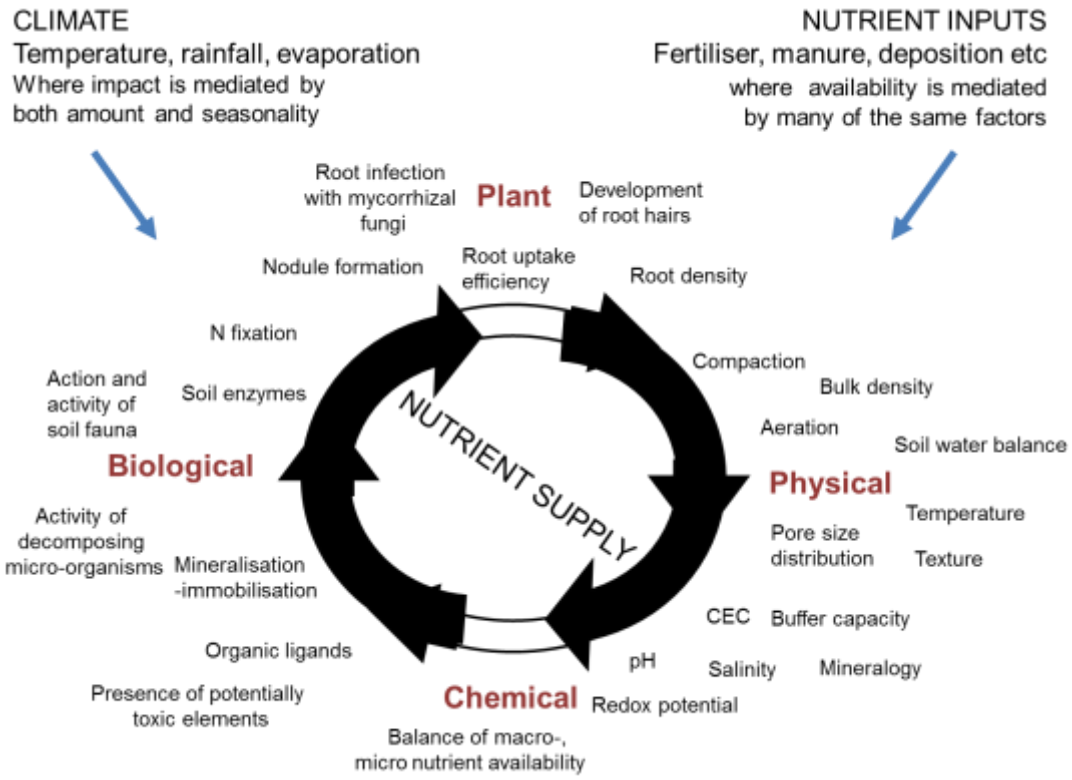

Figure 1.3 Plant root soil-interaction (source: Stockdale et al., 2013).

In forest ecosystems soils have developed under the effect of forest vegetation which also influenced soil formation though their canopy, biomass, litters and roots (Osman, 2013). According to FAO fully developed forest soils (natural soils) are natural bodies with a vertical sequence of layers. On the top is an organic layer (O horizon) usually formed out of litter in different stages of decomposition: undecomposed plant debris (Oi horizon); semi-decomposed, fragmented organic matter (Oe) and humus; amorphous organic matter without mineral material (Oa). Below this surface layer is an organo-mineral surface horizon (A); a subsurface mineral horizon often leached (E); a subsurface mineral horizon with features of accumulation (B horizon); a mineral horizon penetrable by roots (C); and locally hard bedrock (R). The E, B, C, and R horizon may be lacking, or the B horizon may be modified by groundwater or stagnant water (IPCC, n.d.). One major aspect of forest soils is their role in the global C-cycle where soils can act both as carbon sinks and carbon pools, but on global scale they are generally considered carbon sinks as two thirds of the terrestrial C in forest ecosystems is contained in soils (Dixon et al., 1994). The forest soil C stock varies among latitudes, 37% if found in low latitude forests, 14% in mid-latitudes and 49% in high latitudes (Dixon et al., 1994). In the past decades, forest soil nutrient dynamics has been studied intensively leading to the understanding of processes in the major nutrients flow through the

ecosystems. Forests depend on native soil nutrients and on those retained in their own biomasses that are supplied by the litter mineralization (Osman, 2013). Each year nutrients return to the soil especially after the process called litterfall. This process represents a major biological pathway for element transfer from vegetation to soils (Yang et al., 2005) leading to organic matter replenishment and nutrient cycling (Bhat and Jan, 2010). Therefore, the amount of litter and its different nutrient concentrations are very important aspects in the role of forest ecosystems (Osman, 2013). Furthermore the litter decomposition is a complex of different processes such as: the mineralization and humification of lignin, cellulose and other compounds by soil microorganisms followed by leaching in the soil of soluble C and N compounds that are progressively mineralized or immobilized (Berg, 1986).

3.2 Soil management in agroecosystems

The soil management practices used to protect and enhance soil performance are applied with respect to the different land-use ranging from tillage and fertilization in agricultural soils to grazing and harvesting in pastures and forests (Moscatelli et al., 2015). Nevertheless, the impact of management can be an enhancement or a depletion of soil quality described by the soil's physical, chemical or biological properties. These properties are influenced by specific kinds of practice or treatments depending on their intrinsic pedological characteristics and on the response of the soil biota. Particularly negative effects can be buffered by the soil biota's capacity to carry on their functions and reducing these effects on key ecological processes (Moscatelli et al., 2015).

3.2.1 Conservative and conventional agricultural systems

In the past decades the sustainability of conventional agricultural systems has been highly debated in terms of soil quality. In general the conventional agricultural systems have higher yields but also a negative environmental impact on soil. In time, soils under conventional management are subjected to erosion, compaction, nutrient losses and decrease in above and below ground biodiversity. In the last decades, agricultural management shifted from the maximizing profits and high yields strategies towards sustainable practices for maintaining both soil fertility and soil quality. These practices include methods that can be adopted in all the countries independently of their development status, as they are concentrated on low-input systems for cutting the expenses of the high input ones. These practices can be: use of

green manure and dual purpose legumes, careful use of animal manures, reduced tillage, retention of cover residues, targeting planting and farm input use and the use of legumes in rotation (Sánchez and Salinas, 1981).

In the last century food production maximized the yields mainly through biotechnology methods, therefore enabling monopoly over the yields but more importantly over the end product. The same technique could be applied to soil but it would be strenuous as soil cannot be controlled it can only be managed mainly by high inputs of fertilizers, that in a lot of cases are unsustainable. The energy input into an agro-ecosystem will always exceed the energy released and if appropriate management practices are not applied a high risk of energy unbalance increases. Soil fertility is lost once agricultural practices are not well managed promoting soil erosion, nutrient mining, alteration of the soil biota, salinization through improper irrigation, acidification through inappropriate fertilization and deposits of acidifying pollutants (Hopkins and G. Gregorich, 2013). One key practice in preventing soil erosion is a good management of the above-ground vegetation, keeping the soil covered and managing the crop residues. As stated by Turmel et al.(2015) crop residues return organic matter to the soil through a combination of physical, chemical and biological activities that affect SQ. Figure 1.4 shows a simplified cycle of the plant residues inputs transformed by the microorganisms. The residue's biomass can be either consumed by livestock or directly decomposed by the microorganisms, while both decomposition and respiration produce CO_2 and microbial residues that release nutrients (C, N, P, S) available for the plants.

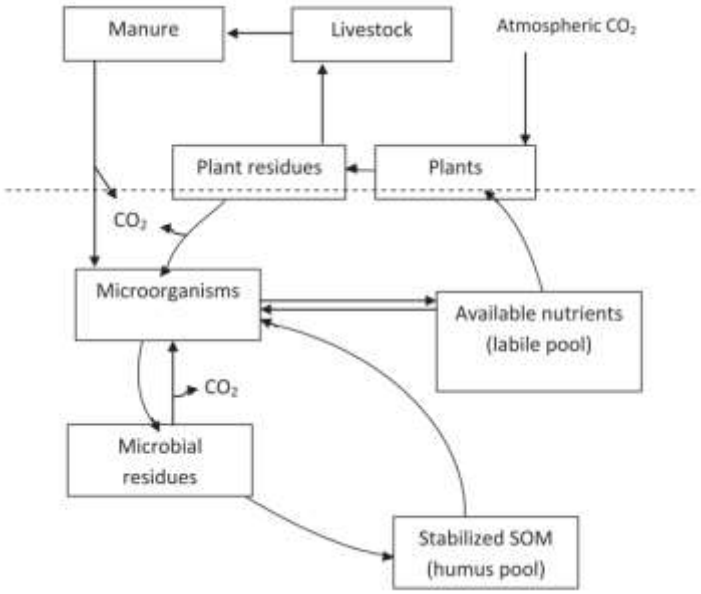

Figure 1.4 Mechanism of plant residues transformation by soil microorganism (source: Turmel et al., 2015)

Climate conditions are one of the most important factors influencing this cycle as it affects decomposition rates. Tillage also influences the decomposition, depending on whether or not the residues are incorporated or left on the soil; in the first case they have a direct contact with the soil microbial biomass therefore a rapid decomposition. Keeping the soil covered during the winter time using cover crops or living mulches have shown a positive effect on soils especially associated with no-tillage which is an important practice for enhancing the soil microbial quality and also the soil organic carbon stocks (Balota et al., 2014).

All of the agricultural management practices presented here (study cases 1 and 2) are known as conservation agriculture (CA) practices. These practices are sustained and described by the major worldwide organisations such as FAO and UNEP and also promoted by several European Projects. For example, the European project SOCO (2009) (Sustainable agriculture and soil conservation) resumed the three pillars of conservation agriculture practices as following:

- minimal soil disturbance (through reduced or no-tillage) in order to preserve soil structure, soil fauna and organic matter;
- permanent soil cover (cover crops, residues and mulches) to protect the soil and contribute to the suppression of weeds;
- diversified crop rotations and crop combinations, which promote soil micro-organisms and disrupt plant pests, weeds and diseases.

CA tries to implement practices that balance the use of farm resources with a positive effect on soil microorganisms, roots and other soil fauna. In this case soil fertility (nutrients and water) is managed through soil cover management, crop rotations and weed management (European Commision, 2009). According to FAO, CA is important for intensifying sustainable agricultural production by integrating good agricultural practices (use of quality seeds, nutrient, water, weed and pest management) with other production sectors of the agroecosystem such as livestock or trees and pastures. These types of practices have been encouraged in the past 20 years, especially the reduced and zero tillage practices, to overcome the negative effects of conventional tillage. The crop residues left on the soil represent a habitat for decomposing organisms; part of the decomposed organic residues will become humus contributing to the stabilization of soil structure. The major benefits of CA are related to the increase of organic carbon stock, biological activity, above- and belowground biodiversity and soil structure that triggers the reduction of soil erosion and run-offs. Moreover, CO_2 emissions are also lowered due to the reduced use of machinery and increased accumulation of organic carbon. SQ indicators in agroecosystems are widely used

in assessing scientific databases used by the stakeholders and also policy makers. The usual parameters described are the physical and chemical ones, whereas biological indicators are the ones that point out the sensitive changes and that can describe the soil in a broader picture (Bastida et al., 2008).

The continuous pressure to produce more food increases the pressures to develop strategies to optimize fertilizers efficiency and improve the recycling or organic resources. This calls for innovative methods to recycle organic matter in nutrients from farms, food processing, household waste and compost etc. (Hopkins and G. Gregorich, 2013).

3.3 Forest management and soil quality

In *forest ecosystems* there is a strong connection between soil, plants and management practices that applied on either of them influences the other. Moreover, tree species' effect on soil microbial processes are generally attributed to differences in quantity and quality of substrate input through litter and root exudates, their specific nutrition, their influence on soil physical characteristics like soil structure and texture, pedoclimate or on the development of an understory vegetation (Malchair and Carnol, 2009).

The forest stands management directly influences the forest soil quality, as argued in this thesis. According to Worrell, (1997), the main impacts of forest management on soils are seen on: soil erosion status, changes in the nutrient dynamics, changes of the quality and quantity of organic matter, physical disturbance of soil profiles and changes in water status and aeration. Sustainable management practices in forest ecosystems are addressed especially for the maintenance and conservation of soil organic matter due to its importance in nutrient and carbon storage, soil physical and hydrological properties and provision of substrates for soil biota (Bauhus et al., 2002). The main influences on soil quality are given by management practices such as: afforestation, reforestation and stand management (thinning and harvesting). Afforestation or reforestation practices increase the C pool in the aboveground biomass and replenish the soil C pool until a new equilibrium between C input (litterfall, rhizodeposition) and C output (respiration, leaching) is reached in the soil (Jandl et al., 2007). In fact, over time these management practices increase C-stocks up to 18%. (Guo and Gifford, 2002). Stand management practices, such as thinning can induce changes in forest microclimate, the decomposition of the litter being stimulated due to warmer and wetter soils (Jandl et al., 2007).

3.3.1 Reforestation with exotic species

For centuries, large areas of world forests were exploited for timber or converted to other land uses. These practices have had a negative impact on climate, soil, hydrology and biodiversity. As these negative impacts worsened in time, to counteract their effects reforestation practices were adopted. These practices can be: plantations for timber, riparian plantings to reduce stream pollutions, upland plantings to reduce soil erosion and salinity, and to increase habitat for native species (Jackson et al., 2005). Moreover reforestation practices restore the biogeochemical cycling of nutrients thus improving biodiversity and increasing ecological resistance to pressure such as climate change (Hooper et. al., 2005). According to Cunningham et al., (2015) after the reforestation practices a range of potential responses of ecosystem structure and function may occur; structure includes the diversity of species in an area (animals, plants, fungi and bacteria) and the spatial arrangement and function includes the biogeochemical processes resulting from interactions between species and the physical environment, such as production, decomposition and nutrient dynamics. Furthermore, the structural complexity after reforestation depends on the planted tree species and the age of the plantations.

In the last decades in Europe restoration of natural woodland has become an important objective on sustainable forestry (Zerbe, 2002). In large parts of Europe many coniferous forests were planted at the end of the 18 th century due to the prior overutilization of broad-leaved woodlands (Zerbe, 2002). For example the Douglas-fir plays an important role in Italian plantation forestry because, within its optimal vegetation zone ranged from 600 to 1000 m a.s.l., no indigenous conifer has similar characteristics of productivity and timber quality (Corona et al., 1998). In terms of soil quality little is known about the effect of this exotic species on soil processes involved in carbon sequestration and nutrients budget, as well as on biogeochemical cycle of elements along soil profiles (Welke and Hope, 2005). In a recent study Antisari et al., (2015) concluded that reforestation with exotic coniferous such as Douglas-fir in comparison to natural beech forests improved C sequestration, N stock and microbial activities. Still, research is needed in order to determine the effects of exotic tree stands across different time classes on soil chemical and biochemical properties, carbon and nitrogen stocks and nutrients dynamics.

3.3.2 Forest stand ages and soil limiting factors

Plant succession and associated space-for-time substitutions are an important and often necessary tool for studying temporal dynamics of soil processes involved in C sequestration across multiple timescales. Natural differences in inputs and transformations of C are likely to occur in stands of different ages due to differences in inputs of above- and belowground litter (both from trees and from ground vegetation), through fall, light, water and nutrient availability (Pignataro, 2012). Usually with stand age the soil carbon stocks rise, as a substantial amount of C accumulates as leaf and stem litter on the forest floor until a steady state between depositing and decomposition is reached (Cunningham et al 2015).For example previous studies on Douglas-fir stand ages, showed that older conifer forests store more total C in the organic layers and in the top 10 cm of mineral soil than do second- and young-growth forests. (Entry and Emmingham, 1998).

Studies on forest chronosequences have shown that a decline in nutrient use in older forests may derive from a lower demand by the slower-growing forests (Binkley and Fisher, 2013). Bauhus et al., (1998) studied the effect of different stand ages on soil microbial biomass and activity and concluded that the soil organic matter quality declined with stand age and the microbial respiration increased, this indicated a decrease in C assimilation efficiency. Also, the soil microbial populations shifted among different stand ages, as bacterial communities dominate the early stages and fungal populations the older stages (Zhu et al., 2010)

There are a large number of limiting factors that can impact the growth and productivity of a forest these depend on the tree species and their geographical position. Some of the limiting factors are: drought, pest and disease, allelopathy, sunlight, forest fires and soil nutrient supplies.

The productivity of a forest can decrease due to the scarce supply of nitrogen (N) and phosphorus (P), as these two nutrients are considered the most common limiting soil nutrients (Binkley and Fisher, 2013). Most forest soils are less fertile than agricultural soils and deficiencies in P and N can limit the growth of most forests. Nitrogen is the primary limiting nutrient in forests productivity (Menge et al., 2012) while P is a limiting factor especially in temperate and tropical forests (Binkley and Fisher, 2013). The biogeochemical cycles of these two elements are linked also to the carbon cycle, since organism require specific proportions of C, N and P from the global to the molecular scale (Bejarano-Castillo et al., 2015).

During the process of pedogenesis, a contrast between abiotic N versus P inputs can be observed; P inputs tend to decline while abiotic N inputs do not change with soil age. The N and P inputs were correlated with the forest net primary production being N-limited in younger soils and P-limited in older soils (Menge et al., 2012). The losses in both elements can be seen firstly in the dissolved organic forms rather than their inorganic forms. In old forests the losses of N are associated with long-term accumulation, humification, mineralization and leaching of soil organic N during ecosystem succession (Hedin et al., 1995). Studies on a different forest stand ages revealed: decreases in available P-forms, increases in N/P ratios in litterfall and decreases in forest primary production in the older forests (Liu et al., 2015). At a microbial level, the activity of soil microorganisms is usually limited by the quality and quantity of C (Wardle, 1992) the microbial growth may be limited by N (Schimel and Weintraub, 2003) and microbial P limitations are common in weathered soils where P is bound to iron or aluminium (Gallardo and Schlesinger, 1994).

4. Assessment of soil quality

According to Gil and Gil, (2011) there are three important aspects involved in soil sustainability: the physical, chemical and biological characteristics of soil that differ as time of response. The latter ones describe soil dynamics in short period of time while the physical and chemical reflect slower changes in soil status. Soil quality is described by using specific indicators that usually resume the complexity and heterogeneity of soil. The outcome of a SQ description is a multivariate structure that describes physical, chemical and biological properties and their interactions, they can also include geophysical (e.g. climatic) and management attributes (e.g. workability) (Hopkins and Gregorich, 2013). As argued by Zornoza et al., (2015), 'SQ is interconnected with management practices, productivity and other ecosystem aspects, showing an interdependence controlled by feedback mechanisms'.
Schjønning et al.,(2004) argues that it is difficult to find a proper threshold of an indicator due to the vast number of soils and ecosystems addressed. He describes that an indicator's threshold links to resilience or a boundary between sustainable and unsustainable values. According to Hopkins and Gregorich, (2013), the main challenge in developing indicator sets for SQ assessment is the identification of indicators that are meaningful, readily measurable, cost-effective and which can be compared with data in existing databases. A SQ indicator is a measurable attribute or a set of attributes that influences the capacity of a soil to carry out a

given function. Furthermore, SQ indicators identify both the condition of the soil resource and the economic and environmental sustainability of land management practices (Doran, 2002). A soil quality index could be defined as the minimum dataset of parameters (MDS) that when interrelated, provides numerical data on the capacity of a soil to carry out one or more functions (Acton and Padbury, 1993). Moreover many soil indicators are strictly interrelated to each other and the value of one is affected by one or more parameters.
There are several studies that determine the selection type of relevant soil indicators from which the following:

a) The American National Soil Survey Center , states that no single soil property can be used as an index of SQ and the selection of indicators should be based on:

- the land use;

- the relationship between an indicator and the soil function being assessed;

- the ease and reliability of the measurement;

- variation between sampling times and variation across the sampling area;

- the sensitivity of the measurement to changes in soil management;

- compatibility with routine sampling and monitoring;

- the skills required for use and interpretation.

b) Karlen et al., (2003) proposes a framework (figure 1.5) for selecting the indicators based on the efficiency in describing critical soil functions such as nutrient cycling, supporting plant growth and development, determined by the specific management goals. Collectively all these indicators represent the minimum data set (MDS).

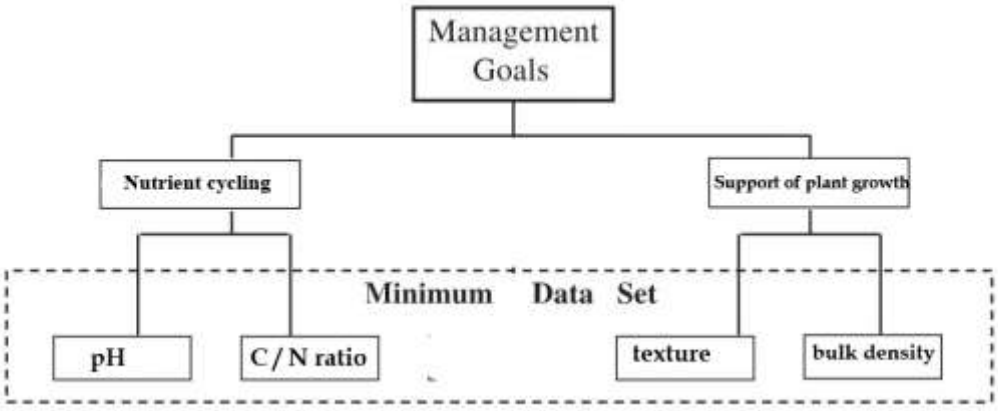

Figure 1.5. Brief example of a framework for selecting indicators for a minimum data set (adapted after Karlenet al 2003).

Quantifying or interpreting a soil indicator can be difficult due to the fact that it can represent a value expressed in common measurement units or it can be a rack in a certain range, they can be combined to express and index of SQ.

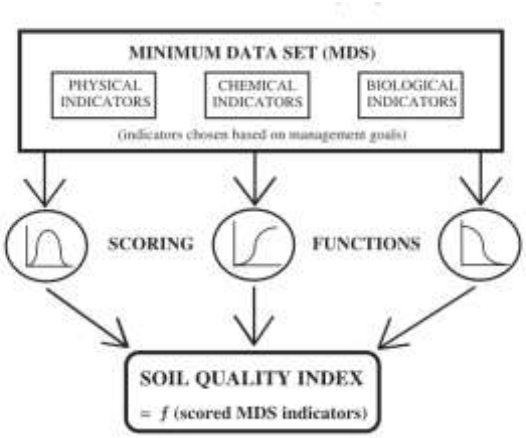

Figure 1.6. Conversion of minimum data set indicators to index values (source: Karlen et al. 2003).

As seen in (fig 1.6) the MDS describes the physical, chemical and biological parameters of data that will be used in the calculation of indexes which can be compared and commented upon. Several multiparametric indexes were constructed for agroecosystems that use parameters such as: SQ indexes that assess aggregate stability, microbial biomass, respiration,

total C and N, bulk density, available water, pH and electrical conductivity (Karlen et al., 1994); Biochemical Soil fertility Index (Koper and Piotrowska, 2003) that encompasses organic C, total N, dehydrogenase activity, alkaline phosphatase, protease activity, amylase activity. Many of these indexes can be found in the scientific literature; depending on which of the soil management parameters are selected. The description of SQ gets more attention every year and new parameters and indexes are found together in mathematical models of prediction especially for soil erosion describing the impact of management practices in detail. The main challenge is identifying the soil indicators that respond rapidly to soil management and show whether these practices have a positive or negative feedback.

4.1 Soil physical and chemical indicators

Soil organic carbon as part of the soil organic matter, is one of the most studied indicators of SQ and is associated with the majority of other soil indicators. It is directly related to the maintenance of soil structure, presence of different groups of microorganisms, mineralization of organic matter, and nutrient availability (Martinez-Salgado et al., 2010). Franzluebbers, (2002), proposed stratification ratios of soil properties, e.g. N and C pools, including total and particulate organic C and N, soil microbial biomass C, and potential C and N mineralization to explain differences respect to soil quality in soils with different managements.
It is almost impossible to describe all the physical and chemical properties as they are extremely vast. A selection of the most important/applied ones, used in the minimum data set is presented in table 1.1.

Table 1.1. List of common soil physical and chemical indicators

Soil physical indicators	Soil chemical indicators
• Texture (sand, silt and clay %)	• pH
• Aggregate stability	• Cation exchange capacity, Base saturation
• Bulk density	• Carbonates content

- Infiltration
- Electrical conductivity
- Colour
- Total organic carbon
- Total nitrogen
- Extractable carbon
- Extractable nitrogen
- P, K, S, Ca, Mg, content
- NO_3^- and NH_4^+ content, as forms of nitrogen

In both forest and agricultural soils physical and chemical indicators are useful in considering the soil's capacity to sustain production and sustainability, maintain nutrient cycling, plant biomass and organic matter (Schoenholtz et al., 2000). Powers et al., (1998) indicated that there are several CI (chemical indicators) that are marginal for agroecosystems and very important for forest growth, as forest ecosystems are highly complex. For example the cation exchange capacity (CEC) is assessed for agrarian soils as a standard indicator whereas it turns out often absent in forest SQ where base saturation is more likely to influence the exchange complex on soil solution chemistry and acidity (Schoenholtz et al., 2000).

Some of the most important chemical indicators and their implications in SQ are:

- pH that correlates with nutrient availability and solubility also influencing the soil biota;
- total organic carbon that affects all the important processes in the soil including microbial activity;
- soil nitrogen one of the most required plant nutrients found in different forms (nitrate, ammonium, organic N or potentially mineralizable N store in the soil organic matter (Cardoso et al., 2013).

4.2 Soil biological indicators

A bioindicator is defined as an organism, part of an organism, product of an organism, collection of organisms or biological processes which can be used to obtain information on the quality of all or part of the environment (Killham and Staddon, 2002). The majority of soil multi-dimensional processes are linked to soil biota; furthermore the soil biological indicators describe phenomena such as the delivery of these processes in ways that other indicators do not (Ritz et al., 2009). Chemical and physical indicators describe soil attributes permanent in time while biological indicators are dynamic and sensitive to changes in soil conditions and management practices (de la Rosa and Sobral, 2008). The processes mediated by soil microbiota have an essential role in cycling of elements and mineralization of organic matter.

Doran and Zeiss (2000), proposed the following principles in selecting soil bioindicators of soil quality:

- sensitivity in variations in management;
- well correlated with beneficial soil functions;
- useful for elucidating ecosystem processes;
- comprehensible and useful for land managers;
- easy and inexpensive to measure.

According to Ritz et al. (2009), the best candidates for biological indicators are: soil microbial taxa and community structure using T-RFLP techniques; soil microbial community structure and biomass from PLFAs; soil respiration from multiple substrate-induced respirations; biochemical processes from multi-enzyme profiling; nematodes; microarthropods and microbial biomass size.

Belowground biodiversity represents 95% of total diversity on Earth but only 5% of it has been classified (Menta et al., 2008). Soil microbial biomass consists of all the organisms generally smaller than 10µm, found inside or on the surface of soil microaggregates, which can be dormant or metabolically active. Microorganisms in the soil can be divided in bacteria, fungi, yeasts, archaea, algae and protozoa. Moreover, the microbial biomass size expressed as biomass carbon (Cmic), is usually comprised of 1-4% of the total organic C (Jenkinson and Ladd, 1981). Microbial carbon is considered a sensitive bioindicator to environmental changes, management or pollution even though different biomass size can occur without direct correlation to SQ as well (Schloter et al., 2003). The soil's intrinsic quality also

influences the amount of Cmic, for example clay soils contain more biomass than sandy soils; forest and grassland soils have also a higher Cmic than agricultural soils. Increases or decreases in the microbial biomass size due to soil management are seen faster than ones on the total soil organic matter content.

Soil biodiversity according to the The Convention on Biological Diversity (CBD) is "the variation in soil life, from genes to communities, and the ecological complexes of which they are part, that is from soil micro-habitats to landscapes". The biodiversity of soil is vital as it is the engine driving soil-based ecosystem services such as food production, nutrient cycling, carbon sequestration and water purification (Turbé et al., 2010). The major part of soil biodiversity comprises soil microorganisms that are studied usually as microbial communities rather than single species. The soil microbial diversity can be studied on different levels such as: ecological diversity, species diversity, functional diversity and genetic diversity (Insam, 2001). Due to the limitations in studying soil genetic diversity, the attention has shifted towards soil microbial functional diversity that is defined as the numbers, types, activities, and rates at which a suite of substrates are utilized by the bacterial community (Zak, 1994). In fact, microbial functional diversity represents “the sum of the ecological processes and/or capacity to use different substrates, developed by the organisms of a community and it can be expressed through species or important groups to maintain several functions in the soil, while the genetic one represents gene and genotype variations” (Insam et al., 1989). Several soil functions are carried out by more than one microbial species and a reduction in any group of species has an insignificant effect on overall soil processes (Nannipieri et al., 2002). Therefore, functional diversity is related to the actual activities and compared to taxonomic diversity may provide greater insight into microbial roles in ecosystems (Zak et al., 1994). Microbial functional diversity represents the capacity to perform different ecological processes and to use a wide array of substrates (Kandeler et al., 2006). This is achieved through different biological processes ranging from oxidative processes (e.g. respiration) to hydrolytic processes (e.g. extracellular enzymes). In the recent years profiling techniques such as multi- enzyme assays and multi-substrate induced respiration methods (Microresp) were widely used as they have a higher degree of sensitivity and discrimination in assessing the functional status of soils (Creamer et al., 2009).

All soils contain enzymes that determine soil metabolic processes (McLaren, 1975) which depend on its physical, chemical, microbiological, biochemical properties.

Soil enzymes are classified in two categories: those present in living cells, intracellular enzymes, and the ones produced by living cells but secreted outside the organism known as

extracellular enzymes(Gianfreda and Rao, 2014). The extracellular enzymes can be either free in the soil solution or adsorbed, linked, anchored or embedded on/in/to solid supports such as clays, clay minerals, organic matter and organo-mineral complexes (Gianfreda and Rao, 2014).

Soil enzymes have key functions in the degradation processes of the soil organic matter, in stabilization of soil structure, the decomposition of organic wastes, organic matter formation, and nutrient cycling (Dick, 1997). Enzymes are released by the soil microflora and they conduct a series of hydrolysis reactions in the degradation of complex polymers to simple monomeric products. The soil hydrolytic enzymes are indicators of microbial activities related to the C, N, P and S cycles. Some examples of soil enzymes related to their specific bio-geo-chemical cycles are: the C cycle enzymes such as β and α- glucosidase, xylanase, cellulase activity; the N cycle enzyme such as amidase, chitinase and urease activity; the P-cycle as alkaline and acid phosphatase activity and S-cycle such as sulphatase.

Soil enzyme activities are often used as indicators of soil fertility, soil microbial processes and they are early indicators of changes in soil quality (Nannipieri et al., 2012). Enzyme activities are influenced by several factors such as: soil physico-chemical characteristics, microbial community structure, vegetation, soil management, succession and pedogenetic factors (Cardoso et al., 2013). The multi-enzyme assays assess the potential enzyme activities under laboratory conditions where pH, temperature and soil moisture are controlled.

Enzyme activities are used as an index of microbial functional diversity and since they includes many metabolic processes a large number of different enzymes should be measured (Nannipieri et al. 2012).

Functional diversity of soil microoganisms can be also assessed by the community level physiological profile (CLPP) determined by the addition of simple C-source substrates to the soils and measuring the respiration response. Different metabolic responses can indicate shifts in the microbial community functional diversity due to various soil management practices, the presence of contaminants, vegetation cover etc. Microresp is a widely used metabolic fingerprinting method (Campbell et al., 2003) based on the addition of 15 sole carbon substrates and water, added directly on the soil, consequently the released CO_2 is easily measured by colorimetric detection. It is hypothesised that the greater the diversity of the microbial community the wider the range of carbon source utilisation (Creamer et al., 2009).

4.3 Ecophysiological indexes

Ecophysiological indices are generated by basing physiological performances (respiration, growth/death, carbon uptake) on the total microbial biomass per unit time (Moscatelli et al., 2005). The indexes are particularly helpful in differentiating the response of soil biota to sustainable soil management practices (Kandeler, 2007).

The ratio of biomass C to soil organic C (Cmic:Corg), also known as *microbial quotient* reflects the contribution of microbial biomass to soil organic carbon (Anderson and Domsch, 1989). It can be used to compare soils with different organic matter fractions, differences between agricultural practices and climate influence. (Bastida et al., 2008). This ratio is related to organic substrate availability and is expected to be higher in a rich soil substrate (Bauhus et al., 1998).

The qCO2 (*metabolic quotient*) is calculated as carbon respired per unit of microbial biomass, environmental impacts usually change this ratio. A high metabolic quotient may indicate a stress response, due to the fact that the microbial community has a high C demand for maintenance (Anderson and Domsch, 2010). The index is usually higher in young sites, lower in monoculture sites due to the low variety of C substrates and is known as a good indicator of alterations of soil due to deforestation, heavy metal contamination, temperature or changes in soil management (Bastida et al 2008).

Based on Odum's theory on bioenergentics of ecosystem development, the metabolic indexes show the developmental stage of microbial community. Hence, at maturity there should be a low value for the qCO2 ratio and high ratio of Cmic:Corg (Anderson and Domsch, 2010).

In the last decades the scientific community proposed a wide range of soil indicators used in SQ assessments. For example, in the case of European soil monitoring, the studies Ritz et al. (2009) and Stone et al. (2015) represent a milestone in identifying soil bioindicators based on their sensitivity and ability to discriminate between land-use. This approaches started from the key soil functions (food and fiber production, environmental interactions and habitats and biodiversity support) associated to the ecological processes and soil. In Ritz et al 2009, a list of bioindicators was assessed and assigned a "factor score" based on pertinence to the defined soil function, applicability and technical criteria (methodology). The top ranking bioindicators were: soil microbial taxa and community structure using T-RFLP methods; soil microbial community structure and biomass using PLFA's; soil respiration and C-cycling from multiple substrate induced respiration (Microresp®), biochemical processes using multi-enzyme profiling methods, nematodes, microarthropodes, (…), and finally microbial

biomass. The cited reference also points out the need to establish how these indicators are sensitive to variations in management. In this thesis the various types of management in both forest and agro-ecosystems using soil chemical and biological indicators are studied first separately in each case studies and then considered all together in order to identify the sensitive indicators that discriminate between the different soil management practices.

II. AIM OF THE STUDY

The general aim of the study was to focus on the sensitive soil quality indicators in order to assess sustainable management in both agricultural and forest ecosystems. Therefore, the main question posed was which soil indicators were more effective in discriminating between management practices in various ecosystems. The five case studies were chosen considering, both agricultural and forest ecosystems. In addition to the general aim, each case study has a specific aim as described below:

Case study 1

Soil ecological impact of tillage in various subsidiary cropping systems across four European climate zones

Aim: To improve the understanding of the winter cover crops use in conservation agriculture systems under different environmental conditions and interactions with management techniques.

Case study 2

Soil quality indicators in a long-term experiment: organic vs. conventional agricultural management

Aim: To study the ecological impact of conventional and organic agricultural systems on soil quality during a 15-year-long experiment.

Case study 3

The impact of exotic tree species (Douglas-fir) reforestation on soil carbon and nutrient dynamics in Northern Apennines – Italy

Aim: To determine the effect of different stand age plantations of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) on soil chemical and biochemical properties, carbon and nitrogen stocks and nutrient dynamics.

Case study 4

Soil microbial diversity as indicator of forest management in Monte Venere- Vico Lake Natural Reserve

Aim: To examine the effects of past beech forest (*Fagus sylvatica L.*) management and consequent variation of carbon stock effect on soil microbial diversity.

Case study 5

The assessment of microbial functional diversity in different soil categories

For the final discussion besides the results of the above 4 case studies presented, the results of 5th case study was considered in order to test two main methodological approaches (enzyme activities and MicroResp®) used to evaluate soil microbial functional diversity and to assess the effectiveness of both methods in discriminating among a vast range of soil categories.

III. MATERIALS AND METHODS

3.1 Experimental designs

a) Case study 1- *Soil ecological impact of tillage in various subsidiary cropping systems across four European climate zones*

This study was founded by the European Union FP7 Programme project- "Optimizing Subsidiary Crop Applications in Rotations (OSCAR)". Part of the data discussed in this thesis were also included in the Final Report of the project that ended on the 31'st March 2016.

The experimental fields were set-up in four European countries: Sweden (SLU), United Kingdom (ORC), Switzerland (ART) and Italy (UNI) (table 3.1). Each experimental trial included a two-year crop rotation with the use of subsidiary crops (SC): leguminous [Commun vetch (*Vicia sativa* L)]; and brassica sp. [(Oilseed radish (*Raphanus sativus)]* as cover crops (CC) and trefoil spp. [white clover (*Trifolium repens* L.*)],* subterranean clover (*Trifolium subterraneum*) as living mulch (LM). The field trials nominated Multi-Environment-Experiments (MEE) were replicated in time and started in 2013 (MEE 1) and 2014 (MEE 2) respectively. Tillage was applied at the end of the SC treatments (spring 2014 and 2015). The above ground biomass of the SC's was either incorporated as green manure in conventional tillage (CT) or left on the soil surface to mineralize (dead mulch) in reduce tillage (RT). Moreover, in each trial control plot, without SC, was considered. All soil samples were collected at plough depth (0-15 cm) after the main crop harvest in 2014 and 2015 respectively. The climate and the geographic position of each experimental field are presented in table 3.2. Climate characteristics, average temperature and annual precipitations data were recorded over a period of 30 years and were taken from the "www.climatedata.eu" site. The environmental zones were identified according to Jongman et al., (2006) environmental stratification of Europe.

Table 3.1: Main characteristics and sampling dates of the experimental fields in case study I and II (Conv= conventional tillage; CC= cover crop; LM= living mulch).

Sites	Climate zone	Experimental design	Tillage	Subsidiary crops	Main Crop(s)	Soil sampling
SLU	Boreal	Split-plot with 4 replications	-Conv. -Reduced tillage (Ecodyn)	Oilseed radish CC Vetch CC White clover LM	Maize	Oct. 2014 Oct. 2015
ORC	Oceanic	Split-plot with 4 replications	-Conv. -Reduced tillage (Ecodyn)	Brassica mix CC Brassica+Yellow trefoil CC Yellow trefoil LM	Barley	Oct. 2014 Oct. 2015
ART	Temperate	Split-plot with 4 replications	-Conv. - No tillage	Oilseed radish CC Hairy Vetch CC Subterranean clover LM	Maize	Oct. 2014 Oct. 2015
UNI	Mediterranean	Split-plot with 4 replications	-Conv. - No tillage	Vetch CC Subclover LM	Tomato	Sept. 2014 Sept. 2015

Table 3.2. The four European experimental sites: climate and geographical coordinates

Studied sites	SLU	ORC	ART	UNI
EU country	Sweden	United Kingdom	Sweden	Italy
Geographical coordinates	59° 49'N, 17° 39'E	51° 23' N, 1° 24' W	47°29'N, 108°54'E	42°25' N, 12° 3'E
Altitude	24 m a.s.l	104 m a.s.l.	504 m a.s.l	303 m a.s.l.
Climate zone	Boreal	Oceanic	Temperate	Mediterranean
Temperature	6.7 °C	10.7 °C	8.8 °C	15.1°C
Rainfall	539 mm	804 mm	1086 mm	876 mm
Aridity Index (AI)	32.3 Slightly humid	38.8 Moderately humid	55.5 Very humid	34.9 Slightly humid
Environmental zone	Boreal	Atlantic central	Continental	North Mediterranean

b) Case study 2- *Soil quality indicators in a long-term experiment: organic vs. conventional agricultural management*

This study was founded also by the European Union FP7 Programme project- "Optimizing Subsidiary Crop Applications in Rotations (OSCAR)" Part of the data discussed in this thesis were also included in the Final Report of the project that ended on the 31'st March 2016.
The second case study was conducted in a long term experiment (LTE) located at Tuscia University experimental farm (Viterbo); the field trials were first established in 2001 with the objective to study conventional vs. organic systems and plowed vs. subsoiled soils. Two soil tillage managements were applied for each system consisting in inversion tillage at 30 cm (CT) and non-inversion tillage (RT). The experimental set-up was a randomized block design with 3 replication. A 3-year crop rotation was established in both cropping systems [pea (*Pisum sativum*), durum wheat (*Triticum durum*) and tomato (*Lycopersicon esculentum*)]. In the organically managed cropping system, the crop rotation was implemented with common vetch (*Vicia sativa*) and sorghum (*Sorghum vulgare*) cover crops, which were green manured

before tomato transplanting and pea sowing, respectively. Since 2008–2009 pea has been substituted with chickpea (*Cicer arietinum*) and sorghum with oilseed rape (*Brassica napus*) (Campiglia et al., 2015). Soil samples from each block were collected at two soil depths: 0-15 cm and 15-30 cm respectively.

c) Case study 3- *The impact of exotic tree species (Douglas-fir) reforestation on soil carbon and nutrient dynamics in Northern Apennines – Italy*

For case study 3, three survey sites of 80 (DOUG 8, 9), 100 (DOUG 10, 11) and 120 years old (DOUG 12) plantations of Douglas-fir were chosen (Figure 3.1). These sites are located in the Vallombrosa Forest, in the Tuscan-Emilian Apennines (Italy). Firstly, a soil profile was opened at each site and taken as reference soil (about 1 m wide and 1 m deep) and genetic horizons were sampled. Secondly, in order to assess soil properties variability, two additional sampling spots were randomly dug in each site 2 m from the representative soil profile. Soil samples were taken from: organo-mineral horizons (0-20cm) and mineral horizons (20-40cm).

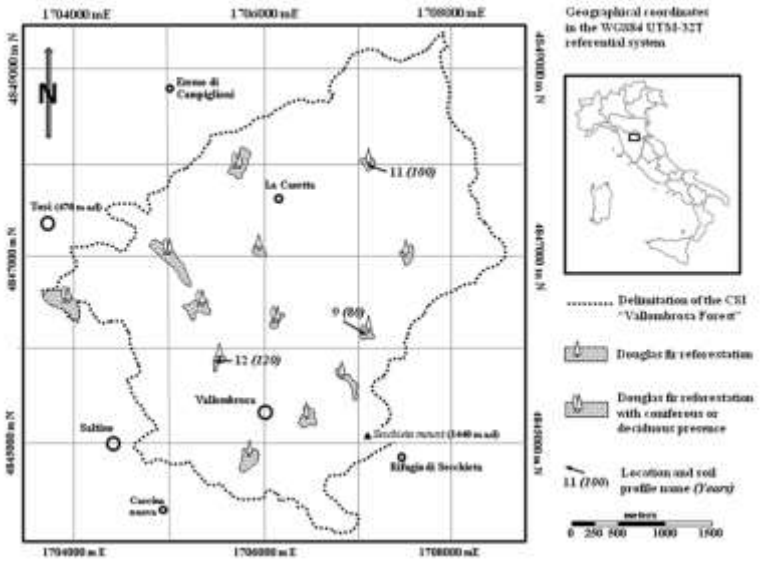

Figure 3.1. Location of soil survey (case study 3).

d) Case study 4- *Soil microbial diversity as indicator of forest management in Monte Venere-Lake Vico Natural Reserve*

The examined area of case study 4 is part of Vico Lake Natural Reserve- Monte Venere, in the Cimini Mountains, Central Italy (Figure 3.2). The beech tree (*Fagus sylvatica* L.) forest is located at 520-838 m a.s.l., the soils are of volcanic origin characterized as Andisols (Typic Hapludands and Entic Hapludands) according to Soil Taxonomy. The whole forest is divided in different management compartments (Figure 3.3A), from which two were selected for this study, 56 and 65 respectively. Soil was sampled within four existing permanent plots (Figure 3.3 B), two in each compartment, designed for stand structure monitoring. The main stand attributes were different in each plot due to past management. In particular, plot 56A is characterized by medium size trees (diameter from 28 to 53 cm) while 56B consists of a higher number of small size individuals (diameter from 17 to 27 cm). In compartment 65, especially in plot 65A, a higher number of large plants (diameter from 52 to 98 cm) is present. Soil samples in 3 replicas were randomly collected from each plot, at 0-5 cm and 5-15 cm, respectively.

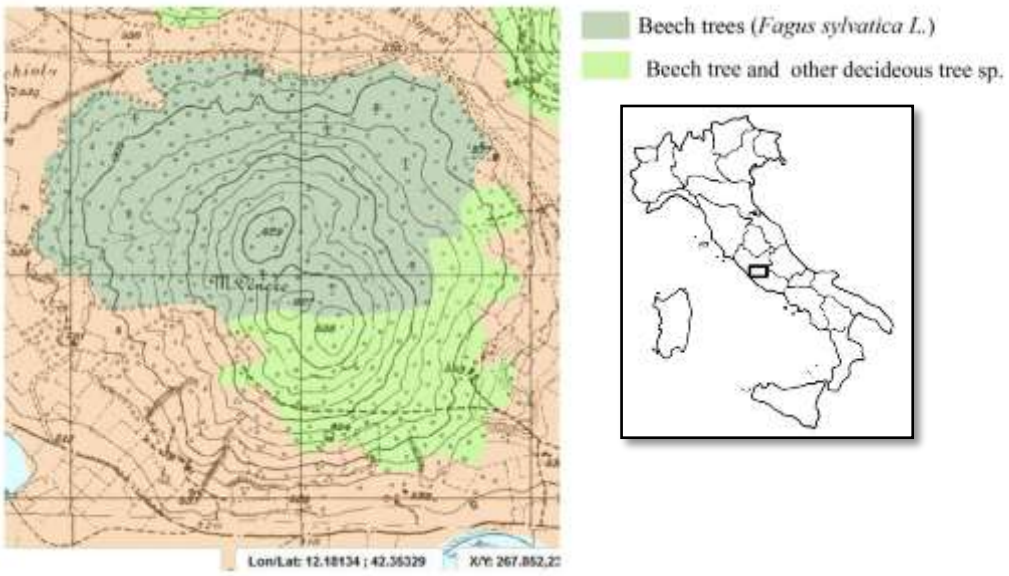

Figure 3.2. Vegetation map and location of Monte Venere forest (source: National Geoportal Italy)

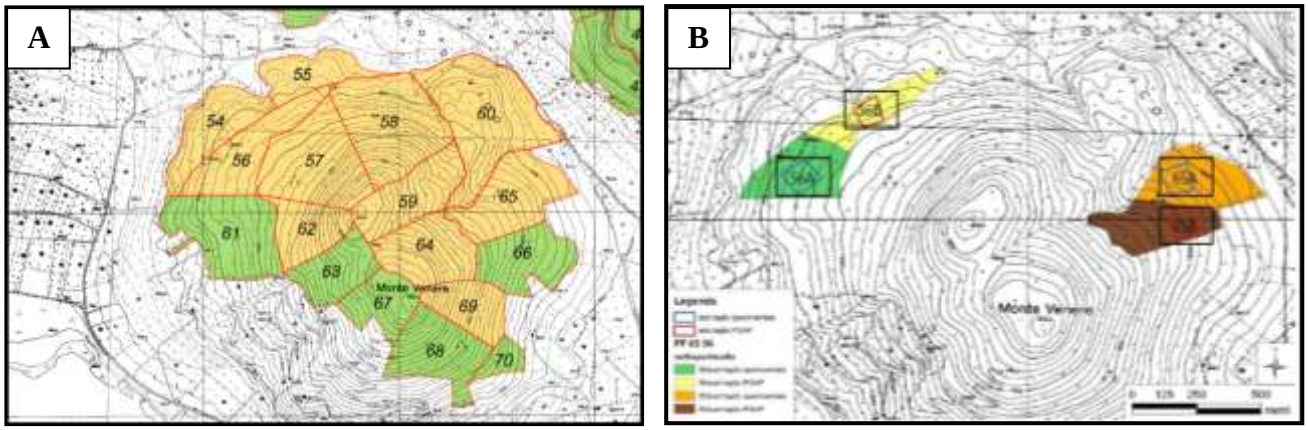

Figure 3.3. Monte Venere forest map divided into management compartments (A); the selected compartments and areas of the study case (B). (source: Lazio region, 2006)

e) Case study 5- The assessment of microbial functional diversity in different soil categories

Microbial functional diversity was measured, by means of enzyme activities and MicroResp^{TM} technique, in a wide range of soils analysed during the last 5 years (2010-2015) in the Laboratory of Chemistry and Biochemistry, University of Tuscia, Viterbo, Italy. The soils were selected from a broad spectrum of key soil properties across different land-uses, wide range of soil pH, soil organic carbon content (Corg), moreover also data from case studies 1 and 3 was included (Table 3.3). The soils were grouped into three main categories with the aim to separate diverse land uses and/or specific conditions. For this purpose, the three groups were termed: F (forest soils, 4 case studies), A (agricultural soils, 5 case studies) and EC (extreme conditions, 6 case studies). The soils samples were: forest soils (F) including different: tree covers, management practices, lithological substrates, afforestation, chronosequences. Soils under agricultural land use (A) are characterized by different managements and/or agricultural practices such as: organic, biodynamic and conventional cropping systems, tillage/no tillage, natural green cover/no cover. The third category (EC) includes soils with peculiar characteristics due to pedoclimatic conditions (saline environments, natural arsenic contamination in rice paddies, highly calcareous soils) or heavy anthropic impact (a multi-element contaminated dump, As contaminated mine, pot experiments with heavy metals etc.). A total of 196 values of microbial functional diversity calculated as Shannon's index assessed by means of enzyme activities and CLPP-MicroResp were used in this study.

Table 3.3. Description of all data sources. Average values of Shannon diversity index (H') measured by means of enzyme activities (H'EA) and MicroResp (H'MR) with standard errors are reported. Reference to data source are provided, when not available a specific acknowledgement to research funding source is added. N.P.=data not published

Soil category	Factor of variation	Corg (%)	pH	H' EA	H' MR	no. of samples	Reference or acknowledgement
Forest (F)	Management (coppiced/aged coppice)	5.9±0.5	6.4±0.1	1.7±0.1	3.7±0.0	12	*Pignataro et al., (2012)*
	Lithological substrate	4.2±0.7	5.8±0.2	2.6±0.1	3.4±0.2	10	*Pignataro et al., (2011)*
	Afforestation (*Fagus* and *Douglasia* spp.)	3.1±0.8	5.6±0.2	2.0±0.1	3.7±0.1	8	*Marinari et al. (2015)*
	Chronosequence (*Fagus* spp.)	4.5±0.5	4.8±0.1	2.2±0.0	3.6±0.1	7	*Papp R. (2016)- Case study 3*
Agricultural (A)	Management (org/conv)	1.5±0.0	7.2±0.0	2.0±0.0	2.6±0.0	30	*Brunetti P. (2014)*
	Management (tillage level)	1.9±0.2	7.2±0.3	1.8±0.1	3.6±0.0	12	*Papp R. (2016) Case study 1*
	Vineyard (natural green cover/no cover)	1.5±0.1	8.1±0.0	2.3±0.0	3.4±0.1	19	n.p. Mania E.
	Management tomato crop (organic/conv)	1.4±0.1	6.6±0.1	2.0±0.0	3.6±0.1	5	n.p. Stazi S.R.
	Vineyard (biodyn/conv)	1.3±0.1	6.7±0.2	2.0±0.0	3.2±0.3	4	n.p. Stazi S.R.

Extreme conditions (EC)	Thallium contamination	6.3±0.8	6.4±0.6	2.2±0.1	3.4±0.3	8	n.p.
	Arsenic contamination	2.7±0.3	5.7±0.2	2.0±0.0	3.5±0.1	14	*Stazi et al. Geomicrobiology(in press)*
	Highly calcareous	1.2±0.1	8.0±0.0	1.3±0.1	4.2±0.3	12	*Italian PRIN 2010JBNLJ7_006*
	Hydromorphous and subaqueous	2.9±0.6	8.2±0.2	1.5±0.3	4.7±0.3	16	*Papp R. et al., (2015)*
	Waterlogged rice paddies+arsenic	1.2±0,1	7.3±0.1	2.3±0.1	2.9±0.1	20	*Italian PRIN 2010JBNLJ7_006*
	Phytoremediation (heavy metals)	1.4±0.0	7.9±0.2	2.4±0.1	3.8±0.0	19	*Emili L., (2013)*

3.2 Soil analysis

Soil analysis of common soil chemical and biochemical characteristics of the five case studies are presented here. For case study 2, 3 and 4 extra analyses were performed in order to better describe the specific aims.

A. Common analysis in all case studies

3.2.1 Soil chemical analysis

All soil samples collected were immediately sieved (<2 mm) after the sampling and air dried at room temperature.

Soil pH was determined in 1:2.5 (w/v) aqueous solution. *The organic carbon (Corg) and nitrogen (TN)* contents were determined by dry combustion using the elemental analyzer (Thermo Soil NC—Flash EA1112). 20 mg of milled dry soil samples were weighed in Ag-foil capsules, with the addition of 40 µl of HCl solution (10%) in order to remove inorganic carbon.

3.2.2 Soil biochemical analysis

The microbial biomass carbon (Cmic) and nitrogen (Nmic) was analysed using the microbial biomass carbon (Cmic) was determined with the fumigation–extraction method (Vance et al., 1987). Before the analysis all the samples were adjusted at 60% of their water holding capacity (WHC) and kept for 3 days in the dark at 25°C. The first portion of the samples was not fumigated and extracted with 80 ml of 0.5 M K_2SO_4 and filtered with Whatman paper (no. 42). The second portion was fumigated for 24 h with ethanol-free chloroform and then extracted as described above. Organic C and N in the extracts were determined with the TOC-V CSN and TNM- 1 analyser (Shimadzu).

The results obtained from non-fumigated soil samples were considered *extractable carbon (Extr. C)* or *extractable N (Extr. N).*

The microbial biomass was calculated as follows: Cmic= EC*kEC, where EC is the C difference between fumigated and non-fumigated soil samples and kEC = 2.64; microbial Nmic= EN*kEN, where EN is the difference between N extracted from fumigated and non-fumigated soil samples and kEN = 2.22. Moreover the labile pools of C and N were calculated using the concentration values of non-fumigated samples (Laudicina et al., 2013).

Soil enzymes were measured following Marx et al., (2001) using fluorogenic methylumbelliferyl (MUF)-substrates. Soils were analyzed for 8 enzymes as follows: (i,ii) *β-cellobiohydrolase* (EC 3.2.1.91) and *α-glucosidase* (EC 3.2.1.21) which are enzymes contributing to the degradation of cellulose; the principal function of cellobiohydrolase is to hydrolyse cellobiose dimmers from the non-reducing ends of cellulose molecules, while α-1,4-glucosidase catalyzes the hydrolysis of cellobiose to glucose; (iii) β-*glucosidase* (EC 3.2.1.20) which contributes to the degradation of starch, specifically it hydrolyses terminal non-reducing 1-4 linked alpha-glucose residues to release glucose molecules; (iv) *β-N-acetyl-glucosaminidase* (chitininase) (EC 3.2.1.30) which plays a role in the degradation of chitin; (v) *xylosidase* (EC 3.2.2.27) which contributes to the degradation of the hemicellulose xylan by removing successive D-xylose residues from its non-reducing end; (vi) *acid-phosphatase* (AP, EC 3.1.3.2) which hydrolyses phosphomonoesters releasing phosphate, (vii) *arylsulphatases* (EC 3.1.6.1) which hydrolyses ester bonds of aryl-sulphate-esters releasing sulphate and is considered a valid measure for sulphur mineralisation in soils and finally (viii) *butyrate esterase* (EC 3.1.1.1) which is considered a proxy of endocellular activity.

The relative fluorogenic substrates were: 4-MUF-β-D-cellobioside, 4-MUF-β-D-glucoside, 4-MUF-N-acetyl-β-glucosaminide, 4-MUF-α-D-glucoside, 4-MUF-phosphate, 4-MUF-7-β-D-

xyloside, 4-MUF-sulphate and 4-MUF-butyrate. All soils prior to the analysis were adjusted at 60% WHC and kept at 25°C in a dark room for 3 days. A soil-homogenous suspension was obtained by homogenizing 2 g of soil (equivalent to 2 g oven-dry material) with 50 ml sterile water with an Ultra Turrax at 9600 rpm for 3 min. Aliquots of 50 µl were withdrawn and dispensed into 96 well black microplates (in 3 analytical replicates).

Finally, 50 µl of Sodium Acetate buffer 0.5 M pH 5.5 and 100 µl of 1 mM substrate solution were added thus obtaining a final substrate concentration of 500 µM. Fluorescence (excitation 360 nm, emission 450 nm) was measured with an automatic fluorimetric plate-reader (Fluoroskan Ascent) and readings were taken after 0, 30, 60, 120 and 180 min of incubation at 30°C (Marinari et al., 2013).

The potential enzyme activity was expressed as nmoles of product (MUF) of each enzymatic reaction released per g of soil per unit of time. Moreover, the enzyme specific activity (per unit of Corg) was calculated in order to keep the amount of organic matter as an internal control (Trasar-Cepeda et al., 2008)

The Synthetic Enzyme Index (SEI) was calculated as the sum of all activities (Dumontet et al., 2001). Synthetic enzyme index for the C-cycle (SEIc) was calculated using the values of the enzymatic activities of β-glucosidase, α-glucosidase, xylosidase, cellobiohydrolase, chitinase. Soil functional diversity was determined by calculating the The Shannon's Diversity Index (H') using the formula: $H' = -\sum pi \log 2\ pi$ where pi is the ratio of the activity of one enzyme to the sum of activities of all enzymes (Bending et al., 2002)

Soil basal respiration was determined using the Microresp® method. 500 mg of soil adjusted at 60% WHC, was weighted in duplicate, randomly arranged in the deepweel and left for 15 days at 25 °C to incubate. The absorbance of the detection plates was read 570 nm before the set-up of the detection plates and after 6 h of incubation at 25°C. The respiration rate (µg CO_2-C $g^{-1}h^{-1}$) was calculated using the difference between the initial absorbance and final absorbance.

Soil microbial indexes were calculated as follows:

Cmic:Corg = µg of biomass C mg total organic $carbon^{-1}$ (Anderson and Domsch, 1989);

qCO2 = (µg C-CO_2 h^{-1} x µg biomass C^{-1}) (Dilly and Munch, 1998).

B. Specific material and methods

Soil bulk density (case studies 2 and 3) was determined on undisturbed soil samples collected using Eijkelkamp® sample ring kit (model A), each metal cylinder having a volume of 100 cm^3. The soil samples were dried at 150 °C for 24 h and the following formula was applied:

BD= TW/VH

where: TW was total oven-dry weight of soil from the metal cylinder; VH was the cylinder volume (100 cm^3).

In case study 2 undisturbed soil samples were collected at 0-5cm and 5-10cm soil depth.
In case study 3 undisturbed soil samples were collected at 0-5; 5-10; 10-15; 15-20cm.

Soil carbon and nitrogen stock was calculated using the following formula (case studies 2 and 3):

C stock (t ha^{-1}) =C org concentration [g kg−1]* BD [g cm−3]•layer thickness [cm]•0.1

The same formula was used for the N stock determination considering the concentration of total nitrogen (TN).

Community level physiological profile (CLPP)

a) *Microresp®(case studies 3,4,5)* method was performed as described in Campbell et al., (2003). This method measures microbial respiration rates induced by a range of carbon sources, defined as Multiple Substrate Induced Respiration (MSIR). The colorimetric method is based on pH change given by the reaction between bicarbonate and CO_2 in the presence of an indicator dye (creasol red). 15 carbon substrates were selected depending on their ecological relevance to soil and their solubility in water (table 3.3). Water was added as well in order to assess the basal respiration.

Table 3.3. Substrates used for CLPP analysis

Substrates	Type	C delivered (mg C ml $^{-1}$*)	Abbreviation
d-Glucose	Carbohydrate	30	G
d-Galactose	Carbohydrate	30	GA
d-Fructose	Carbohydrate	30	FR
l-Arabinose	Carbohydrate	30	ARA
N-acetyl-	Carbohydrate	7.5	NAG
Citric acid	Carboxylic acid	30	CIT
Oxalic acid	Carboxylic acid	7.5	OX
Ascorbic acid	Carboxylic acid	30	ASC
Vanillic acid	Phenolic acid	0.3	VAN
Syringic acid	Phenolic acid	0.3	SYR
Aspartic acid	Amino acid	7.5	ASP
l-Leucine	Amino acid	3	LEU
Glicine	Amino acid	7.5	GLI
γ-Amino-butyric	Amino acid	30	BUT
Arginine	Amino acid	7.5	ARG

(*soil water)

Prior to the substrate addition each soil sample was adjusted at 30% of the water holding capacity (WHC). Soil was placed in 96 deepwell plates by using the Microresp filling device provided in the kit. Approximately 0.4g of soil was added to each well. 25 µl from each C substrate was dispensed on the soil, in 3 replicates randomly while avoiding the edge effect. The CO_2 detection plates were prepared 5 days before the analysis and kept in a glass desiccator with soda-lime and water. Each well of the detection plate contained: creasol red (12.5 ppm, *w/w*) as indicator dye; KCl (150mM); $NaHCO_3$ (2.5mM) and all reagents were dissolved in 150µl (1%) agar. The absorbance at 570nm was determined with LT-4000 Microplate reader immediately before (T0) and after 6h (T6) of incubation at 22°C. Respiration rates (mg CO_2-C $g^{-1}h^{-1}$) were calculated from the absorbance data subtracting from the values at T6 the values of basal respiration.

Fungiresp (Case study 4) method was applied as described by Sassi et al., (2012). The same technique as Microresp® was considered but with the use of specific substrates and an

antibiotic in order to assess the catabolic fingerprint of only the soil fungal community. 9 carbon sources were used as substrates and divided as follows: carbohydrates (glucose, sucrose, trehalose, mannose, dextrin and cellobiose); amino acids (glycine and alanine) and carboxylic acid (malic acid). Each substrate was prepared in order to deliver 30 mg of C/mL of soil water. Furthermore, in order to inhibit the bacterial population different doses of Bronopol were tested. Petri dishes were prepared containing culture media for fungi (Potato Dextrose Agar) and bacteria (Plate Count Agar) and different Bronopol concentrations were also added as follows: 5, 10, 20, 40, 50, 75, 150 and 300 μg, respectively. 100 μl soil aqueous solution (2:3 w/v) at different dilutions (1:10; 1:50; 1:100) was dispensed on all Petri dishes. Finally the colony forming units (CFU) were counted after a 3 and 7 days of incubation at 28°C. The optimal concentration of Bronopol was identified to be 20 μg g^{-1}. Each soil sample was distributed into the deepweel plates together with the substrates and the antibiotic and was left for one hour or so that the antibiotic would react. The absorbance was determined at 570 nm before and after 6 hours of incubation, and the mg CO_2-C $g^{-1}h^{-1}$ respiration rates were determined exactly as in the Microresp method.

Soil microbial community structure - Ester- linked fatty acid methyl ester (EL-FAME) analysis (Case study 4*).* was performed according to Schutter and Dick, (2002). The method uses a alkaline methanolysis that extracts the ester-linked fatty acids but not free fatty acids. 3 analytical replicas were extracted from the soil samples previously stored at 20°C. 5 g of soil together with 16.6 ml KOH (0.2M) dissolved in methanol and 150 μl of methylnonadecanoate 19:0 (internal standard) were added into a 50 ml sterile tube. The tubes were incubated for 2 h in a hot water bath at 37°C and vortexed for 10 s every 10 minutes during which ester-linked fatty acids were released and methylated. After the incubation, pH in each tube was neutralized by adding 6.6 ml of acetic acid 1M. Furthermore, FAME's were separated into an organic phase, by the addition of 10ml of hexane and centrifuged (4500 rpm) at 4°C for 20 min. The hexane layer was transferred into a GC sterile glass tubes, dehydrated using Na_2SO_4 and evaporated under a steam of N_2. Finally, FAMEs were dissolved in 150μl of hexan and transferred to an amber vial for GC quantification analysis. Each FAME was identifiedby the use of Master GC (Dany Instruments, Italy) equipped with an RXI-5 ms capillary column (internat diameter 0.25 mm, 30 m, film thickness 0.25μm) (Restek) and a flame ionization detector (FED-GC). The temperature program ramped from 89°C (for 2 min); from 89°C to 280°C at 6°C/min with 5 min at 280°C. 14 methylated fatty acids (FA) were recorded according to their retention time and using BAME 24 (47080 U) and 37 FAME Mix (47885-U, Sigma–Aldrich) as chemical standards. Data was expressed as

nmol g^{-1} dry weight. Methylnonadecanoate, C19:0, at known concentrations, was used as internal standard. Standard nomenclature is used to describe FAs, which are designated by the total number of carbon atoms/number of double bonds, followed by the position of the double bond from the methyl (aliphatic) end (ω) of the molecule. The prefixes "a" and "i" refer to ante-iso and iso-branched FAs. The prefix "10Me" indicates a methyl group on the tenth carbon atom from the carboxyl end of the molecule and "cy" indicates cyclopropane FAs. Microbial groups assigned to ester linked fatty acids are listed in

Table 3.4. Microbial groups with respect to the FA biomarkers and the scientific literature reference

Microbial groups	*References*
G +	
i 14:0 i 15:0 a 15:0 i 16:0 i 17:0 a 17:0	
General	
14:0 15:0 16:0 17:0 18:0	Frostegård et al., (1993); Waldrop et al., (2000); Fierer et al., (2003)
G -	
16:1 ω7c cy 17:0 18:1 ω9t 18:1 ω7 cy 19:0	
Actinomycetes	
10Me 16:0 10Me 18:0	Frostegård et al., (1993); Allison et al., (2007).
Fungi	
18:1 ω 9c 18:2 ω6,9	Frostegård et al. (1993); Zelles, (1999) Hill et al., (2000); Högberg, (2006); Joergensen and Wichern, (2008);
Arbuscular mycorrhizal fungi	
16:1 ω 5c	Pacovsky and Fuller, (1988) Olsson et al., (1995)

The sum of total bacteria (total general bacteria, G+ and G-), ratios G+/G- and fungi/total bacteria were calculated using the quantity expressed in nmol $g^{-1}$of the biomarkers.
Shannon's diversity index was calculated as: $H' = -\sum pi \log 2\ pi$ where pi is the concentration of one FA over the sum of all FA's concentration.

Soil texture (case study 1 and 3) was obtained by the pipette method after dispersion of the sample with a sodium hexametaphosphate solution (Gee and Bauder, 1986).

The cation exchange capacity (CEC) *(case study 3)* was determined after exchange with 0.05 N cobalthexamine chloride solution (Orsini and Rémy, 1976 modified by Ciesielski et al., 1997). The exchange acidity was determined in KCl 1M.

The total element concentrations (Fe, Al and Ca) *(case study 3)* were measured by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES, Ametek, Spectro) after HNO_3:HCl (1:3 v:v, suprapure Merck) microwave digestion of soil samples (Vittori Antisari et al., 2011).

Soil carbon pools (case study 3)

For each genetic horizon the organic C was (TEC) extracted with a solution of 0.1 M NaOH and 0.1 M $Na_4P_2O_7$ at 65 °C for 24 h. The humic acids (HA) were separated from TEC by acidification (pH<2) and centrifugation, while fulvic acids (FA) were separated from the non-humified organic material by solid chromatography with polyvinyl pyrrolidone resin (Vittori Antisari et al., 2010). The organic C content in the TEC, HA and FA fractions were determined by wet oxidation at 160 °C with $K_2Cr_2O_7$ 1/3 M, according to the method of Springer and Klee, (1954).

Foliar analysis (case study 3)

Three samples, each including three mature sunny leaves, were collected per species on each half-plot. Total N and microelement (Fe, Mn, Al) contents have been determined on these samples using Elemental combustion Analysis (EA-1110 Thermo Scientific Lab) and ICP-OES after HNO_3:H_2O_2 (3:1.5 v:v, suprapure Merck) microwave digestion of samples, respectively. Both data were expressed on dry leaf mass basis. Moreover, each nutrient was expressed on N-ratio basis as suggested by Linder, (1995). The method is based on two main assumptions: (i) within a wide range, the concentration of a nutrient element per se is not essential to the "vitality" of a plant; the proportions of elements relative to nitrogen are at

least just as important; and (ii) the optimal proportion between nutrient elements is similar for all vascular plants and can be defined in relation to nitrogen.

3.2.3 Data analysis

Statistical analyses were performed using JMP 9 software (SAS Institute Inc., Cary, NC), and STATA 14 software (StataCorp LP) for the quantile regression analysis.

3.2.4 Arbuscular mycorrhizal fungi identification

For a period of 3 months (September-December 2015) I was a PhD visiting student at the James Hutton Institute (Dundee-Scotland) under the supervision of Dr. Tim Daniell and his staff. During the three months I was taught a molecular technique for the identification and characterization of AMF.

The technique applied related to the application of molecular techniques to assess the presence and characterization of the structural diversity of arbuscular mycorrhizal fungi (AMF) by combination of sequencing (to obtain phylogenetic information) and Terminal Restriction Fragment Length Polymorphism (T-RFLP). The techniques were applied in order to attain high-throughput analysis of AMF. The plant-root samples were taken for the long term experiment presented in Case study 2, considering five time points from April to August 2015. Samples were collected in triplicates from each plot for a total of 189 samples. The aim of the study was to identify different AMF patterns in time comparing ORG and CONV managements. The protocol followed five steps: DNA-extraction, PCR 18sRNA small subunit analysis of AMF, cloning, sequencing and designing a directed T-RFLP strategy.

The DNA extraction of AMF was done approximately 20 mg of dry powder tomato root/ sample followed by PCR analysis using primer NS31 (5'- TTGGAGGGCAA-GTCTGGTGCC-3') as forward primer and AML2 (5`- GAACCCAAACACTTTGGTTTCC -3`) as reverse primer.

The cloning procedure was done on 8 PCR products selected from different time points. The PCR products were ligated and into pGEM^{R}-T Easy vector (Promega), 3 µl of the ligation was added to *Escherichia coli* competent cells and transformed using thermic shock. Cell suspension was plated onto LB agar and incubated at 37°C over-night; white and light blue

colonies were picked and added into 384 well plates and further incubated over night at 37°C. Plasmids were extracted using MultiScreenHTS 96-well filter plates (Merck Millipore, Watford, UK) following the manufacturer's instructions. Sequencing was done on 192 clones using the Sanger method using T7 and SP6 primers. The sequence alignments were obtained using ClustalW tool (http:// www.genome.jp /tools/clustalw/). Where both T7 and SP6 primers were used and sequences were overlapped using Sequencer 4.9 software (Gene Codes Corporation, MI, USA). Finally a phylogenetic tree was generated using both the sequences of the present study and additional sequences downloaded from the Marjaam database of Glomeromycota DNA sequences (Opik et al., 2010). Finally, the obtained clone sequences were clustered in 7 different groups of AMF.

A directed T-RFLP strategy was designed starting with the identification of the specific digestion strategy (restriction enzymes selection) that could be used in T-RFLP analysis to effectively separate the expected sequence types represented in the clone library (directed T-RFLP). Initial screening of suitable restriction enzymes in order to separate the identified sequence groups was performed using the open-source program Directed Terminal Restriction Analysis Tool (DRAT; Roberts et al., 2012). The set of enzymes selected offered the best combination of resolving power, within-group fidelity and compatibility of reaction conditions. Afterwards, sequences were imported into GeneDoc software and "in silico" digestions were set using the recognition sites of the enzymes previously suggested by DRAT. Before applying the enzymes digestion to all the samples it was validated on clones and clone mixes appertaining to each AMF group identified.

The T-RFLP analysis implied an enzyme digestion on PCR products obtained with the use of one fluorescent primer NS31 FAM (5'- TTGGAGGGCAAGTCTGGTGCC-3') and AML2 (5`- GAACCCAAACACTTTGGTTTCC -3`) and the obtained DNA fragments were separated by high-resolution automated capillary electrophoresis utilizing internal size standards (LIZ 600). Genotyping was carried out on a 3730 DNA Analyzer capillary sequencer (Applied Biosystems).

3.2.5 Identification of the sensitive soil quality indicators

The data included in the analysis for the identification of the sensitive indicators was taken from the four case studies after dividing the soils with respect to the management practices. The analysis takes in consideration the following indicators: specific soil enzyme activities (per unit of Corg) of cellulase, chitinase, acid-phosphatase, b-glucosidase, arylsulphatase,

xylosidase, a-glucosidase and the microbial quotient (Cmic:Corg). Each indicator and Cmic:Corg were considered dependent variables tested with respect to total nitrogen (TN) and soil pH. These soil chemical characteristics were chosen due to their crucial importance in the microbial biomass activity. Soil nitrogen is considered a limiting factor plant growth whereas its presence in soil was widely associated with soil management practices in agriculture (tillage intensity, organic farming etc.). Moreover in forest ecosystems the N concentration can reflect changes in soil quality induced by tree species composition and stand ages. Soil pH is an intrinsic soil characteristic that affects the soil quality indicators (chemical and biological) and also affects availability of nutrients for plant growth. Soil analysis data from case studies 1-4 were divided in two groups; group one- agricultural soils (case study 1 and 2) and group two- forest soil (case study 3 and 4). The agricultural soils (group 1) were subsequently divided and analysed with respect to the management practice as following: conventional tilled soils (CT) vs reduced tilled soils (RT) and conventionally managed soils (CONV) vs organic managed soils (ORG). The forest soils (group two) were divided into the different management practices: soils from Douglas-fir forests with different stand ages (case study 3) and Beech tree forest soils from different forest stand structures (case study 4).

The existence of association between each of the soil indicators and soil management type was determined by estimating quantile regression models (QRM). This analysis was applied separately for each case study.

In case studies 1-4 in order to assess the models each soil indicator has to relate to a management type. Indeed, quantile regression offers the possibility to highlight how the effect of the independent variables changes throughout the entire distribution of the dependent variable. In our case shows how pH and TN influence the selected soil indicators across all of theirs statistical distribution from the low values (Q25) until the higher values (Q75).

To estimate the relationships (association) between the dependent variables and the set of selected regressors the classical OLS (Ordinary Least Squares) regressions can be applied; but the accuracy of this method can be limited to a normal distribution of the data. All the data obtained from the case studies tends to be skewed so OLS models risk describing false relationships. On the other hand, QRM can provide a better picture of the relationship between variables estimating changes from the minimum to the maximum responses (Cade and Noon, 2003). The quantile regression model specifies the conditional quantile of the dependent variable *y* as a linear function of covariates (Koenker, 2005):

$$Q_\theta \left(y_i \left| \mathbf{x}_i \right. \right) = \mathbf{x}_i^{'} \boldsymbol{\beta}_\theta + \varepsilon_{i\theta}$$

where y_i (i=1,...,n) is the dependent variable represented, in turn, by, x_i is a sequence of k-vector of regressors, β_θ is an unknown vector of regression parameters associated with the θ_{th} quantile and $\varepsilon_{i\theta}$ is an unknown error term.

A second statistical analysis was applied in order assess through the Wilcoxon or Kruskal-Wallis non-parametric tests that show the discriminatory ability of the selected dependend variable (specific enzyme activities and microbial quotient) divided by management within different classes of pH a Next/TN (independent variables). In this case Next/TN was selected due to the fact that the extractable nitrogen pool has a higher turn-over rate than total N and has a very important role in soil ecological processes such as N mineralization. This test was performed among soil indicators under different types of management within different classes of pH and Extr-N/TN for case studies 1-4.

The data of the 5th case study comes as a deepening of the knowledge on soil microbial functional diversity by comparing how the microbial diversity measured as Shannon's Index with two different methodological approaches (Microresp and Multi-enzyme analysis) discriminates in a high variety of soils with different concentration of soil Corg and pH. The same statistical approach was applied as for the soils from case studies 1 and 4. The QRM has taken in consideration the various soils (agricultural, forest and extreme conditions) and related them to the Shannon diversity index (calculated in terms of enzyme activities and CLPP) with respect to different values of pH and Corg. Furthermore, the Kruskal-Wallis non parametric test was used to test if and to what extent the two indexes made possible to distinguish the various type of soils with different peculiarities in terms of Corg and pH.

IV. RESULTS and DISCUSSION

4.1 Soil ecological impact of tillage in various subsidiary cropping systems across four European climatic zones (case study 1)

4.1.1 Results

a) Description of pedoclimatic data

The soil samples were collected at the harvesting of the main crop at both MEE crop cycles, in 2014 and 2015, respectively. The pedoclimatic description includes the average climate data for rainfall and temperature together with the aridity index calculated according to Mancinelli et al., (2013), moreover the initial soil properties (before the crop cycle) are shown in table 4.1.1.

Table 4.1.1 : Pedoclimatic description of the four European experimental sites.

		Climate description (*)			Soil properties							
Crop cycles	Sites	Rainfall (avg mm y^{-1})	Temperature (avg °C y^{-1})	AI	Clay (%)	Silt (%)	Sand (%)	Soil texture (USDA)	pH (H_20) 1:2.5 w:v	Total carbonate	Corg	TN
I (2013-2014)	ART	1111	9.5	3.74	19	35	46	Loam	7.1	1.0	20.0	2.3
	ORC	628	10.8	1.19	58	20	22	Clay	7.5	4.6	20.0	1.9
	SLU	598	8.2	2.66	10	60	24	Silt loam	5.7	1.4	30.8	2.6
	UNITUS	845	11.6	2.84	23	21	55	Sandy-Clay-loam	6.7	0.4	12.3	1.0
II (2014-2015)	ART	1259	10.6	4.80	22	35	43	Loam	6.9	4.5	21.6	1.4
	ORC	352	10.5	0.95	58	20	22	Clay	7.2	2.8	23.2	2.2
	SLU	526	7.3	1.18	20	63	14	Silt loam	6.1	3.0	28.6	2.2
	UNITUS	614	11.2	0.86	15	22	63	Sandy-loam	6.7	1.0	12.4	2.7

*Averages of rainfall and temperature are calculated considering data record of 12 months before soil sampling date, AI= aridity indices calculated on a monthly basis (30 days before soil sampling).

b) Soil chemical and biochemical characteristics

Soil chemical and biochemical properties were statistically analysed in order to find the significant difference among various management types (cover crops C, tillage levels T and their interaction TxC). In particular, through ANOVA, significant differences were analysed between: 1. reduced and conventional tillage, 2. Leguminous and brassica spp. and living mulch LM (table 4.1.2). by comparing various climatic zones, the minor effects of management were observed in the very humid site (ART) where annual precipitation was abundant (1086 mm). The effects of treatments were significant, especially for the extractable C and N pool and for soil biochemical properties, such as microbial biomass and enzyme activities. Moreover, no significant differences were found in terms of soil total organic C (Corg), total nitrogen (TN) and C/N ratio. In general three of the four European experimental sites (SLU, UNI and ORC) showed more significant effects. The tillage effect was less evident than cover effect in all countries at both MEE's. The significant effect due to the interaction between cover crop (C) and tillage (T) was registered in terms of microbial biomass carbon (Cmic) and nitrogen (Nmic), both expressed per gram of soil and per unit of organic carbon (Cmic:Corg) or total nitrogen (Nmic:TN). Regarding soil enzyme, specific activity was chosen in order to compare various sites with very different soil organic matter content as background value. In this study, the enzyme activities involved in C and N cycles showed significant interactions between T and C at ORC, SLU and UNI sites at both MEEs. Conversely, at ART site the effect of management on soil specific enzyme activity (per unit of organic carbon) was not significant. The functional diversity calculated using the Shannon's index (H') showed significant differences only at ORC and UNI sites (Table 4.1.2).

The principal component analysis PCA (Figure 4.1.1), indicated that the two MEEs of all sites were usually separated and no differences were seen between conventional and reduced tilled soil. With respect to the PC1, 33.7 % of variance was explained; moreover, MEE1 showed negative scores compared to MEE2. In addition, along PC1, SLU, ART and UNI were separated from ORC in MEE1; while in MEE2, ORC and UNI were separated from SLU and ART. The PC2 explained 18.9% of variance distinguishing ART, ORC, UNI from SLU in MEE1 and UNI, ORC from SLU and ART in MEE2, respectively. In conclusion, the scores of MEE1 were clustered in four groups one for each site, while the scores of MEE2 were divided into two groups UNI-ORC and ART-SLU.

The variables discriminating on PC1 (loading values higher than 0.5) were the microbial quotient (Cmic:Corg), enzyme specific activities (expressed per unit of Corg) involved in C, N, S, and P cycles, and the basal microbial respiration (BR). Conversely, for PC2, the variables were: labile pools of N (extractable and microbial) expressed per unit of TN, Shannon's index (H') and clay content (Figure 4.1.1). The differences between the two tillage levels were particularly evident in terms of soil biochemical properties, therefore the obtained results on soil microbial biomass, synthetic index of the C-cycle enzymes and chitinase were showed with respect to the different SC (leguminous, living mulch and Brassica sp.) and MEEs in figures 4.1.2-4.1.4.

Table 4.1.2: Statistical analysis (ANOVA) of soil chemical and biochemical properties in the four European experimental sites (* p<0.05; ** p<0.01; *** p<0.001) after the main crop

Site	Cycle		TN	Corg	C/N	Extr-C	Extr- N	ExtrC/ Corg	ExtrN/ TN	Cmic	Nmic	Cmic/ Nmic	Cmic/Corg	Nmic/ TN	SEIc	SEIc/ Corg	Chit	Chit/ Corg	Pho	Pho/ Corg	Aryl	Aryl/ Corg	H'
ART	2014	T				*		*															
		C							*	*					**	*	*		*				
		TxC																					
	2015	T															*				**	**	
		C					*		*	**	*		**	*		*							
		TxC																					
ORC	2014	T									*					*	*	**		*	**	***	
		C				**	**	*		***	***	***	***	*			***	**			**	*	*
		TxC				*		**		***	***	**	***	**	**	**							
	2015	T				*		*				*									*		
		C				*		*				*			***	**	**	*	***	**	***	**	
		TxC						**	**		*	**	*	**	**	**	*	*	*			**	
SLU	2014	T								**	***		*	*									
		C								***	***	*	***	***	**	***	***	***	**	*			
		TxC								***	*	***	***	*			***	***					
	2015	T					*			***	***		*	*	*						*	*	
		C					*			***	***		***	***	**	**			**	*	*	*	
		TxC									***			*	*		*	*		*	*	*	
UNITUS	2014	T																		*			
		C					**			***	***	***		***	**		**	**	**		**		*
		TxC							*	**		*		**	***	**	**	**		*		*	
	2015	T								*	*					*		*		*		*	*
		C					*			***	**	***	***	*	*	*	**	**	*			*	
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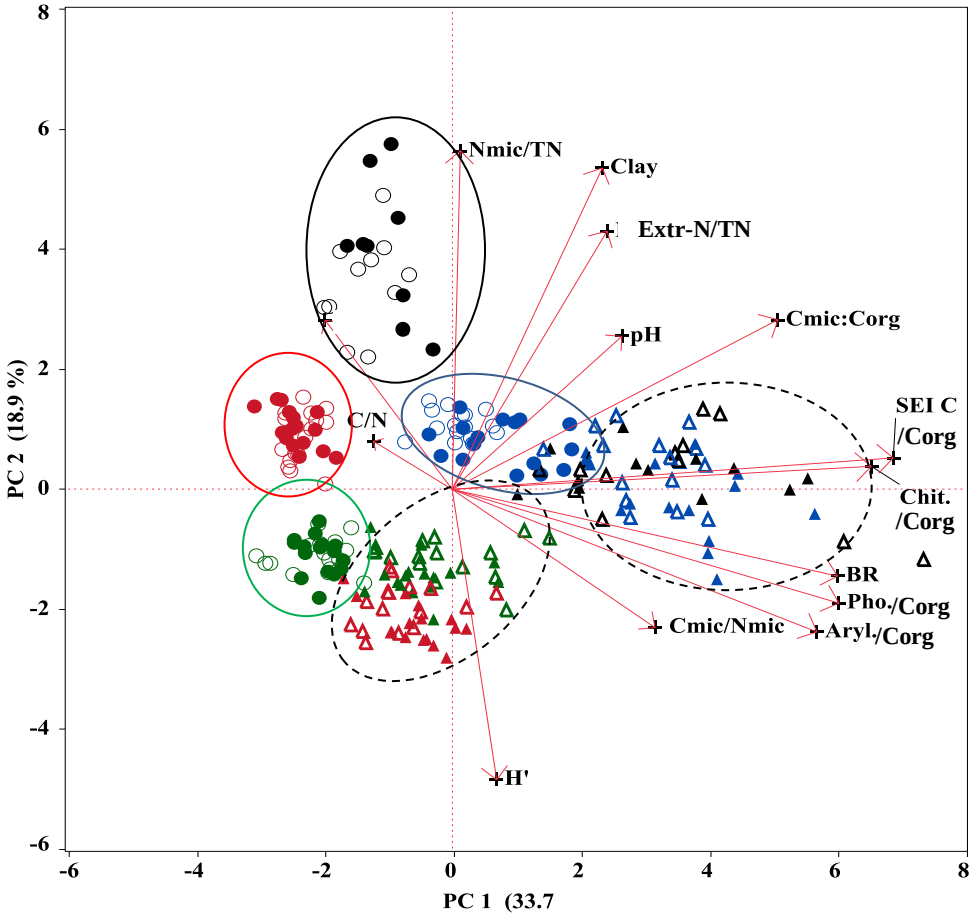


	PC 1	PC 2
C/N	-0.17	0.11
Cmic:Corg	**0.69**	0.38
Nmic/TN	0.02	**0.77**
Extr-C/Corg	-0.27	0.38
Extr-N/TN	0.33	**0.59**
Cmic/Nmic	0.43	-0.31
SEI C/Corg	**0.94**	0.07
Chit./Corg	**0.89**	0.05
Pho./Corg	**0.82**	-0.26
Aryl./Corg	**0.77**	-0.32
H'	0.09	**-0.66**
BR	**0.82**	-0.20
pH	0.36	0.35
Clay	0.32	**0.73**

Figure 4.1.1 PCA and loadings values of each site at MEE1 (cycles) and MEE 2 (triangles). Different colour markers are used for various sites: red ART, blue ORC, green SLU and black UNI; full and hollow markers are used to distinguish conventional (CT) and reduced tillage (RT), respectively. In table bold values are the significant factor variables (> 0.5).

The Cmic content in MEE1 and MEE2 was generally higher at ORC site followed by SLU, UNI and ART (Figure 4.1.2). In particular, in MEE1 the conventional tillage (CT) caused a significant reduction of Cmic at ORC under leguminous mix CC (Figure 4.1.2 A) and at UNI under the living mulch (Figure 4.1.2 B). Conversely, a higher content of Cmic was found in CT at UNI under leguminous CC, at ART under LM and at ORC under brassica sp CC (Figure 4.1.2 C).

The highest value of microbial quotient (Cmic:Corg), at both MEEs, was recorded at ORC under leguminous mix and brassica spp. CC and at UNI under LM. Conventional tillage, caused at both MEEs, a significant decrease of microbial quotient at ART under leguminous CC (Figure 4.1.2 A) and increase of Cmic:Corg at ORC under Brassica CC (Figure 4.1.2 C). The sum of all enzyme activities involved in C-cycle (SEI C) expressed per unit of Corg, in MEE1 was highest at ORC site in all SC treatments, whereas at MEE 2, the highest activity was registered at UNI site under leguminous CC (Figure 4.1.2 D) and living mulch (Figure 4.1.2 E) treatments. ORC showed significant higher values of SEI C in leguminous CC under CT (Figure 4.1.3 A) and brassica CC at MEE 1 (Figure 4.1.3 C). In MEE 2 SEI C was significantly higher at UNI under leguminous CC and LM as well as at ORC under LM (Figure 4.1.3 D-E).

The specific chitinase activity showed a similar trend of SEI-C, the highest activity was registered in soils of MEE 1 at ORC site, while UNI showed the highest values in leguminous CC and LM at MEE2. Moreover, tillage significantly increased the chitinase activity at UNI for leguminous cover crops (Figure 4.1.4 A) and at ORC for leguminous and brassica CC at the MEE 1 (Figure 4.1.4 A and C). On the other hand, tillage at ART and SLU caused a decrease of chitinase activity under brassica CC (Figure 4.1.4 C). At ORC, SLU and UNI MEE 2, the leguminous showed a significantly lower chitinase activity with respect to the other CC (Figure 4.1.4. D). Finally in the living mulch treatments, CT was lower at ART and ORC and higher at SLU (Figure 4.1.4 E).

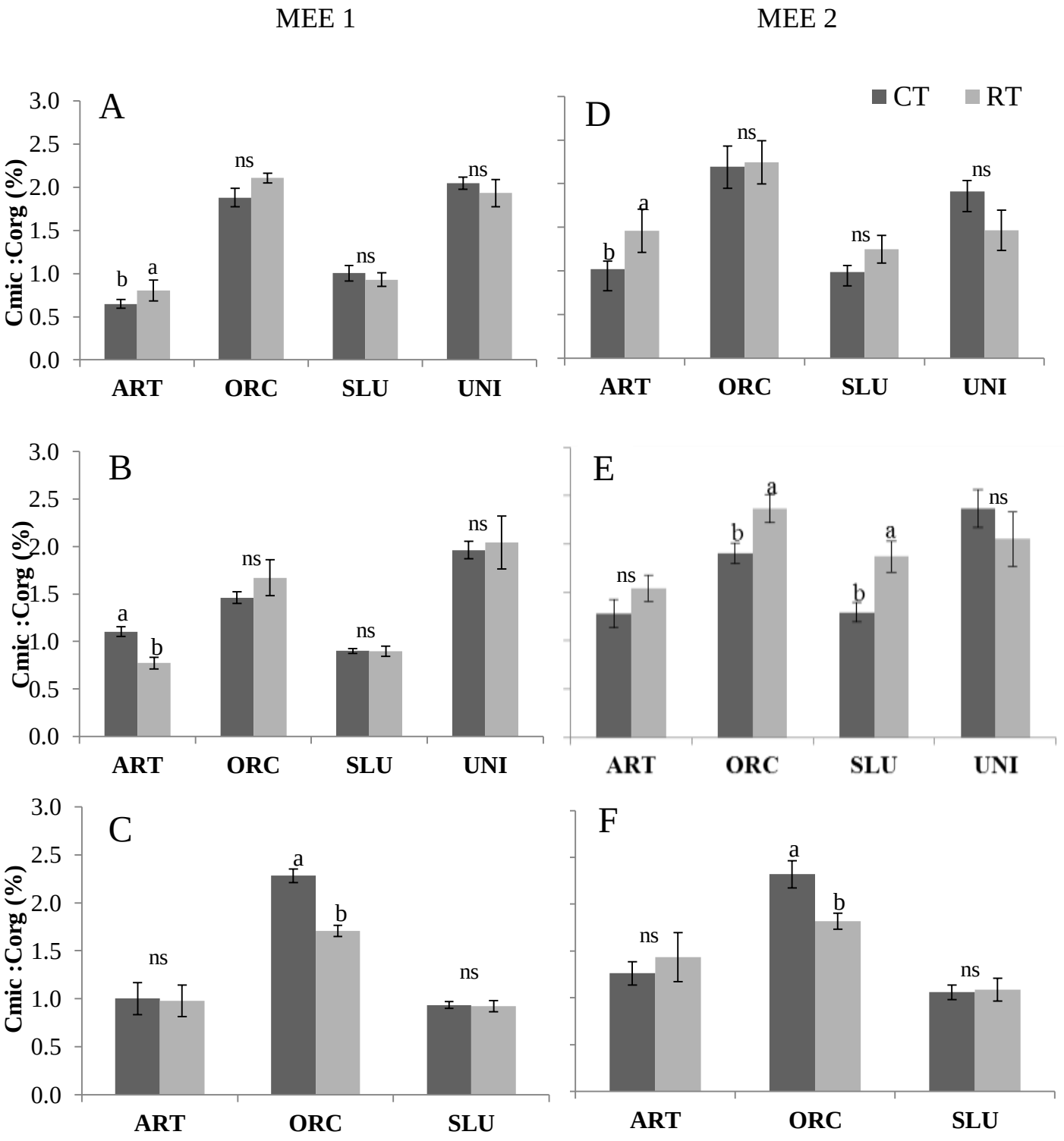


Figure 4.1.2 Microbial quotient (Cmic:Corg) of each SC at MEE 1 and MEE 2 under conventional tillage (CT) and reduced tillage (RT) for each subsidiary crop: A and D- leguminous and leguminous mix, B and E- living mulch and C and F- Brassica sp. Different letters represent significant differences between tillage levels according to LSD ($p<0.05$) and ns is not statistically significant.

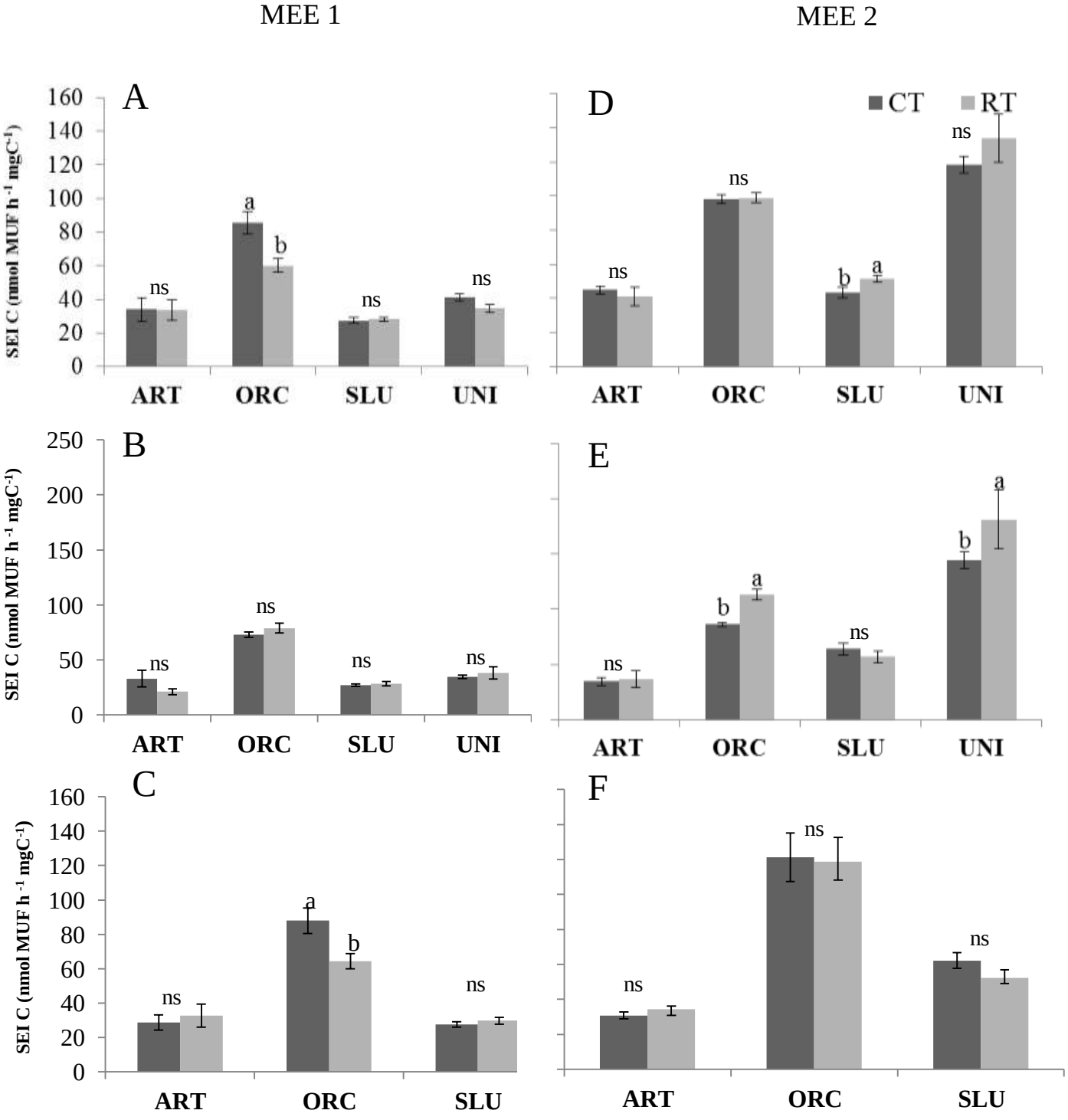


Figure 4.1.3 Synthetic index of C-cycle enzymes (expressed per unit of Corg) of each SC at MEE 1 and MEE 2 under conventional tillage (CT) and reduced tillage (RT) for each subsidiary crop: A and D- leguminous and leguminous mix, B and E- living mulch and C and F- Brassica sp. Different letters represent significant differences between tillage levels according to LSD ($p<0.05$) and ns is not statistically significant.

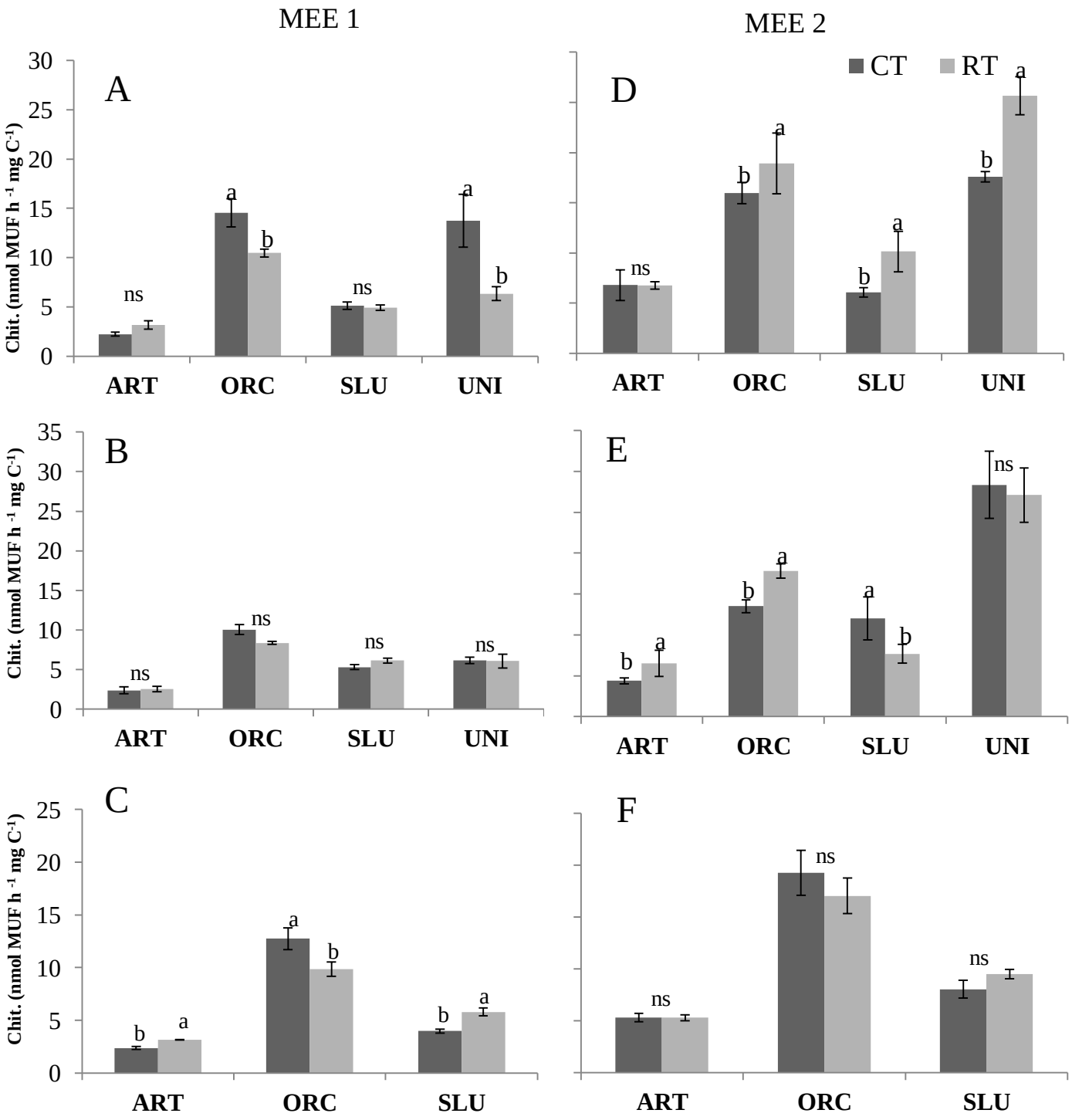


Figure 4.1.4 Chitinase (expressed per unit of Corg) of at MEE 1 and MEE 2 under conventional tillage (CT) and reduced tillage (RT) for each subsidiary crop A and D- leguminous and leguminous mix, B and E- living mulch; C and F- Brassica sp. Different letters represent significant differences between tillage levels according to LSD ($p<0.05$) and ns is not statistically significant.

4.1.2 Discussion

In this study the soil ecological impact of agricultural management (tillage and use of subsidiary crop) was found to be strongly related to climatic conditions. The four climate zones (Boreal, Continental, Oceanic and Mediterranean) showed different interactions between tillage level and cropping system. It is known that climate and seasonal variations have a major influence on the soil organic matter dynamics, especially on the decomposition of plant detritus and substrate availability for the soil microbial biomass (Davidson and Janssens, 2006; Marinari et al., 2015). Furthermore tillage accelerates the process of decomposition by stimulating the growth of microbial communities with high metabolic rates (Pankhurst et al., 2002; Spedding et al., 2004) and conservation practices such as the cover crop use and reduced tillage may delay microbial biomass response by temporary immobilisation of nutrients (Pankhurst et al., 2002). In this study the different behaviour of microbial biomass and activity, observed in MEE1 and MEE2 respectively, suggests a significant interaction with seasonal conditions occurred in spring-summer 2014 and 2015, respectively. Moreover, the values of the microbial quotient, especially in the upper soil levels, tend to increase when reduced tillage is applied, showing a higher substrate availability and a better quality of organic matter thus improving food supply for the soil microflora (van Capelle et al., 2012). In this study, the soil microbial quotient (Cmic:Corg) was a sensitive indicator of tillage ecological impact because at both MEEs reduced tillage showed a positive effect of leguminous CC at ART and a negative effect of Brassica at ORC. For this reason, it can be supposed an opposite effect of reduced tillage in leguminous and brassica CC grown in continental and in oceanic climatic zones, respectively. Moreover, the specific activity was chosen in order to compare various sites with different background of soil organic matter. In this context, the specific activity may represent an indicator of the organic matter nutritional status present from the perspective of the microbial community (Boerner et al., 2005). In fact, the specific activity (per unit of organic carbon) could be related to the quality of soil organic matter (Trasar-Cepeda et al., 2008) showing how much the present organic substrates are involved in hydrolytic biochemical reactions. In this study, the experimental site with a very humid climate (continental zone), presented the lowest soil specific enzyme activity. Moreover, at this site the effect of agricultural management on soil specific enzyme activity was less evident than the other sites where the precipitations are lower.

The response of soil enzyme activity to tillage level depended on seasonal variations in climate; in fact the effect of tillage on specific enzyme activity (per unit of organic carbon) was different between MEE1 and MEE2 because of changes in average temperature and rainfall in 2014 and 2015, respectively. This result supports the findings of Mancinelli et al., 2013 who argues the need of discussing the mineralization of soil organic matter, produced by different cover crops, in relation with climatic conditions. Furthermore the enzyme activity tends to be higher in soils with reduced tillage compared to the conventional one (Curci et al., 2007; Roldan et al., 2003). Soil under reduced tillage as well as soil with SC, showed a higher soil enzyme activity compared to conventional tillage and control soil without SC. These results might be due to the input of plant residues and to the subsequent increase of decomposable organic matter for soil microorganisms. Several studies reported that the enzyme activities involved in C, N, S and P cycles were enhanced by SC cropping system and by no-tillage of soil (Hamido and Kpomblekou-A, 2009; Mbuthia et al., 2015; van Capelle et al., 2012). In this study, the microbial activity measured in terms of enzyme activities involved in C and N cycles (SEI C and chitinase), was more sensitive to seasonal variation than microbial quotient. In fact, an opposite effect of reduced tillage in brassica and leguminous CC at ORC, SLU and UNI was observed comparing data obtained at MEE1 and MEE2, respectively. In the second MEE especially at ORC and UNI sites leguminous SC (leguminous CC and living mulch), showed significantly higher values of SEI C and chitinase under RT compared to brassica spp. It is a known fact that legumes, due to their biomass composition with a low C/N ratio, promote soil enzyme activity causing a faster mineralization process with respect to non-legume CC species (Balota et al., 2014; Mancinelli et al., 2013). In this study the increase of soil microbial activity in RT soil was still evident at the main crop harvesting, after few months from SC suppression. This result could be explained by a slower process of legume litter decomposition, considering that in RT the plant detritus is left on the soil surface and not incorporated as in CT. ORC and UNI sites had similar microbial biomass quotients and enzyme activities, these two sites also had similar temperature and rainfall as an annual average in the past 30 years, suggesting that in this case the climate affects the soil response to tillage in a similar way.

No change of total soil organic carbon and nitrogen occurred after SC suppression and tillage of the soil in different climatic zones. Therefore, on a short-term it is hardly possible to detect changes in soil organic matter after the implementation of a new management practice (Alvarez et al., 2000).

4.1.3 Conclusions

In this study the microbial quotient (Cmic:Corg) was the most sensitive indicator of tillage effect on soil quality, however it was less sensitive to seasonal variations than soil specific enzyme activities. The reduced tillage caused an opposite effect in terms of microbial quotient change in leguminous and brassica CC at ART and ORC, respectively. Therefore, the effect of reduced tillage was depending on the type of CC and climate zone. In particular, the very humid climate inhibited the effect of management on soil biochemical properties. Finally, the specific activity of enzymes involved in C and N cycles were mainly influenced by season climate conditions. The soil in the Oceanic and Mediterranean climate zones showed similar response to the tillage and SC in terms of microbial biomass content change as well as its activity. This similarity could be attributed to the fact that both sites also had analogous annual rainfall which seems to mask the effect of management on soil organic matter quality and dynamics.

4.2 Soil quality indicators in a long-term experiment: organic vs. conventional agricultural management (case study 2)

4.2.1 Results

In this study organic and conventional agricultural managements were compared in terms of soil quality change after 14 years of field experiment in a three years crop rotation system. The tillage level was included as second factor in the experimental design, for this reason two soil depths were analyzed (0-15 cm and 15-30 cm). The effects of agricultural management (CONV vs. ORG) and soil tillage level (RT vs CT) were particularly evident in the upper soil layers (0-15 cm) for several soil chemical and biochemical properties (table 4.2.2). First of all, soil total nitrogen (TN) and the C/N ratio were influenced by the management system while the total organic carbon (Corg) was influenced by both management system and tillage level. The soil carbon stock was significantly different among management and tillage systems according to the following order: CONV CT< CONV RT< BIO CT< BIO RT (Table 4.2.2). Conversely, the soil nitrogen stock as well as the extractable carbon and nitrogen (Extr-C and Extr-N, respectively) were not significantly different among management and tillage systems. However, significant differences were found when the labile soil nutrient pools were expressed per unit of Corg and TN in the upper soil layers. The Extr-C/Corg was significant different between the two management systems (CONV>ORG), while the Extr-N/TN was significantly higher in ORG- RT than in CONV- CT (Figure 4.2.1 A).

The microbial biomass carbon (Cmic) and nitrogen (Nmic) were sensitive indicators of the agricultural management and tillage level. In particular, the highest quantity of Cmic was found under RT in the upper layers (0-15 cm) of ORG soil, while in the deeper layers (15-30 cm) the values were influenced only by the tillage regardless of the management system (Figure 4.2.2. A). The microbial quotient (Cmic: Corg), at both soil depths followed the same trend of Cmic (Figure 4.2.3.A). The enzyme activities were expressed per gram of soil and per unit of carbon (specific enzyme activity) in order to overlook the effect of Corg quantity, but focusing more on soil organic matter quality. The sum of enzyme activity involved in the C-cycle (SEI-C) at both soil depths, showed the greatest values under RT in the ORG system (Figure 4.2.2 B). Moreover, the C-cycle specific enzyme activity (Figure 4.2.3 B) was influenced by tillage at 0-15 cm with higher values for the RT plots compared to CT.

Table.4.2.1. Statistical analysis (ANOVA) of soil chemical and biochemical properties in the LTE at 0-15 cm depth and at 15-30 cm (* p<0.05; ** p<0.01; *** p<0.001)

	TN	Corg	C/N	Extr C	Extr N	ExtrC /Corg	ExtrN /TN	Cmic	Nmic	Cmic/ Nmic	Cmic/ Corg	Nmic /TN	SEI C	SEIc/ Corg	Chit	Chit/ Corg	Pho	Pho/ Corg	Aryl	Aryl/ Corg	H’
Soil depth 0-15 cm																					
SYSTEM (S)	*	***				*		**	**		*		***		*		*				
TILLAGE (T)		**	*				**	*	**			**	***	***	***	***	**	*			**
S x T															*	*					
Soil depth 15-30 cm																					
SYSTEM (S)				*									*	*			**	*			
TILLAGE (T)					*			*			*										
S x T													*	*							

The chemical properties are: total nitrogen (TN); organic carbon (Corg); extractable C (Extr.C); Extractable N (Extr. N). The biochemical properties are: microbial carbon (Cmic); microbial nitrogen (Nmic); Synthetic index of the C-cycle enzymes (SEI C-cycle), acid phosphatase (Pho), arylsulphatase (Aryl), Shannon’s diversity index (H’).

The ORG management showed a significant increase of specific C-cycle enzyme activities at 15-30 cm soil depth. Chitinase activity was considered as enzyme involved to both carbon and nitrogen cycles. Also this enzyme was expressed per gram of soil and per unit of Corg (Figure 4.2.2 C and 4.2.3 C), in both cases significant differences were found only at the first soil depth. Moreover, the highest chitinase activity was registered in ORG-RT management. Furthermore, management systems and tillage revealed a significant effect on the soil acid phosphatase activity (Pho). The most positive effect of tillage and management system on Pho was reached in the upper layers of ORG-RT plots. However, in the deeper layers the Pho activity was influenced by the type management with higher values in ORG compared to CONV system (Figure 4.2.2.D). The Pho specific activity was positively influenced by RT at 0-15 cm while the ORG system registered the highest values at 15-30 cm (Figure 4.2.3.D). The RT, in both CONV and ORG systems, caused a positive effect on microbial functional diversity, showed by the Shannon's index (H') (Figure 4.2.4).

Table 4.2.2. Soil carbon and nitrogen stocks after 14 years of organic and conventional managements under tillage (CT) and reduced tillage (RT) (n=9) at 0-15 cm soil depth. Upper case letters represent significant differences between the management systems, while lower case letters represent differences between the tillage (LSD, $p < 0.05$).

	C stock		N stock	
	Mg C ha $^{-1}$		Mg N ha $^{-1}$	
System	CT	RT	CT	RT
ORG	16.0 Ab	17.5 Aa	1.8 Aa	1.7 Aa
CONV	14.6 Bb	15.7 Ba	1.6 Aa	1.6 Aa

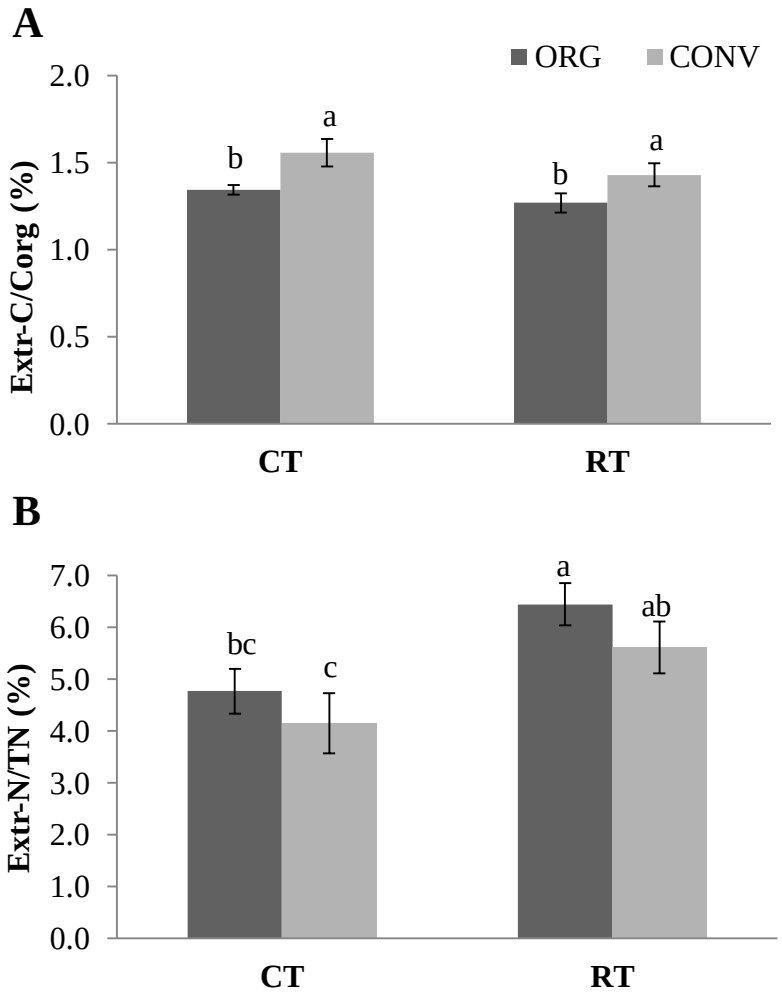


Fig. 4.2.1.Extractable carbon (Extr-C) to total organic carbon (Corg) percentage (A); extractable N (Extr-N) to total nitrogen (TN) percentage (B), at 0-15 cm. Different letters represent significant differences according to LSD ($p<0.05$).

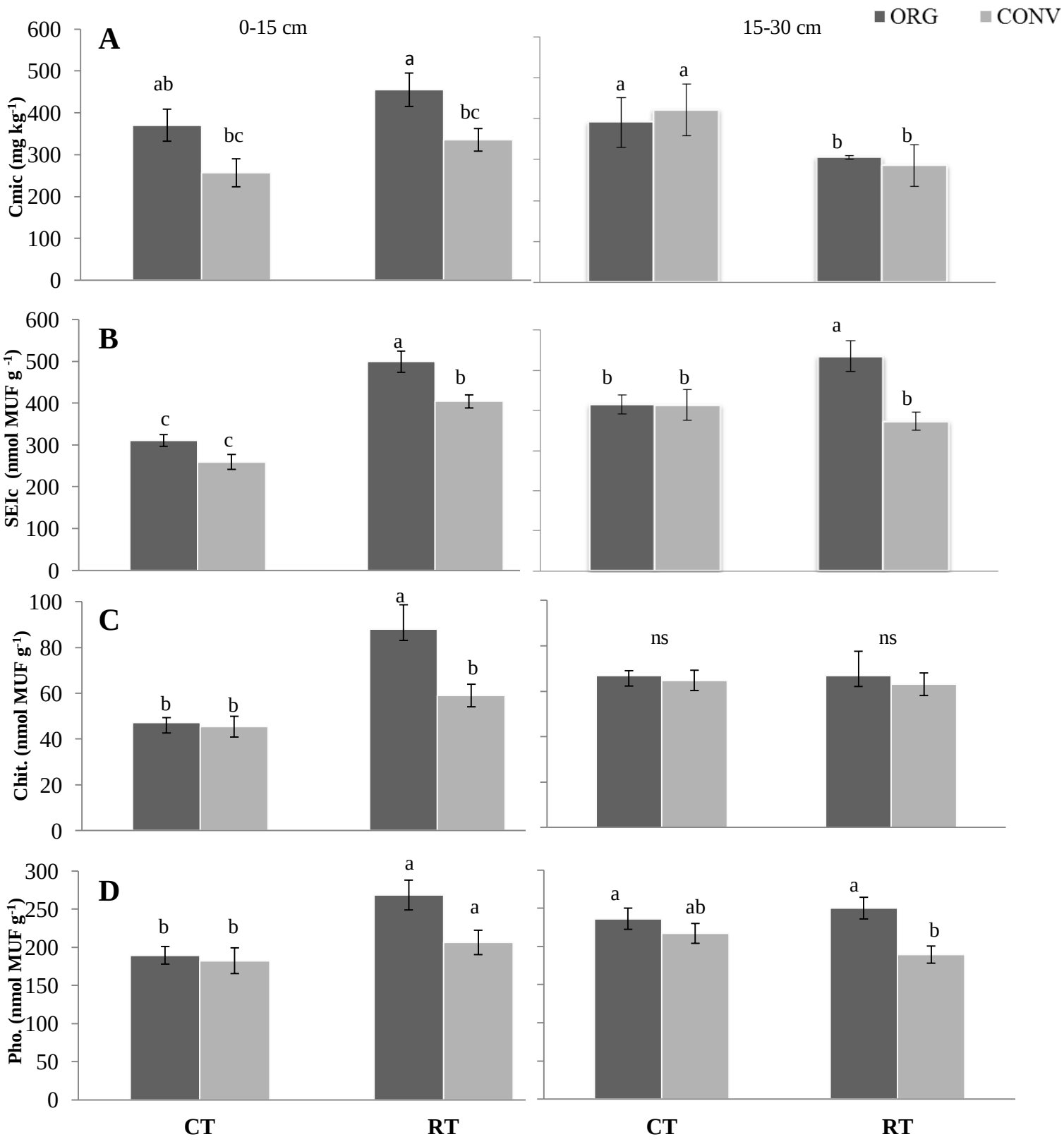


Fig. 4.2.2. Microbial biomass and enzyme activities expressed per gram of soil, at both soil depths in organic (ORG) and conventional (CONV) management under conventional tillage (CT) and reduced tillage (RT). Different letters represent significant differences according to LSD ($p<0.05$)

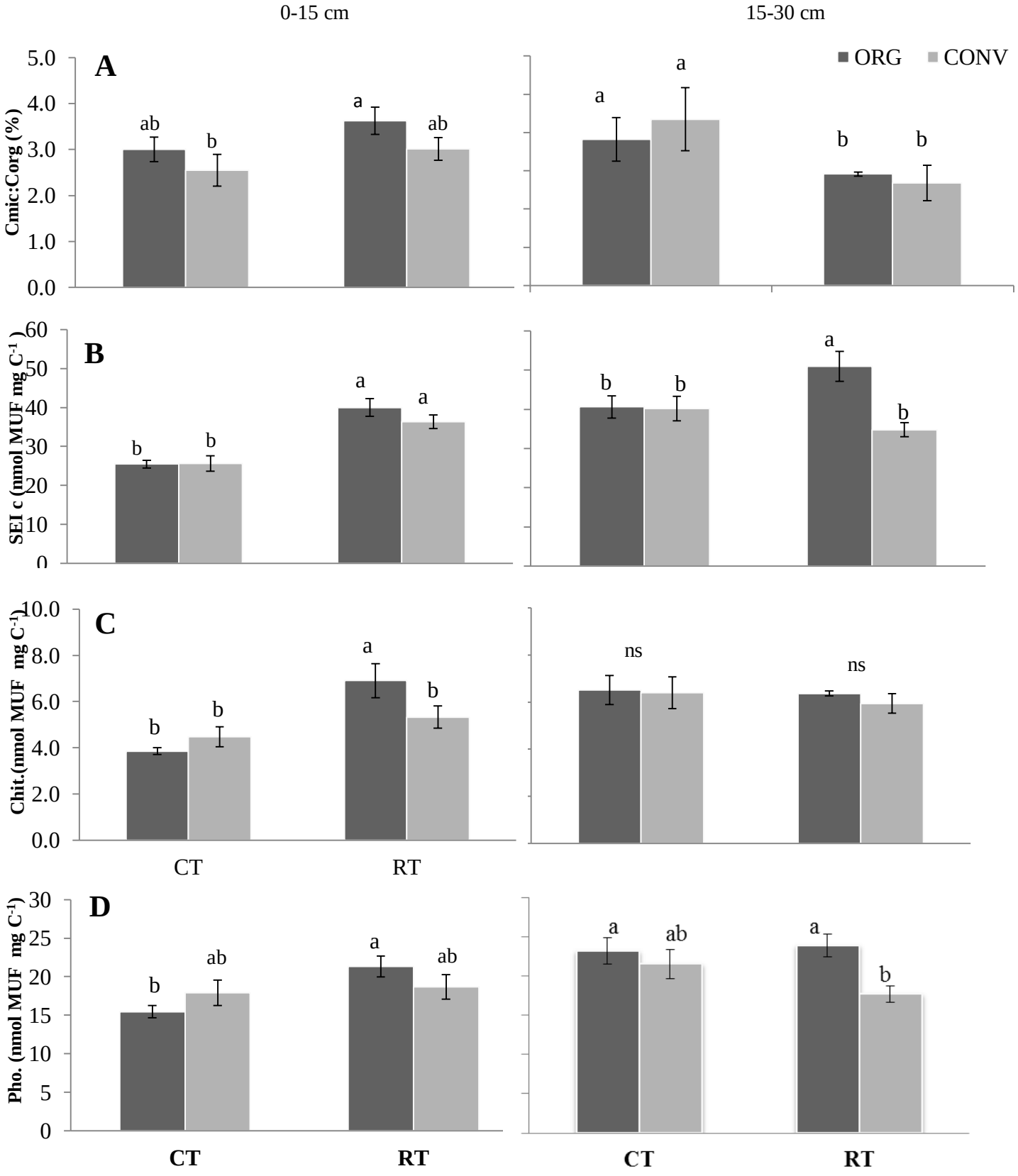


Fig.4.2.3. Microbial quotient and enzyme activities expressed per mg of Corg (specific activity), at both soil depths in organic (ORG) and conventional (CONV) management under conventional tillage (CT) and reduced tillage (RT). Different letters represent significant differences according to LSD ($p<0.05$)

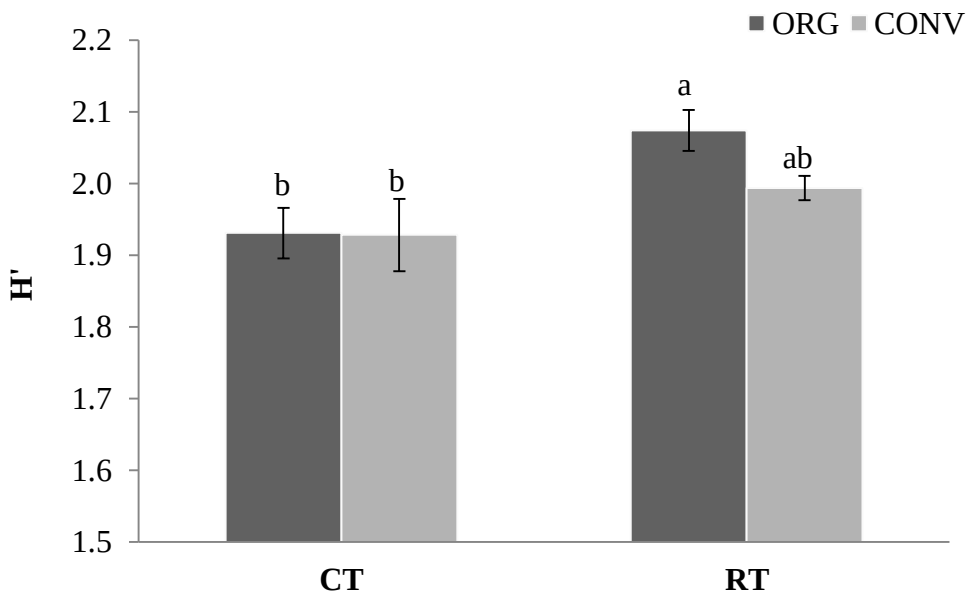


Fig.4.2.4. Soil microbial functional diversity expressed as Shannon index (H') at 0-15 cm. Different letters represent significant differences according to LSD ($p<0.05$)

A directed T-RFLP strategy was chosen for the identification of different colonization patterns of the arbuscular mycorrhizal (AMF) over time. Firstly, optimal restriction enzymes were identified by an in-silico digestion of 96 clones using DRAT software. Bse 1 and Hinf 1 were the restriction enzymes that gave the best discrimination between the AMF groups. In order to confirm the effectiveness of these enzymes, a T-RFLP was performed on 18 clones appertaining to the AM fungi groups; the analysis identified 7 AMF groups according to their terminal-restriction fragments (Table 4.2.3).

Table 4.2.3. The groups AMF identified and their corresponding virtual taxon name (VT); family and genus of AM fungi from Maarjam Database

Group number	Corresponding family and genus name of AM fungi and VT number
1	Glomeraceae Glomus VTX00105 Glomeraceae Glomus sp. VTX00113
2	Glomeraceae Glomus VTX00113
3	Glomeraceae Glomus VTX00067
4	Glomeraceae Glomus VTX00067
5	Claroideoglomeraceae Claroideoglomus sp. VTX00193
6	Glomeraceae Glomus VTX00334
7	Glomeraceae Glomus VTX00155

Each group had a specific terminal restriction fragment length (TR-F) that was identified either by Bse 1 or Hinf 1 enzymes. (table 4.2.4.)

Table 4.2.4. TR-F's for each groups produced by Bse 1 (yellow) Hinf (green), X marks the specific cut length of the enzyme, 0 marks cut sites that did not generate a terminal fragment

Group	119 bp	129 bp	133 bp	141 bp	189 bp	194 bp	279 bp	524 bp	565 bp (uncut)
1	x	0	0	0	0	0		0	
2		x	0	0	0			0	
3							x	0	
4								x	
5			x				0	0	
6								x	
7					x	0	0	0	

Finally, the T-RFLP applied on all tomato root samples was successful for 140 samples out of 180. A principal component analysis was performed in order to identify which of the TR-F's explain better the variation between the samples. The PCA coordinates showed 37%, 29% and 22 % of the total variation. The loadings for PC1 had the highest explanatory strength for the 129 bp, 189 bp and 281 bp TR-F's respectively, PC 2 was characterized by the 189 bp and 281 bp TR-F's and PC 3 has high factor loadings for the 129 bp and 565 bp (uncut samples). Furthermore, Anova was performed on the scores of each PC considering system, tillage and sampling date as main factors (table 4.2.5).

4.2.5. Anova table for the main factors (System, Tillage and Sampling date) and their interactions (bold values represent significant differences according to LSD)

	PC1 (37%)			PC2 (29%)			PC3 (22%)		
	df	F value	*p*	*df*	F value	*p*	*df*	F value	*p*
System (S)	1	6.74	**0.011**	1	0.51	0.476	1	1.12	1
Tillage (T)	1	0.36	0.551	1	0.04	0.849	1	0.1	0.755
Sampling date (SD)	4	2.41	0.054	4	2.17	0.077	4	3.94	**0.005**
S x T	1	0.86	0.356	1	2.85	0.094	1	0.18	0.669
S x SD	4	1.86	0.123	4	2.72	**0.033**	4	0.86	0.489
T x SD	4	1.28	0.283	4	0.91	0.461	4	0.78	0.543
S x T x SD	4	0.28	0.889	4	2	0.1	4	1.9	0.116

Differences in agricultural system ($p<0.05$) were seen for PC 1 scores. PC 2 showed a significant interaction between system and sampling date ($p<0.05$) and, finally, PC 3 describes a significant difference between the five sampling dates ($p<0.001$). The tillage level was not significant for any of the PC's. The scores plot of the means according to sampling date factor of PC 2 and PC 3 (Figure 4.2.5) show that the ORG AMF community shifts in time while the CONV AMF community is more static. Moreover, a temporal variation can be seem in the ORG community from the second to the third sampling date, ending in August 2015, with the presence or 565 bp TR-F corresponding to uncut samples.

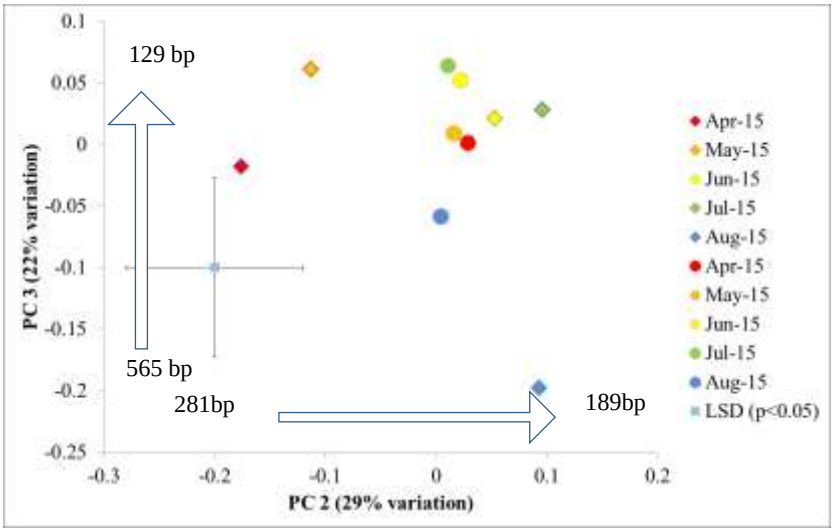


Figure. 4.2.5. Average values of the scores according to sampling date

4.2.2 Discussion

Long-term experiments represent an important tool in assessing the sustainability of agricultural management practices and can also provide information on C-sequestration over time. The rate of C-sequestration in agro-ecosystems depends on climate, soil type, and past management (Freibauer et al., 2004). Organic farming together with reduced tillage and plant residue incorporation may contribute, in time, to build-up total soil organic carbon or to slow down the processes of Corg loss (Freibauer et al., 2004; Leifeld and Fuhrer, 2010). In Mediterranean areas the climate conditions combined to conventional tillage could be major limiting factors on C-sequestration with respect to northern cold climate region (Melero et al., 2009). However, previous studies in Mediterranean area

showed that the adoption of reduced tillage is an effective soil management technique that enhances C-sequestration (Lal, 2004). In our experimental site, a previous study (Lagomarsino et al., 2009), reported that after 4 years of organic management the amount of soil Corg slightly increased over time under ORG but significant differences were not yet found between CONV and ORG managements. In this study, after 14 years of different management, the ORG-RT soil showed an increase of 20% of C stock with respect to the CONV-CT management. Moreover, over 14 years of management, in the ORG-RT system there was an increase of approximately 3.75 Mg C ha^{-1} with respect to the CONV-CT. In fact, Gattinger et al. (2012) in a meta-analysis comparing organic and conventional farming systems, reported that organically managed soils after a period of 14 years registered 3.5 ±1.08 Mg C ha^{-1} more than conventional managed soils.

The soil microbial biomass and the microbial quotient were positively influenced by the ORG system, probably due to the cover crop incorporation as green manure, which was not used in the CONV system. Previous work carried out in the same field also reported similar results arguing that the green manure is a source of easily available carbon fraction for the soil microflora (Lagomarsino et al., 2009; Marinari et al., 2006). Moreover, previous studies reported that organically managed soils have a higher enzyme activity than conventionally managed soils (García-Ruiz et al., 2008; Maeder et al., 2002). Furthermore, the greater accumulation of inorganic nutrients in reduced tillage tends to increase enzyme activities (Melero et al., 2009). In this study, enzyme activities reflected the effect of system management when expressed per unit of soil while the tillage effect was registered only for the specific enzyme activity expressed per unit of Corg. This suggests that the increase in Corg under the ORG systems had a major impact on C, N and P cycle enzymes. Our results are consistent with other studies where the amount of Corg was the major driver in soil enzyme activities (Hendriksen et al., 2016). Moreover, the tillage effect was recorded when the enzyme activity was expressed per unit of C, and the high activity values found under RT showed a better quality of soil organic matter for the soil microflora (Boerner et al., 2005). The effect of the management system (ORG vs. CONV) was also marked at 15-30 cm soil depth in terms of SEI C and Pho activities, expressed both on mass soil base and per unit of organic carbon. These results suggest that these enzyme activities could be sensitive indicators of organic matter changes as quantity and as well as quality in deeper layers of organically managed soil. The fact that soil organic matter quality changes under different management was also confirmed by the significant variations of extractable C expressed per unit of Corg, resulting higher in CONV than in ORG. Therefore, the ORG system reduced the labile C form and promoted the organic matter stabilization

as driving force of C sequestration. Conversely, the tillage level caused a significant change of organic matter quality in terms of labile pool with respect to the total nitrogen content (Extr- N/TN). Moreover, the highest value of Extr-N/TN, registered in ORG-RT, was probably related to the highest enzyme activity, especially of chitinase, detected in ORG-RT. Finally, a positive effect of RT on microbial community functional diversity was observed. This result soils could be attributed to the retention of residues on the soil surface which modulate the soil temperature and water content (Schomberg et al., 1994) producing a favorable environment (microclimate) for microbial activity. Moreover, tillage may disturb the microbial biomass, especially fungi trough the interruption of soil continuum (Beare et al., 1997). The different pattern in the composition of the AMF suggested that ORG compared to the CONV system induced the presence of a more dynamic AMF community. The shift in the AMF composition could be attributed to the better soil conditions found in the ORG plots in terms of microbial size and activity (Oehl et al., 2004).

4.2.3 Conclusions

The organic management over 14 years period under Mediterranean environment determined 0.27 Mg ha^{-1} yr^{-1} soil C sequestration rate, reaching an increase of soil C-stock of 3.75 Mg C ha^{-1} with respect to conventional system. Moreover, the soil quality bioindicators such as microbial biomass size and activity responded promptly to changes in quantity and quality of the soil organic matter induced by the different agricultural management and tillage level. In particular, in the organic systems soil enzyme activities on soil mass base were enhanced by the increase of the amount of organic carbon, while the specific enzyme activities (per unit of organic C) were a sensitive indicator of different management suggesting significant changes of soil organic matter quantity and quality. Concerning this aspect, the organic management showed the main effect on C sequestration rate. Conversely, tillage levels mainly caused an effect on soil organic matter quality by an increase of labile nutrients pool, which suggested an intensification of the mineralization process.

4.3 The impact of exotic tree species (Douglas-fir) reforestation on soil carbon and nutrient dynamics in Northern Apennines – Italy (case study 3)

This study was made in collaboration with University of Bologna: prof. Livia Vittori Antisari, Dr. Serena Carbone, Dr. Anna Graziani and prof. Gilmo Vianello.

4.3.1 Results

a) Soil properties of stand age classes

The soils showed a limited thickness with a lithic contact within 50 cm of the mineral soil surface. A progressive deepening of organo-mineral horizons was observed and for DOUG11 and 12 pedons the sum of A horizons was higher than 20 cm. Under wet conditions the colour of the surface layers varies from dark brown to brown and from dark reddish to brown in deeper horizons (Table 4.3.1). The A1 and A2 horizons (epipedon) showed a value and chroma ≤ 3, (Table 4.3.1). The main physicochemical properties of investigated pedons are shown in Table 2. The pH values increased with pedon depth according to the stand age classes (ranging from 4.4 to 5.4) while the base saturation was less than 50% in all horizons. The soil texture was predominantly sandy. The organic C decreased with depth of soil profiles, in the epipedon was always higher than 20 g kg^{-1}. According to the Soil Taxonomy the pedon DOUG 9 is classified as Dystrudepts, while pedons11 and 12 are Humudepts (Soil Survey Staff, 2014) due to the clear identification of Umbric horizon (Sanesi and Certini, 2005). In these last soil profiles a differentiation of organic layers (Oi, Oe, Oa), deepening of organo-minerals horizons and an increase of organic C content in the epipedon were observed.

Table 4.3.1. Description of soil profiles according to Schoeneberger et al., (2012)

Reforestation age	Profile	Horizons		Boundary		Color Munsell		Structure			Texture	Consistence				Roots		Rock fragments		
		Master	Depth (cm)	D	T	dry	moist	G	S	T		D	M	S	P	Q	S	S	V%	R
80 years	9	Oi	5 - 3	A	S															
		Oe/Oa	3 - 0	C	S															
		A1	0 – 5	C	W	10YR 4/3	5YR 3/3	1	f	SBK	l	S	VFR	(w) so	(w) ps	2	f/m		0	
		A2	5 - 16	C	S	10YR 4/4	5YR 3/3	1	f	SBK	l	S	VFR	(w) ss	(w) p	2	f/m	FGR	2	3
		Bw	16 - 26	C	S	10YR 5/4	5YR 6/4	1	f	SBK	sl/l	SH	FR	(w) s	(w) p	0	f	FGR	6	3
		C	26 - 40+	U		10YR 6/4	5YR 5/8	0	m	SG	sl	MH	FR	(w) so	(w) ps	0	m	MGR	16	2
100 years	11	Oi	6 - 5	A	S															
		Oe	5 - 2	A	S															
		Oa	2 - 0	A	S	10YR 3/3	10YR 3/2													
		A1	0 – 7	C	W	10YR 4/3	10YR 3/3	1	f	GR	l	S	VFR	(w) so	(w) ps	2	f/m	MGR	2	1
		A2	7 - 22	C	W	10YR 4/4	5YR 4/4	1	f	SBK	l	S	VFR	(w) so	(w) ps	1	f/m	FGR	2	3
		Bw	22 - 30	A	S	10YR 5/4	2.5YR 4/8	1	f	ABK	sl	S	FR	(w) ss	(w) ps	0	f	FGR	8	1
		C	30 - 52+	U		10YR 6/4	7.5YR 5/8	0	m	SG	l	MH	FI	(w) ss	(w) p			MGR	20	1
120 years	12	Oi	5 - 4	A	S															
		Oe	4 - 2.5	A	S															
		Oa	2.5 - 0	A	S	10YR 3/2	5YR 3/1													
		A1	0 – 8	A	W	10YR 4/3	5YR 3/2	1	f/m	GR	sl	S	VFR	(w) so	(w) ps	3	f/m	CGR	7	2
		A2	8 - 25	C	W	10YR 4/4	5YR 4/4	1	f	SBK	sl	S	VFR	(w) so	(w) ps	2	f	MGR	8	2
		Bw	25 - 31	C	S	10YR 5/4	5YR 6/4	1	f/m	ABK	sl	SH	FR	(w) so	(w) ps	0	f	FGR	7	1
		C	31 - 60+	U		10YR 6/4	7.5YR 5/8	0	m	SG	l	SH	FR	(w) so	(w) ps					

Horizon Boundary. (**D**) Distinctness: **A**=abrupt, **C**=clear, **G**=gradual, **D**=diffuse - (**T**) Topography: **S**=smooth, **W**=wavy, **I**=irregular, **U**=unknown

Structure. (**G**) Grade: **0**=structureless, **1**=weak, **2**=moderate (**S**) Size: **vf**=very fine, **f**=fine, **m**=medium**, co**=coarse **T**) Type: **GR**=granular, PL=platy, **ABK**==angular blocky, **SBK**=subangular blocky, **SG**=single grain .

Texture. Field estimation: s = sand, ls = loamy sand, l = loam, sil = silt loam, sl = sandy loam.

Consistence. Rupture resistance: (**D**) Dry: **S**= soft, **SH**=slightly hard, **MH**=moderate hard - (**M**) Moist: **VFR**=very friable, **FR**=friable, **FI**=firm - (**S**) Stickiness: **(w)so**=non-sticky, **(w)ss**=slightly sticky, **(w)s**=moderately sticky – (**P**) Plasticity: (w) po = non-plastic, (w) ps = slightly plastic, (w)p = moderately plastic

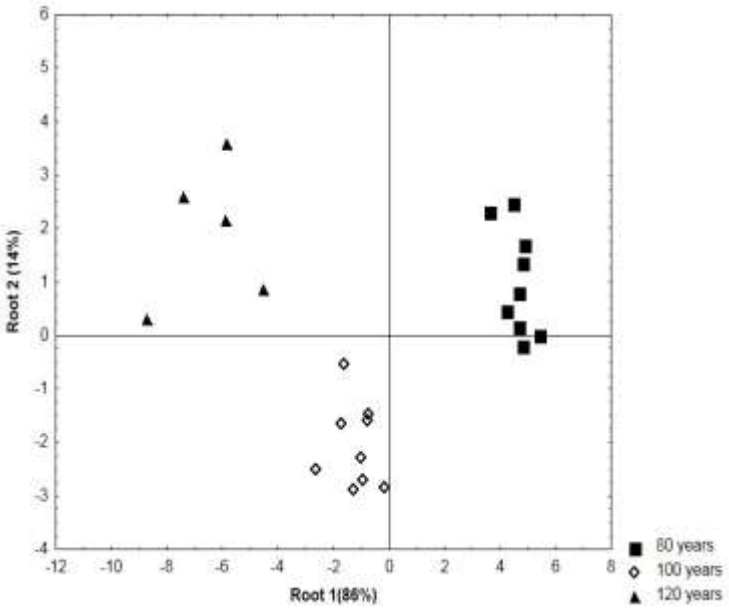
Roots. (**Q**) Quantity: **0**=very few, **1**=few, **2**=common, **3**=many – (**S**) Size: **vf**=very fine, **f**=fine, **m**=medium, **co**=coarse.

Rock fragments. (**S**) Size: FGR = fine gravely, MGR=medium gravely; CGR=coarse gravely - (**V%**) Fragment content % by volume – (**R**) Roundness: 1 = angular, 2 = subangular, 3 = subrounded

Table 4.3.2. Main physico-chemical properties of investigated pedons. Values are mean ± standard error (n=3).

		pH		Corg		Sand		Silt		Clay		CEC		BS	
Aging	Horizons			g kg^{-1}		g kg^{-1}		g kg^{-1}		g kg^{-1}		$Cmol_{(+)}$ kg^{-1}		%	
80	A1	4.4	*±0.0*	48.9	*±1.6*	341	*±33*	404	*±64*	255	*±31*	16.8	*±1.1*	22.0	*±1.1*
	A2	4.4	*±0.3*	31.8	*±4.5*	374	*±47*	389	*±76*	237	*±28*	12.8	*±1.6*	11.0	*±8.2*
	Bw	4.5	*±0.2*	25.6	*±6.3*	640	*±117*	297	*±95*	64	*±24*	9.8	*±2.8*	8.7	*±3.2*
	C	4.7	*±0.1*	20.3	*±7.3*	676	*±86*	263	*±71*	61	*±14*	7.4	*±2.4*	7.0	*±1.3*
100	A1	5.1	*±0.1*	56.8	*±4.8*	419	*±70*	393	*±38*	189	*±32*	18.7	*±1.1*	45.5	*±3.0*
	A2	5.0	*±0.1*	31.6	*±3.3*	428	*±95*	385	*±43*	187	*±52*	18.2	*±0.5*	33.8	*±7.6*
	Bw	5.2	*±0.4*	20.5	*±2.7*	628	*±34*	301	*±30*	71	*±3*	9.9	*±2.2*	35.0	*±1.2*
	C	5.4	*±0.4*	8.6	*±5.2*	610	*±35*	295	*±90*	96	*±60*	9.8	*±2.4*	16.8	*±0.4*
120	A1	5.2	*±0.1*	91.2	*±2.2*	620	*±36*	239	*±42*	141	*±26*	38.9	*±1.1*	43.8	*±1.1*
	A2	5.4	*±0.1*	34.7	*±3.4*	639	*±72*	209	*±63*	152	*±33*	26.4	*±0.8*	42.4	*±2.6*
	Bw	5.4	*±0.3*	43.9	*±1.6*	662	*±34*	217	*±42*	121	*±20*	19.5	*±0.9*	45.3	*±1.5*
	C	5.0	*±0.2*	18.3	*±2.8*	473	*±38*	383	*±79*	144	*±8*	8.0	*±2.1*	34.1	*±1.8*

Various lines of independent evidence are essential to justify the space-for-time assumption to study soil temporal dynamics, and to discern what characteristics change over time. For this reason, the trajectory of soil properties across stand age classes was measured using Discriminant Function Analysis (DFA). DFA showed separated groups for the three stand age classes (Figure 4.3.1),. The soil chemical and biochemical properties drew a trajectory from 80 to 120 years according to soil pH, Al and Ca contents, which were negatively correlated with root 1 of the DFA and explained 86% of total variance (Figure 4.3.1). Moreover, iron and non-humic substances contents of soil were two additional properties which significantly correlated with root 2 of DFA explaining additional variance (14%). Soil pH and Ca content in the soil upper layers increased from 80 to 120 years old plantation, while Al content increased from 100 to 120 years old plantation (Figure 4.3.2.). Moreover, the base saturation increased from 80 to 120 years old plantation, while a slight increase of the Synthetic Enzyme Index was observed in soil of 100 years old plantation (Figure 4.3.3). Furthermore, the soil enzyme activity, expressed as acid phosphatase: chitinase ratio, decreased from 80 to 120 years old plantation, (Figure 4.3.4).



	Root 1	Root 2
Percentage of variance	86%	14%
pH	-.8070***	ns
Aluminum	-.5489**	ns
Phosphorus	ns	ns
Iron	ns	.4405*
Calcium	-.6636***	ns
SEI	ns	ns
T.E.C.	ns	ns
Sulfur	ns	ns
Nitrogen	ns	ns
Fulvic acids	ns	ns
Humic acids	ns	ns
Non humic substances	ns	.4250*

Figure 4.3.1. Discriminant Function Analysis of chemical and biochemical properties of soil profiles under Douglas-fir stand at different aging and the relative linear correlation (p-values) between the scores of Root 1 and 2 and the initial values.

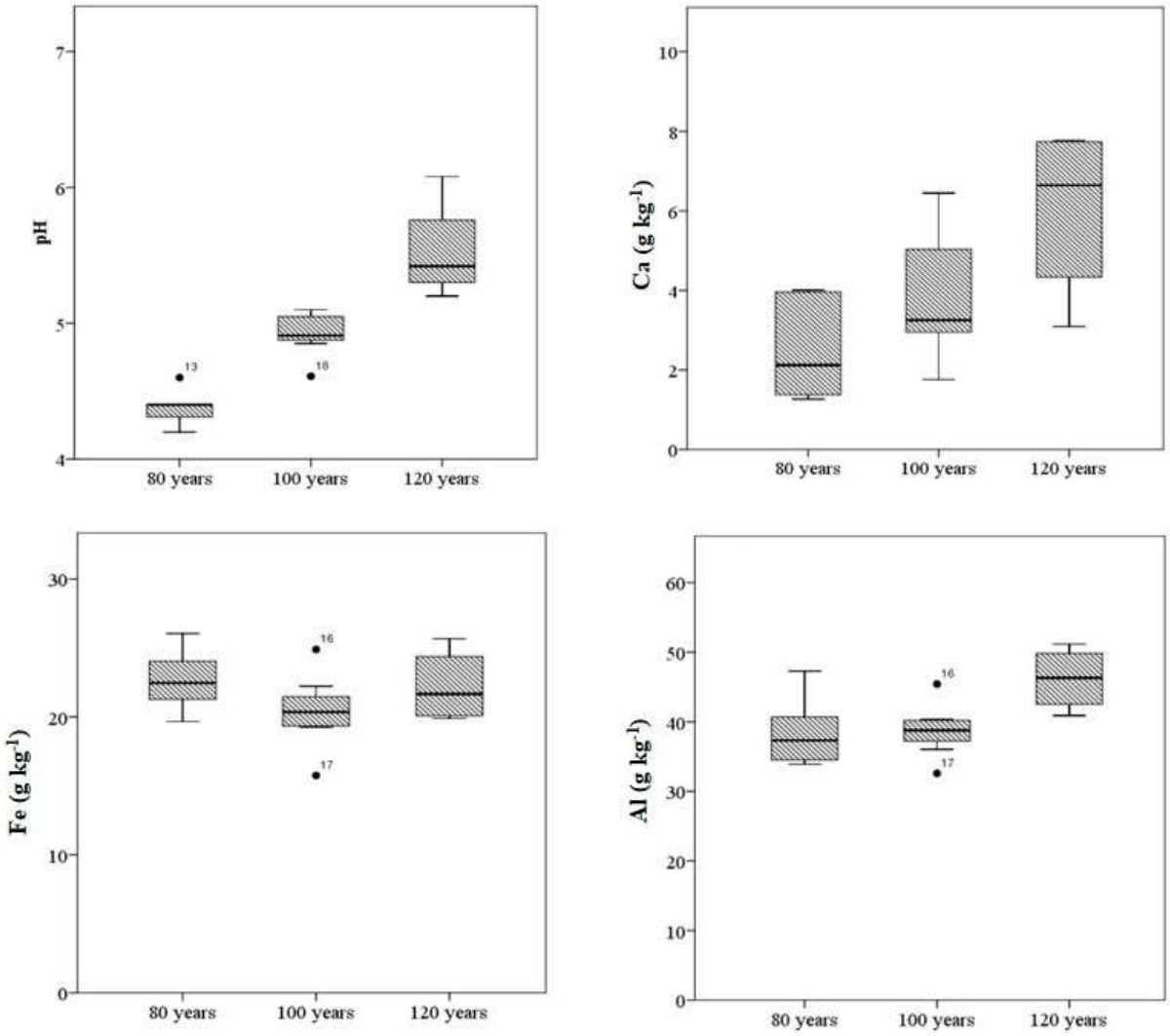


Figure 4.3.2 pH, calcium, iron and aluminium content of soil. The boxplot includes the *upper layers* data of soil profile.

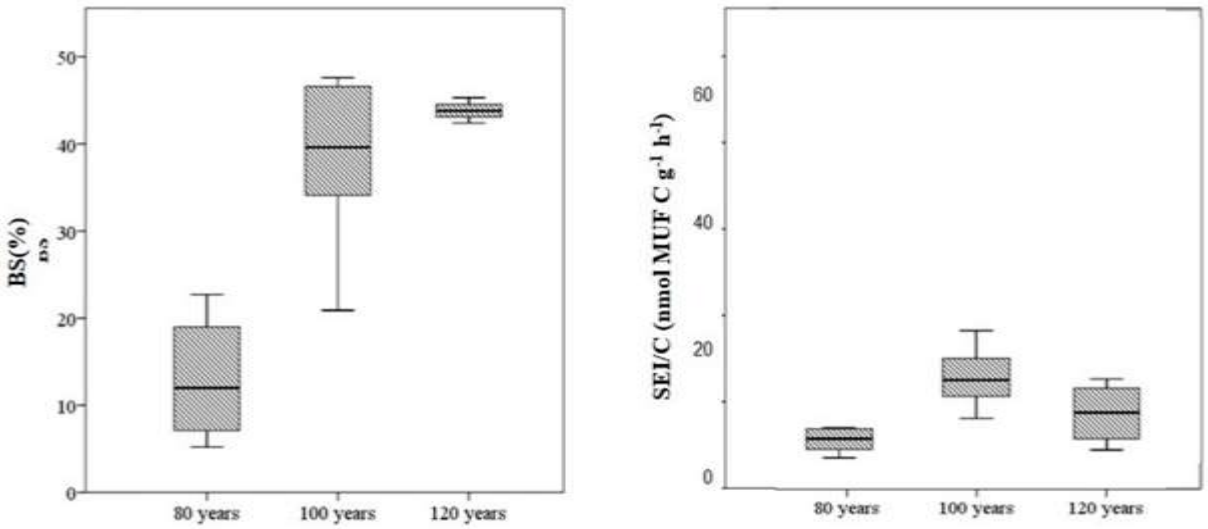


Figure 4.3.3: Base saturation (BS) and Synthetic Enzyme Index per unit of organic carbon (SEI/C) of soil. The boxplot include the *deep layers* data of soil profile.

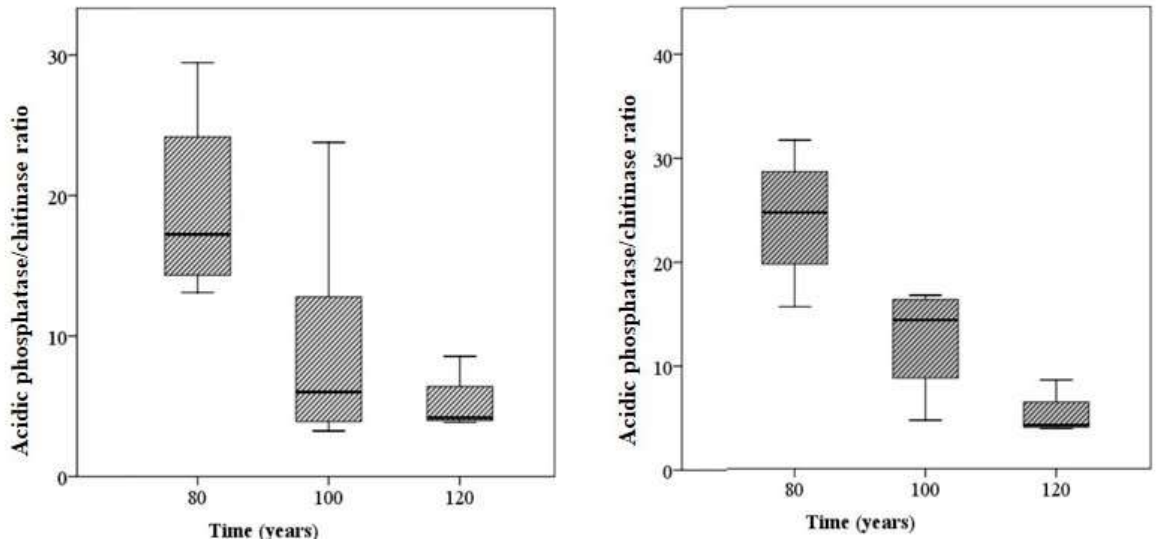


Figure 4.3.4. Acid phosphatase to chitinase activity ratio in *organic* and *organo-mineral horizons* of soil profile. The boxplot include data of soil profile.

b) Foliar analysis

The foliar content of Fe, Al and Mn increased as the age of plantation increased. Conversely the N content of leaves decreased with increasing stand class age (Table 4.3.3). Therefore, when the elements were expressed on N-ratio (Table 4.3.3), the values were slightly reduced by the age of the plantation. The Fe/N and Al/N ratios ranged from 72 to 256 and 144 to 372, respectively.

Table 4.3.3: Leaf nutrient content expressed as mean value ± standard error (n=3)

Year	Al	Fe	Mn	N	Al/N	Fe/N	Mn/N
				mg kg-1			
80	237± *80*	118± *36*	184± *75*	64± *0*	144± *29*	72± *8*	112± *27*
100	309± *100*	149± *56*	399± *191*	39± *0*	222± *53*	107± *28*	287± *103*
120	522 ± *267*	358± *191*	273± *114*	40± *0*	372± *38*	256 ± *162*	195 ± *162*

c) Humic substances and soil carbon, nitrogen stocks

In the 20 cm depth of pedons humic substances stock (e.g humic acids, fulvic acids and humin) increased from 80 to 100 years old plantation. Conversely, a decrease of humic substances stock was detected in the highest class of age (120 years) with an increase of non-humic substances pool (Figure 4.3.5).

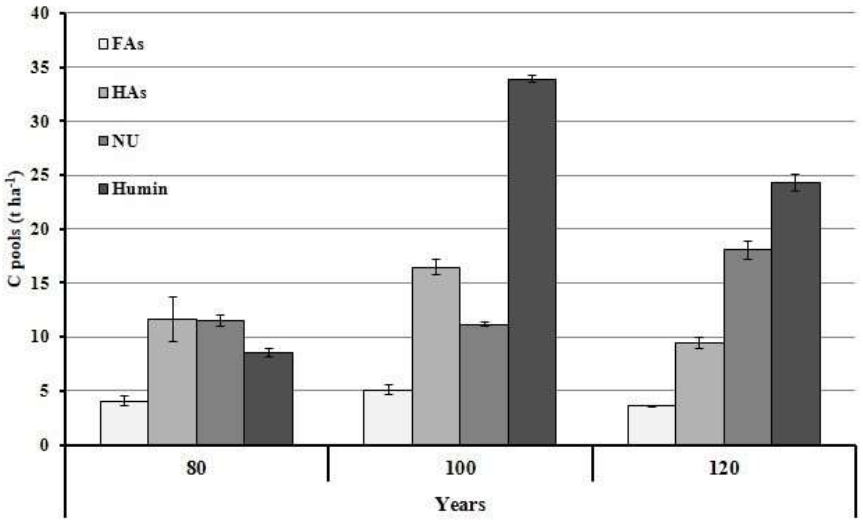


Figure 4.3.5. Carbon pools in the 0-20 cm soil depth: humin, humic acids (HAs), fulvic acids (FAs) and no-humic substances (NU). Bars are standard deviations within soil horizons of profile.

Soil C stocks increased in both organic and organo-mineral layers with increasing stand class age (Figure 4.3.6 a and b). Nitrogen stock showed a slight increase in the soil organo-mineral horizon from 60 to 100 yrs stand class age, while a steady state was observed from 100 to 120 yrs old plantation (Figure 4.3.6 b)

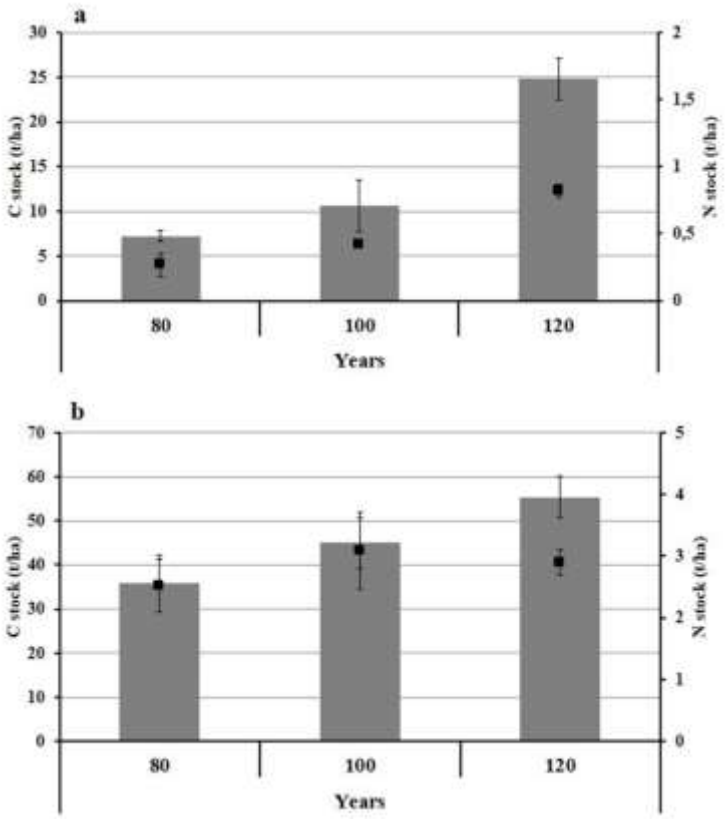


Figure 4.3.6. Stock of organic C (bars) and total N (squares) in *organic (a)* and *organo-mineral horizons (b)* obtained with mean values between the pedons at the same Douglas-fir stand age.

d) Community level physiological profile (CLPP)

The CLPP was measured using the Microresp method. The A horizons of each soil profile were analyzed then the average values of SIR were calculated for each type of C-source substrate groups (Fig. 4.3.7.). An increasing trend of SIR was noticed in soil from 80 to 120 years stands. However, the CLPP of soil under 120 yrs old stand showed a decrease in microbial response after the addition

of amino acids. On the other hand, the catabolic activity for the other substrate categories (carboxylic acids, carbohydrates and phenolic acids), was higher in the oldest stand.

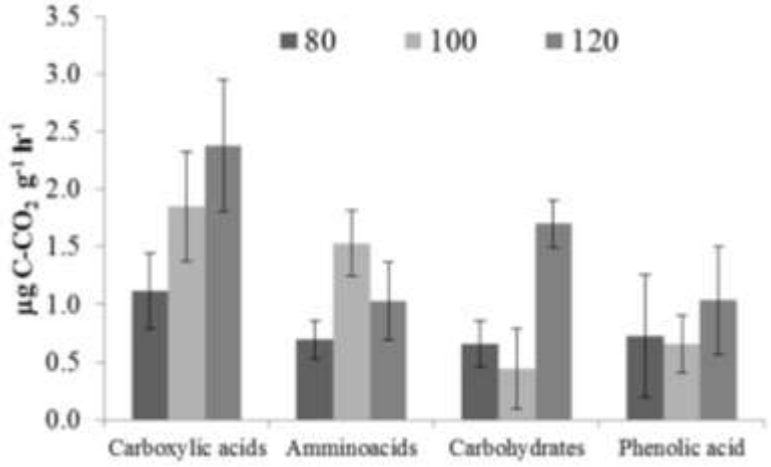


Fig 4.3.7. CLPP for C-substrates classes among the different stand ages: carboxylic acids (CIT, OX, ASC); amino acids (LEU, ARG, GLY, ASP, BUT); Carbohydrates (G, NAG, GA, FR, ARA) and phenolic acids (SIR, VAN).

e) Functional diversity of the microbial biomass

The Shannon's Index was calculated using the values of both, enzyme activities (H' Enz) and substrate induced respiration (H'SIR). The functional diversity in terms of SIR increased with the stand ages; while for the enzyme activities the 100 and 120 forest stands showed a similar value (Table 4.3.4).

Table 4.3.4. Shannon's Index (H') for the enzyme activities (Enz) and substrate induced respiration (SIR)

Years	H'SIR	H'Enz
80	3.42	2.08
100	3.60	2.30
120	3.78	2.27

4.3.2 Discussion

In the North Apennine region, before the Second World War (yr 1940) the reforestation with the Douglas-fir had the main purpose of occupying the degraded areas from grazing or from excessive cuts made especially during the First World War. In this situation the soil became strongly eroded showing impoverished *epipedon* with low nutrients content and humified organic matter. The Douglas-fir was then the principal practice to make up for this deficiency. In the following years reforestation activities had the main purpose of providing work and marginal area usage of forest with low productivity.

In regards to the soil organic matter deposition, a considerable change was observed passing from 80 to 100 years age classes due to the clear identification of Umbric horizon only in the last two age classes (100 and 120 yrs). Moreover, humin, humic acids and fulvic acids increased with the age of plantation even if, in the last class age (120 yrs) a decline of humic substances have may occurred due to a soil nitrogen limitation that decreased the N stocks in the soil organic and organo-mineral layers. Nitrogen is the primary limiting nutrient in Douglas-fir plantation (Perry, 1994) in this study a critical gap for soil nitrogen was found in the over 100 yrs old plantation. This gap could be due to a higher nitrogen mineralization with respect to the immobilization process. However, higher nitrogen mineralization rates in the forest floor could be found in older coniferous stands (Côté et al., 2000). In addition, a slight increase of soil specific enzyme activities, expressed per unit of organic carbon, was observed mainly from 80 to 100 years old plantations, therefore the activity of mineralizing microflora probably changes according to nitrogen availability. This result suggested a different level of potentially SOM hydrolysing activity depending on nitrogen depletions with respect to carbon stock.

A relationship between phosphatase to chitinase ratio and soil age has been observed in previous studies (Marinari et al., 2013; Olander and Vitousek, 2000). However, in this study the obtained results were not consistent with values reported in previous work (Caldwell, 2005; Olander and Vitousek, 2000), where in modern, 300-year-old soil, the phosphatase to chitinase ratios in the mineral soil horizons were 2.85, while in the oldest soil (20,000 yrs) the ratios increased to 18.6. In this study, the decrease of this ratio, from 24 at 80 yrs to 6 at 120 yrs, was mainly due to the enrichment of soil chitinase activity with increasing stand age, probably because the limitation of

nitrogen occurred in soil at plantations over 100 yrs of age. On the other hand, the highest phosphatase activity in the most acidic soils at the youngest sites may be the result of the increase in substrate availability; phosphorus pools shift from easily soluble phosphatic minerals such as apatites (Eger et al., 2011), to organic phosphates that govern P availability in more leached acidic soils (Tiessen et al., 1984). Even if coniferous litter is usually low in bases and broadleaves, hardwood forest typically returns to the soil a large amount of bases (Bonneau, 1988). Douglas-fir compared with domestic coniferous species, has lower acidifying effects on upper soil layers and contributes to better humus forms, recycling nutrients more effectively and producing litter which can be easily decomposed (Kupka et al., 2013). In this study the increase of soil pH, calcium and base saturation from 80 to 120 yrs, suggests the topsoil accumulation of alkaline elements. Furthermore, according to the stand class age, the slight increase of Al content in both, needles and soil organic layers, suggest a positive effect of Douglas-fir on the biogeochemical cycle of these elements producing their accumulation in the topsoil.

Regarding the foliar element content, the optimal proportion between nutrients is similar for almost all vascular plants, and can be defined as a nitrogen ratio (Linder, 1995). In this study, the increase of element/N ratios with the increase of the plantation age suggests that the soil nitrogen limitation to plant growth do not necessarily reduce the requirements for other nutrients.

The response in substrate utilization increased with Douglas-fir stand age, with the exception of amino-acids substrate in the oldest plantation (120 yrs). Similar results were also obtained by Gartzia-Bengoetxea et al., (2016) showing an increase of C substrate utilization in coniferous forests with the exception of amino acids. In our case, the decrease of amino acid use can reflect a microbial community shift that may have been induced by the soil N limitation in the oldest stand. The oldest stand also showed a higher Corg, pH and Ca content that could induce a change in microbial community physiological profile. Moreover, Corg and pH were also recognized to be the dominant factors influencing CLPP in a vast gradient of soils (Creamer et al., 2015b). For this reason the soils of the oldest stands (120 yrs) with higher Corg and pH compared to the 80 and 100 yrs stands may had have the highest CLPP. Furthermore, the microbial functional diversity, measured in terms of SIR (H'SIR), showed similar trend of soil Corg and pH, while when expressed in terms of enzyme activities (H'Enz) did not discriminate between the 100 and 120 yrs old stands.

4.3.3 Conclusions

The analysis of soil properties and the use of soil indicators proved to be a useful tool to discriminate the differences related to Douglas-fir stand aging in forest soil. A general evidence was found on topsoil element accumulation due to Douglas-fir plantation. A more conspicuous accumulation of alkaline element with respect to Al was hypothesized due to the increase of soil pH along the Douglas-fir stand age classes. Soil organic matter deposition became sufficient for Umbric horizon definition when Douglas-fir plantation reached the age of 100 years. Over this class age of plant also a limitation of soil nitrogen occurred. Organic substrate utilization of the microbial biomass increased with forest age.

4.4 Soil microbial diversity as indicator of forest management of Vico Lake Natural Reserve (case study 4)

4.4.1 Results

a) Chemical analysis

Significant differences were found for the majority of soil chemical properties (table 4.4.1). At both soil depths a higher quantity of organic carbon (Corg) was found in the 65 compartment, particularly 65A plot, compared to the 56 compartment. Also the C-stock was significantly higher in 65 areas compared to the 56 areas at first soil depth (0-5 cm) while at the second soil depth (5-15cm) 65A and 56A had a similar C-stock. The highest values of total nitrogen (TN) and N-stock were seen in the 56A beech stand at both soil depths. Moreover, the 56 compartment registered the highest pH value at 0-5 cm while at 15-10 cm differences were not significant.

Table 4.4.1. Average values and standard errors of the soil main chemical properties at 0-5cm and 10-15cm soil depth (n=3). Different letters represent significant differences according to Tukey's test at $p<0.05$

	Corg	TN	C-stock	N-stock	pH
	mg g $^{-1}$		Mg ha $^{-1}$		
0-5 cm					
56A	211.6 ^{b}	37.7 ^{a}	58.5 ^{b}	10.7 ^{a}	6.5 ^{a}
56B	219.6 ^{b}	13.5 ^{b}	67.3 ^{ab}	4.1 ^{b}	6.2 ^{ab}
65A	293.2 ^{a}	15.3 ^{b}	89.2 ^{a}	4.6 ^{b}	6.1 ^{b}
65B	289.3 ^{a}	14.2 ^{b}	72.8 ^{ab}	3.6 ^{b}	6.1 ^{b}
5-15 cm					
56A	78.1 ^{ab}	16.9 ^{a}	25.0 ^{ab}	5.4 ^{a}	6.0
56B	52.6 ^{b}	4.0 ^{c}	18.3 ^{b}	1.4 ^{b}	5.9
65A	104.0 ^{a}	7.2 ^{b}	29.7 ^{a}	2.0 ^{b}	5.6
65B	78.8 ^{ab}	6.0 ^{bc}	17.8 ^{b}	1.4 ^{b}	5.8

The extractable carbon (Extr-C) and extractable nitrogen (Extr-N) labile pools showed significant differences only in the upper layers. In both compartments the A plots showed higher quantities Extr-C compared to the B plots (Figure.4.4.1 A). A high value of Extr-N was seen in the upper layers of the 56A, followed by the 65A whereas 56B and 65B had similar values. (Figure.4.4.1 B)

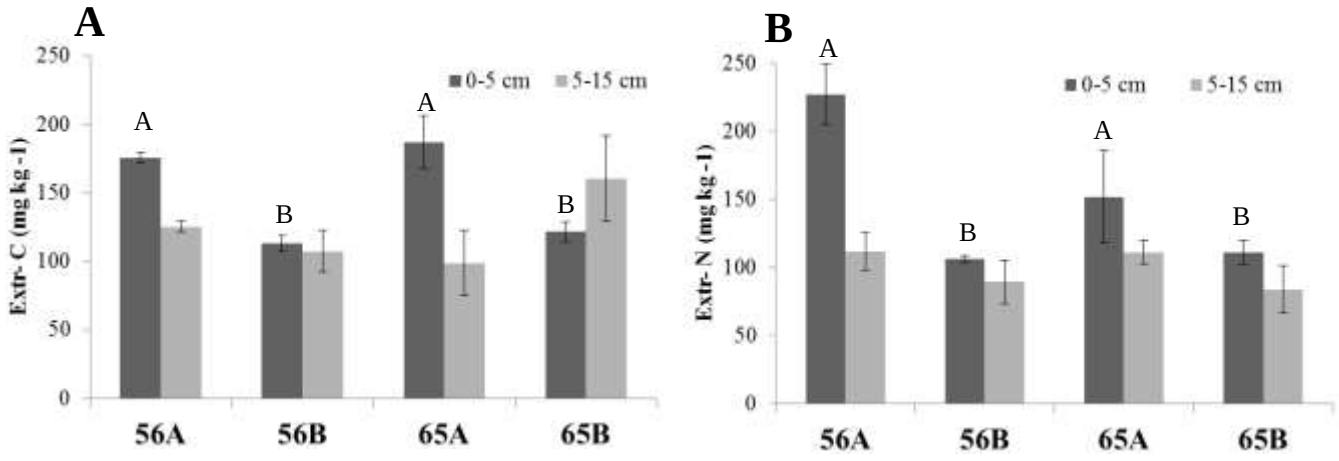


Figure 4.4.1 . Extr-C (A) and Extr-N (B) at both soil depths. Different letters represent significant differences according to Tukey's test at $p<0.05$.

b) Microbial biomass size and enzyme activity

The microbial carbon (Cmic) was generally high in the upper layers of 56A and B and 65B plots while at the second soil depth (5-15 cm) Cmic was significantly low in the 56B and 65A plots (Figure 4.4.2). The microbial quotient (Cmic:Corg) showed high values at the second soil depth but no significant differences were found among plots (Figure 4.4.3 A). Moreover, in the upper layers the 56 compartments showed significantly higher values compared to the 65 compartments. The enzyme activity expressed as the Synthetic Enzyme Index (SEI) resulted higher in the upper horizons (0-5 cm) particularly in the 65A plot. Conversely, a minor activity was registered in the deeper horizons (5-15 cm) of the plot 56 B. (Figure.4.4.3 B).

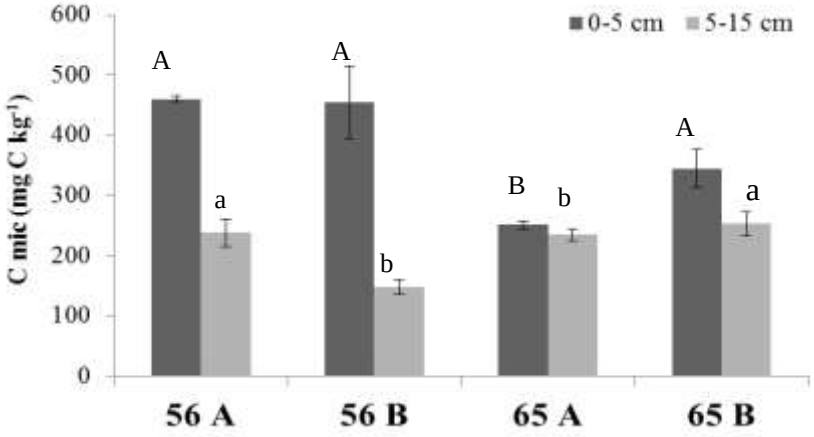


Figure 4.4.2. Microbial carbon in the different plots. Different letters represent significant differences according to Tukey's test at $p<0.05$. Upper case letters represent differences between the superficial layers (0-5 cm) and lower case letters between the deeper layers (5-10 cm)

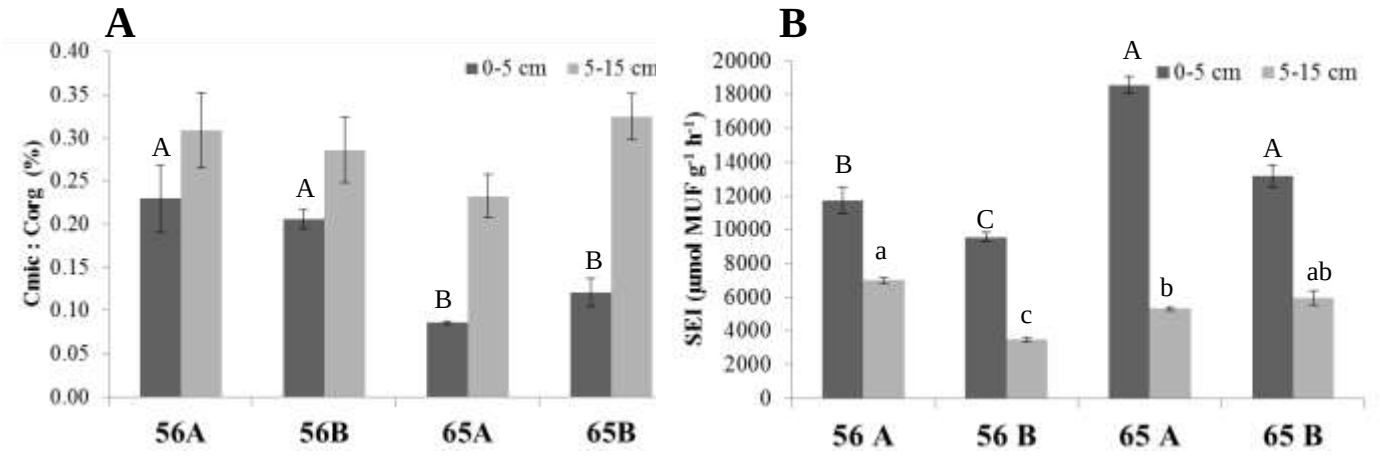


Figure 4.4.3. Microbial quotient (A) and Synthetic enzyme index (SEI) (B) at 0-5cm and 5-15 cm soil layers. Different letters represent significant differences at $p<0.05$.Upper case letters represent differences between the superficial layers (0-5 cm) and lower case letters between the deeper layers (5-10 cm).

c) Community level physiological profiles

The microbial respiration rate induced by the addition of different C substrates (Microresp) was higher in both soil layers of the 56A plot (Figure. 4.4.4 A). Conversely in the 5-15 cm layers of 65B the catabolic response that resulted was significantly lower compared to the other plots. For the specific respiratory response of the fungi population (Fungiresp), a significantly high use of substrates was registered in 65B at the first soil depth and in plot 65A for the second soil depth (Figure 4.4.4 B).

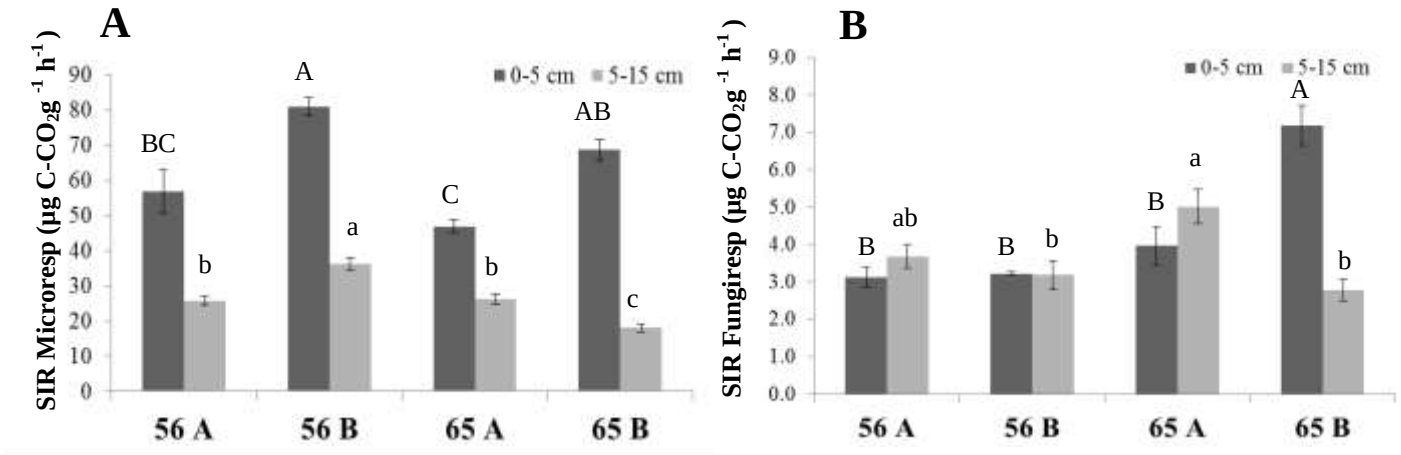


Figure 4.4.4. Soil induced respiration by the addition of various C substrates using Microresp (A) and Fungiresp method (B). Different letters represent significant differences according to Tukey's test $p<0.05$. Upper case letters represent differences between the superficial layers (0-5 cm) and lower case letters between the deeper layers (5-10 cm).

d) Microbial structural diversity

The structural diversity had more significant results at the first soil depth. As shown in table 4.4.2., the majority of the biomarkers in plot 56B, 65A and 65B had similar FA concentrations while plot 56A showed significantly lower values with the exception of AMF biomarkers. At the second soil depth (5-15 cm), actynomicetes, fungi and total bacteria were higher in the 56 compartment

compared with the 65 compartments. The ratios G+/G- have not shown shifts in bacteria populations and the ratio fungi to total bacteria evidenced higher ratios in the 56 B, 65A and 65 B plots.

Table. 4.4.2. EL-FAME biomarkers: total GB (total general bacteria); G+ (Gram- positive bacteria); G- (Gram-negative bacteria); Actyno. (actynomicetes); Prot. (Protozoa); AMF (Arbuscular mycorrhizal fungi); TB (total bacteria). Different letters represent significant differences according to Tukey's test $p<0.05$

	Total GB	G+	G-	Actyno.	Fungi	Prot	AMF	TB	G+/G-	Fungi/TB
	nmol g^{-1}								%	
0-5 cm										
56A	682	323^{b}	553^{b}	169^{ab}	188^{b}	30	28^{a}	1727	0.6	0.1^{b}
56B	983	539^{a}	867^{a}	210^{a}	440^{a}	32	7^{b}	2600	0.6	0.2^{a}
65A	721	424^{ab}	731^{ab}	182^{ab}	353^{a}	26	26^{a}	2059	0.6	0.2^{a}
65B	741	399^{ab}	712^{ab}	119^{b}	333^{a}	28	27^{a}	1967	0.6	0.2^{a}
5-15 cm										
56A	307	289	306	131^{ab}	161^{a}	13	4	1032^{a}	0.9	0.2
56B	296	320	267	145^{a}	149^{ab}	12	4	1028^{a}	1.2	0.1
65A	175	156	164	79^{b}	77^{b}	9	6	573^{b}	1.0	0.1
65B	199	206	176	89^{b}	89^{ab}	9	3	668^{b}	1.2	0.1

d) Functional and structural diversity indexes

The Shannon-Weaver (H') diversity index calculated with the enzyme activity, substrate induced respiration (Microresp and Fungiresp) and EL-FAME data showed different results in soil of all four beech forest plots (Table 4.4.3.). In particularly the 65 compartments showed significantly higher indexes in terms of structural and functional diversity compared to the 56 compartments at the first soil depth. The diversity index calculated with the CLPP data indicated significant differences only at 5-15 cm and plot 65 A registered the highest value.

Table 4.4.3. Shannon's diversity index calculated with data from 3 different methods. Different letters represent significant differences according to Tukey's test $p<0.05$

soil depth	Forest compartments				Method
	56		65		
	A	B	A	B	
0-5 cm	2.52^{b}	2.67^{a}	2.70^{a}	2.67^{a}	EL-FAME
5-15 cm	2.70	2.76	2.80	2.80	
0-5 cm	3.80	3.78	3.83	3.83	MICRORESP
5-15 cm	3.61^{b}	3.57^{bc}	3.75^{a}	3.43^{c}	
0-5 cm	2.43^{c}	2.35^{bc}	2.56^{a}	2.50^{ab}	ENZYMES
5-15 cm	2.20	2.24	2.44	2.26	

4.4.2 Discussion

The area 65A showed a changed of forest structure with different distribution of plant diameter classes with higher density of larger classes. For this reason, the soil organic matter in this area is expected to be richer in lignin content and other complex polymers than the litter produced by the younger forest trees with smaller diameter. Therefore, the forest stands composed of plants belonging to the area 56B, indicated the presence of younger plants with lower soil organic depositions (litter). Consequently, the different forest stands could explain the different enzyme activity in the upper soil layers of the 65A area. In fact, the initial phase of the humification process consists in the degradation of complex biopolymers that determines the productions of monomers/oligomers as precursors of the process. In this study, the increased enzyme activity in the upper horizons of the 65A area, as well as the carbon accumulation in the deeper horizons (5-15 cm), suggested a higher degradative activity dependent on the superficial organic deposits as initial phase of the humification process. Conversely, the lower enzyme activity was registered in the 5-15

cm layer of the area 56B where the total organic carbon accumulation was lower. This result confirms what has been widely reported in the literature, that the soil enzyme activities are usually strongly related to the quantity and quality of organic matter (García et al., 1994; Lagomarsino et al., 2009). On the other hand, the compartment 56, with a younger forest stand, probably produced a less complex organic matter with a lower request of enzyme activity as degradative action on litter.

The soil microbial biomass was lowest at 5-15 cm soil depth of plot 56B and showing the same trend as the enzyme activities. However, in the compartment 56 the microbial quotient (Cmic:Corg) was significantly higher than in compartment 65, suggesting an increase of the immobilization rather than mineralization process. Moreover, the microbial quotient is often used as an early indicator of soil organic matter accumulation (Sparling, 1997), also as an index of organic substrates availability for the soil microflora (Anderson and Domsch, 1989). The Cmic:Corg values were lower than those reported in several other studies of different forest soils (Merino et al., 2004; Moscatelli et al., 2007; Ross et al., 1999), suggesting a scarce organic substrate availability for soil microbial biomass. The quotient was higher in the 5-15 cm soil layer than in the upper layers suggesting a greater immobilization process by the soil microorganisms. Particularly in the plot 65 A and B the average range values was 0.09-0.12%, while in plot 56 the quotient values were double, reaching 0.23-0.21%.

The forest stand age in the two compartments could not be defined with certainty due to the fact that the tree populations were heterogeneous. However, as average estimation, the beech trees age in the compartment 65 was about 120 years, while in the compartment 56 was about 50 years. In the compartment 65 the carbon accumulation probably reached a steady state condition, conversely in the compartment 56 this steady state was not yet reached, this was also supported by the high microbial quotient. Indeed, it is known that in any type of soil subjected to constant management the steady state of organic matter accumulation is usually reached over 50 years (Greenland, 1995).

Concerning the soil microbial diversity, our results indicate a significant effect of forest stand on soil microbial community structure and functions. In fact, in the upper layers of 56B a high respiratory response of the microbial biomass was found probably because more abundant community of bacteria (Gram + and Gram -), actynomicetes and fungi were recorded. Conversely, in the other plots there was no clear relationship between the catabolic response and the presence of soil microbial community abundance measured according to the EL-FAME technique. The Fungiresp data showed a significantly higher respiratory response of soil in the 65B than soil of the other plots even if, in this plot, the fungi biomarkers did not reveal a more abundant fungal

population. A discrepancy between microbial function and structure was also found in terms of enzyme activities (SEI). For example, the plot that registered the highest value of SEI, such as the 65A, did not show the highest total amount of fatty acids (FA). As reported in previous research conducted in forest soil (Purahong et al., 2014), a disconnection between microbial community structure and metabolic function could be found, when the latter is measured in terms of hydrolytic activities, since enzymes are functionally redundant and are immobilized in soil matrix and widely spread among different microbial groups. Furthermore according to Leckie (2005), in forest soils a relationship between diversity and function is hard to demonstrate because microbial composition provide little information about microbial functions and which of the present microorganisms are active. Fungi and bacteria are the major decomposers in forest soils and their ratio can be used as indicator of forest management practices impact. Fungi are capable to decompose recalcitrant litter and also dominate the decomposition of cellulose and hemi-cellulose. Therefore a soil system dominated by higher proportion of fungi may lead to greater C-sequestration (Strickland and Rousk, 2010). In this study the lowest ratio (0.1) was found in the plot 56A at the upper soil layer, while in the other plots the ratio of fungi with respect to bacteria was 0.2. Moreover, according to Bardgett et al. (1996), an increase of fungi / bacteria ratio could point out to that the ecosystem started to return to its original status prior to a disturbance. Nevertheless, this ratio can also decrease after an increase of soil pH, that promotes the bacteria growth (Rousk et al., 2009). Our finding is also in accordance with microbial response to soil pH, since in plot 56A the highest pH and the lowest fungal / bacteria ratio was found. Finally, the Shannon's Index (H'), calculated with enzyme activities and EL-FAME data, resulted significantly higher at the first soil depth in the compartment 65 (especially in 65B). Conversely, in the deeper soil layer the CLPP data provided the highest value of H' in plot 65A. It is known that land use and management systems which determine a loss of organic matter may also induce a decline of soil microbial diversity (Degens et al., 2000). In this study a close relationship between soil microbial diversity and total organic carbon was found among different tree stand structure generated by different past forest management. A higher microbial functional and structural diversity was observed in the compartment 65 where the Corg was also significantly higher than in the compartment 56. Purahong et al. (2014) have also studied the effects of management in different beech trees stands and concluded that the microbial communities were sensitive to management practices, rates of litter decomposition and seasonal factors. In general, in forest soils, microbial communities are influenced by the quantity and quality of plant litter. Forest litter production increases with stand ages (Lebret et al., 2001) this process could have influenced

the development of different microbial communities in the older stands (65 A and B) compared to the younger ones (56 A and B).

This study highlights a relationship between forest stand structure and soil microbial functions. This relationship as well as the link between different forest stands and soil biology influenced by different types of vegetation and microclimate conditions was also highly documented in the scientific literature. For example Closa and Goicoechea, (2012) revealed how the soil microflora can be significantly influenced by microclimate conditions generated by beech trees size and densities. The authors also indicated that tree density was even more decisive than tree size in determining the microclimatic conditions within younger beech tree stands.

4.4.3 Conclusions

In this study the different forest managements over time caused a significant effect on soil carbon sequestration and microbial functional ad structural diversity. The methodological approach applied to assess microbial diversity (enzyme activities, substrate induced respiration and EL-FAME biomarkers) revealed that microbial metabolic functions and community structure are giving different responses to soil forest management. In conclusion catabolic and enzyme activity of soil microflora were sensitive indicators for the soil organic matter transformation processes produced in the forest floor of different beech tree stand ages.

4.5. Soil indicators set up to assess sustainable management in various agricultural and forest ecosystems

4.5.1 An overview of soil sensitive indicators in various ecosystems management: four case studies

This part was performed in collaboration with prof. Luca Secondi

In this study, a wide range of soil quality indicators was studied across both agricultural and forest soils, but only some indicators were common throughout all the case studies. In order to apply the final statistical analysis only the common soil indicators were considered: total organic C (Corg), total nitrogen (TN), pH, extractable C (Extr-C) and extractable N (Extr-N) as chemical indicators; and microbial C (Cmic), absolute enzyme activities (per unit of soil) and specific enzyme activities (per unit of organic carbon) as biochemical indicators.

From the chemical indicators listed above the pools of nitrogen (total nitrogen and extractable nitrogen) were chosen together with soil pH furthermore these parameters were also considered independent variables in the applied statistical model. Moreover the biochemical indicators chosen for the final analysis were enzyme activity and microbial biomass expressed per unit of organic carbon, hence specific enzyme activity and microbial quotient (Cmic:Corg) that were considered dependent variables in the statistical model applied. Expressing biochemical indicators per unit of Corg better highlighted the changes induced by soil management on the quality of the organic matter rather than on the quantity; in addition this approach eliminates the possible differences given by the various Corg concentrations among the studied soils.

The selection of the indicators was based on characteristics related to nutrient availability and microbial biomass size and activity that are strongly influenced by management on a short time scale, in contrast with soil properties such as total organic carbon C-stock or total nitrogen and pH that are influenced by soil management practices at longer time scales. All the collected soil data were firstly divided by land use in agricultural and forest soils and secondly divided with respect to the different management types. The Wilcoxon non-parametric test was performed among soil indicators under different types of management, within different classes of pH and soil nitrogen availability expressed as percentage of extractable to total nitrogen (Extr-N/TN).

This statistical analysis focused on to what extent the selected soil biochemical properties respond to the various types of management according to the different ranges of soil pH and nitrogen availability. Moreover, the quantile regression model (QRM) was applied separately on the management practices in agricultural and forest ecosystems. All the statistical outputs of the QRM can be found in Appendix 1 of the thesis.

a) Agroecosystems

The Wilcoxon non-parametric test was performed in case study 1 and 2 considering the following three soil pH classes: 5.0-6.8 (slightly acid/neutral); 6.8-7.2 (neutral) and 7.2- 7.7 (slightly alkaline). The results with the level of significance among the various ranges of pH are presented in Figure 4.5.1.A. The selected soil bioindicators, with the exception of cellulase, under a neutral pH significantly differentiate the different types of agricultural management.

The only enzyme activity significantly sensitive to agricultural management at a slightly alkaline soil pH was xylosidase. In sub-acid/acid and sub-alkaline/alkaline soil pH the specific enzyme activities and the microbial quotient did not differentiate the types of agricultural management. The Wilcoxon non-parametric test was also performed on soil indicators considering three range of soil available nitrogen (Extr-N/TN): 0-3%; 3-6% and 6-10%.

The results presented in Figure 4.5.1 B show that the majority of specific enzyme activities and the microbial quotient significantly responded to the various agricultural managements across all ranges of Extr-N/TN with the exception of cellulase at low level of available nitrogen.

The quantile regression model (QRM) was applied on the agricultural soil properties that were previously divided into two categories of soil tillage (conventional vs. reduced tillage) and into two agricultural system (organic vs. conventional). In general, the soil indicators were more sensitive to pH and TN in the tillage (CT and RT) than in the management system (ORG and CONV).

In the CT and RT soils the arylsuphatase activity was negatively related to pH ($p<0.001$) across all the quantiles distribution, while a positive relationship with pH was reported for the xylosidase activity at the median (Q50) and upper (Q75) quartiles.

Moreover, TN had a positive relationship with acid phosphatase, arylsuphatase and xylosidase ($p<0.001$) across all the quartiles, conversely TN had a negative relationship with low and medium values of α-glucosidase and β-glucosidase (Q25, Q50) ($p<0.001$). A negative relationship

characterizes the high values of arylsuphatase (Q75) in RT soils compared to the CT soils which represented the reference category. The microbial quotient was positively influenced by soil pH in the second half of the distribution (Q50 and Q75), while TN showed a negative effect on microbial quotient in these two quartiles.

The comparison of the two agricultural management system (ORG vs.CONV), the QRM showed a negative relationship between acid phosphatase and soil pH across all quantiles distribution. Moreover, the same negative relationship with soil pH was reported at values Q75 of arylsuphatase ($p<0.05$) and xylosidase ($p<0.01$). Organically managed soils with respect to the conventional ones registered a negative relationship in the upper quantile (Q75) for the acid-phosphatase, β-glucosidase and xylosidase specific activity.

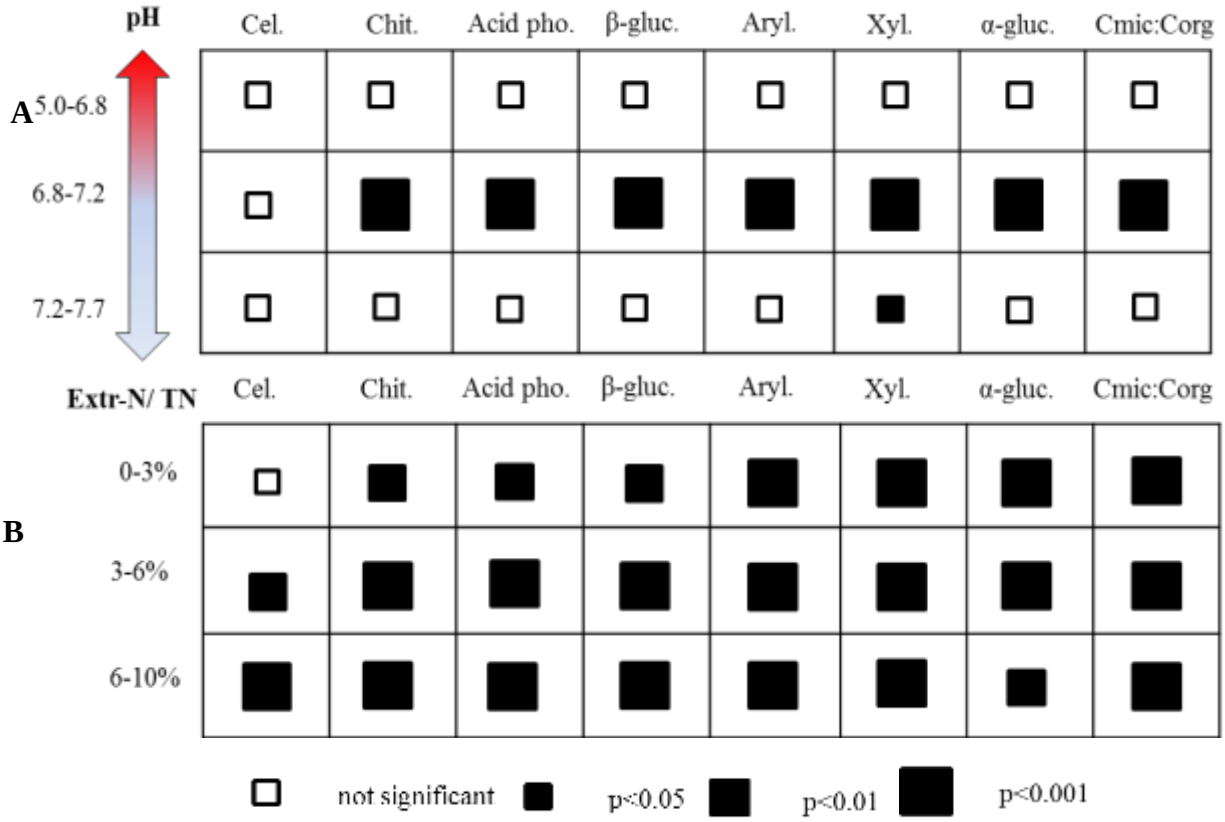


Figure. 4.5.1. Results of Chi-squared statistics X^2 and p-values obtained by various specific enzyme activities: cellulase (Cel.), chitinase (Chit.), acid. pho (Acid phosphatase), β-gluc (β-glucosidase), aryl.(Arylsuphatase), xyl. (Xylosidase), α-gluc (α-glucosidase) and the microbial quotient Cmic:Corg performed among agricultural soils within classes of pH (A) and Extr-N/TN (B).

b) Forest ecosystems

The following soil pH ranges were considered for the Wilcoxon non-parametric test: 3.9-4.5 (very strongly acid); 4.5-5.5 (strongly acid) and 5.5- 6.5 (slightly acid- neutral). The soil enzymes specific activities chitinase, β-glucosidase and α-glucosidase at 4.4-5.5 pH showed significant differences among the forest management practices. The microbial quotient significantly distinguished the management practices at a slightly-acid, neutral pH. In the 3-6 % range of Extr-N/TN (Figure 4.5.2 B) only cellulase, chitinase and α-glucosidase distinguished among the different management practices. Moreover the microbial quotient significantly discriminated only at low values of Extr-N/TN.

The results of the QRM of the different Douglas-fir stand ages showed that TN had a significant positive relationship with cellulase ($p<0.001$) and chitinase ($p<0.05$) in the lower quantile (Q25) and a significant negative relationship with arylsuphatase ($p<0.05$) in the upper quantiles. pH had decreased the activity of β-glucosidase ($p<0.05$) and acid phosphatase ($p<0.001$) in the lower quantiles and upper quantiles respectively. A positive relationship between the older tree stands and younger tree stands was reported at high values (Q50, Q75) of the specific activity of acid phosphatase, β-glucosidase, chitinase and xylosidase. The microbial quotient was negatively influenced by TN ($p<0.001$) in the upper and median quartiles. Moreover, a positive relationship between the 100 yrs old stands and 80 yrs old stands was registered at high values of the microbial quotient. The second QRM analysis applied on data from forest soils was performed for the beech tree stands. In this case the QRM did not reveal any significant relationships between the soil indicators pH and TN, but a positive relationship of older beech tree stands compared with the younger ones registered in Q25 for the specific activity of chitinase and β-glucosidase. As for the xylosidase specific activity at Q50 and Q75, a negative relationship was found between the two stand ages.

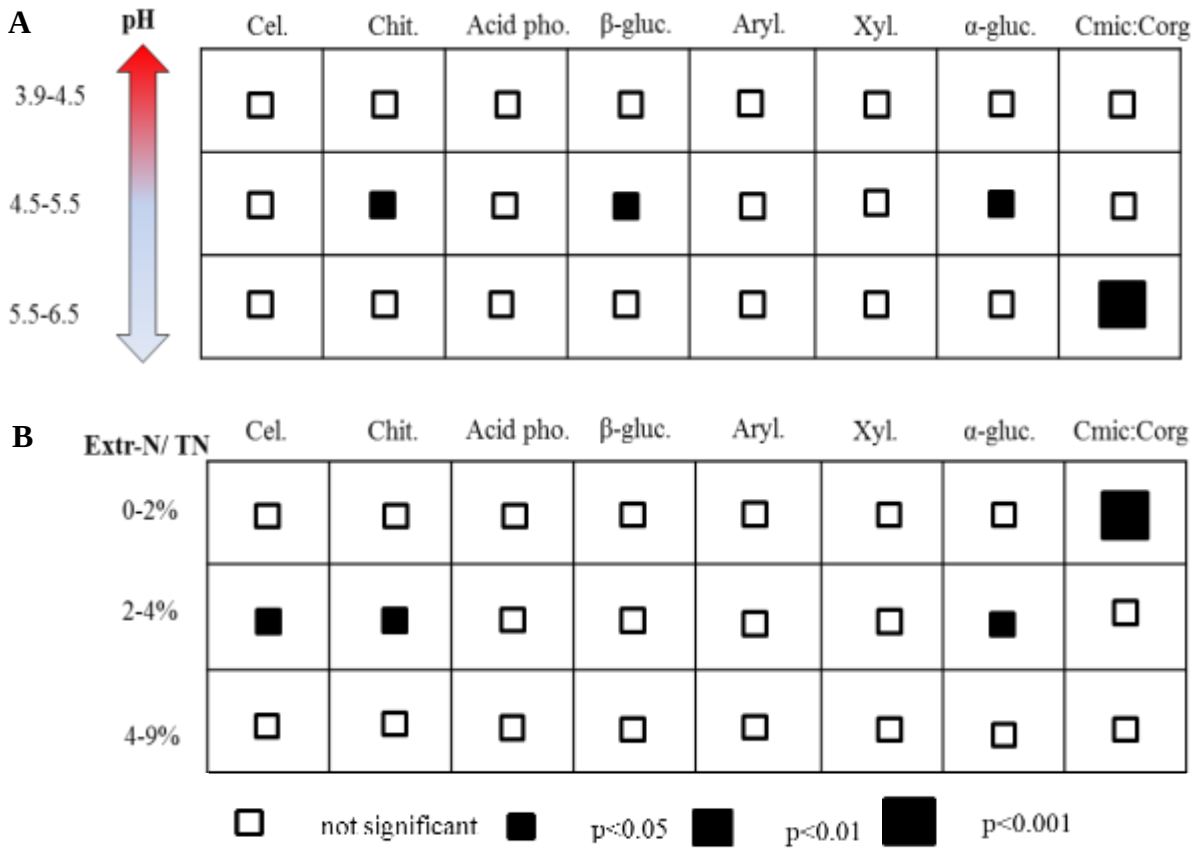


Figure 4.5.2. Results of Chi-squared statistics X^2 and p-values obtained by various specific enzyme: activities cellulase (Cel.), chitinase (Chit.), acid. pho (Acid phosphatase), β-gluc (β-glucosidase), Aryl. (arylsuphatase), Xyl. (xylosidase), α-gluc (α-glucosidase) and the microbial quotient Cmic:Corg performed among forest soils within classes of pH (A) and Extr-N/TN (B).

The response of soil quality to management practices is based on a feedback mechanism where soil chemical, physical and biological indicators highlight the sustainable management (Zornoza et al., 2015). Therefore, as soil properties changes over time they can reflect if soil quality, with a certain land use and under a specific management system, is improving, is constant or declining (Shukla et al., 2006). In this study, the soil biological indicators such as microbial biomass size and functions have been selected as bioindicators that rapidly respond to management practices and can forecast early any environmental disturbance (Cardoso et al., 2013). These bioindicators are known to be the most sensitive to changes of soil organic matter quantity/quality induced by land-use and management practices (Álvaro-Fuentes et al., 2013).

In this study, land use was considered to be the first criteria to separate soil types due to the different frequencies of disturbance induced by management practices, producing different soil organic matter turn-over. Agricultural ecosystems are frequently disturbed by soil management, especially tillage which accelerates the mineralization of soil organic matter compared to forest ecosystems where SOM have a slow turn-over and tend to accumulate. In fact, in this study, the first obvious results showed the difference between agricultural and forest ecosystems especially in terms of specific enzyme activities and microbial quotient. In the agricultural ecosystems there were far more significant differences between the management practices than in the forest ecosystems. Particularly in agroecosystems, only in soils under neutral pH did the biochemical parameters differentiate between the types of soil management (tilled vs. not tilled soils and conventionally vs organically managed systems). The enzymes' capacity for discrimination among different managements in agroecosystems was highly constrained by soil pH, showing the most sensitive response in soil with neutral values of pH. The only specific enzyme that discriminated at a sub-alkaline pH was xylosidase even if its optimum pH range between 5.0 and 7.0 (Turner, 2010). According to Sinsabaugh et al. (2008) soil pH has direct effects on enzymes activity, especially on the ones immobilized in the soil matrix. Moreover, soil pH induces changes in nutrient availability and therefore has an indirect effect on soil microbial activity. In this study, almost all soil enzymes related to the C, N, P and S bio-geochemical cycles discriminated soil among different management systems. Several previous studies reported that enzyme activities such as β-glucosidase, chitinase, arylsuphatase, xylosidase and acid phosphatase promptly responds to land-use change (Moscatelli et al., 2007) and agricultural managements such as conventional and reduce tillage, use of cover crop and residues incorporation (Bandick and Dick, 1999; Hendriksen et al., 2016; Mbuthia et al., 2015; Trasar-Cepeda et al., 2008). The biochemical indicators became highly sensitive in discriminating soil management practices in agricultural soils at various ranges of soil nitrogen availability (expressed as percentage of Extr-N to TN). This result suggests that the nitrogen availability in agroecosystems may changes with management and it might be related to microbial size and activity. In fact, Extr-N represents a more dynamic soil pool compared to TN. Soil Extr-N pool is controlled and replenished by organic matter turnover and is therefore linked to microbial activity (Kalbitz et al., 2000) . It also consists in readily biodegradable substrates that can be consumed by soil microorganisms. However, several soil properties such as TN and pH, as well as seasonal variations, land-use (agricultural vs. forest soils), agricultural management practices (tillage, green manure application) can all influence the quantity of soil Extr-N pool (Ros et al., 2009). For this

reason, the effect of management on soil bioindicators were analysed considering three soil categories with different ranges of pH and TN content. The QRM results showed that in the tilled/not tilled soils the high specific activity of cellulase, chitinase, xylosidase, arylsuphatase and the microbial quotient were influenced by soil pH. Furthermore, in the comparison of conventional and organic cropping systems, soil pH had a negative influence on Q75 values of arylsuphatase, acid phosphatase and xylosidase activity. Moreover, soil TN in the tilled/not tilled soils positively influenced the activity of arylsuphatase, acid phosphatase and xylosidase while β and α-glucosidase activities were negatively influenced. The effect of TN and soil pH on soil biochemical indicator in the conventional vs. organic cropping systems was not evident since the case study 2 considered it just a single range of both pH and TN.

In the forest ecosystems with more acid soil pH, only chitinase, α- glucosidase and β-glucosidase specific activities significantly differentiated ($p<0.05$) between the management practices. This results was probably due to the fact that, the pH range 4.5-5.5 corresponds to the optimum pH reported in the literature for α- glucosidase and β-glucosidase, with the exception of chitinase where the optimum pH is between 4.0 and 5.0 (Turner, 2010). It is interesting to note that all the above enzymes are related to the C and N-cycle, mainly involved in the degradation of starch, cellulose and chitin. In forest soils the Extr-N/TN percentage tends to be lower compared to the agroecosystems, which could be a result of higher immobilization rates of N in the microbial biomass that decomposes more complex C-rich compounds (Ros et al., 2009). The forest management practices were significantly discriminated at 4.5-5.5% Extr-N/TN by cellulase, chitinase and α-glucosidase. In both, pH and Extr-N/TN ranges, only the C and N-cycle enzymes were sensitive indicators of forest management. Moreover, the microbial quotient was a sensitive indicator especially when soil pH was not extremely acid and when Extr-N was particularly low. The QRM model applied for the different stand ages of Douglas-fir showed that cellulase, chitinase and β-glucosidase specific activities were positively influenced by TN. Conversely, the arylsulphatase and acid phosphatase specific activities and the microbial quotient were negatively influenced by TN. Soil pH had just one negative influence on high values of chitinase. In the soils from the beech tree forest stands no significant relationships were found between the distribution of studied soil indicators and soil pH and soil TN content. The results obtained in this study suggest that in forest ecosystems the soil biochemical properties that change due to different management are less evident than in the agroecosystems. This result is explained by the fact that forest soil has a

higher level of organic matter which participates in the improvement of soil resilience and homeostasis (Lal, 1997).

4.5.2 The assessment of microbial functional diversity in different soil categories (case study 5)

This part was performed in collaboration with prof. Maria Cristina Moscatelli and prof. Luca Secondi

In order to deepen the knowledge on soil microbial functional diversity and on how it can be influenced by soil characteristics such as organic carbon content and soil pH, the same statistical models as in the previous case were assessed. In this case study the soils were selected from different environments (agro-ecosystems, forest ecosystems and extreme condition ecosystems). The microbial functional diversity was assessed using two of the most popular methods: multi-enzyme assays and the multi-substrate induced respiration method (Microresp). The data from both methods were used for the calculation of Shannon's diversity index (H'). The aim of the study was to explore, through a quantile regression approach (QRM), the possible relationships between each of the two methods, to explore a set of selected explicative variables Corg soil type and pH and to assess the efficacy of both methods in capturing differences among the various types of soil when different levels of pH (H_2O) and Corg are considered. The results of the QRM analysis are presented in appendix 1.

The results of the QRMs provided us with useful information concerning the magnitude and signs of the relationships between explicative variables throughout the entire distribution of the dependent variables. Apart from a slight significance for H' Enzyme Activities (EA) (Q50 for medium and high classes of Corg), the soil Corg content did not show any significant relation for either of the indices. Conversely, pH values were negatively related to the H'EA measures in the lower part of the distribution (i.e. for low values of the dependent variable H'EA) while a positive relationship was observed in the highest quantiles of the distribution (i.e. for high values of the dependent variable H'EA). On the other hand, pH values are positively related with H'Microresp (MR) only in the middle part of the distribution (Q50).

The soil type is an important factor in distinguishing the values of the two measures. In particular, the sign and magnitude of the relationship characterizing A-type soils varies according on the basis of both quantile analysed and measures considered. For H'EA the relationship is positive and strogly

significant in the lower part of the distribution (Q25) while we found a negative relationship in the upper part (Q75). A negative relationship characterizes this type of soil (A) and the H'MR measure in the first half of the distribution, (Q25 and 50) compared to the EC-type soil.

According to the results of the Kruskal-Wallis test, both H'EA and H'MR significantly differentiated the various types of soil when Corg or pH was considered. However, H'MR showed a greater capability than H'EA according to the p-values reported in Figure 4.5.3. In fact, while H'EA discriminates soils only for Corg values <1.5 - 3%> and pH values <6.5 - 7.4>, H'MR is significantly effective along all ranges for both soil properties.

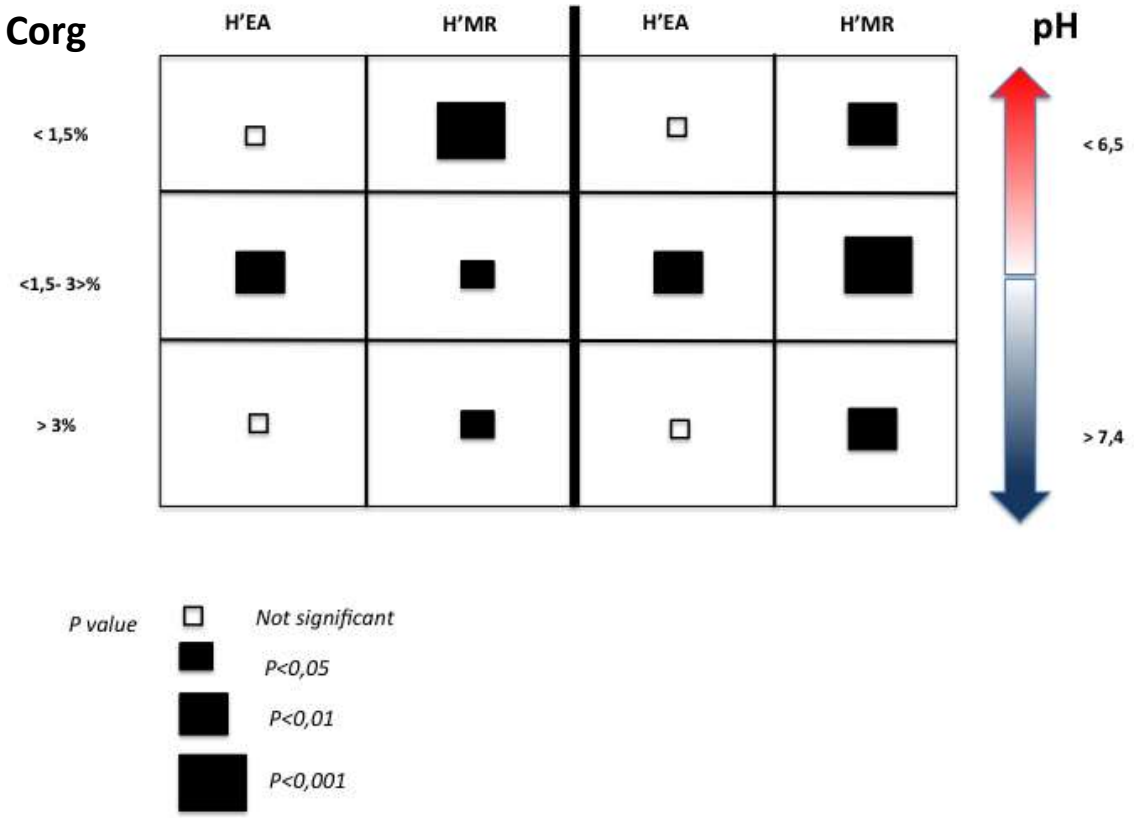


Figure 4.5.3 – Results of Chi-squared statistics X^2 and p-values obtained with Kruskal-Wallis rank test performed among soils within classes of Corg and pH measured by H'EA and H'MR

The Quantile Regression Model (QRM) helped if and to what extend the role of selected co-variates (relevant soil properties such as Corg and pH) change throughout the entire distribution of each dependent variable (H'EA and H'MR). The QRM showed that both diversity indexes depended

more on soil pH than on Corg content, indicating soil reaction as the property which mostly affects microbial diversity (Zhalnina et al., 2015).

In particular, no significant relationship linked the two indexes to Corg values, no matter the quantile considered. Conversely, H'EA showed a negative and positive relationship to pH for Q25 and Q75 while H'MR a positive one for Q50.

Microbial functional diversity expresses the capacity of microbial biomass to perform different processes and to metabolize diverse substrates. Soil pH variations can induce, more than Corg , significant changes within the microbial biomass structure in terms of species and related functional patterns. Microbial processes are usually strictly dependent on pH values that control the majority of the reactions occurring in the soil. Fierer and Jackson, (2006) reported soil pH as the best predictor of microbial diversity and richness. Other authors such as Thomson et al. (2015) and (Griffiths et al., 2011) reported that bacterial diversity is mainly correlated with soil pH. However, the nature of this relationship is controversial: in a similar study Fierer and Jackson (2006), showed a unimodal distribution of bacterial diversity, reaching possibly a plateau at near neutral pH. In our study H'EA values in the Q75 showed a positive relationship to pH as well as the H'MR values in Q50. Conversely a negative relationship was found in the Q25 of H'EA, in accordance with the decline of diversity found by Fierer and Jackson, (2006) at acidic pH.

The Kruskal-Wallis test showed that CLPP-MicroResp was a more powerful technique than enzyme activities in highlighting differences among soils and treatments. Enzyme activities were effective only within a certain range of Corg and pH values (<1,5-3%> and <6,5-7,4>, respectively), MicroResp was able to discriminate soils along a wide range of Corg and pH values therefore representing an effective tool for evaluating microbial functional diversity changes. However, in relation to the lack of significant response of EA to pH variations we should keep in mind that the enzymes determination was performed using NaAc buffer pH 5.5 as standardized in the protocol proposed by Marx et al. (2001). It is thus possible that the lower discriminant capacity of enzyme activities across a wide range of pH values may be due to this methodological constraint. However, since also Corg values do not significantly affect H'EA, except in the range <1,5-3%>, we can conclude that MicroResp shows a higher discrimination capacity among soil uses and managements.

It is widely demonstrated that the agricultural land use, particularly under conventional management characterized by monocultures, high fertilizers inputs and tillage, reduces the variety of carbon inputs to soil, thus a lower differentiation of available substrates will reduce the potential spectrum of microbial processes. Creamer et al. (2016) showed the greater utilisation of carboxylic acid based

substrates in arable sites using CLPP-MicroResp in a study across the European continent (and associated islands). They concluded that the impact of management practices in arable systems reduces the catabolic functional capacity of the microbial community.

V. GENERAL CONCLUSIONS

In this thesis the impact of forest and agricultural management on soil quality was assessed using various chemical and biochemical indicators. In particular, the sensitive soil quality indicators were pointed out for each ecosystem, because the response of the indicators usually depends not only on environmental conditions (e.g. climate) but also on land-use. Moreover, it is important to consider the effect of management over time, since some agricultural practices are usually more frequent than the forest ones. For instance, the sustainable soil management increases the C-stock in both agricultural and forest ecosystems, but these changes are usually evident after several years of management. For this reason, the soil C-stock is an important indicator of sustainable management in a long term-period. Conversely, other soil bioindicators, such as microbial biomass and its activity, usually promptly respond to soil management therefore they are sensitive to soil quality changes in a short-term period.

In this study, the short-term agricultural management influenced the quality of soil organic matter while over a long period both quality and quantity of soil organic matter were influenced. In the first case study two years crop rotation including two levels of tillage (conventional and reduced) and three types of subsidiary crops (leguminous spp, brassica spp. and living mulch) were considered as short term experiments across four European climate zones. The soil chemical indicators such as total organic carbon and total nitrogen did not vary with respect to tillage and type of subsidiary crops. Conversely, soil biochemical indicators promptly responded to soil management. In particular, the microbial quotient was sensitive to the tillage level, while the specific enzyme activities were mainly affected by the cover crops and showed sensitivity toward climatic seasonal variations. In the long-term experiment of case study 2 (conventional vs. organic agricultural systems), the increase of C-stock in the organic systems and reduced tillage confirmed the sustainability of these managements. The absolute soil enzyme activities (per g of soil) were enhanced by the increase of the amount of organic carbon. Conversely, the specific enzyme activities (per unit of organic C) were sensitive indicators of different management suggesting significant changes on soil organic matter quality.

In the forest ecosystems (case studies 3 and 4) the response of soil microbial biomass and its activity was different with respect to various tree stand ages and to the forest management over time.

In the Douglas-fir stands at different age classes, the microbial physiological profile assessed with the Microresp® technique, increased in the oldest stands. Moreover, at this age class (120 yrs) , the microbial community showed a different physiological profile probably due to different soil nutrient availability and nitrogen limitation. Finally, the different management of the Beech forest in Monte Venere (Central Italy) produced heterogeneous forest structure which triggered a significant effect on soil carbon sequestration and on microbial functional and structural diversity.

Moreover, the application of the quantile regression model (QRM) represented an innovative approach to study the relationship between soil quality indicators across different types of soil management practice. However, the statistical method was more effective where soils from different areas and diverse characteristics were analysed. The Wilcoxon and Kruskal-Wallis non parametric tests made possible the distinction of the various soil types with different ranges of pH, nitrogen availability and total organic carbon. For instance, in all ecosystems (forest and agricultural), the effectiveness of soil specific enzyme activities to discriminate among various soil managements highly depended on soil pH. Moreover, in agroecosystems the specific soil enzymes activities and microbial quotient were sensitive indicators of soil management depending also on nitrogen availability. Conversely, in forest ecosystems the same bioindicators were not responsive to management according to the level of nitrogen availability.

Soil microorganisms and their functions play an essential role in maintaining soil ecosystem stability providing a wide range of services that contribute to the sustainability of the ecosystems. Microbial functional diversity has a crucial role in facing natural and anthropogenic pressures and in maintaining the capacity for resilience of the soil ecosystem. Moreover, soil functional diversity is highly sensitive to land use and management practices therefore it has to be taken into consideration in the assessment of the soil management impact. For this reason in this study, two methodological approaches (community level physiological profile CLPP and enzyme activities) were used to assess soil microbial functional diversity using Shannon Index in various soil types. The diversity index calculated with data from the CLPP provided a generally higher discrimination capacity between various land uses and soil managements than the diversity index calculated in terms of enzyme activities. In this study the identification of the sensitive indicators of soil management was performed considering some soil properties such as: pH, organic carbon and nitrogen availability.

In conclusion, considering the main question posed in this study, the soil specific enzyme activity (per unit of soil organic carbon) and the microbial quotient can be considered effective soil indicators to assess management sustainability in both agricultural and forest ecosystem. However, a

different behaviour of these biondicators was observed in the two type of ecosystems. For instance, in the agricultural system they were more sensitive to management when the soil was in the neutral range of pH, and they were strongly related to the wide range of available nitrogen pool. Conversely, in the forest ecosystems the response of the selected biondicators to soil management was not strongly affected by soil pH and nitrogen availability. For future studies this approach could be extended to other intrinsic soil characteristics such as soil texture as independent variables in the QRM and Kruskal-Wallis statistical analysis.

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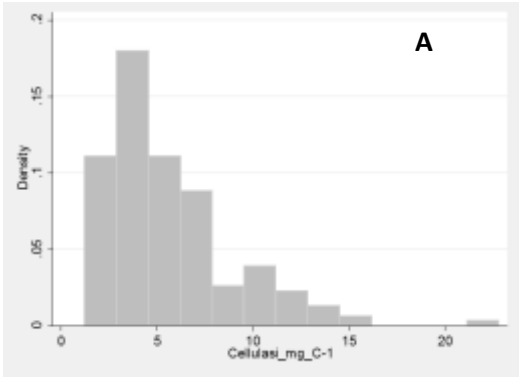
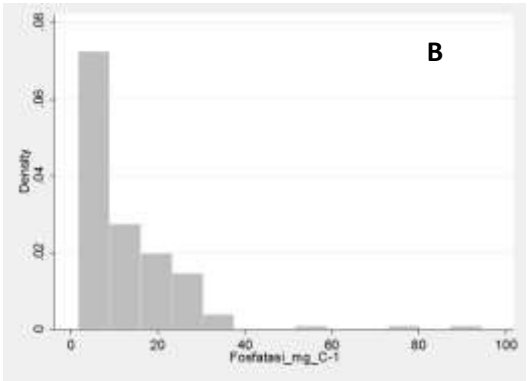
APPENDIX

I. Agricultural soils

Table1. Basic statistical description of the agricultural soils

Measure	**Mean**	**SD**	**CV**	**Min**	**Max**	**Skewness**
Cellulase	5.59	3.31	0.59	1.23	22.81	1.53
Chitinase	12.34	8.05	0.65	3.55	64.94	2.30
Acid phosphatase	12.46	11.73	0.94	1.76	94.47	3.14
β -glucosidase	1.72	0.96	0.56	0.24	4.38	0.43
Arylsulphatase	97.1	91.03	0.93	3.53	356.17	0.75
Xylosidase	37.2	53.99	1.45	2.99	513.24	5.04
α-glucosidase	0.59	0.34	0.59	0.12	1.76	0.83
Cmic:Corg	2.13	1.33	0.62	0.58	8.08	1.64





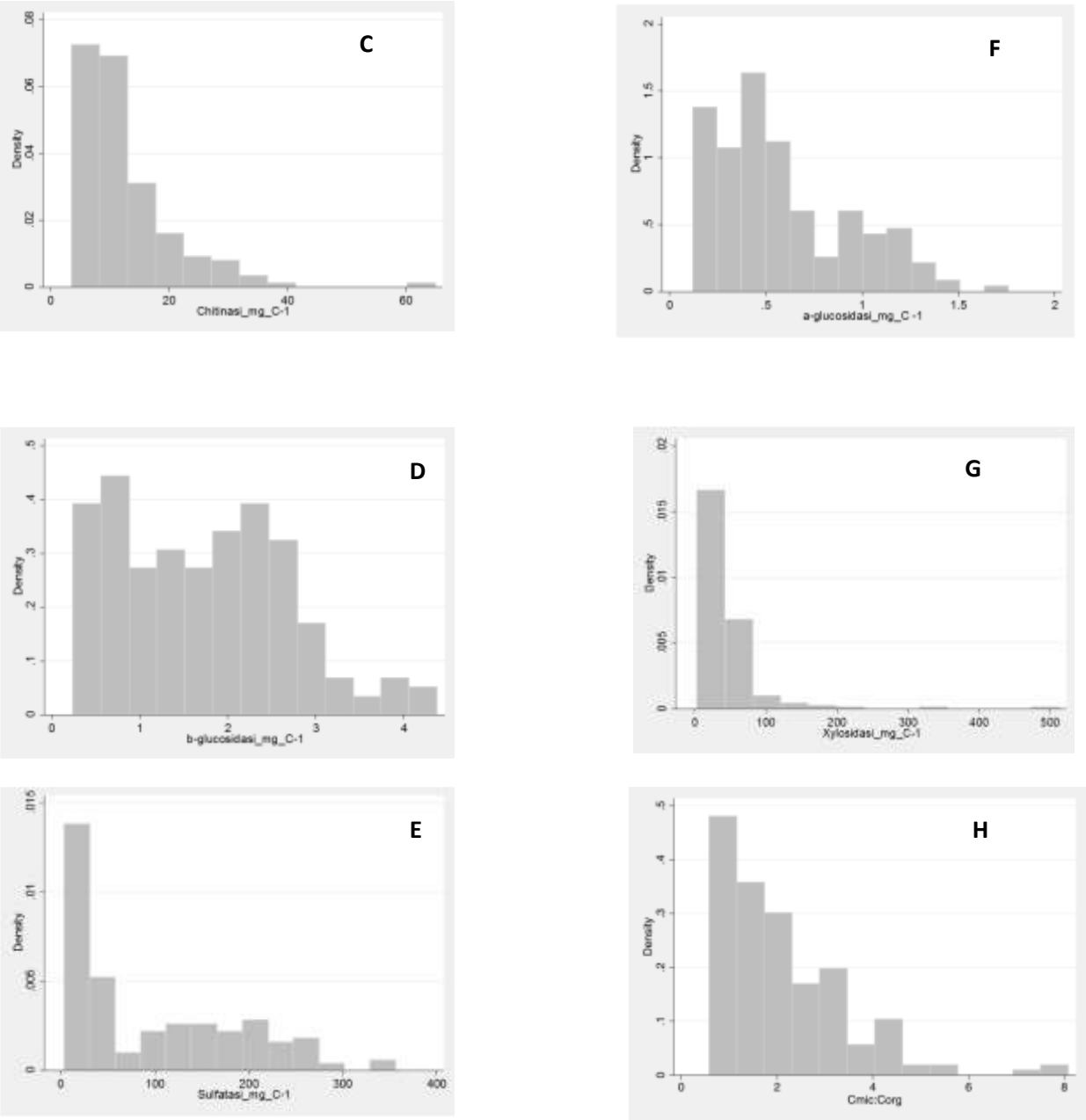


Figure. 1 Distribution of specific enzyme activities (nmol MUF mg C^{-1} h^{-1}) and microbial quotient [Cellulase (A), Acid phosphatase (B), Chitinase (C), β-glucosidase (D), Arylsuphatase (E), α-glucosidase (F), Xylosidase (G) and the microbial quotient Cmic:Corg (H)]

1.1 The Quantile regression model (STATA software output) for case study 2, the codes represent **1-convetional tillage and-2 reduced tillage**. Model (1) represents the regression without considering the management practice while Model (2) considers the management practice (this model was discussed in the thesis).

a) Cellulase (nmol MUF mg C^{-1} h^{-1})

	(1) cellulasi_~1	(2) cellulasi_~1
q25		
ph	-1.208*	-1.140
	(-2.21)	(-1.92)
tn	-2.441***	-2.097***
	(-7.11)	(-5.06)
1bn.codice		.
		.
2.codice		-0.673
		(-1.05)
_cons	16.59***	15.98***
	(4.60)	(4.05)
q50		
ph	3.443	3.416
	(1.57)	(1.90)
tn	-0.234	-0.271
	(-0.23)	(-0.35)
1bn.codice		.
		.
2.codice		-1.052
		(-1.91)
_cons	-15.99	-15.10
	(-1.02)	(-1.19)
q75		
ph	4.043***	3.862***
	(6.39)	(4.89)
tn	-0.995	-1.081
	(-1.29)	(-1.23)
1bn.codice		.
		.
2.codice		-0.231
		(-0.22)
_cons	-16.66**	-15.14*
	(-3.22)	(-2.36)
N	112	112

b) Chitinase (nmol MUF mg C^{-1} h^{-1})

	(1) chitinasi_~1	(2) chitinasi_~1
q25		
ph	-2.712**	-0.933
	(-2.72)	(-0.65)
tn	-0.0106	0.0171
	(-0.02)	(0.02)
1bn.codice		.
		.
2.codice		1.277
		(1.50)
_cons	27.85***	15.14
	(4.13)	(1.59)
q50		
ph	2.641	1.922
	(0.65)	(0.44)
tn	0.717	0.380
	(0.35)	(0.21)
1bn.codice		.
		.
2.codice		0.970
		(0.55)
_cons	-4.793	0.494
	(-0.17)	(0.02)
q75		
ph	6.322***	6.322***
	(4.72)	(3.79)
tn	2.920	2.920*
	(1.91)	(2.31)
1bn.codice		.
		.
2.codice		0.166
		(0.09)
_cons	-29.28**	-29.44*
	(-2.84)	(-2.37)
N	112	112

c) β-glucosidase (nmol MUF mg C^{-1} h^{-1})

	(1) bglucosida~1	(2) bglucosida~1
q25		
ph	-0.201	-0.306
	(-1.14)	(-1.55)
tn	-0.424**	-0.349**
	(-3.07)	(-2.76)
1bn.codice		.
		.
2.codice		-0.175
		(-0.89)
_cons	3.912**	4.541**
	(2.91)	(3.13)
q50		
ph	-0.104	-0.104
	(-0.63)	(-0.43)
tn	-0.220	-0.276
	(-1.16)	(-1.56)
1bn.codice		.
		.
2.codice		-0.147
		(-0.78)
_cons	3.379**	3.526*
	(2.72)	(2.04)
q75		
ph	0.0827	0.0616
	(0.38)	(0.27)
tn	-0.352	-0.374
	(-0.98)	(-0.94)
1bn.codice		.
		.
2.codice		-0.0374
		(-0.16)
_cons	2.786	2.985
	(1.46)	(1.36)
N	112	112

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

d) Arylsuphatase (nmol MUF mg C^{-1} h^{-1})

	(1) sulfatasi_~1	(2) sulfatasi_~1
q25		
ph	-23.42*	-23.14***
	(-2.58)	(-3.44)
tn	92.97***	94.26***
	(11.24)	(12.38)
1bn.codice		.
		.
2.codice		-7.586
		(-0.82)
_cons	101.9	104.8*
	(1.81)	(2.46)
q50		
ph	-33.21**	-36.79***
	(-2.94)	(-4.40)
tn	104.7***	107.1***
	(18.60)	(15.83)
1bn.codice		.
		.
2.codice		-10.26
		(-1.02)
_cons	172.1*	198.0***
	(2.23)	(3.68)
q75		
ph	-42.51**	-33.46***
	(-3.03)	(-5.08)
tn	126.0***	120.5***
	(19.42)	(15.76)
1bn.codice		.
		.
2.codice		-18.05*
		(-2.44)
_cons	223.0*	179.2***
	(2.23)	(4.94)
N	112	112

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

e) α –glucosidase (nmol MUF mg C^{-1} h^{-1})

	(1) aglucosida~1	(2) aglucosida~1
q25		
ph	-0.0351	-0.0349
	(-0.78)	(-0.85)
tn	-0.149*	-0.146**
	(-1.99)	(-2.65)
1bn.codice		.
		.
2.codice		-0.00715
		(-0.23)
_cons	1.038*	1.034**
	(2.50)	(2.91)
q50		
ph	0.0744	0.0689
	(0.56)	(0.66)
tn	-0.223**	-0.229***
	(-2.64)	(-4.86)
1bn.codice		.
		.
2.codice		-0.0123
		(-0.24)
_cons	0.624	0.678
	(0.66)	(0.97)
q75		
ph	0.309*	0.265
	(1.98)	(1.55)
tn	-0.0565	-0.0926
	(-0.51)	(-1.01)
1bn.codice		.
		.
2.codice		-0.0643
		(-0.64)
_cons	-0.968	-0.585
	(-0.91)	(-0.53)
N	112	112

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

f) Acid phosphatase (nmol MUF mg C^{-1} h^{-1})

	(1) fosfatasi_~1	(2) fosfatasi_~1
q25		
ph	-4.093	-3.031
	(-1.41)	(-1.50)
tn	4.369***	4.460***
	(3.60)	(6.91)
1bn.codice		.
		.
2.codice		-1.819
		(-1.76)
_cons	30.46	23.87
	(1.62)	(1.79)
q50		
ph	-0.961	-0.934
	(-0.60)	(-0.55)
tn	8.603***	8.674***
	(9.31)	(9.16)
1bn.codice		.
		.
2.codice		0.171
		(0.17)
_cons	6.817	6.484
	(0.62)	(0.54)
q75		
ph	2.918	3.158
	(1.40)	(1.30)
tn	12.08***	11.96***
	(12.09)	(10.32)
1bn.codice		.
		.
2.codice		0.513
		(0.37)
_cons	-21.70	-23.21
	(-1.47)	(-1.33)
N	112	112

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

g) Xylosidase (nmol MUF mg C^{-1} h^{-1})

	(1) xylosidasi~1	(2) xylosidasi~1
q25		
ph	**-0.161**	**1.909**
	(-0.03)	**(0.27)**
tn	**25.91*** **	**26.02*** **
	(9.14)	**(7.42)**
1bn.codice		.
		.
2.codice		**-2.022**
		(-0.52)
_cons	**-15.00**	**-27.68**
	(-0.43)	**(-0.56)**
q50		
ph	**14.25**	**17.40** *
	(1.73)	**(2.98)**
tn	**35.22*** **	**36.07*** **
	(10.21)	**(19.47)**
1bn.codice		.
		.
2.codice		**-3.898**
		(-1.54)
_cons	**-117.6***	**-138.3*** **
	(-2.03)	**(-3.39)**
q75		
ph	**23.89** *	**26.19***
	(3.16)	**(2.49)**
tn	**47.81*** **	**48.71*** **
	(8.89)	**(11.33)**
1bn.codice		.
		.
2.codice		**-3.179**
		(-0.61)
_cons	**-191.1*** **	**-206.5** *
	(-3.66)	**(-2.76)**
N	**112**	**112**

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

h) Microbial quotient (Cmic:Corg)

	(1) cmiccorg	(2) cmiccorg
q25		
ph	**0.0383**	**0.0358**
	(1.21)	**(0.24)**
tn	**-0.352*** **	**-0.351*** **
	(-3.55)	**(-4.89)**
1bn.codice		.
		.
2.codice		**0.00303**
		(0.02)
_cons	**1.474*** **	**1.486**
	(5.68)	**(1.40)**
q50		
ph	**0.304** *	**0.316***
	(3.04)	**(2.19)**
tn	**-0.484*** **	**-0.475*** **
	(-3.64)	**(-3.60)**
1bn.codice		.
		.
2.codice		**0.0719**
		(0.63)
_cons	**0.291**	**0.189**
	(0.40)	**(0.17)**
q75		
ph	**0.510*** **	**0.506*** **
	(4.93)	**(4.85)**
tn	**-0.453*** **	**-0.491*** **
	(-3.60)	**(-6.08)**
1bn.codice		.
		.
2.codice		**0.0909**
		(1.13)
_cons	**-0.832**	**-0.777**
	(-1.00)	**(-0.99)**
N	**112**	**112**

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

1.2. The Quantile regression model (STATA software output) for case study 2, the codes represent **1- convetional system and-2 organic system**. Model (1) represents the regression without considering the management practice while Model (2) considers the management practice (this model was discussed in the thesis).

a) Cellulase (nmol MUF mg C^{-1} h^{-1})

	(1) cellulasi_~1	(2) cellulasi_~1
q25		
ph	1.965	2.422
	(0.71)	(0.51)
tn	-1.269	-1.680
	(-1.02)	(-1.53)
3bn.codice		.
		.
4.codice		0.250
		(0.53)
_cons	-9.189	-12.02
	(-0.46)	(-0.36)
q50		
ph	-1.071	-1.136
	(-0.47)	(-0.20)
tn	-1.286	-1.261
	(-0.59)	(-0.54)
3bn.codice		.
		.
4.codice		-0.00966
		(-0.02)
_cons	12.87	13.30
	(0.89)	(0.35)
q75		
ph	-7.490	-9.623
	(-1.98)	(-1.23)
tn	3.962	3.211
	(1.19)	(0.86)
3bn.codice		.
		.
4.codice		-0.176
		(-0.14)
_cons	53.19	69.23
	(1.97)	(1.25)
N	72	72

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

b) Chitinase (nmol MUF mg C^{-1} h^{-1})

	(1) chitinasi_~1	(2) chitinasi_~1
q25		
ph	-2.969	-4.687
	(-0.78)	(-0.68)
tn	1.620	1.356
	(0.78)	(0.79)
3bn.codice		.
		.
4.codice		-0.637
		(-0.58)
_cons	24.29	36.88
	(0.93)	(0.76)
q50		
ph	-4.023	-4.659
	(-1.05)	(-0.65)
tn	5.509	5.297
	(1.40)	(1.23)
3bn.codice		.
		.
4.codice		-0.142
		(-0.13)
_cons	28.25	33.05
	(1.06)	(0.64)
q75		
ph	-7.942	-2.244
	(-1.68)	(-0.19)
tn	8.931	8.931
	(1.93)	(1.74)
3bn.codice		.
		.
4.codice		0.954
		(0.70)
_cons	53.78	13.34
	(1.62)	(0.16)
N	72	72

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

c) β-glucosidase (nmol MUF mg C^{-1} h^{-1})

	(1) bglucosida~1	(2) bglucosida~1
q25		
ph	**-0.325**	**0.0926**
	(-0.30)	**(0.08)**
tn	**-0.164**	**-0.179**
	(-0.53)	**(-0.65)**
3bn.codice		.
		.
4.codice		**0.0758**
		(0.84)
_cons	**2.986**	**0.0192**
	(0.39)	**(0.00)**
q50		
ph	**-0.0192**	**-0.594**
	(-0.02)	**(-0.42)**
tn	**-0.121**	**-0.0617**
	(-0.24)	**(-0.21)**
3bn.codice		.
		.
4.codice		**-0.141**
		(-0.96)
_cons	**0.984**	**5.055**
	(0.11)	**(0.50)**
q75		
ph	**-0.919**	**-2.786**
	(-0.62)	**(-1.45)**
tn	**-0.128**	**0**
	(-0.15)	**(0.00)**
3bn.codice		.
		.
4.codice		**-0.463***
		(-2.28)
_cons	**7.637**	**20.89**
	(0.70)	**(1.50)**
N	**72**	**72**

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

d) Arylsuphatase (nmol MUF mg C^{-1} h^{-1})

	(1) sulfatasi_~1	(2) sulfatasi_~1
q25		
ph	**-3.867**	**-1.249**
	(-0.30)	**(-0.08)**
tn	**-0.0783**	**-1.561**
	(-0.01)	**(-0.32)**
3bn.codice		.
		.
4.codice		**1.531**
		(0.61)
_cons	**35.78**	**18.62**
	(0.38)	**(0.16)**
q50		
ph	**-0.761**	**-13.30**
	(-0.07)	**(-1.13)**
tn	**-3.142**	**-2.371**
	(-0.32)	**(-0.68)**
3bn.codice		.
		.
4.codice		**-2.211**
		(-1.26)
_cons	**21.09**	**109.9**
	(0.27)	**(1.32)**
q75		
ph	**-30.01**	**-63.30***
	(-1.64)	**(-2.18)**
tn	**-3.372**	**-3.622**
	(-0.25)	**(-0.33)**
3bn.codice		.
		.
4.codice		**-7.336**
		(-1.79)
_cons	**232.3**	**470.6***
	(1.71)	**(2.31)**
N	**72**	**72**

t statistics in parentheses

e) α –glucosidase (nmol MUF mg C^{-1} h^{-1})

	(1) aglucosida~1	(2) aglucosida~1
q25		
ph	**-0.281**	**-0.289**
	(-1.64)	**(-0.95)**
tn	**-0.0472**	**-0.0399**
	(-0.48)	**(-0.35)**
3bn.codice		.
		.
4.codice		**-0.00362**
		(-0.09)
_cons	**2.238**	**2.288**
	(1.83)	**(1.09)**
q50		
ph	**-0.221**	**-0.288**
	(-1.31)	**(-0.59)**
tn	**0.103**	**0.0868**
	(0.82)	**(0.54)**
3bn.codice		.
		.
4.codice		**0.00612**
		(0.08)
_cons	**1.690**	**2.177**
	(1.44)	**(0.62)**
q75		
ph	**-0.175**	**-0.200**
	(-0.41)	**(-0.27)**
tn	**-0.138**	**-0.141**
	(-0.66)	**(-0.64)**
3bn.codice		.
		.
4.codice		**-0.00412**
		(-0.03)
_cons	**1.754**	**1.940**
	(0.56)	**(0.38)**
N	**72**	**72**

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

f) Acid phosphatase (nmol MUF mg C^{-1} h^{-1})

	(1) fosfatasi_~1	(2) fosfatasi_~1
q25		
ph	**-7.069***	**-11.81***
	(-2.02)	**(-2.22)**
tn	**-1.444**	**-1.444**
	(-0.92)	**(-0.74)**
3bn.codice		.
		.
4.codice		**-0.758**
		(-0.92)
_cons	**54.36***	**88.25***
	(2.19)	**(2.36)**
q50		
ph	**-11.56*****	**-14.65****
	(-5.16)	**(-3.08)**
tn	**-1.414**	**-0.316**
	(-0.67)	**(-0.17)**
3bn.codice		.
		.
4.codice		**-0.753**
		(-0.75)
_cons	**87.14*****	**107.9****
	(5.38)	**(3.17)**
q75		
ph	**-6.919**	**-25.43*****
	(-0.81)	**(-3.67)**
tn	**-0.549**	**-0.578**
	(-0.16)	**(-0.37)**
3bn.codice		.
		.
4.codice		**-2.856***
		(-2.38)
_cons	**54.27**	**186.6*****
	(0.85)	**(3.75)**
N	**72**	**72**

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

g) Xylosidase (nmol MUF mg C^{-1} h^{-1})

	(1) xylosidasi~1	(2) xylosidasi~1
q25		
ph	-1.980	-3.759
	(-0.38)	(-0.31)
tn	2.120	1.965
	(0.56)	(0.45)
3bn.codice		.
		.
4.codice		-0.495
		(-0.30)
_cons	16.33	29.49
	(0.45)	(0.34)
q50		
ph	-8.550	-18.12
	(-1.26)	(-1.50)
tn	6.636	5.269
	(1.65)	(1.15)
3bn.codice		.
		.
4.codice		-1.806
		(-0.83)
_cons	59.34	129.4
	(1.19)	(1.46)
q75		
ph	-4.409	-38.92**
	(-0.29)	(-3.05)
tn	5.079	5.046
	(0.66)	(1.01)
3bn.codice		.
		.
4.codice		-6.873***
		(-3.45)
_cons	35.09	281.9**
	(0.31)	(3.00)
N	72	72

h) Microbial quotient (Cmic:Corg)

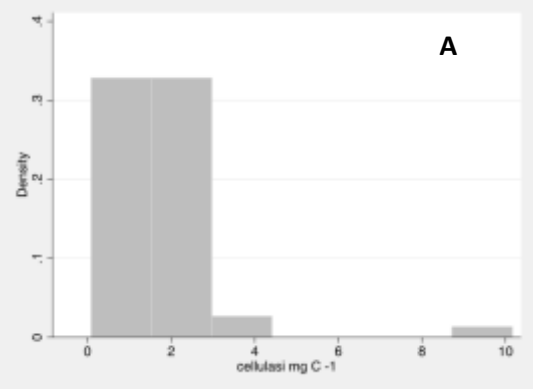
	(1) cmiccorg	(2) cmiccorg
q25		
ph	-1.195	4.406
	(-0.35)	(1.24)
tn	0.0686	-0.842
	(0.07)	(-0.71)
3bn.codice		.
		.
4.codice		1.279***
		(4.42)
_cons	10.76	-28.32
	(0.44)	(-1.14)
q50		
ph	-2.139	0.921
	(-1.29)	(0.33)
tn	-0.594	-0.752
	(-0.70)	(-1.00)
3bn.codice		.
		.
4.codice		0.555
		(1.37)
_cons	18.89	-2.800
	(1.56)	(-0.14)
q75		
ph	-4.306	-7.708
	(-1.36)	(-1.37)
tn	-2.248	-1.863
	(-1.59)	(-1.08)
3bn.codice		.
		.
4.codice		-0.344
		(-0.40)
_cons	37.01	60.45
	(1.61)	(1.49)
N	72	72

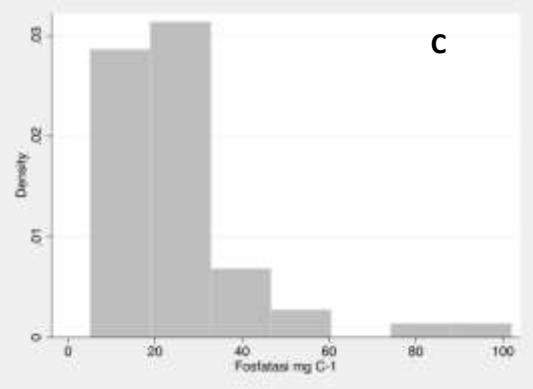
t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

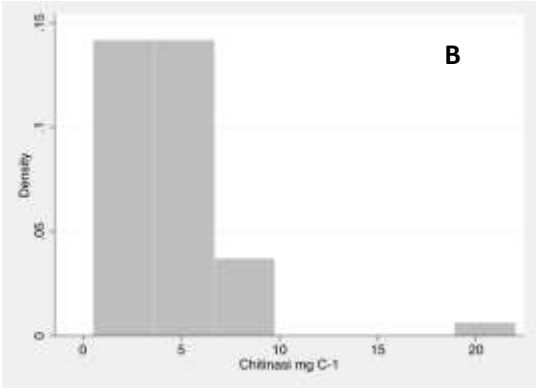
II. Forest soils

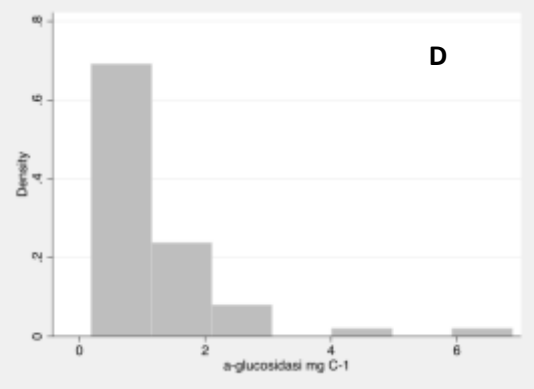
Table 2. Basic statistical description of the forest soils

Measure	Mean	SD	CV	Min	Max	Skewness
Cellulase	1.72	1.49	0.87	0.09	10.2	10.16
Chitinase	4.05	3.42	0.84	0.54	22	21.99
Acid phosphatase	25.2	17.09	0.68	5.1	102	101.9
β -glucosidase	7.19	6.79	0.94	1.17	49	18.98
Arylsulphatase	8.78	6.64	0.76	2.33	36.23	36.23
Xylosidase	2.94	1.86	0.63	0.86	7.48	7.48
α-glucosidase	1.15	1.11	0.97	0.19	6.88	6.88
Cmic:Corg	1.53	1.88	1.23	0.18	12.4	12.4









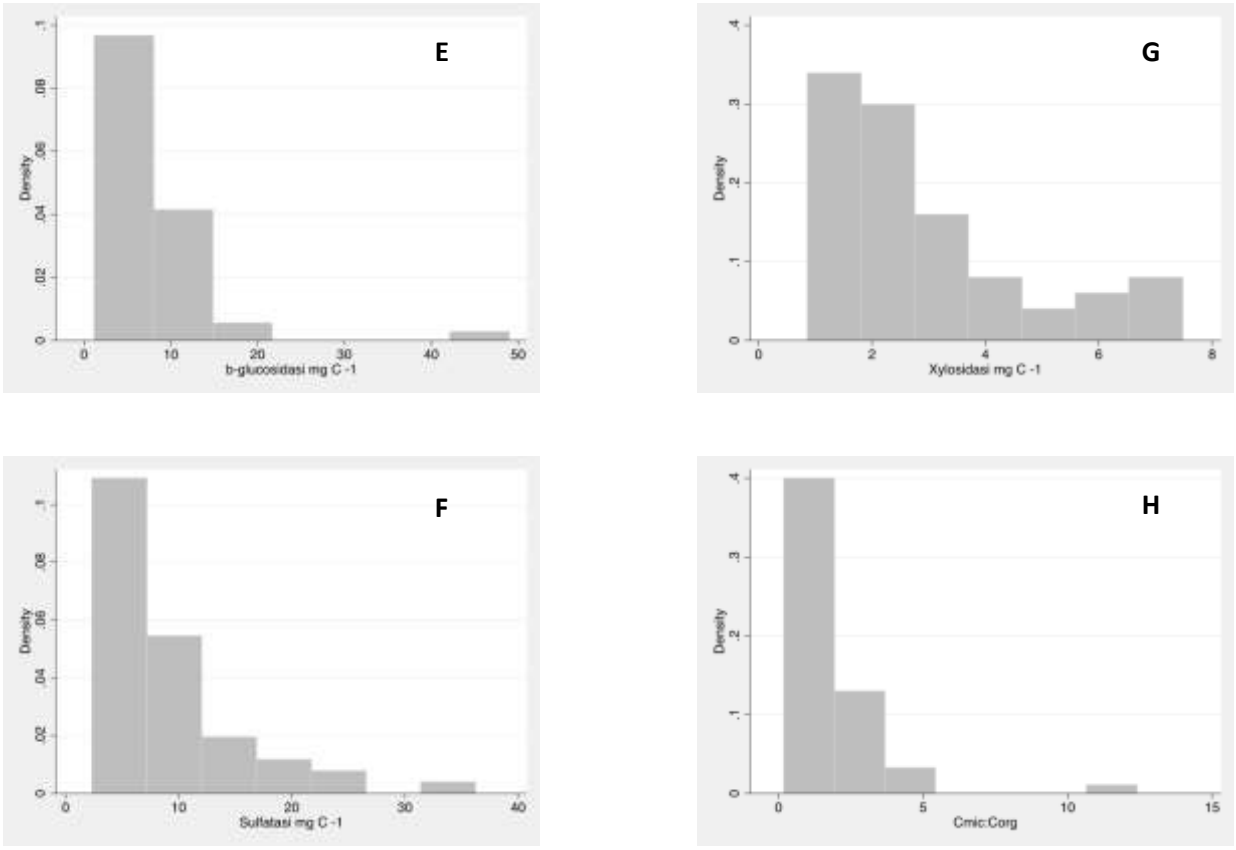


Figure 2. Distribution of specific enzyme activities (nmol MUF mg C^{-1} h^{-1}) and microbial quotient [Cellulase (A), Chitinase (B), Acid phosphatase (C), α-glucosidase (D), β-glucosidase (E), Arylsuphatase (F), Xylosidase (G) and the microbial quotient Cmic:Corg (H)]

2.1. The Quantile regression model (STATA software output) for case study 3, the codes represent 80, 100 and 120 yrs old **Douglas-fir stand ages**. Model (1) represents the regression without considering the management practice while Model (2) considers the management practice (this model was discussed in the thesis).

a) Cellulase (nmol MUF mg C^{-1} h^{-1})

	(1) cellulasim~1	(2) cellulasim~1
q25		
ph	0.232	-0.331
	(0.66)	(-0.75)
tn	0.212***	0.199***
	(4.11)	(4.14)
80bn.var20		.
		.
100.var20		0.663
		(1.54)
120.var20		0.478
		(0.76)
_cons	-1.274	1.234
	(-0.77)	(0.60)
q50		
ph	0.782	-0.295
	(1.83)	(-0.35)
tn	0.236*	0.192
	(2.43)	(1.42)
80bn.var20		.
		.
100.var20		0.912
		(1.76)
120.var20		1.249
		(1.70)
_cons	-3.661	1.228
	(-1.84)	(0.29)
q75		
ph	0.582	0.0215
	(1.11)	(0.02)
tn	0.161	0.167
	(0.85)	(0.98)
80bn.var20		.
		.
100.var20		1.642
		(1.97)
120.var20		1.805
		(1.51)
_cons	-1.648	-0.0800
	(-0.71)	(-0.02)
N	29	29

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

b) Chitinase (nmol MUF mg C^{-1} h^{-1})

	(1) chitinasim~1	(2) chitinasim~1
q25		
ph	0.865	-1.129
	(1.11)	(-0.74)
tn	0.423***	0.305*
	(3.86)	(2.33)
80bn.var20		.
		.
100.var20		1.616
		(1.23)
120.var20		1.858
		(0.77)
_cons	-4.217	4.977
	(-1.20)	(0.70)
q50		
ph	1.220	-1.552
	(0.56)	(-0.88)
tn	0.362	0.143
	(0.96)	(1.03)
80bn.var20		.
		.
100.var20		2.415*
		(2.18)
120.var20		3.608
		(1.36)
_cons	-5.430	7.663
	(-0.58)	(0.94)
q75		
ph	3.128	-0.286
	(0.86)	(-0.12)
tn	0.487	0.231
	(0.79)	(0.51)
80bn.var20		.
		.
100.var20		5.546*
		(2.60)
120.var20		5.132
		(1.73)
_cons	-13.36	1.673
	(-0.78)	(0.15)
N	29	29

c) β-glucosidase (nmol MUF mg C^{-1} h^{-1})

	(1) bglucosida~1	(2) bglucosida~1
q25		
ph	1.017	-1.589
	(1.47)	(-0.41)
tn	1.162**	1.204
	(2.97)	(1.69)
80bn.var20		.
		.
100.var20		2.978
		(0.79)
120.var20		2.278
		(0.43)
_cons	-4.709	6.918
	(-1.57)	(0.38)
q50		
ph	-0.121	-2.737
	(-0.06)	(-0.84)
tn	1.339*	1.191
	(2.22)	(1.88)
80bn.var20		.
		.
100.var20		4.912*
		(2.16)
120.var20		2.272
		(0.59)
_cons	1.702	13.26
	(0.17)	(0.84)
q75		
ph	-0.453	-6.813*
	(-0.14)	(-2.61)
tn	0.909	1.517**
	(0.92)	(3.20)
80bn.var20		.
		.
100.var20		8.162
		(0.57)
120.var20		9.103*
		(2.68)
_cons	8.286	32.18*
	(0.44)	(2.60)
N	29	29

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

d) Arylsuphatase (nmol MUF mg C^{-1} h^{-1})

	(1) sulfatasim~1	(2) sulfatasim~1
q25		
ph	-0.209	-0.166
	(-0.21)	(-0.03)
tn	-0.844	-1.001
	(-1.94)	(-1.31)
80bn.var20		.
		.
100.var20		1.976
		(0.35)
120.var20		0.360
		(0.05)
_cons	9.819	9.483
	(1.64)	(0.35)
q50		
ph	-0.322	-6.738
	(-0.23)	(-0.85)
tn	-1.884**	-1.881
	(-2.79)	(-1.94)
80bn.var20		.
		.
100.var20		7.215
		(1.04)
120.var20		4.987
		(0.54)
_cons	17.21	45.27
	(1.92)	(1.27)
q75		
ph	-2.910	-7.271
	(-0.68)	(-0.96)
tn	-2.704**	-2.772*
	(-3.24)	(-2.74)
80bn.var20		.
		.
100.var20		7.262
		(1.16)
120.var20		5.210
		(0.60)
_cons	37.09	55.58
	(1.78)	(1.62)
N	29	29

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

e) α –glucosidase (nmol MUF mg C^{-1} h^{-1})

	(1) aglucosida~1	(2) aglucosida~1
q25		
ph	0.135	-0.199
	(0.64)	(-0.23)
tn	0.0534	0.0702
	(0.71)	(0.58)
80bn.var20		.
		.
100.var20		0.717
		(1.04)
120.var20		0.396
		(0.42)
_cons	0.127	1.391
	(0.13)	(0.33)
q50		
ph	0.0857	-0.757
	(0.22)	(-1.12)
tn	0.154*	0.0268
	(2.07)	(0.21)
80bn.var20		.
		.
100.var20		1.548*
		(2.48)
120.var20		0.783
		(0.94)
_cons	0.298	4.322
	(0.17)	(1.36)
q75		
ph	0.236	-1.555
	(0.32)	(-0.95)
tn	-0.0814	0.0645
	(-0.59)	(0.60)
80bn.var20		.
		.
100.var20		2.276
		(1.11)
120.var20		2.290
		(1.27)
_cons	1.015	8.044
	(0.24)	(1.08)
N	29	29

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

f) Acid phosphatase (nmol MUF mg C^{-1} h^{-1})

	(1) fosfatasim~1	(2) fosfatasim~1
q25		
ph	-3.184	-14.10
	(-0.91)	(-1.02)
tn	-0.389	-1.889
	(-0.36)	(-1.23)
80bn.var20		.
		.
100.var20		13.93
		(0.94)
120.var20		18.32
		(0.94)
_cons	37.39*	86.18
	(2.11)	(1.36)
q50		
ph	-4.832	-18.05
	(-1.07)	(-1.14)
tn	-1.515	-0.451
	(-0.85)	(-0.23)
80bn.var20		.
		.
100.var20		22.40
		(1.74)
120.var20		23.36
		(1.23)
_cons	55.34*	105.5
	(2.14)	(1.41)
q75		
ph	-0.471	-38.92*
	(-0.03)	(-2.72)
tn	-4.416	-1.790
	(-1.62)	(-0.75)
80bn.var20		.
		.
100.var20		41.28**
		(3.10)
120.var20		55.18*
		(2.66)
_cons	55.11	208.3**
	(0.72)	(3.00)
N	29	29

g) Xylosidase (nmol MUF mg C^{-1} h^{-1})

	(1) xylosidasi~1	(2) xylosidasi~1
q25		
ph	-0.173	-0.435
	(-0.69)	(-0.48)
tn	-0.0366	-0.0276
	(-0.61)	(-0.23)
80bn.var20		.
		.
100.var20		0.324
		(0.36)
120.var20		0.205
		(0.22)
_cons	2.298	3.432
	(1.61)	(0.88)
q50		
ph	-0.325	-1.028
	(-0.98)	(-0.70)
tn	-0.0953	-0.0925
	(-1.00)	(-0.53)
80bn.var20		.
		.
100.var20		1.548
		(1.43)
120.var20		0.662
		(0.46)
_cons	3.618	6.622
	(1.84)	(0.96)
q75		
ph	-0.459	-2.507
	(-0.41)	(-1.84)
tn	-0.273	-0.272
	(-1.29)	(-1.30)
80bn.var20		.
		.
100.var20		3.402**
		(3.00)
120.var20		2.415
		(1.46)
_cons	5.751	14.08*
	(0.91)	(2.24)
N	29	29

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

h) Microbial quotient (Cmic:Corg)

	(1) cmiccorg	(2) cmiccorg
q25		
ph	0.278	0.0446
	(1.11)	(0.06)
tn	-0.391***	-0.407***
	(-4.81)	(-4.05)
80bn.var20		.
		.
100.var20		0.312
		(0.57)
120.var20		0.0754
		(0.08)
_cons	1.717	2.819
	(1.23)	(0.75)
q50		
ph	0.116	-0.391
	(0.48)	(-0.80)
tn	-0.417***	-0.485***
	(-6.35)	(-6.46)
80bn.var20		.
		.
100.var20		0.527
		(1.12)
120.var20		1.121
		(1.42)
_cons	2.857*	5.242*
	(2.48)	(2.33)
q75		
ph	0.448	-0.525
	(0.81)	(-0.40)
tn	-0.493***	-0.483
	(-5.74)	(-1.26)
80bn.var20		.
		.
100.var20		1.377***
		(4.10)
120.var20		1.171
		(1.14)
_cons	1.850	5.913
	(0.61)	(1.27)
N	29	29

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

2.2 The Quantile regression model (STATA software output) for case study 4, code 11 represents the compartment 65 and code 12 represents compartment 56 of the **Beech tree forest** Model (1) represents the regression without considering the management practice while Model (2) considers the management practice (this model was discussed in the thesis).

a) Cellulase (nmol MUF mg C^{-1} h^{-1})

	(1) cellulasim~1	(2) cellulasim~1
q25		
ph	-0.0235	-0.0915
	(-0.03)	(-0.11)
tn	-0.00615	-0.00513
	(-0.16)	(-0.33)
11bn.codice		.
		.
12.codice		-0.0548
		(-0.13)
_cons	1.701	2.104
	(0.44)	(0.42)
q50		
ph	0.649	0.673
	(1.92)	(1.46)
tn	-0.0223	-0.0206
	(-0.62)	(-0.90)
11bn.codice		.
		.
12.codice		0.0825
		(0.22)
_cons	-1.695	-1.917
	(-0.89)	(-0.67)
q75		
ph	0.393	-0.0868
	(0.66)	(-0.12)
tn	0.0141	0.0193
	(0.32)	(0.58)
11bn.codice		.
		.
12.codice		-0.296
		(-1.08)
_cons	-0.383	2.690
	(-0.10)	(0.60)
N	24	24

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

b) Chitinase (nmol MUF mg C^{-1} h^{-1})

	(1) chitinasim~1	(2) chitinasim~1
q25		
ph	0.130	0.260
	(0.07)	(0.18)
tn	0.00181	0.0495
	(0.02)	(0.51)
11bn.codice		.
		.
12.codice		1.842**
		(3.04)
_cons	3.165	0.353
	(0.32)	(0.05)
q50		
ph	0.889	2.059
	(0.36)	(0.74)
tn	-0.0320	-0.0390
	(-0.25)	(-0.36)
11bn.codice		.
		.
12.codice		1.306
		(0.97)
_cons	-0.548	-7.931
	(-0.04)	(-0.48)
q75		
ph	1.401	2.310
	(0.72)	(0.93)
tn	-0.0221	0.0384
	(-0.21)	(0.36)
11bn.codice		.
		.
12.codice		0.900
		(0.55)
_cons	-2.080	-8.624
	(-0.18)	(-0.57)
N	24	24

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

c) β-glucosidase (nmol MUF mg C^{-1} h^{-1})

	(1) bglucosida~1	(2) bglucosida~1
q25		
ph	**-0.740**	**2.522**
	(-0.19)	**(0.69)**
tn	**0.0537**	**0.0405**
	(0.62)	**(0.31)**
11bn.codice		.
		.
12.codice		**3.736***
		(2.70)
_cons	**7.668**	**-13.06**
	(0.34)	**(-0.62)**
q50		
ph	**2.306**	**0.738**
	(0.49)	**(0.16)**
tn	**-0.0696**	**0.0434**
	(-0.42)	**(0.42)**
11bn.codice		.
		.
12.codice		**2.799**
		(1.42)
_cons	**-7.329**	**-1.245**
	(-0.27)	**(-0.05)**
q75		
ph	**2.390**	**4.321**
	(0.45)	**(1.46)**
tn	**-0.0548**	**-0.143**
	(-0.42)	**(-1.01)**
11bn.codice		.
		.
12.codice		**1.436**
		(0.69)
_cons	**-5.980**	**-17.68**
	(-0.19)	**(-1.03)**
N	**24**	**24**

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

d) Arylsuphatase (nmol MUF mg C^{-1} h^{-1})

	(1) sulfatasim~1	(2) sulfatasim~1
q25		
ph	**0.796**	**1.319**
	(0.34)	**(0.46)**
tn	**-0.0459**	**-0.0520**
	(-0.45)	**(-0.38)**
11bn.codice		.
		.
12.codice		**0.478**
		(0.55)
_cons	**-0.291**	**-3.465**
	(-0.02)	**(-0.22)**
q50		
ph	**2.493**	**1.414**
	(0.46)	**(0.27)**
tn	**-0.147**	**-0.128**
	(-0.81)	**(-0.79)**
11bn.codice		.
		.
12.codice		**-0.237**
		(-0.15)
_cons	**-7.430**	**-1.046**
	(-0.24)	**(-0.03)**
q75		
ph	**-4.939**	**-0.947**
	(-0.65)	**(-0.11)**
tn	**-0.0749**	**-0.192**
	(-0.33)	**(-1.07)**
11bn.codice		.
		.
12.codice		**-1.916**
		(-0.82)
_cons	**38.47**	**16.73**
	(0.89)	**(0.35)**
N	**24**	**24**

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

e) α –glucosidase (nmol MUF mg C^{-1} h^{-1})

	(1) aglucosida~1	(2) aglucosida~1
q25		
ph	-0.314	-0.314
	(-1.33)	(-0.76)
tn	0.00425	0.00425
	(0.63)	(0.29)
11bn.codice		.
		.
12.codice		0.0253
		(0.20)
_cons	2.188	2.188
	(1.57)	(0.90)
q50		
ph	-0.703*	-0.703
	(-2.45)	(-1.75)
tn	0.00889	0.00889
	(0.73)	(0.95)
11bn.codice		.
		.
12.codice		0.0129
		(0.11)
_cons	4.615*	4.615
	(2.74)	(1.92)
q75		
ph	-0.409	-0.517
	(-0.80)	(-1.34)
tn	-0.00699	-0.00695
	(-0.31)	(-0.42)
11bn.codice		.
		.
12.codice		-0.138
		(-0.94)
_cons	3.278	3.982
	(1.18)	(1.74)
N	24	24

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

f) Acid phosphatase (nmol MUF mg C^{-1} h^{-1})

	(1) fosfatasim~1	(2) fosfatasim~1
q25		
ph	-5.336	5.761
	(-0.42)	(0.20)
tn	0.142	-0.480
	(0.50)	(-0.94)
11bn.codice		.
		.
12.codice		10.60
		(1.36)
_cons	40.40	-23.49
	(0.54)	(-0.13)
q50		
ph	-8.699	-0.519
	(-0.65)	(-0.02)
tn	-0.158	-0.0191
	(-0.47)	(-0.04)
11bn.codice		.
		.
12.codice		8.680
		(0.90)
_cons	73.91	15.27
	(0.95)	(0.11)
q75		
ph	-16.44	-17.71
	(-1.90)	(-1.20)
tn	-0.135	-0.306
	(-0.30)	(-0.82)
11bn.codice		.
		.
12.codice		-6.223
		(-1.07)
_cons	124.2*	138.7
	(2.56)	(1.56)
N	24	24

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

g) Xylosidase (nmol MUF mg C^{-1} h^{-1})

	(1) xylosidasi~1	(2) xylosidasi~1
q25		
ph	**-0.599**	**-0.806**
	(**-0.40**)	(**-0.46**)
tn	**-0.0106**	**-0.0215**
	(**-0.10**)	(**-0.31**)
11bn.codice		.
		.
12.codice		**-0.599**
		(**-0.66**)
_cons	**6.464**	**8.232**
	(**0.77**)	(**0.81**)
q50		
ph	**0.933**	**-1.286**
	(**0.33**)	(**-0.72**)
tn	**-0.0736**	**-0.0799**
	(**-1.07**)	(**-1.37**)
11bn.codice		.
		.
12.codice		**-1.865****
		(**-3.61**)
_cons	**-1.073**	**13.61**
	(**-0.06**)	(**1.28**)
q75		
ph	**-1.011**	**-1.177**
	(**-0.27**)	(**-0.80**)
tn	**-0.0682**	**-0.0853**
	(**-0.66**)	(**-1.10**)
11bn.codice		.
		.
12.codice		**-2.243***
		(**-2.80**)
_cons	**12.04**	**13.76**
	(**0.55**)	(**1.54**)
N	**24**	**24**

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

h) Microbial quotient (Cmic:Corg)

	(1) cmiccorg	(2) cmiccorg
q25		
ph	**-0.0104**	**0.0247**
	(**-0.05**)	(**0.15**)
tn	**0.00902**	**0.00810**
	(**0.96**)	(**1.22**)
11bn.codice		.
		.
12.codice		**-0.138**
		(**-1.33**)
_cons	**0.314**	**0.206**
	(**0.25**)	(**0.22**)
q50		
ph	**0.142**	**0.149**
	(**0.94**)	(**1.22**)
tn	**0.00695**	**0.00553**
	(**1.33**)	(**1.13**)
11bn.codice		.
		.
12.codice		**-0.155**
		(**-1.36**)
_cons	**-0.512**	**-0.425**
	(**-0.59**)	(**-0.55**)
q75		
ph	**0.294**	**0.0408**
	(**2.04**)	(**0.19**)
tn	**0.00966**	**0.00919**
	(**1.25**)	(**1.46**)
11bn.codice		.
		.
12.codice		**-0.261**
		(**-1.76**)
_cons	**-1.365**	**0.306**
	(**-1.69**)	(**0.23**)
N	**24**	**24**

t statistics in parentheses
* p<0.05, ** p<0.01, *** p<0.001

III. The assessment of microbial functional diversity in different soil categories

Table 3. Descriptive statistics of the two measures- Shannon index for the enzyme activities

Measure	**Mean**	**Q25**	**Q50**	**Q75**	**SD**	**CV**	**Min**	**Max**	**Skewness**
H'EA	2.014	1.835	2.049	2.320	0.522	0.259	0.250	5.490	0.775
H'MR	3.465	2.876	3.554	3.750	0.780	0.225	1.537	6.720	1.281

Table 4. Estimation results of QRMs (0.25, 0.50, 0.75 quantiles). SE= standard error, * Significant at the 10% level ** Significant at the 5% level *** Significant at the 1% level

	H'EA			**H'MR**		
	Coef.	*SE*	*Sign.*	*Coef*	*SE*	*Sign.*
Quantile 0.25						
TOC values (*ref. Low: <1.5%*)						
Medium: 1.5≤TOC<3	0.030	0.056		-0.043	0.050	
High: TOC ≥3%	0.093	0.181		-0.026	0.298	
pH	-0.181	0.036	***	0.146	0.103	
Soil type (*ref. EC*)						
F	0.005	0.243		0.668	0.218	***
A	0.321	0.085	***	-0.544	0.161	***
Constant	2.780	0.230	***	2.150	0.805	***
Quantile 0.50						
TOC values (*ref. Low: <1.5%*)						
Medium: 1.5≤TOC<3	-0.096	0.054	*	-0.060	0.128	
High: TOC ≥3%	-0.249	0.145	*	-0.0625	0.1995	
pH	-0.002	0.042		0.105	0.0395	***
Soil type (*ref. EC*)						
F	0.101	0.121		0.269	0.176	
A	-0.099	0.117		-0.584	0.189	***
Constant	2.210	0.269	***	2.887	0.311	***
Quantile 0.75						
TOC values (*ref. Low: <1.5%*)						
Medium: 1.5≤TOC<3	-0.084	0.061		0.037	0.124	
High: TOC ≥3%	-0.107	0.110		-0.029	0.310	
pH	0.089	0.041	**	0.081	0.054	
Soil type (*ref. EC*)						
F	0.194	0.145		0.098	0.379	
A	-0.194	0.088	**	-0.282	0.232	
Constant	1.754	0.296	***	3.212	0.414	***