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SOIL ENZYME ACTIVITIES AND BIOCHEMICAL INDEXES TO ASSESS SOIL QUALITY IN AGRONOMIC AND FORESTED ECOSYSTEMS

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TABLE OF CONTENTS

ABSTRACT

Soil has the ability to carry out a wide range of functions, from being the support to food production to being a natural filter that naturalize pollutant and therefore, it is important study the effects of land use on soil quality in order to provide most sustainable practices.

Three field trial have been considered to assess soil quality and functionality after human alteration of the systems, and to determine the power of soil enzymatic activities, biochemical indexes and mathematical model in the evaluation of soil status. The first agronomic experiment (MoLTE, Tuscany region) was characterized by conventional and organic management in which were tested also the effects of three tillage. The second (QUABIO, Marche region) was characterized by the comparison between conventional, organic and agro-ecological management. Finally, the third was a beech forest (Veneto region) where was tested the effects of N deposition on soil organic carbon sequestration.

Results highlight that both enzyme activities and biochemical indexes could be valid parameters for the evaluation of soil quality and for the differentiation of different management. Specifically, conventional management and plowing negatively affected soil quality and functionality with intensive tillage that lead to the downturn of microbial biomass and activity. On the other hand, both organic and agro-ecological management revealed to be good practices for the maintenance of soil functionality with better microbial activity and metabolic efficiency. This in turn positively affected also soil organic carbon content. At the forested site, even if enzyme activities and biochemical indexes positively respond to the treatments, one year of experimentation resulted to be not enough time to observe variation in soil organic carbon content especially because the site was a eutrophic forest. Mathematical models and biochemical indicators resulted to be valid tools for assess soil quality, nonetheless it would be better including the microbial component (i.e. enzymatic activity) in the mathematical model and it appears better to consider more than one index if the aim of the work is to evaluate the overall quality and functionality of a soil. Concluding, as expected the forest site is the richest one in terms of organic carbon, microbial biomass and activity while, within the agronomic management the organic and the agro-ecological seem to be the more sustainable but without taking in consideration the yield.

Keywords: *soil enzyme activities, biochemical indexes, agronomic management, forest soil, soil quality, long-term experiment*

GENERAL INTRODUCTION

The soil is a complex and heterogeneous system in which several biochemical cycles occur such as the water cycle, the organic carbon fluxes, the greenhouse gasses production and storage, and the nutrient cycles. Therefore, the soil is an open and dynamic system which represents the junction between all the environmental compartments interacting with atmosphere, minerals, water, and living organisms (Voroney, 2007). The capacity to interact with the other environmental systems gives to the soil the ability to carry out a wide range of functions that go from being the support to food production to being a natural filter that naturalize pollutant. Because of this, it represents a fundamental support to natural and human activities that are at the base of plants, animals and human life (Rao and Gianfreda, 2014).

Thus, it appears to be of particular importance study soil physical, chemical and biological properties to understand the dynamics of the bio-geo-chemical interactions (Voroney, 2007) and to assess its quality. Specifically, soil quality could be defined as "*the capacity of soil to function sustaining plant and animal productivity, maintaining or enhancing water and air quality, and supporting human health and habitation*" (Karlen et al., 1997). For these reasons humans have all the advantages in maintaining or enhancing soil quality if there is the interest to preserve agricultural productivity and environmental quality also for feature generations (Reeves, 1997).

The growing concern about the decreasing of soil quality derives mainly from the greater awareness on the impact of intensive agriculture, industry, urbanization, infrastructure constructions, that release pollutants in the environment affecting the related ecosystem services, and reduce soil available for agriculture.

Indeed, after the World War II there has been an increasing need of food that enhance the technological development for the production of more food (Karlen et al., 1997; Tilman et al., 2002). This fundamental human need of food leaded to the adoption of soil and crop management strategies without perceiving that there could have been consequences on productivity and environmental quality (Karlen et al., 1997). Over time, the strategies chosen to improve crops productivity lead to soil degradation not only from a physical point of view (i.e. soil erosion) but also biochemical, thus inducing a process that lowers soil capacity to produce goods or services (Reeves, 1997). For at least thirty years and still today agriculture and research are working to slow down and stop the soil degradation process, adopting more sustainable agronomic practices and studying the biochemical

processes related to soil quality and fertility. Maintaining and improving soil fertility and quality is essential for the sustainability of agricultural productivity and environmental quality (Reeves et al., 1997) and therefore, strategies to maintain soil ability to supports agricultural production have to be applied. Consequently, even if still nowadays it is important to consider that there is the need to maximize soil productivity, it is also fundamental to maintain to a minimum level the environmental effects of intensive agricultural practices. Therefore, it is important to study the effects of land use on soil quality in order to provide most sustainable agricultural management (Trasar-Cepeda et al., 2008), where the concept of sustainability includes both the maintenance of high productivity and the adoption of agricultural practices with acceptable environmental impacts (Tilman et al., 2002).

Soil Organic Matter (SOM) content and Soil Organic Carbon (SOC) content are main soil properties useful to assess the soil quality and fertility, and intensive soil cultivation generally cause significant SOM and SOC loss over time (Reeves, 1997; Lal, 2004; Paul, 2016). Soil microorganisms playing a central role in C, N and nutrient cycles (Nannipieri et al., 2003) are another important soil component impacted, in terms of biomass, biochemical activity and diversity, by the agricultural practices, due to the negative actions of lower SOC available for the microbial metabolism and the negative selection of microorganisms sensitive to changed soil conditions and eventual presence of pollutants. As compared to the SOM changes, microbial responses are much faster as microorganisms quickly adapt to environmental changes (Dick and Tabatabai, 1992). Therefore, responses of microbial biomass, microbial activity (i.e. microbial quotient) and microbial diversity can be used as indicators on changes in soil properties and soil fertility and overall soil quality (Piotrowska-Długosz, 2014).

Soil enzymes activities represent the main mechanism of nutrient acquisition by soil microorganisms and drive the SOM decomposition and mineralization (Schimel and Weintraub, 2003), and generally different enzymes act synergistically during the complex SOC decomposition pathways (Moorhead and Sinsabaugh, 2006), produced by microorganisms in ecological successions. Specifically: *i*) the opportunists which rapidly colonizing the fresh litter, mainly producing hydrolytic enzymes; *ii*) decomposers that degrade cellulose and lignocellulose of plant residues producing both hydrolytic and oxidative enzymes, and *iii*) miners which degrade phenols, humified organic matter and recalcitrant material, producing mainly oxidative enzymes. Therefore, shifts in enzymes production and activity can indicate fundamental changes in microorganisms nutrient use (Schimel and Schaeffer, 2012), with possible reflections on the SOC and SOM quality (Nannipieri et al., 2003).

Enzymes are proteins that act as catalysts of biochemical reactions acting on specific substrates increasing the reaction rate (Kandeler, 2007). In the soil environment, microorganisms are the main source of enzymes (Asakawa et al., 2011; Nannipieri et al., 2003) that can be active inside the living cells, in the cell periplasmic space or be stabilized in active forms by forming organo-mineral associations (Tabatabai and Dick, 2002). Extracellular enzymes degrade either fresh or stabilized high molecular weight SOM (e.g. lipids, proteins, carbohydrates, nucleic acids, humic substances), forming smaller molecules that microbial cells are able to use and mineralize to inorganic carbon (C), nitrogen (N), phosphorus (P) and sulfur (S) (Asakawa et al., 2011; Kandeler, 2007; Tabatabai and Dick, 2002). Collectively, enzymes represent a large number of proteins which have been categorized by the IUPAC Enzyme Commission (EC) in various classes, based on the catalytic reaction mechanism.

Hydrolases (EC 3) are the most common studied class of soil enzymes. They catalyze the hydrolysis of several C compounds (i.e. cellulase, lipase and glucosidases), N compounds (i.e. amidases and ureases), P compounds (i.e. phosphatase) and S compounds (i.e. and arylsulfatase) (Gregorich and Janzen, 2011). Numerous enzymes are involved in carbohydrates (the most abundant class of biomolecules) hydrolysis, breaking down large and complex organic compounds (i.e. cellulose, xylanase and chitinase), or hydrolyzing di- and oligosaccharides in simple monosaccharides (Deng and Popova, 2011).

The cellobiosidase (EC 3.2.1.91) releases cellobiose from the non-reducing end of the cellulose chains (Deng and Popova, 2011), while glucosidases (3.2.1.20/21) hydrolyze the degradation products of amylase (α-glucosidase) and cellulase (β-glucosidase), a major source of glucose for microbial growth and activity (Deng and Popova, 2011; Piotrowska-Długosz, 2014).

The enzyme N-Acetyl-β-glucosaminidase (EC 3.2.1.30) is involved in the hydrolysis of the chitooligosaccharides thus playing an important role in both soil C and N cycling (Ekenler and Tabatabai, 2003; Parham and Deng, 2000).

Phosphohydrolases (also called phosphatases) are enzymes that hydrolyze organic P-esters compounds, releasing inorganic P available for uptake by plants and microorganisms (Piotrowska-Długosz, 2014). Among phosphohydrolases, the phosphomonoesterases (EC 3.1.3.2) are the most studied for the evaluation of the P cycle, and an increase of phosphomonoesterase activity could be observed in P-limited soil (Acosta-Martínez and Tabatabai, 2011).

The arylsulfatase (EC 3.1.6.1) are sulfatases involved in the mineralization of organic S-ester in soil, releasing phenols and sulfate-S (Klose et al., 2011; Piotrowska-Długosz, 2014).
Lipases (triacylglycerol acylhydrolase; EC 3.1.1.3) are enzymes catalizing the hydrolysis of soil lipids (tri-, di-, mono-glycerides, fatty acids and glycerol) from plant and animal tissue (Cooper and Morgan, 1981; Veeraragavan, 1990).

Oxidoreductases (EC 1) are enzymes that catalyze redox reactions. Within the oxidoreductases, the dehydrogenase activity (EC 1.1.1) is the main studied intracellular oxidative activity, as it represents the global cellular oxidative activity, therefore an integrative indicator of soil microbial activity (Piotrowska-Długosz, 2014; Prosser et al., 2011). Specifically, dehydrogenases act transferring electrons and protons from organic substrates to inorganic acceptors (Piotrowska-Dlugosz, 2014).
Phenol oxidase and peroxidase are oxidoreductase that mediate important decomposition processes such as lignin degradation, humification, C mineralization, and act also for mitigate the potential toxicity of phenolic compounds (McGuire and Treseder, 2010; Sinsabaugh, 2010).
Soil phenol oxidases oxidize phenolic compounds with oxygen as electrons acceptor (Bach et al., 2013; Baldrian, 2006). The two main studied phenol oxidases are laccases (EC 1.10.3.2) and tyrosinases (EC 1.14.18.1), which share highly similar characteristics that make possible to differentiate between their activities mainly on the specific oxidized substrate (Bach et al., 2013; Baldrian, 2006). It has been demonstrated that laccases are the enzymes with the highest oxidizing potential as it widely ranges between 450-790 mV with relatively small changes over the pH 2.7-11 (Xu, 1997), while tyrosinases show a redox potential of 260 mV (Bach et al., 2013; Baldrian, 2006). Therefore, it is supposed that laccases could be able to initiate the degradation of soil compounds characterized with high recalcitrance such as lignin. Laccases production is generally associated to wood-rotting basidiomycetes, white-rot and brown-rot fungi which are species strictly related to lignin degradation processes (Baldrian, 2006; Sinsabaugh, 2010).

Soil enzymes production and activity are regulated by biotic and abiotic factors including soil microbial biomass, availability of water, C, N, P and S, microbial community composition, presence of cell debris, clay minerals and humic substances (Burns, 1982; Kandeler, 2007). In soil studies, oxidoreductases and hydrolases are the most studied enzyme activities for their direct involvement in soil nutrient cycles (Nannipieri et al., 2002; Rao and Gianfreda, 2014; Sinsabaugh et al., 2008).

AIM OF THE WORK

The overall aim of the research was to determine soil quality in agronomic and forest soils where anthropogenic factors could significantly alter soil functionality using enzymatic activities and biochemical indexes as indicators of soil status.

Specifically, the objectives were:

(I) Investigate the impact of agricultural management and tillage on soil quality and evaluate which one represent the most impacting factor on soil biochemical indexes and soil fertility.

(II) Determine the effect of nitrogen deposition on soil quality and carbon stock in a beech forest.

(III) Apply a mathematical model for the evaluation of soil carbon storage in a long term experiment.

(IV) Evaluate the use of enzyme activities and biochemical indexes as indicators of soil quality changes and examine the possibility to condense all the biochemical parameters into a unique index.

In the following chapters specific aims will be defined in accordance to the field experiment considered.

CHAPTER 1

MATERIALS AND METHODS FOR SOIL ANALYSIS

Materials and methods are here described to avoid repetition in the thesis as they were the same for all the field experiment considered. Instead, experimental sites description will be presented in each specific chapter.

1.1 Soil pH and water content

Soil pH was measured using a glass electrode in 1:2.5 (m:v) suspension of air dried soil in both Milli-Q® analytical grade water and in 1M KCl (ISO 10390, 2005). Soil water content was determined by drying soil samples at 105 °C for 24 hours.

1.2 Soil organic carbon and total nitrogen

Soil organic carbon (SOC) and total nitrogen (TN) were analyzed on 10 mg of air dried and finely ground soil subsamples using an elemental analyzer (Flash 2000, Thermo Fisher Scientific). On the same subsamples has been determined the stable isotopes (^{15}N and ^{13}C) using an elemental analyser coupled with isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher Scientific).

1.3 Soil microbial biomass

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the chloroform-fumigation extraction method (Vance et al., 1987). Five grams of field moist soil were fumigated at room temperature for 24 h in a desiccator with chloroform. For the extraction, 20 mL of 0.5M K_2SO_4 were added to both fumigated and unfumigated soil samples, which were extracted on a horizontal shaker for 1 h. Extracts were filtered with Whatman no. 42 filter paper and analyzed with an elemental analyzer (Flash 2000, Thermo Fisher Scientific). MBC was calculated as the difference between organic C in the fumigated and organic C in the unfumigated soil extracts. The organic C content of the unfumigated extracts was used as an estimation of soil extractable C (C_{ext}). Similarly, soil microbial biomass N (MBN) and soil extractable N (N_{ext}) were calculated. Both C and N pools were expressed as mg of C or N contained in 1 kg of dried soil (mg kg_{ds}^{-1}).

1.4 Available phosphorous

The available phosphorous content (P_{Olsen}) was determined according to Olsen et al. (1954). Two grams of dried soil were weighted and 0.5 g of active carbon and 40 mL of sodium bicarbonate extract solution were added. After 30 minutes of extraction on a horizontal shaker, the extracts were filtered with Whatman no. 42 filter paper. One drop of *p*-nitrophenol and sulphuric acid were added to an aliquot of 10 mL of extracts, and 3.2 mL of sulfomolybdic reactive solution were added. The final volume was reached at 50 mL with the addition of Milli-Q® analytical grade water. The product of the colorimetric reaction was determined using a spectrophotometer (Jasko V-530 UV/VIS Spectrophotometer) at 882 nm and the P content was expressed as $mg_{POlsen}\ kg_{ds}^{-1}$.

1.5 Soil basal respiration

Soil basal respiration (SBR) was determined according to Isermeyer (1952). An equivalent of 20 g of dried soil was weighted into glass jars. Field moist soil was adjusted for water content to 50% of water holding capacity (WHC) and samples were pre-incubated for 7 days at 20°C. After the pre-incubation, into the jars was placed a plastic vials containing 1 mL of 0.5M KOH that served as trap for CO_2 produced by the system. After 24 h of incubation at 20°C, 2 mL of 0.75M $BaCl_2$ were added to the KOH for the precipitation of carbonate and the titration has been done with 0.05M HNO_3. The measure was repeated every day for one week and the SBR was expressed as the quantity of CO_2 produced by 1kg of dried soil in the incubation time ($mg_{C\text{-}CO2}\ kg_{ds}^{-1}\ h^{-1}$).

1.6 Soil enzymatic activities

Soil enzymes activities determination is based on the degradation of synthetic substrates in presence of a buffer solution at precise temperature and time incubation conditions (Nannipieri et al., 2002). The analytical method allows to work with optimal condition, which, however, are not the natural conditions of the soil system and therefore it is more correct to refer to potential soil enzymatic activity (Nannipieri et al., 2002). Anyway, in this work has been decided for clarity and simplicity to refer to the potential enzymatic activities as "enzymatic activities".

1.6.1 Oxidative enzymes – Spectrophotometric assay

Oxidative enzyme activities were assayed on triplicate field-moist soil samples using the traditional colorimetric methods. For each enzyme in Table 1.1 is reported the specific substrate and the concentration used.

The reaction product was measured using a spectrophotometer (Jasko V-530 UV/VIS Spectrophotometer) each time set to a specific wavelength.

Table 1.1 - Oxidative enzymes considered in the study and the relative abbreviation, enzyme commission number (EC) and substrate used for the determination of their activities.

Enzyme	Abbreviation	EC	Substrate	Concentration
Dehydrogenase	Dehy	1.1.1	2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride	0.2 % (w:v)
Tyrosinase	Tyr	1.14.18.1	L-3,4-dihydroxyphenylalanine	10 mM
Laccase	Lac	1.10.3.2	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)	0.1 M

The dehydrogenase activity (Dehy) was measured according to von Mersi and Schinner (1991). The reaction (Figure 1.1) consists in the reduction of the 2-*p*-iodo-nitrophenyl tetrazolium chloride (INT) into 2-*p*-iodo-nitrophenyl formazan (INTF). One gram of field moist soil was incubated with 3.5 mL of 0.2% (w:v) INT solution, previously prepared with 1 M tris(hydroximethyl)aminomethane buffer (TRIS, pH 7.0), in a thermostatic bath at 37°C for 2 h. The extraction has been done at dark for 1 h after the addition of 10 mL of the extract solution made of N,N-Dimetilformamide and ethanol 1:1 (v:v). Released *p*-nitrophenol (INTF) was measured at 464 nm and the activity was expressed as $nmol_{INTF}$ g_{ds}^{-1} h^{-1}.

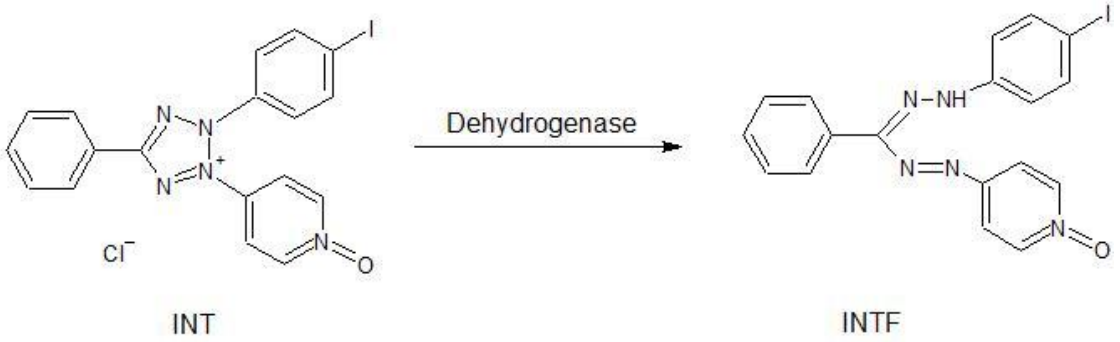

Figure 1.1 - Reduction of the 2-p-iodo-nitrophenyl tetrazolium chloride (INT) into 2-p-iodo-nitrophenyl formazan (INTF).

The determination of the tyrosinase activity (Tyr) is based on the reaction of the amino acid L-3,4-dihydroxyphenylalanine (L-DOPA) whose product, being not stable, is spontaneously converted to the red pigment dopachrome (Figure 1.2) that could be determined using the spectrophotometer (Pind et al., 1994). The tyrosinase activity was measured according to Sinsabaugh et al. (1999). To 0.5 g of field moist soil were added 3.0 mL of 50 mM acetate buffer solution (pH 5.0) and 2.0 mL of 10 mM substrate solution. The incubation proceeded for 30 minutes at 25°C at the end of which samples were centrifuged 5 min x 12000 rpm at 5°C to stop reaction. Dopachrome absorbance was measured at 475 nm and the activity was expressed as $\mu mol_{Dopachrome}\ g_{ds}^{-1} h^{-1}$.

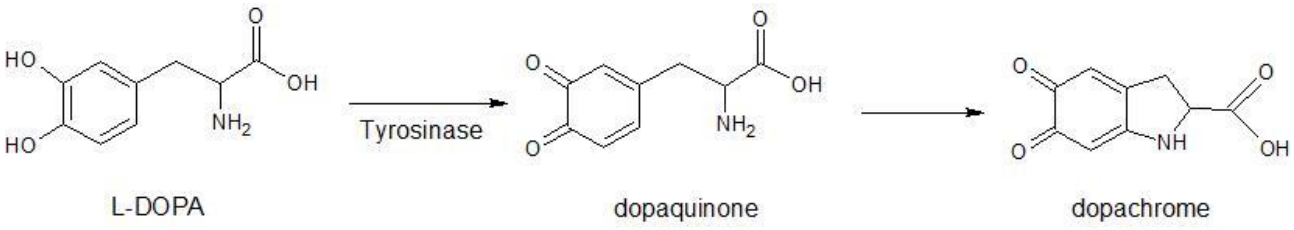

Figure 1.2 - Reaction of the amino acid L-3,4-dihydroxyphenylalanine (L-DOPA) into dopachrome.

The laccase activity (Lac) was determined according to Floch et al. (2007). The reaction is based on the oxidation of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic) acid (ABTS) and the subsequent measurement of the reaction product ($ABTS^{+\cdot}$) has been done using a spectrophotometer (Figure 1.3). ABTS substrate has been choose for laccase determination because it is considered as a substrate that is not oxidized also by tyrosinase (Eichlerová et al., 2012). Differently from the method considered, it has been used higher values of buffer pH with the aim to better simulate soil natural conditions rather than measure the activity at its optimal pH. This choice has some implication

because as the pH increases the laccase activity could decrease considering the possible OH^- inhibition on the T2/T3 center, but anyway the ABTS oxidation occur up to the pH of 7.5 in any soil (Bach et al., 2013; Xu, 1997). Moreover, different type of laccases could be detected into the soil and the one produced by *Phellinus ribis* has catalytic features typical for laccases but it lacks the T1 center and contains one Mn atom per molecule thus leading to relatively higher pH optimum for ABTS oxidation (pH of 5 rather than 2-4 as observed for other laccases) (Baldrian, 2006; Min et al., 2001). Therefore, for laccase activity assay, 0.1 g of field moist soil were weighted and incubated for 5 minutes at 30°C with 10 mL of Modified Universal Buffer (MUB, at pH 7.0 for the agronomic experiment and at pH 5.0 for the forest site) and 200 µL of 0.1 M substrate solution. To stop the reaction, samples were immediately centrifuged 2 min x 12000 rpm at 4°C. The absorbance was measured at 420 nm and the activity was expressed as $\mu mol_{ABTS+\cdot}\ g_{ds}^{-1} min^{-1}$.

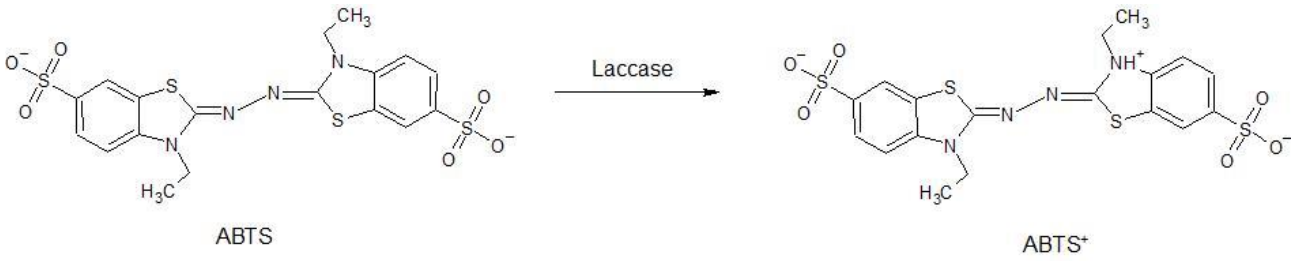

Figure 1.3 - Oxidation reaction of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic) acid (ABTS).

1.6.2 Hydrolytic enzymes – Microplate assay

The microplate assay allows to simultaneously estimate the potential activity of multiple enzymes on one soil sample. For the determination of hydrolytic enzyme activities, the method is based on the measurement of fluorescence emitted by 4-methylumbelliferone (MUF) following the enzymatic hydrolysis of MUF conjugated artificial substrates (Giacometti et al., 2014; Marx et al., 2001; Vepsäläinen et al., 2001) (Figure 1.4).

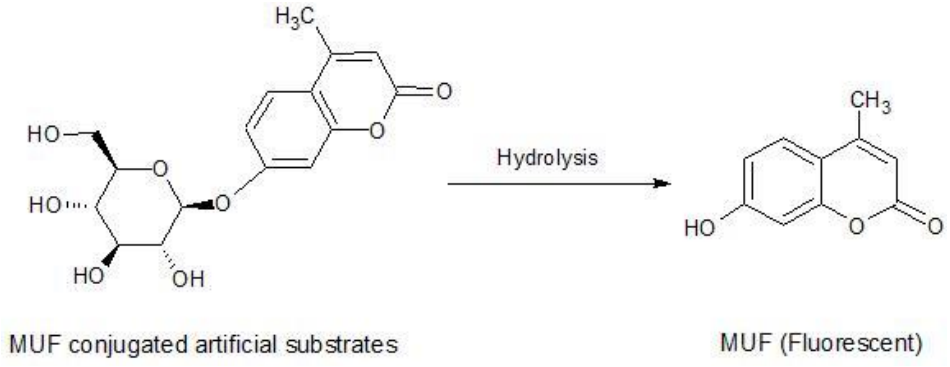

Figure 1.4 - Hydrolysis of a MUF conjugated substrate in the fluorescent product 4-methylumbelliferone (MUF).

With the MUF-based assays the fluorescence could be measured directly in the reaction mixture, the only negative aspect is that soil particles and soil phenolic compounds exercise a quenching effect reducing the fluorescence signal (Giacometti et al., 2014) and for this reason, it is fundamental to use some wells of each plate as quench controls. To minimize variations due to the chemical stability of the MUF-conjugate enzymatic substrates, all reagents and substrates were prepared on the same day of the assay and were kept in dark until use; glassware, buffers and Milli-Q® analytical grade water were sterilized in autoclave at 121°C for 20 minutes to prevent microbial contamination. As for traditional assays the buffer solution is needed to dilute standard, substrates and soil samples. In the case of this assay a 0.5 M sodium acetate buffer solution was prepared with sodium acetate anhydrous and analytical grade water. Buffer solution pH was regulated to 5.5 with glacial acetic acid (ISO/TS 22939, 2010).

In this study were considered eight enzyme activities (Table 1.2) and each corresponding substrate was dissolved in dimethyl sulfoxide (DMSO) before usage and subsequently brought to the desired final concentration with acetate buffer. The calibration curves for the fluorescence was made starting from a 5 mM 7-hydroxyl-4-methylcoumarin (MUF) standard solution (stock) prepared with a solution of methanol and water (1:1, v:v). The stock solution was than diluted to 100 µM (work solution) in sodium acetate buffer, and this work solution was additionally diluted in buffer solution to obtain a final concentration per well corresponding to 1.00, 2.00, 4.00, 10.0, 20.0, 30.0, 40.0 µM.

Table 1.2 - Hydrolytic enzymes considered in the study and the relative abbreviation, enzyme commission number (EC) and substrate used for the determination of their activities.

Enzyme	Abbreviation	EC	Substrate
β-1,4-glucosidase	β-Glu	3.2.1.21	4-MUF- β-D-glucoside
α-1,4-glucosidase	α-Glu	3.2.1.20	4-MUF- α-D-glucoside
N-acetyl- β-glucosaminidase	NAG	3.2.1.30	4-MUF- *N*-acetyl- β-D-glucosamide
β-1,4-xylosidase	β-Xyl	3.2.1.37	4-MUF- β-D-xyloside
β-1,4-cellobiosidase	β-Cel	3.2.1.91	4-MUF- β-D-cellobioside
Arylsufatase	AS	3.1.6.1	4-MUF-sulfate
Phosphomonoesterase	PME	3.1.3.2	4-MUF-phosphate
Lipase	LIP	3.1.1.3	4-MUF-heptanoate

Due to the differences that generally occur between agronomic and forest soils has been decided to use different substrate concentration (Table 1.3). Moreover, for the forest soil samples has been used different substrate concentration also in accordance to the soil horizon tested.

Table 1.3 - Concentration of the substrate used for the determination of the hydrolytic enzyme activities for the different experimental site considered.

	Agronomic sites	Forest site	
Substrate	Concentration (μM)	A horizon Concentration (μM)	AE horizon Concentration (μM)
4-MUF- β-D-glucoside	200	400	200
4-MUF- α-D-glucoside	400	400	200
4-MUF- *N*-acetyl- β-D-glucosamide	200	600	300
4-MUF- β-D-xyloside	400	800	400
4-MUF- β-D-cellobioside	100	400	200
4-MUF-sulfate	2000	2000	2000
4-MUF-phosphate	200	800	400
4-MUF-heptanoate	300	500	300

The soil slurries were prepared weighting a mass of moist soil corresponding to 2.00 g of dry soil into sterilized Pyrex beckers, and adding 100 mL of 0.5 M acetate buffer (pH 5.5). The slurries were mixed

using an Ultra Turrax IKA mixer for 2 min at 9000rpm (IKA-Werke, Staufen, DE), and then soils were kept under continuous stirring until dispensation in 96-wells microplates.

The assays were done using black polystyrene 96-well microplates with a well capacity of 350 µL and the plate set-up here presented (Figure 1.5 – 1.6) is for the measurement of enzyme activities rates. The aliquots of buffer, standard solutions, substrate solution and soil slurries were dispensed in that order according to the scheme and the quantities reported in figures 1.5 and 1.6 respectively for the soil plate and for the controls plate. Finally, the total volume of the reaction mixture per well was 200 µL and the addition of the soil slurries was considered the starting point of the incubation time which corresponded to 3 hours and half, in the dark at 30°C.

	Soil assay								Quench controls						
													Soil	Buffer	std
	β-Glu	α-Glu	NAG	β-Xyl	β-Cel	AS	PME	LIP	rep.1	rep.2	rep.3	rep.4	µL	µL	µL
A													100	100	0
B													100	50	50
C													100	50	50
D													100	50	50
E													100	50	50
F													100	50	50
G													100	50	50
H													100	50	50
Soil µL	100	100	100	100	100	100	100	100							
Sub. µL	100	100	100	100	100	100	100	100							

Figure 1.5 - Example of soil plate organization.

	Substrate controls								Reference standards				Buffer	std
	β-Glu	α-Glu	NAG	β-Xyl	β-Cel	AS	PME	LIP	rep.1	rep.2	rep.3	rep.4	µL	µL
A													200	0
B													150	50
C													150	50
D													150	50
E													150	50
F													150	50
G													150	50
H													150	50
Buffer µL	100	100	100	100	100	100	100	100						
Sub. µL	100	100	100	100	100	100	100	100						

Figure 1.6 - Example of substrate controls and reference standards plate organization.

The fluorescence intensity was measured using a microplate fluorimeter (Infinite200, TECAN, Männedorf, CH) with excitation and emission filters of 365 nm and 450 nm respectively. Before each measurement (one every 30 minutes) the microplates were shaken for 5 s in order to homogenize the reaction mixture. The enzyme activities were expressed as $nmol_{MUF}\ g_{ds}^{-1}\ h^{-1}$ and for calculation were used the rate of fluorescence increase according to the following equations (German et al., 2011; Marx et al., 2001).

$$\text{Enzyme activity}\left(\text{nmol}_{\text{MUF}}\ \text{g}^{-1}\text{h}^{-1}\right)=\frac{\text{Net fluorescence}\left(\text{RUF min}^{-1}\right)*\text{V}_{\text{f}}\ (\mu\text{L})*\text{V}_{\text{e}}\ (\text{mL})*60\ (\text{min h}^{-1})}{\text{Emission coefficient}\left(\frac{\text{RUF}}{\mu\text{mol}}\text{L}^{-1}\right)*\text{V}_{\text{a}}\ (\mu\text{L})*\text{Soil dry mass (g)}}$$

Where:

$$\text{Net fluorescence}\left(\text{RUF min}^{-1}\right)=\left(\frac{\text{Assay slope - Soil control slope}}{\text{Quench coefficient}}\right)-\text{Substrate control slope}$$

$$\text{Quench coefficient}=\frac{\text{Quench controls curve slope}\left(\frac{\text{RUF}}{\mu\text{mol}}\text{L}^{-1}\right)}{\text{Reference standards curve slope}\left(\frac{\text{RUF}}{\mu\text{mol}}\text{L}^{-1}\right)}$$

$$\text{Emission coefficient } \left(\frac{\text{RUF}}{\mu\text{mol}}\text{L}^{-1}\right) = \text{Reference standards curve slope}$$

RUF = Relative Units of Fluorescence

V_f = final volume in well (200 µL)

V_e = soil slurry extraction volume (100 mL)

V_a = Volume of soil slurry per well (100 µL)

1.7 Soil biochemical indexes

The microbial quotient (q_{mic}), one eco-physiological parameter used as indicator of organic matter availability changes (Haynes, 1999), was calculated by dividing the MBC by SOC. The metabolic quotient (qCO_2), an index that highlight soil microbial biomass efficiency in utilizing C resources (Hartman and Richardson, 2013; Wardle and Ghani, 1995), was calculated by dividing SBR by MBC and expressed as $mg_{C\text{-}CO2}\ g_C^{-1}$. The metabolic index (MI), that could be considered a good indicator of the microbial metabolism efficiency (Masciandaro et al., 1998), was obtained by dividing the dehydrogenase activity by the C_{ext} and was expressed as $\mu mol_{INTF}\ g_C^{-1}\ h^{-1}$. Finally, specific soil enzymatic activity, which can be considered a simple index of soil quality (Gil-Sotres et al., 2005; Kandeler and Eder, 1993; Trasar-Cepeda et al., 2008), have been calculated by dividing the enzymatic activity values with the MBC.

1.8 Statistical analysis

The assumptions of the ANOVA were tested using the Bartlett's test for the homogeneity of variances, and the Shapiro-Wilk's test for the normally distribution of data (R Core Team, 2019). Whereas the assumptions were not always respected, data has been transformed based on BoxCox procedure (Fox and Weisberg, 2011). The analysis of the variance has been performed with the 'aov' function (R Core Team, 2019). Means were separated calculating Tukey's Honestly Significance Difference (HSD) at the significant level $P \leq 0.05$ (de Mendiburu, 2017).

Pearson's correlation coefficients (r) were also calculated and here reported in a correlation plot obtained with the 'corrplot' package (Wei and Simko, 2017). In the corrplot would be displayed only significant ($P \leq 0.05$) positive and negative correlations with different colors; the level of significance would be represented by bullet of different diameter (the greater the diameter the more significant the correlation will be).

Principal component analysis (PCA) was carried out using the 'princomp' function (R Core Team, 2019). Within the PCA has been carried out also the correlation with the 'rcorr' function (Harrell Jr. et al., 2019) between the scores of each principal component and the variables considered. When the PCA gave no significant results a canonical correspondence analysis (CCA) was done using the 'vegan' package (Oksanen et al., 2018).

Finally, a dendrogram analysis for the visualization of the hierarchical clustering was done using the 'dendextend' package (Galili, 2015).

CHAPTER 2

AGRONOMIC MANAGEMENT AND TILLAGE EFFECTS ON SOIL QUALITY IN A LONG TERM EXPERIMENT

2.1 State of the art

Increasingly population density with the scarcity of available land for food production lead to an agriculture that evolve to continuous cropping of the same parcel of land (Reeves, 1997) and all these intensive agricultural practices in the long-term lead to ecosystems degradation and loss of productivity (Hartmann et al., 2015). Indeed, the inappropriate use of agricultural practices as for example, fertilization, soil tillage (i.e. deep plowing), the use of machinery and harvesting (Trasar-Cepeda et al., 2008) could negatively affect soil quality contributing to soil degradation (López-Garrido et al., 2014), microbial diversity and activity downturn (Bonanomi et al., 2016), and losses of soil organic carbon (SOC) (Lal, 2004). Therefore, it is fundamental study the effects of land use on soil quality in order to provide most sustainable agricultural management (Trasar-Cepeda et al., 2008). Indeed, agricultural evolution finds in the "sustainability" the new keyword towards which directs all the agricultural methods (Tilman et al., 2002). Specifically, the concept of agricultural sustainability includes both the maintenance of high productivity and the adoption of agricultural practices with acceptable environmental impacts (Tilman et al., 2002).

2.1.1 Management strategy

Mainly in the past but also todays, nutrient management strategies have been applied to maintain high crop production (Giacometti et al., 2013; Zhang et al., 2019) which is the main purpose of agriculture. The use of mineral fertilizers and pesticides could be considered the main practice that characterize agricultural conventional management strategy (Hartmann et al., 2015). Fertilization practices principally aim to maintain soil nitrogen (N) and phosphorous (P) content and this could lead to an increase in plant biomass production which consequently enhance also the amount of plant residue returned to the soil (Dick and Tabatabai, 1992). On the other hand, considering the dynamic connections between soil ecosystem with the other environmental sphere, mineral fertilizers application could substantially contribute to the degradation of water quality due to runoff, erosion and/or leachate (Lavelle, 2013). Furthermore, mineral fertilizers may also significantly and negatively affect soil properties (Dick, 1992) related to its functionality and this in turn has the potential to significantly alter the soil microbial community structure and activity (Carreiro et al., 2000; Fog, 1988; Sinsabaugh et al., 2002). Many studies (Carreiro et al., 2000; Dick, 1992; Giacometti et al., 2013; Hartmann et al., 2015; Nannipieri et al., 2002; Sinsabaugh et al., 2002) have been done

on the effects that mineral fertilizers application could have on soil biochemistry. To achieve this object, soil enzymatic activities are useful measurements as they quickly respond to soil alteration and give a picture on soil microbial activity reaction to the fertilization.

For example, it has been found that long-term N addition in conventionally-managed agricultural soils could increase hydrolytic enzyme activities involved in labile C breakdown (Chen et al., 2018; Zhang et al., 2019) and reduce the activity of the enzymes involved in N mineralization (Bowles et al., 2014). These changes in soil enzymatic activities could significantly affect the processes related to C-cycle that in turn have consequence on SOC sequestration. Therefore, considering all the environmental consequence that intensive agriculture produces and the will of develop towards a sustainable agriculture (Tilman et al., 2002), organic farming could represent the right way to achieve this purpose. Hence, organic agriculture is defined as ''*a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects.*'' (IFOAM, 2012). Indeed, organic farming aim to reach the needed yield production maintaining soil fertility with the adoption of crop rotation with cover crops, reduced tillage (Tilman et al., 2002, Hartmann et al., 2015) and the application of organic fertilizer such as animal and green manures and off-farm organic wastes (Drinkwater et al., 1995). From an ecosystem services perspective, organic agriculture is more sustainable than the conventional once which, as previously seen, degrades some of the fundamental soil's ecosystem services (Luo et al., 2019). Moreover, organic agriculture is able to significantly contribute to global food supply (Luo et al., 2019; Sandhu et al., 2010) but this is not the general opinion as some studies reported that yields obtained with organic farming are usually lower than in conventional agriculture (Bonanomi et al., 2016; De Ponti et al., 2012). However, in general, organically managed lands show better values of soil physical parameters, aggregate stability, soil organic matter (SOM) content and moisture content than the conventional systems (Drinkwater et al., 1995; Luo et al., 2019).

2.1.2 Soil tillage practices

Within the management strategy adopted, the choice of the tillage practice represents another possible source of variation in soil quality and fertility. The primary purpose of soil tillage is to control weeds and prepare the seedbed to enhance crop production. There are two main categories of tillage according to its intensity: the more intense tillage that usually is plowing which consists in the fully

inversion of the soil, and the less intensive tillage that vertically disrupts soil without inversion (Zuber and Villamil, 2016). Anyway, tillage directly affects soil increasing the risk of erosion, degrading soil structure, dispersing soil macro-aggregates and, in general, altering the physico-chemical, biochemical and microbiological soil properties (Balota et al., 2004; Piotrowska-Długosz, 2014; Six et al., 2000; Zuber and Villamil, 2016). Moreover, tillage changes soil temperature, moisture and aeration conditions (Roldán et al., 2005). This has consequence on soil nutrient content and availability which in turn induce decreases in SOM and SOC due to accelerated decomposition and mineralization processes (Zuber and Villamil, 2016; Six et al., 2000; Balota et al., 2004) changing, in the end, soil fertility. The major problem related to soil intensive tillage is that it causes the exposure of new surfaces as soil aggregates are broken (Piotrowska-Dlugosz, 2014; Roldán et al., 2005; Trasar-Cepeda et al., 2008) and, considering that aggregates represent the main structures for SOM protection and maintenance, it induces also the exposure of SOM to microbial attack (Balota et al., 2004; Zuber et al., 2017).

As for the management practices, soil microbial parameters and enzymatic activities could be considered useful variables for study tillage effects on soil microbial community. Indeed, they are considered in a great amount of studies on tillage effects on soil properties. Specifically, intensive or conventional tillage induce a reduction of the microbial biomass C (Mikanová et al., 2009) and a downturn of the ratio between microbial biomass C and microbial biomass N, thus indicating a shift from fungi to bacteria (Laudicina et al., 2011). Moreover, intensive tillage caused the increase in the ratio between soil respiration and microbial biomass C (the so-called metabolic quotient, qCO_2) thus, indicating stressing condition for the microbial community (Laudicina et al., 2011, Balota et al., 2004). Regarding enzymatic activities reaction to tillage, previous studies generally found lower hydrolytic enzyme activities in correspondence to tilled and intensively tilled soils (Murillo et al., 2009; Roldán et al., 2005; Wanjiru et al., 2015) while the oxidative enzyme activities showed lower or no differences in respect to the applied tillage (Mangalassery et al., 2015). Also dehydrogenase activity, which is the most measured intracellular enzyme activity, showed the same trends (Murillo et al., 2009; Roldán et al., 2005) with some exception in which reduced tillage lowered this activity (Laudicina et al., 2011). Reduced or no tillage practices have been show to significantly increase the activities of phosphatase and arylsulphatase (Laudicina et al., 2011), β-Glucosidase (Finn et al., 2017; López-Garrido et al., 2014) and phenol oxidase (Mangalassary et al., 2015). The greater enzymatic activities detected under no-till or reduced tillage indicates that potentially there is also greater functional diversity

(Zuber and Villamil, 2016). Moreover, the absence of tillage let plant residues remain on soil surface leading to a slower decomposition and to an accumulation of the organic matter (Mikanová et al., 2009; Zuber et al., 2017) which in turn stimulates the biological activity (Mikanova et al., 2009). Indeed, all these changes related to C-cycle processes lead to a higher SOC storage under both no tilled or reduced tilled soils (Finn et al., 2017).

However, the no tillage practice has some constraints as, depending on the climate of the area, soil temperature and texture, it not always produce the sufficient yields to be a real sustainable practice (Lal, 2007). Therefore, reduced tillage appear to be the best management practice for both yields (Lopez-Garrido et al., 2014) and soil fertility (Laudicina et al., 2011).

2.1.3 Aim of the work

In Mediterranean agroecosystems, tillage and N fertilization (which represent the main strategy in conventional management) are key agronomic practices (Álvaro-Fuentes et al., 2013) and many studies have been done to assess their impact on soil physical, chemical and biological properties (Laudicina et al., 2011; López-Garrido et al., 2014; Mikanová et al., 2009; Six et al., 2000; Zuber and Villamil, 2016). Moreover, long-term studies showed that continuous cropping induce a downturn of SOC and therefore, it is essential to adopt management practices with lower impacts on soil fertility (Reeves et al., 1997). Developing more suitable agricultural soil management practices require a continuous monitoring of soil properties to assess the long-term effects on soil quality and fertility (Giacometti et al., 2013; Murillo et al., 2009).

Therefore, in this study has been taken in consideration a long-term agricultural experiment located in a Mediterranean area (Tuscany region, Italy) in which both system management (conventional versus organic) and tillage practice (plowing, harrowing and ripping) have been tested together.

The aim of the work was to define the combined effects of management strategies and tillage at different intensity on soil functionality.

Specifically, the hypotheses were to observe (i) an increase of C-pools content with the organic management strategies (ii) a positive response of the biochemical parameters related to the microbial biomass under the organic strategy (iii) a decrease of the enzymatic activities with the plowing as tillage (iv) a positive response of soil biochemical properties under the less impacting reduced tillage (harrowing) and (iv) that the combination of conventional management with the plowing represent the worst practice for both soil fertility and functionality.

2.2 Experimental site description

2.2.1 Area of study

The site considered is part of the project called Montepaldi Long-Term Experiment (MoLTE, http://www.dispaa.unifi.it/vp-463-molte.html), which started in 1991 by the Department of Agrifood Production and Environmental Sciences and is part of the experimental farm of the University of Florence. MoLTE experimental site (Figure 2.1) is located in Montepaldi, San Casciano Val di Pesa (FI), Tuscany, central Italy (43.66° N, 11.14° E, and 90 m a.s.l.). It covers a lightly sloped area of approximately 15 ha that is characterized by the presence of lower hills at North-East and of river Pesa at South-West (Bedini et al., 2013).

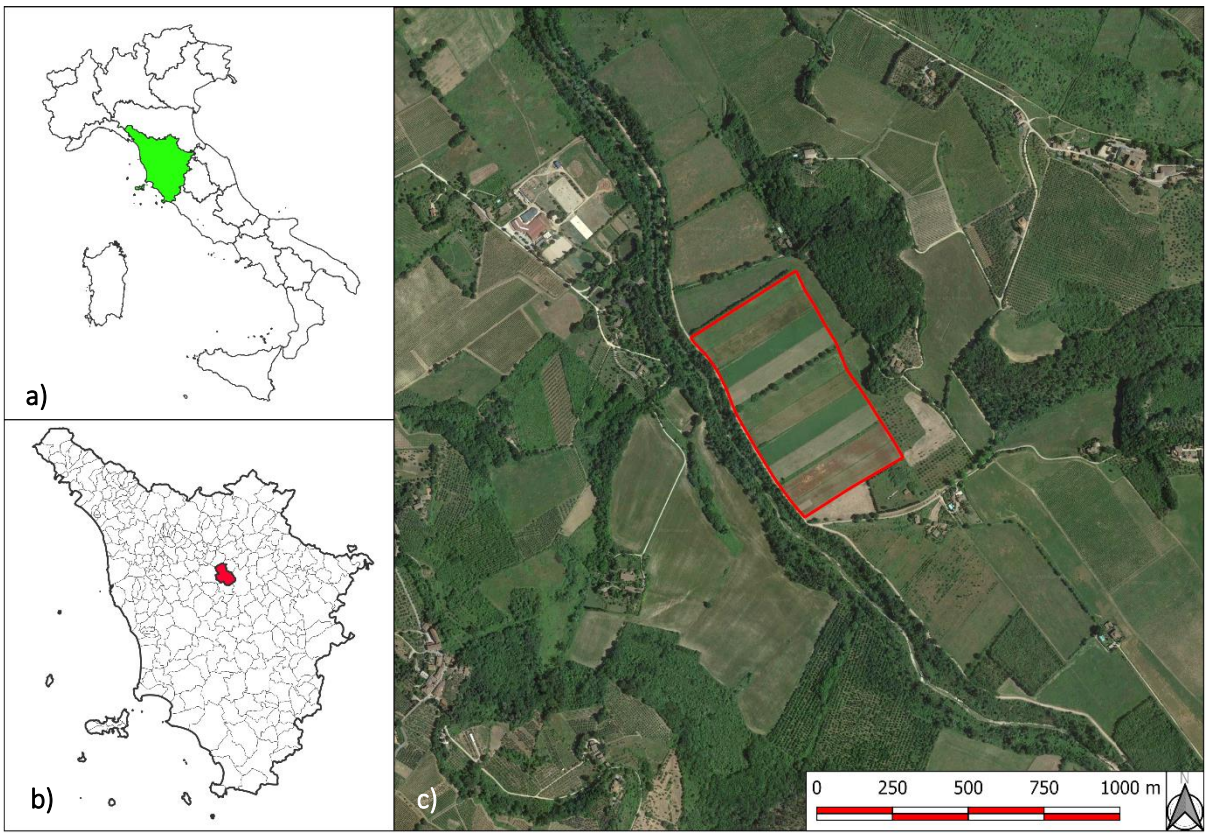

Figure 2.1 – a) Italian map including Tuscany region colored in green; b) MoLTE location indicated by the red area; c) Detail of the experimental site surrounded by a red square (Google orthophoto).

The climatic conditions of the area are typical of the Mediterranean sub-Apennine zone (Bedini et al., 2013), characterized by hot, dry summers and mild-cool, wet winters with cumulative annual precipitation and mean annual temperature referred to 2016 that are 856 mm and 16.1 °C respectively (Figure 2.2).

The soil in this area is classified as Fluventic Xerochrepts (Migliorini et al., 2014) and it is composed of parent rock material derived from Pliocene sediments (slopes) and river Pesa fluvial deposits from the Holocene (plane). The soil texture ranges between silt-loam and loam.

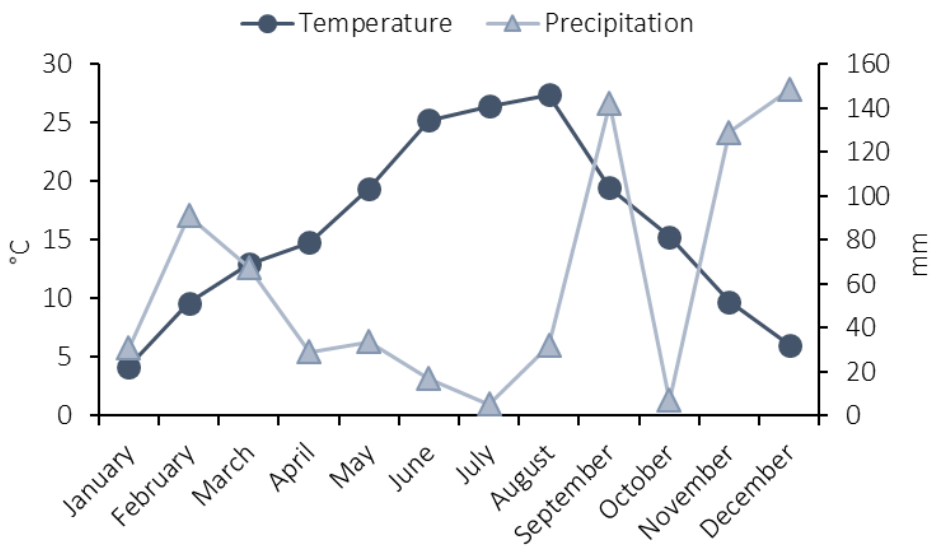

Figure 2.2 - Temperature (°C) and precipitation (mm) data referred to the year 2017. Data has been provided by Consorzio LAMMA.

2.2.2 The Experimental design

In the flat area of the agricultural farm, along river Pesa, is located the experimental site with a design that take in consideration two organic management systems, "Old Organic" and "New Organic", and one "Conventional" system (Figure 2.3).

The "Conventional" management (CONV) is spread out on 2.3 ha that are divided in two sub-areas of 1.15 ha each one. These areas are cultivated with barley and sunflower in a biennial rotation since 2015.

The New Organic management (not taken in consideration) is spread out on 5.2 ha that are divided in four sub-areas of 1.3 ha each one. These areas are cultivated with barley, chickpea, sunflower and

lentil in a four-year rotation since 2001 (until 2001 it was managed with the integrated agriculture system).

The Old Organic management (ORG) is spread out on 5.2 ha that are divided in four sub-areas of 1.3 ha each one. These areas are cultivated with barley, chickpea, sunflower and lentil in a four-year rotation since 2015.

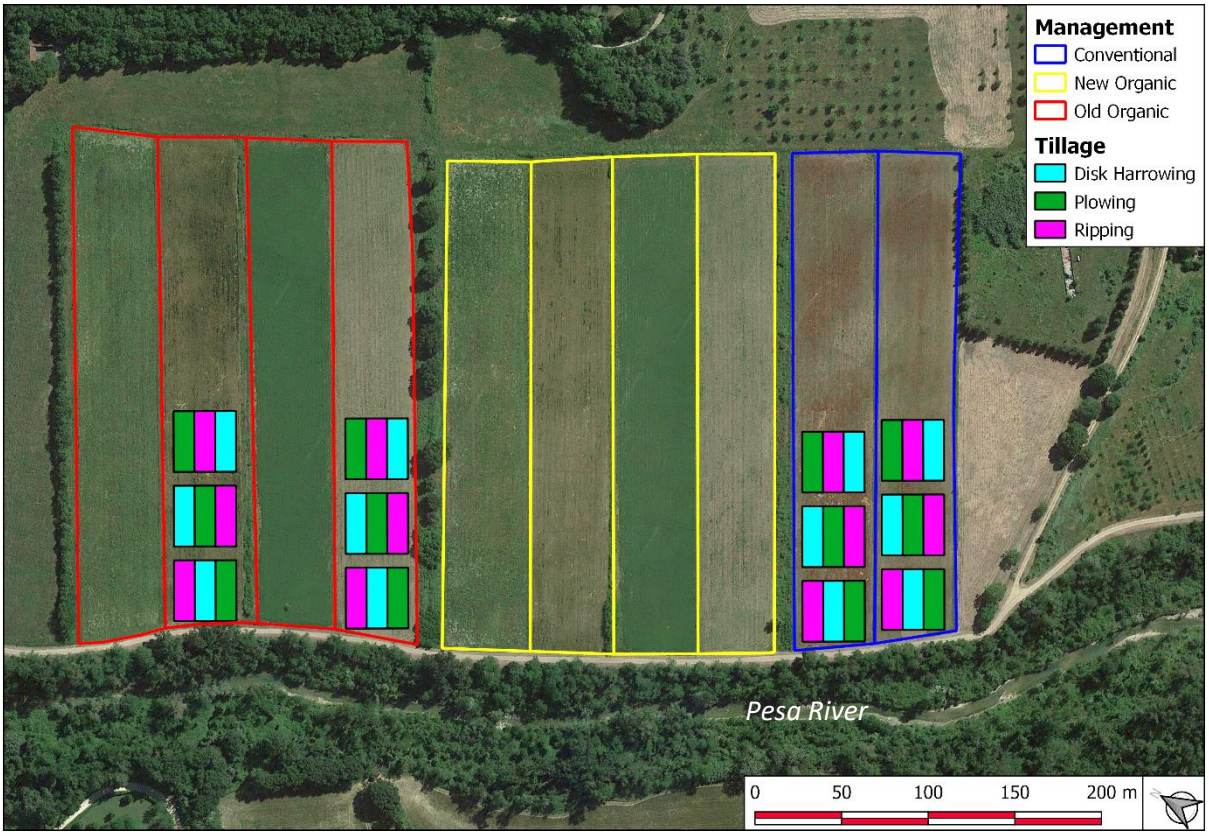

Figure 2.3 - Scheme of the experimental site of MoLTE with the three management and tillage. The experimental design is a randomized block design

Therefore, it has been chosen to compare the Old Organic management system with the Conventional one, and samples has been collected in the parcels where barley and sunflower (the only two crops considered for the conventional management) were cultivated in that moment.

Each field is divided in two parts and in the southern section (the part nearest to Pesa River) different tillage treatments are carried out to observe their impact on soil properties. Tillage practices adopted were one conventional tillage (plowing, P) and two reduce tillage (disk harrowing, DH and ripping, R).

In this experimental site, there were not control plots or no tillage plots as the aim of this evaluation was to compare different tillage treatments.

Gathering the factors considered (management and tillage) and the structure of the experimentation, the resulting is a complex experimental scheme that can be summarized as in the Figure 2.3.

As it is possible to observe the experimentation was established arranging three replicates for each "factors combination" with the aim to consider also field variability once looking at the data (i.e. barley crop managed in organic and tilled with plow is replicated three times).

Therefore, for the statistical analysis has been performed a two-way analysis of variance (ANOVA) for a randomized block design with management and tillage as the two main factors. The significance level was set at $P \leq 0.05$.

Table 2.1 - Agricultural operation performed during the year 2016 for each management considered in this study.

	Organic						*Conventional*					
	Barley			*Sunflower*			*Barley*			*Sunflower*		
	P	*R*	*DH*	*P*	*R*	*DH*	*P*	*R*	*DH*	*P*	*R*	*DH*
End of October	Harrowing						Harrowing					
Start of November	Sowing						Fertilization Sowing					
End of February							Fertilization Weeding					
End of March				Harrowing						Harrowing		
Start of April				Disk Harrowing Sowing			Fertilization			Disk Harrowing Sowing Fertilization Weeding		
End of April				Hoeing						Hoeing Fertilization		
Start of July	Harvest						Harvest					
Helf September				Harvest Shredding						Harvest Shredding		

This experimental design has implied that agricultural practices had to be different depending on management, crop and tillage. A detailed table that summarize agricultural operation established has

been reported (Table 2.1). For which concern tillage operations, each plot was tilled with plow, harrow or ripper as reported in the experimental scheme (Figure 2.4) during the second half of September.

Finally, for which concern the conventional management, those plots received fertilization and weeding, and seeds were treated before sowing. It is possible to observe (Table 2.2) that comparing the two crops considered, barley needs more fertilization than sunflower and that those fertilizations were made with different product.

Table 2.2 - Chemical treatments and doses used in the conventional management system for the two crops cultivated in the plots considered in this study.

	Sunflower		*Barley*	
	Product	*Dose*	*Product*	*Dose*
Fertilization			18-46	192 kg ha^{-1}
Sowing	Seed treated with apron-xl p.a metalaxil-m		Seed treated with redigo (pure prothioconazole 8.7%)	
Fertilization			Ammonium Nitrate (27N)	150 kg ha^{-1}
Weeding	Goal	500 ml ha^{-1}	Axial + ready Axial + Logran	1 lt ha^{-1} + 750 ml ha^{-1} + 37 gr ha^{-1}
Fertilization	20.10.10	150 kg ha^{-1}	Urea	150 kg ha^{-1}

2.2.3 Soil sampling

In May 2017, during the growing season, soil samples from the top 15 cm soil profile were taken from the middle of each replicate (for a total of 36 soil samples). Fresh samples were kept in a cooler for transportation to the laboratory where they were sieved through to 2 mm; roots and plant residues were carefully removed by forceps. Samples were then homogenized and divided into three aliquots: one was air-dried, one was stored in plastic bags at 4 °C and the third was frozen.

2.3 Results

2.3.1 Soil chemical and biochemical properties

Soil reaction was significantly affected only by the management with higher values in correspondence of the organic managed plots (Table 2.3). Nevertheless, the arithmetical difference is so tiny considering the parameter that in fact it is possible to suppose no variation in the pH values.

Table 2.3 - Marginal means of soil active rection (pH), extractable carbon to nitrogen ratio ($C:N_{ext}$), microbial carbon to nitrogen ratio ($C:N_{mic}$), total nitrogen (TN, g kg_{ds}^{-1}), ^{13}C stable isotope ($\delta^{13}C$, ‰), ^{15}N stable isotope ($\delta^{15}N$, ‰), soil basal respiration (SBR, mg $C\text{-}CO_2$ kg_{ds}^{-1} h^{-1}). Differences between treatments has been considered significant for $P<0.05$ and has been indicated with different lowercase letters.

	pH	$C:N_{ext}$	$C:N_{mic}$	TN	$\delta^{13}C$	$\delta^{15}N$	SBR
System							
Org	8.45[a]	8.78	5.38	1.32	-26.4	5.35	0.643
Conv	8.35[b]	5.26	5.59	1.20	-26.3	5.54	0.588
p-value	*0.004*	*0.094*	*0.462*	*0.116*	*0.668*	*0.055*	*0.323*
Tillage							
Plowing (P)	8.40	6.91	6.16	1.30	-26.5	5.64[a]	0.558
Harrowing (DH)	8.38	6.88	5.07	1.24	-26.4	5.40[ab]	0.691
Ripping (R)	8.42	7.29	5.21	1.23	-26.2	5.29[b]	0.597
p-value	*0.395*	*0.828*	*0.126*	*0.990*	*0.189*	*0.013*	*0.304*
System x Tillage							
Org (P)	8.49	8.78	5.43	1.44	-26.5	5.46	0.554
Org (DH)	8.42	9.02	5.31	1.26	-26.4	5.39	0.726
Org (R)	8.45	8.55	5.40	1.25	-26.3	5.19	0.648
Conv (P)	8.32	5.03	6.89	1.17	-26.5	5.82	0.562
Conv (DH)	8.34	4.73	4.84	1.21	-26.4	5.40	0.655
Conv (R)	8.39	6.03	5.03	1.21	-26.1	5.38	0.546
p-value	*0.133*	*0.486*	*0.210*	*0.446*	*0.889*	*0.287*	*0.803*

Organic management system promoted the increase of extractable C (+12%) and, even if not significantly, of the soil organic C (+12%) (Figure 2.5). On the other hand, the conventional system

significantly enhanced available P content (+81%) and, even if not significantly, it leads an increase also in the extractable N content (+49%) (Figure 2.5).

The adopted tillage did not affect those parameters. Otherwise, plowing significantly increase the ^{15}N stable isotope value (Table 2.3) which highlight differences also between the two reduce tillage considered with lower values measured in correspondence of the ripped plots. Almost the same trend is observable also for N_{ext} (+14% with plowing compared with the reduced tillage) and SOC content (Figure 2.5) without differences between the two reduce tillage.

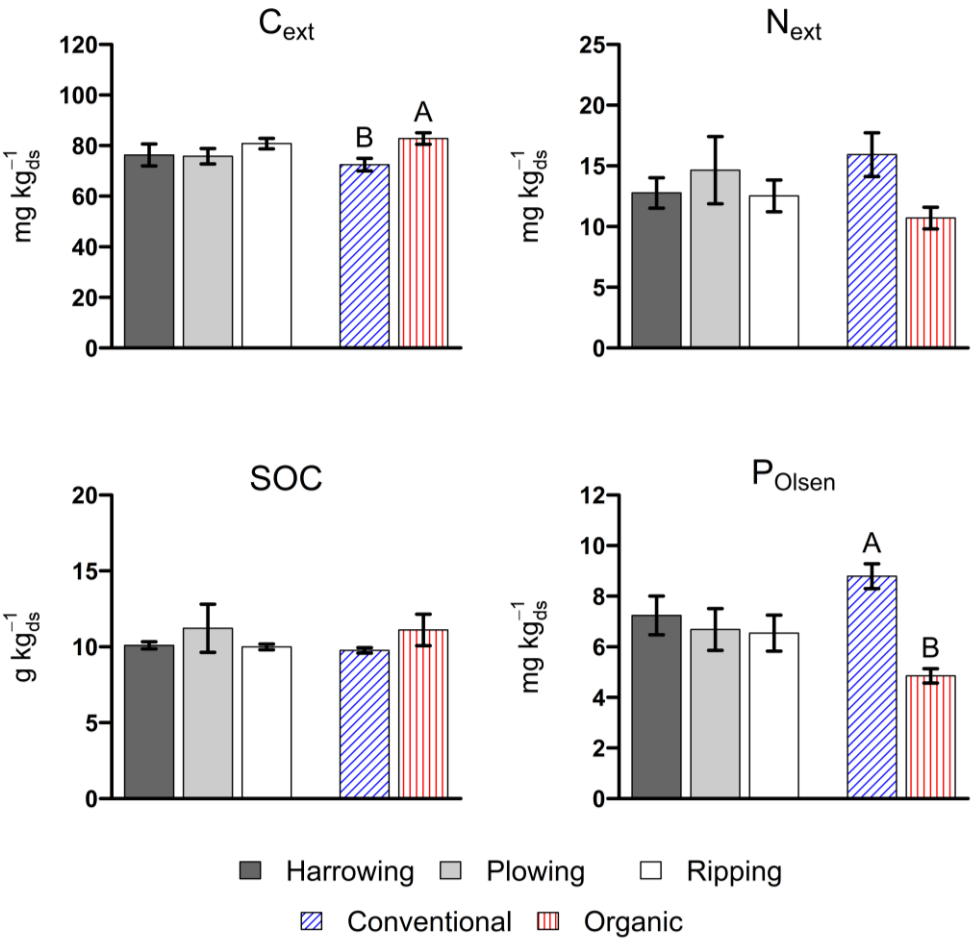

Figure 2.5 - Marginal means of extractable C (C_{ext}), extractable N (N_{ext}), soil organic carbon (SOC) and available phosphorus (P_{Olsen}). Error bars represent the standard error for each treatment. Different lowercase letters represent significant differences ($P< 0.05$) between tillage and uppercase letters represent significant differences ($P< 0.05$) between management.

Microbial biomass resulted significantly affected by the tillage practice adopted (Figure 2.6). Specifically, plowing induce a reduction of the microbial C and N content (-37% and -43% respectively) and the means of MBC highlight also significant differences between the two minimum tillage considered with higher values in correspondence of the harrowing. The MBC resulted significantly affected also by the management with higher values (+22%) in correspondence of the organic once. The same trend has been observed also for the MBN (+17%) (Figure 3.6).

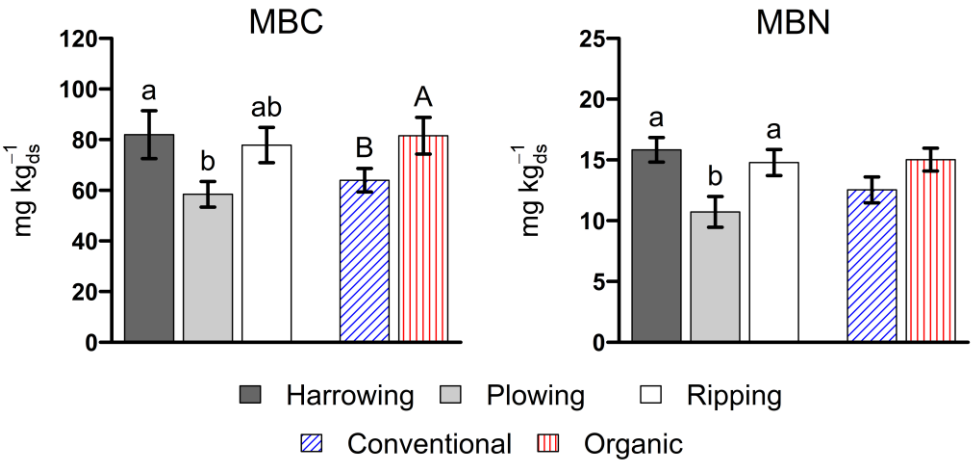

Figure 2.6 - Marginal means of microbial biomass C (MBC) and microbial biomass N (MBN). Error bars represent the standard error for each treatment. Different lowercase letters represent significant differences (P< 0.05) between tillage and uppercase letters represent significant differences (P< 0.05) between management.

2.3.2 Soil enzyme activities

In figure 2.7 are reported the activities of the three oxidative enzymes measured. After 26 years of agricultural management, the activities of dehydrogenase and tyrosinase resulted significantly improved by the organic system (+25% and +36% respectively) but laccase activity resulted not affect by the management system adopted. Considering tillage practices, plowing significantly downturn dehydrogenase activity (-37%) and, even if not significantly, the same trend has been observed for tyrosinase activity. As for the management, also the tillage did not induce any change in laccase activity (Figure 2.7).

In figure 2.8 has been reported the measured hydrolytic enzyme activities. The activities of β-xylosidase and β-cellobiosidase resulted significantly affected by the adopted management with higher values in correspondence of the conventional once (+15% and +24% respectively).

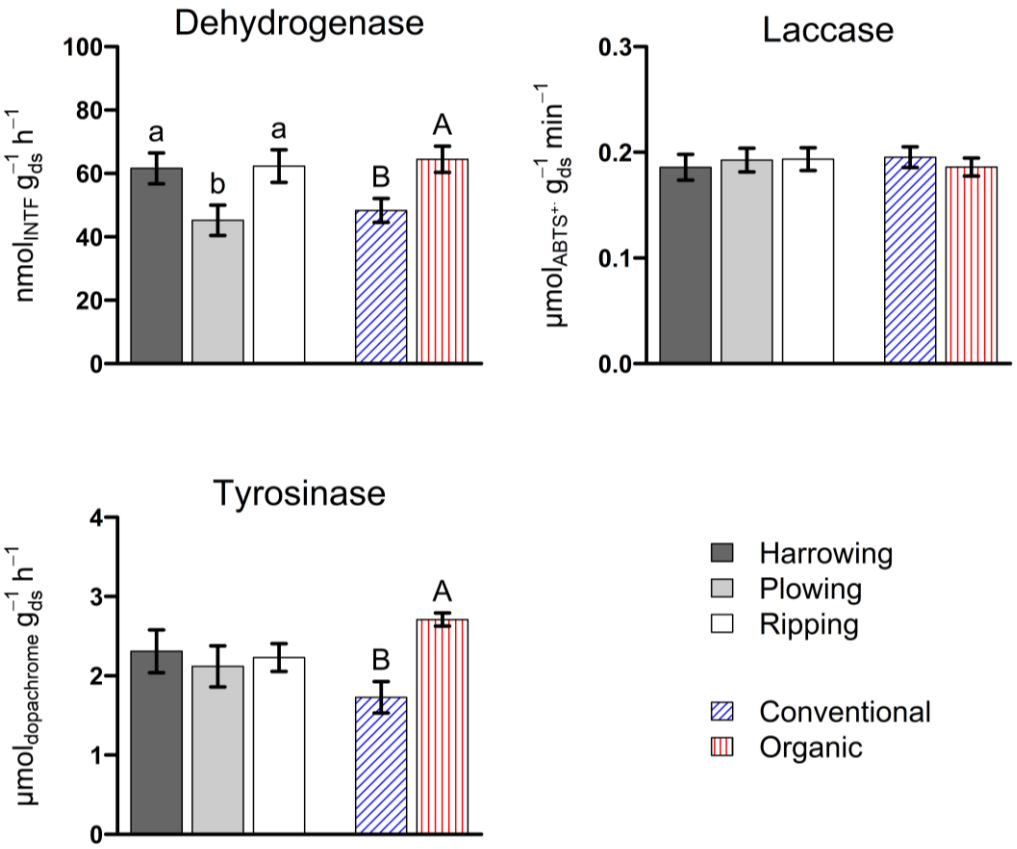

Figure 2.7 - Marginal means of dehydrogenase, laccase and tyrosinase activities Error bars represent the standard error for each treatment. Different lowercase letters represent significant differences (P< 0.05) between tillage and uppercase letters represent significant differences (P< 0.05) between management.

The same trend has been observed also for β-glucosidase, α-glucosidase and lipase activities (+15%, +12%, +20% respectively), indeed these five enzymes are all involved in C cycle processes and thus the same trend was expected. Continuing on management effects, the phosphomonoesterase activity resulted significantly increased by the organic management, while the activities of N-acetyl-β-glucosaminidase and of arylsulfatase appeared not affected by the type of management adopted (Figure 2.8). The most significant effect of tillage on the hydrolytic enzyme activities is that of the plowing (Figure 2.8). Indeed, plowing significantly downturn the activities of β–glucosidase (-21%), β –xylosidase (-28%), β–cellobiosidase (-36%) and phosphomonoesterase (-17%). Even if not significantly, the same trend has been observed for the other hydrolytic enzyme activities with the exception of lipase. Indeed, has been measured a downturn of 22% for the α-glucosidase activity, 17% for N-acetyl-β-glucosaminidase activity and 12% for arylsulfatase activity. Results did not highlight any difference between the two reduced tillage.

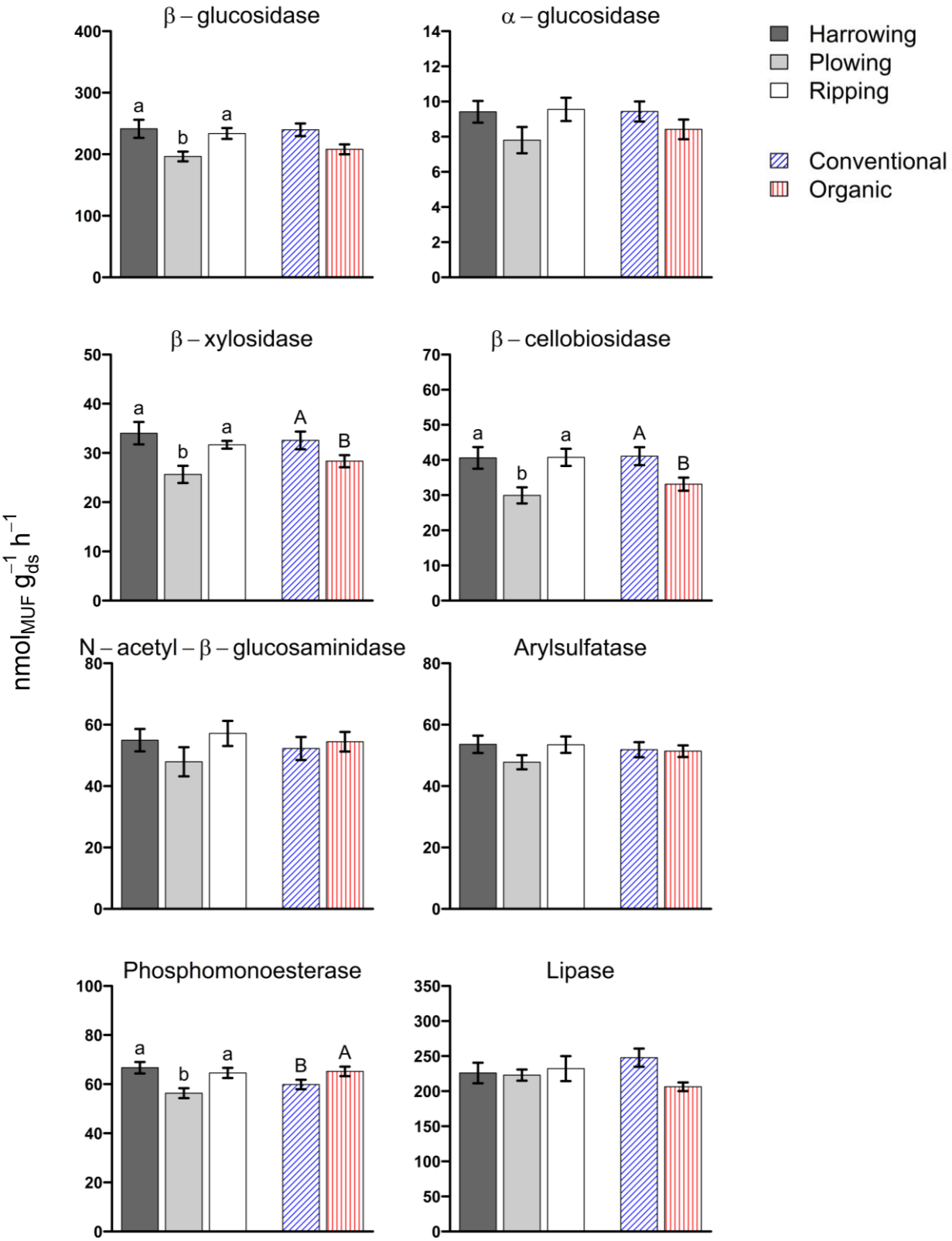

Figure 2.8 - Marginal means of β-glucosidase, α-glucosidase, β-xylosidase, β-cellobiosidase, N-acetyl-β-glucosaminidase, arylsuphatase, phosphomonoesterase and lipase activities. Error bars represent the standard error for each treatment. Different lowercase letters represent significant differences (P< 0.05) between tillage and uppercase letters represent significant differences (P< 0.05) between management.

2.3.3 Soil biochemical indexes

The tillage practices significantly affects the microbial quotient and the metabolic index (Figure 2.9) and, specifically, the plowing tillage induced a reduction of these biochemical indexes (-38% and -34% respectively). The calculated values of the qmic highlight also significant differences between the two minimum tillage considered, with higher values in correspondence of the harrowed plots. Regarding the metabolic quotient, no significant differences has been observed. Nevertheless, it showed the higher values in correspondence of the plowed plots (+18%) (Figure 2.9) and, looking at the management, the higher metabolic quotient has been registered for the conventional system (+10%) where the other two biochemical indexes (qmic and MI) resulted to have the lower values (-17% and -14% respectively).

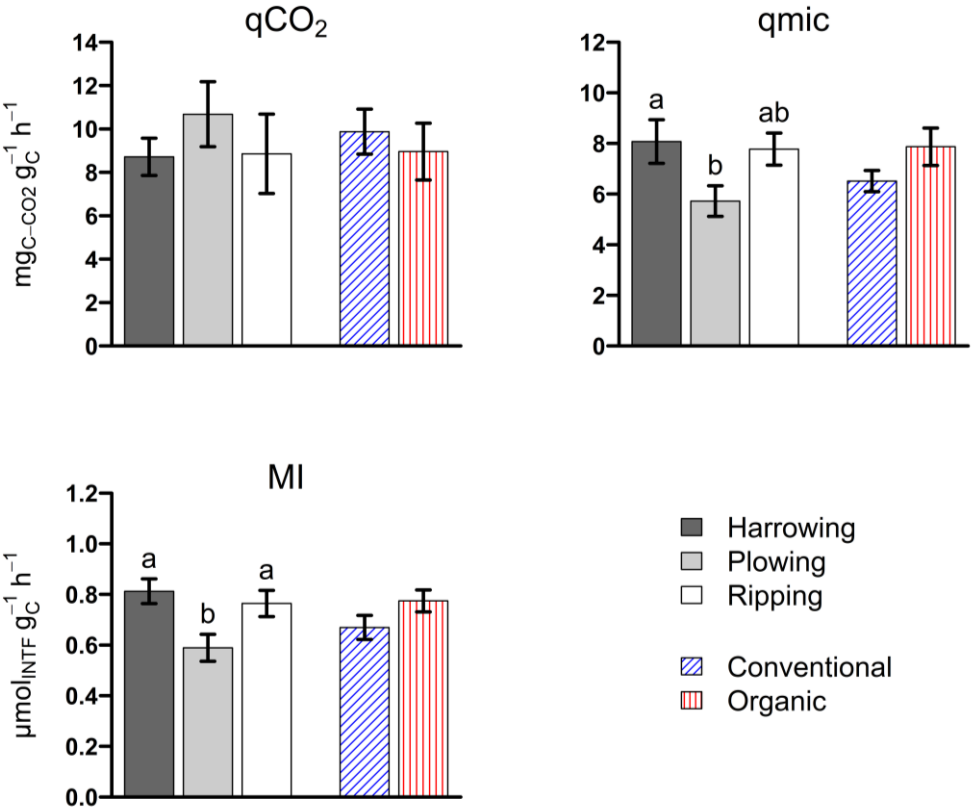

Figure 2.9 - Marginal means of the metabolic quotient (qCO_2), the microbial quotient (qmic) and the metabolic index (MI). Error bars represent the standard error for each treatment. Different lowercase letters represent significant differences ($P< 0.05$) between tillage and uppercase letters represent significant differences ($P< 0.05$) between management.

For which regard specific enzyme activities (Table 2.4), only specific β-xylosidase, β-cellobiosidase and arylsufatase activity resulted significantly affected by the management with higher values in correspondence of the conventional plots (+36%, +48% and +24% respectively). Even if not significant, the trend was the same also for the other specific enzyme activities with the exception of dehydrogenase and tyrosinase (-15% and -29% respectively).

Regarding tillage, only specific laccase and lipase activities resulted significantly affected by plowing tillage which induced an increase of these specific enzyme activities (+30% and + 22% respectively). Nevertheless, also for the other specific enzyme activities the trend was the same with the higher values in correspondence of the plowed plots. The only exceptions were the dehydrogenase and the β-cellobiosidase specific activities for which the higher values have been reported in correspondence of the ripped plots. Moreover, specific dehydrogenase, α-glucosidase and N-acetyl-β-glucosaminidase specific activities resulted significantly affected also by the interaction between system management and tillage. Specifically, α-glucosidase and N-acetyl-β-glucosaminidase specific activities resulted significantly higher in the conventional plowed samples and this trend is observable also for the other specific enzyme activities. There are only two exceptions: specific dehydrogenase activity, which showed the higher values in the organic ripped samples, and specific tyrosinase activity that was higher in the organic plowed plots.

Table 2.4 - Marginal means of dehydrogenase ($Dehy_{MBC}$, $\mu mol_{INTF}\ mg_{C}^{-1}h^{-1}$), laccase ($Lac_{MBC}$, $\mu mol_{ABTS}\ mg_{C}^{-1}\ min^{-1}$), tirosinase ($Tyr_{MBC}$, $\mu mol_{DOPA}\ mg_{C}^{-1}\ h^{-1}$), β-glucosidase (β-$Glu_{MBC}$, $\mu mol_{MUF}\ mg_{C}^{-1}h^{-1}$), α-glucosidase (α-$Glu_{MBC}$, $\mu mol_{MUF}\ mg_{C}^{-1}h^{-1}$), β-xylosidase (β-$Xyl_{MBC}$, $\mu mol_{MUF}\ mg_{C}^{-1}h^{-1}$), β-cellobiosidase (β-$Cel_{MBC}$, $\mu mol_{MUF}\ mg_{C}^{-1}h^{-1}$), N-acetyl-β-glucosaminidase (NAG_{MBC}, $\mu mol_{MUF}\ mg_{C}^{-1}h^{-1}$), arylsuphatase ($AS_{MBC}$, $\mu mol_{MUF}\ mg_{C}^{-1}h^{-1}$), phosphomonoesterase ($PME_{MBC}$, $\mu mol_{MUF}\ mg_{C}^{-1}h^{-1}$) and lipase ($LIP_{MBC}$, $\mu mol_{MUF}\ mg_{C}^{-1}h^{-1}$) specific activities. Differences between treatments has been considered significant for $P<0.05$ and has been indicated with different lowercase letters.

	$Dehy_{MBC}$	Lac_{MBC}	Tyr_{MBC}	β-Glu_{MBC}	α-Glu_{MBC}	NAG_{MBC}	β-Xyl_{MBC}	β-Cel_{MBC}	AS_{MBC}	PME_{MBC}	LIP_{MBC}
System											
Org	0.942	2.36	38.1	2.99	0.121	0.767	0.403^{b}	0.470^{b}	0.732^{b}	0.921	3.01
Conv	0.805	3.22	27.3	4.10	0.159	0.893	0.548^{a}	0.695^{a}	0.906^{a}	1.03	4.38
p-value	*0.689*	*0.223*	*0.194*	*0.062*	*0.089*	*0.389*	*0.022*	*0.034*	*0.040*	*0.348*	*0.099*
Tillage											
Plowing (P)	0.806	3.49^{a}	36.0	3.79	0.148	0.898	0.490	0.584	0.925	1.07	4.34^{a}
Harrowing (DH)	0.852	2.36^{b}	30.2	3.32	0.128	0.758	0.462	0.556	0.741	0.914	3.23^{b}
Ripping (R)	0.962	2.53^{b}	31.9	3.53	0.144	0.835	0.475	0.608	0.790	0.947	3.52^{ab}
p-value	*0.904*	*0.042*	*0.392*	*0.639*	*0.661*	*0.505*	*0.928*	*0.827*	*0.349*	*0.392*	*0.050*
System x Tillage											
Org (P)	0.708^{b}	2.82	39.7	2.74	0.100^{b}	0.681^{d}	0.346	0.391	0.706	0.855	3.10
Org (DH)	0.877^{ab}	1.85	36.0	2.60	0.102^{b}	0.649^{d}	0.359	0.421	0.624	0.866	2.56
Org (R)	1.24^{a}	2.42	38.7	3.62	0.161^{ab}	0.973^{b}	0.503	0.598	0.866	1.04	3.37
Conv (P)	0.904^{ab}	4.16	32.2	4.83	0.197^{a}	1.12^{a}	0.633	0.777	1.14	1.29	5.58
Conv (DH)	0.828^{ab}	2.87	24.4	4.04	0.155^{ab}	0.867^{c}	0.565	0.691	0.858	0.963	3.89
Conv (R)	0.683^{b}	2.63	25.2	3.44	0.126^{b}	0.697^{d}	0.447	0.617	0.715	0.851	3.66
p-value	*0.019*	*0.081*	*0.771*	*0.082*	*0.025*	*0.020*	*0.065*	*0.106*	*0.089*	*0.051*	*0.066*

2.3.4 Correlation between measured parameters

A correlation plot including all the considered soil properties is reported in figure 2.10. Soil reaction is positively correlated only with the tyrosinase activity. For which regard the biochemical indexes, the microbial quotient (qmic) resulted negatively correlated with the metabolic quotient and with laccase and lipase activities, instead it is positively correlated with the metabolic index and tyrosinase, dehydrogenase and phosphomonoesterase activities. The metabolic quotient (qCO_2) resulted negatively correlated with microbial biomass C and N and presented no significant correlation with any of the enzyme activities measured. Finally, the metabolic index (MI) is the one that most correlated with the enzyme activities showing a positive correlation with the activities of tyrosinase, dehydrogenase, N-acetyl-β-glucosaminidase, phosphomonoesterase and β-xylosidase.

The oxidative enzymes showed a different behavior indeed laccase and tyrosinase activities resulted negatively correlated. Moreover, while the laccase activity resulted negatively correlated with microbial biomass C and N, the tyrosinase activity showed a positive correlation with these parameters.

With the exception of arylsulfatase and lipase, the other hydrolytic enzyme activities resulted negatively correlated with both SOC content and total nitrogen content. Moreover, the hydrolytic activities generally showed no correlation with the other C and N pools with some exception: N-acetyl-β-glucosaminidase is positively correlated with C_{ext} as the phosphomonoesterase that is positively correlated also with MBC and MBN. The lipase activity resulted negatively correlated with the MBC and the MBN but it showed a positive correlation with the P_{Olsen} content. P_{Olsen} resulted positively correlated also with β-cellobiosidase, α-glucosidase, β-glucosidase and β-xylosidase activities.

Finally, regarding the correlation between oxidative and hydrolytic enzyme activities, the correlation plot highlight that laccase correlate negatively only with lipase while tyrosinase showed positive correlation with the dehydrogenase and negative correlation with β-glucosidase and lipase.

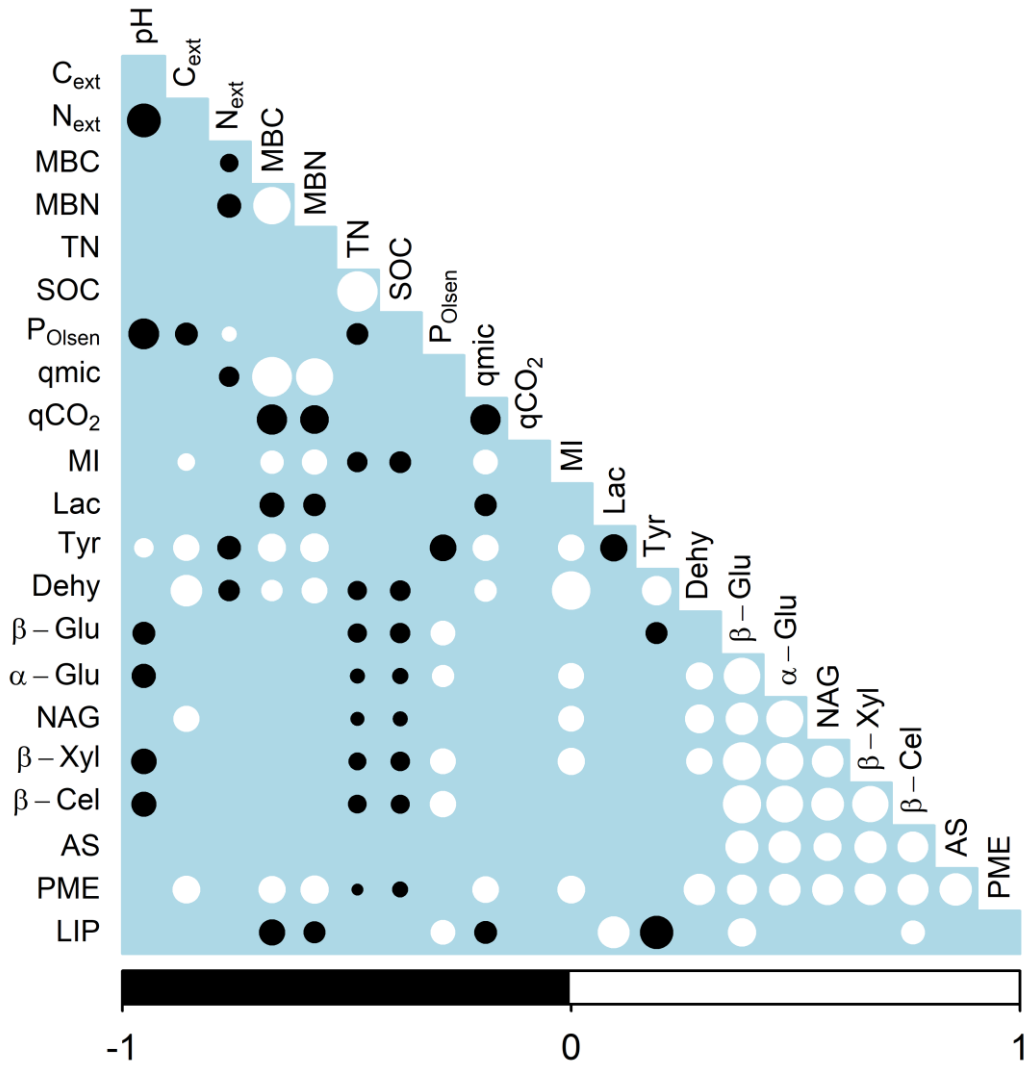

Figure 2.10 - Correlation plot including all the considered soil properties.

2.3.5 Principal Component Analysis

A principal component analysis (PCA) was carried out with C and N pools, available P and the enzyme activities (Figure 2.11) for the two management considered (the PCA carried out for the tillage and for the interaction of the two factors did not give significant results).

The first two principal components accounted for the 32% and 20% of the total variance, respectively. Conventional and organic management clearly separate and were respectively in the negative and in the positive part of the component 2.

The PCA analysis highlight that while the organic management is mainly characterized by microbial biomass, extractable and organic carbon, total nitrogen, tyrosinase and dehydrogenase activities, the conventional management denotes the application of chemical fertilizers being characterized by extractable N, available P, hydrolytic enzyme activities involved in C-cycle and laccase activity.

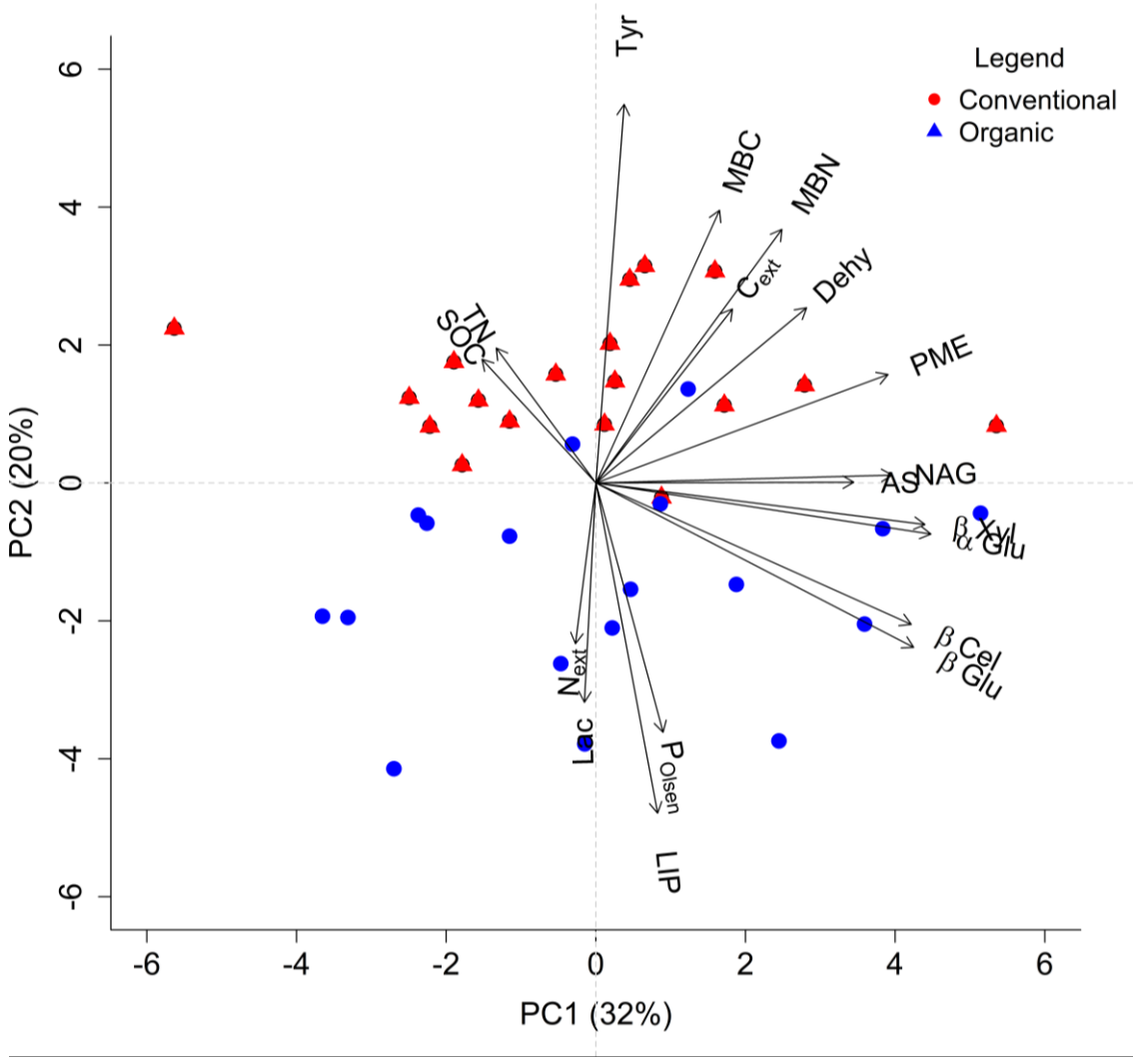

Figure 2.11 - PCA plot with C and N pools, available P, and the enzyme activities for the management considered.

Table 2.5 - Correlation between the two principal component of the PCA and the parameters included in the PCA analysis.

	PC1	PC2
C_{ext}	0.36*	0.40*
N_{ext}	-0.05	-0.37*
MBC	0.33	0.63***
MBN	0.50**	0.59***
SOC	-0.30	0.29
TN	-0.27	0.31
P_{Olsen}	0.18	-0.58***
Lac	-0.03	-0.51**
Tyr	0.08	0.87***
Dhey	0.56***	0.40*
β-Glu	0.85***	-0.38*
α-Glu	0.90***	-0.12
NAG	0.79***	0.02
β-Xyl	0.88***	-0.10
β-Cel	0.84***	-0.33
AS	0.69***	0.00
PME	0.78***	0.25
LIP	0.16	-0.76***

Correlation between PCs and original variables has been done (table reported with Figure 2.11) and results highlight that positive correlation occurred for PC1 with the hydrolytic enzyme activities and for PC2 with microbial biomass C and N and oxidative enzyme activities indicating that, for the PC2, these variables contributed more to the separation of the samples for the applied management practice.

2.4 Discussion

As expected the mineral fertilizers applied within the conventional management lead to significant increase in the available P (P_{Olsen}) and N (N_{ext}) content (Figure 2.5) thus inducing enhanced hydrolytic enzyme activities relative to the C-cycle (Figure 2.8). Indeed, the increase in the P and N content generated a disequilibrium in the microbial stoichiometry balance which drive to a higher production of those enzymes involved in C recover as previously observed also in other studies (Chen et al., 2018; Zhang et al., 2019). At the same time, contrary to what has been observed by Bowles et al. (2014), the N content increase lead to no variations in NAG activities, while the increase in available P content induced a decrease of the PME activities in correspondence of the conventional management. However, to the increased C-cycle enzymatic activities did not correspond an increase of the microbial biomass content (MBC and MBN) with the conventional management (Figure 2.6) thus indicating that the enhanced activity was not related to an increase of the microbial community size. This hypothesis could be supported also by the specific enzyme activities data (Table 2.4) which showed higher specific hydrolytic activities in relation to the conventional management. Moreover, the tendency to increase of the qCO_2 index and to decrease of the qmic and MI indexes in correspondence of the conventional management (Figure 2.9) let suppose that the microbial community is in a stressed (Laudicina et al., 2011; Balota et al., 2004) and less efficient (Masciandaro et al., 1998) condition. Therefore, even if the conventional management supplied to the soil the needed content of N and P, it creates an unbalanced condition to the microbial community that in the long-term could undermine soil fertility.

On the other hand, the organic management positively favors all the C pools (Figures 2.5 and 2.6) inducing an increase of the content of C_{ext}, MBC and, even if not statistically significant, of SOC. Even if the soil C-pools content increased under organic management, it seems that this upturn did not affected the C:N microbial stoichiometry balance as the NAG activity did not show (Figure 2.8) differences among managements while a higher PME activity has been measured in correspondence of the organic management (Figure 2.8). This probably could mean that with the organic management there is a lack of available P but not of N, which probably enter the soil through the leguminous crops included in the rotation plan for the organic management (Lazzerini et al., 2014; Migliorini et al., 2014) and maybe this N amount is enough for microbial community wellness.

A particular behavior came to light observing the oxidative enzymes data (Figure 2.7). Indeed, no many references has been found in literature concerning oxidative enzymes under different

management, especially for which concern the laccase activity. Nevertheless, looking at both oxidative and specific oxidative enzymatic activities (Table 2.4), even if differences were not always significant, it is possible to notice that while dehydrogenase (Dehy) and tyrosinase (Tyr) showed higher values under the organic management, laccase (Lac) had slightly higher values in correspondence of the conventional management. Indeed, both Dehy and Tyr resulted positively correlated (Figure 2.10) with both MBC and C_{ext} content while Lac showed a negative correlation with both MBC content and Tyr activity. Moreover, observing the principal component analysis (PCA) results (Figure 2.11), come evident that Lac and Tyr went to the opposite direction, the first characterizing the conventional management and the second one defining the organic management. The PCA confirmed correlation results concerning Dehy and Tyr activities in relation to the C pools thus indicating that effectively C_{ext} and MBC could be considered the drivers for these enzymatic activities. Deduction on laccase activity behavior are not so easy as it showed the only positive correlation with lipase activity. However, looking at the PCA results it would come to mind a possible correlation with the N_{ext} content as possible element which changes could drive variations also in laccase activity, but obtained results do not support this hypothesis. Anyhow, Sinsabaugh (2010) sustained that one of the factors controlling phenol oxidazing enzyme activities is N availability which lead to variations that are opposite for hydrolytic and oxidative enzymes.

Concerning tillage effects on soil functionality, as attended (Balota et al., 2004; Mikanová et al., 2009; Piotrowska-Dlugosz, 2014) plowing induce the downturn of all the measured variables related to the microbial community (MBC, MBN, qmic and MI) (Figure 2.6 and 2.9) and almost all the enzymatic activities (Figure 2.8) with the exception of the specific laccase and lipase activities which, instead, resulted significantly enhanced by this intensive tillage practice (Table 2.4). Therefore, the aggregate disruption induced by plowing (Six et al., 2000) and the related consequence on soil biochemical properties (Roldán et al., 2005; Zuber and Villamil, 2016), clearly affected soil microbial community. Moreover, plowing, favoring the alteration of soil structure lead to a slightly increase in N_{ext} and SOC content (Figure 2.5) and in this situation they could be more exposed to microbial degradation processes (Balota et al., 2004; Zuber et al., 2017), thus inducing a higher production of laccase enzymes. Indeed, as seen before, N_{ext} could be a driven for laccase activity and the decomposition of SOC, which represent a recalcitrant form of soil C, is generally associated to these enzymes (Sinsabaugh, 2010) that have the ability to encompass its redox potential through the production of specific redox mediators (Bach et al., 2013). Finally, qCO_2 increase after plowing (Figure 2.9)

confirmed that the microbial community resulted negatively affected by this tillage as the increase of this biochemical index, coupled with the decrease in the MI, let suppose that microorganisms were in a situation of higher stress and lower efficiency as founded for the conventional management.

Regarding the two reduced tillage considered, significant differences have been detected only in relation to the MBC content (Figure 2.5) and to the qmic index (Figure 2.9) with higher values in correspondence of the harrowing. Therefore, it could be hypothesized that between the two reduced tillage harrowing is the one that less impacted the microbial community. Indeed, slightly indication about that come also from the specific enzyme activities (Table 2.4). Therefore, in correspondence of the harrowing tillage has been measured the lower specific activities of Lac, Tyr, β-Glu, α-Glu, NAG and Lip thus indicating that even if the microbial community size increased there were not an increase in the production/activation of these enzymes. This could be interpreted as that harrowing was favoring almost sufficient soil condition for microbial community wellness.

The interaction between management and tillage did not give many significant result and the PCA (data not showed) only differentiate for the two management. Only the specific α-Glu and NAG activities showed significant differences for the interaction with higher values in correspondence of the conventional plowed plots. Nevertheless, results highlight that in general both conventional management and plowing negatively affected soil biochemistry and functionality thus leading to the deduction that also the combination of these two agricultural practices could downturn soil quality and fertility.

Finally, as previously observed (Saiya-Cork et al., 2002; Sinsabaugh et al., 2002; Sinsabaugh, 2010; Zeglin et al., 2007), there were no correlation between hydrolytic and oxidative enzymes and the PCA analysis placed highlighted that the first were correlated with the PC1 and the second with the PC2, thus indicating a clear differentiation of these two groups of enzymes. Moreover, only tyrosinase and peroxidase activities measurements have been generally included in previous studies without considering laccases. Instead, these results confirmed that between tyrosinase and laccase activities exist a large difference as they are neither positively correlated and therefore, consider more than two oxidative enzyme activities could be an important point for feature studies on soil functionality.

2.5 Conclusion

The organic management had a positive effect on soil carbon pools inducing an increase in MBC, C_{ext} and SOC content. Moreover, the organic management positively affected the biochemical parameters and indexes measured thus confirming its beneficial impact on soil quality and fertility. Furthermore, the absence of difference in the NAG activities between the two managements let suppose that the organic one is able also to cover the N demand for the microbial community.

As expected plowing significantly depress the enzymatic activities with the exception of laccase and lipase for which behavior deeper investigation are needed. It was also expected to observe differences between the two reduced tillage but results showed only slightly variations between harrowing and ripping for just some of the measured parameters.

Therefore, it is possible to conclude that surely the conventional management in combination with plowing negatively affect soil functionality and fertility. On the other hand, the organic management resulted to be a good agricultural choice for maintain and improve soil quality, independently form the reduced tillage applied. Moreover, organic management with reduced tillage could be considered a sustainable agricultural practices from the soil quality point of view, however it remains to consider the yields reached with these practices to be sure of their complete sustainability.

Finally, through this study it is possible to confirm that soil enzymatic activities respond both to management and tillage agricultural practices and that it is necessary to consider a wide range of enzymes to have a complete view on microbial activity response.

CHAPTER 3

THE ICBM MODEL APPLIED TO MONTEPALDI EXPERIMENTAL SITE

3.1 State of the art

Soil organic carbon (SOC) conservation is fundamental in agricultural system where it has a key role in maintaining soil structure and quality for crop production and soil protection (Clivot et al., 2019; Lal, 2004; Luo et al., 2019; Reeves, 1997). In general, SOC dynamics vary with soil type, climatic condition and, in agricultural systems, with the agronomic management practices adopted (Luo et al., 2019). SOC dynamics are dependent on C inputs (i.e. crop residues and organic amendments) and outputs (i.e. organic matter decomposition and erosion) (Clivot et al., 2019), and C inputs are the predominant factor influencing the SOC balance (Luo et al, 2019). For maintaining and increasing soil fertility, many agricultural studies and practices aim at improving soil organic carbon (SOC) storage. However, changes in SOC content happen over a time scale of decades, and to detect them we need experiments on the same scale.

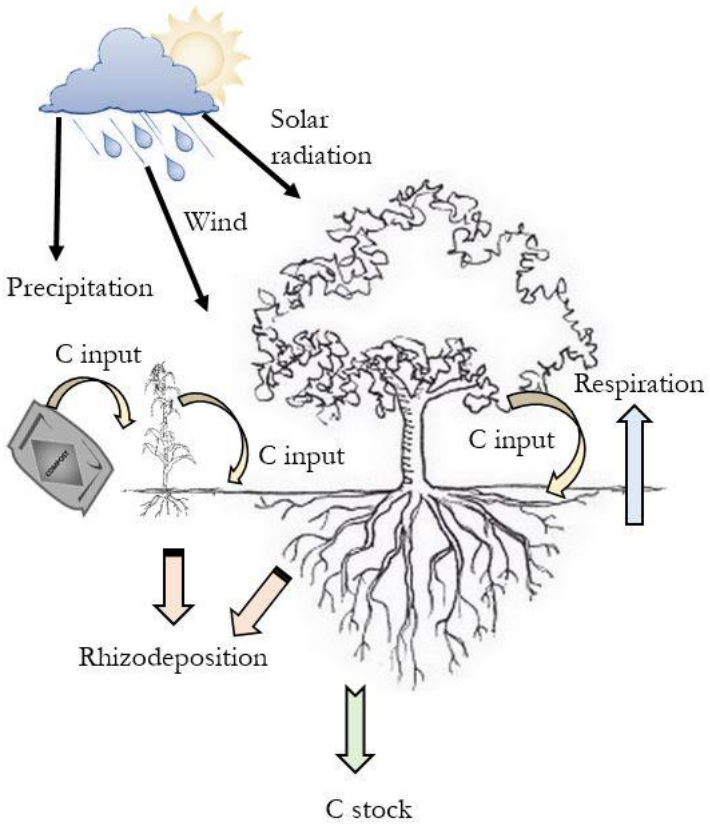

Figure 3.1 - Schematic representation of the factors that influence C dynamics in agro-ecosystems.

But soil is a very complex system characterized by many abiotic and biotic processes that could occur simultaneously and on much shorter time scales, and is influenced by different factors (Figure 3.1) that should all be accounted for when running field experiments. Specifically, considering the processes involved in C sequestration, one of the most important factors determining SOC stocks is

the balance between C outputs and the C inputs coming to the soil such as litter and root deposition, the addition of compost or amendments. Outputs from the system are represented by soil respiration process and, in the agronomic contest, by the part of the crops harvested. All these fluxes are influenced by soil type and climatic conditions of the area that determine soil moisture through the soil hydrologic balance. In order to deal with field-scale complexity we need to relate all the factors involved in SOC cycling with mathematical functions, building a model.

The development of mathematical models able to reproduce SOC dynamics and predict future SOC evolutions needs data to calibrate and validate them that can be produced only by long-term experiments (Clivot et al., 2019). In fact, mathematical models represent a useful tool to extrapolate future trends from the information in these data, a tool with which is possible to have an overview on SOC sequestration leading to a better understand of the driving factors and test hypotheses (Clivot et al., 2019).

Clearly, the application of mathematical models means that involved processes would be abstracted and represented by equations over time and this implies a choice on what to represent. As previously said, soil biochemical processes are really complex and dependent on many biotic and abiotic factors that might be active at different scales and therefore any modelling approach must approximate some of them. These approximations will be based on various trade-off dependent on the model intended application, and after all a model will still be only as good as the data used to build it. A correctly calibrated models could represent a good compromise between complexity, robustness and practical value in order to be used as support to help managing SOC in arable systems (Clivot et al., 2019). Moreover, SOC models in agricultural systems permit to investigate the impact of alternative management scenarios, climate variability and future climate change effects on SOC dynamics (Luo et al., 2019).

In this context, with the aim to evaluate SOC storage at the Montepaldi Long Term Experiment (MoLTE) it has been decided to use the Introductory Carbon Balance Model (ICBM), which has been defined and implemented by Andrén and Kätterer at the Ecology Department of the SLU, Uppsala University. The ICBM model allows to determine if a system is losing or sequestering soil carbon and moreover, it could be utilized also for future prediction also with different scenarios such as an increase of the mean annual soil temperature or a change in the annual carbon input (Andrén, 1997). This model has all the elements common to the many first-order SOC models developed over the last decades, but is simple enough to be easily solvable analytically and can be modified in a relatively

manageable way. The presence of an analytical solution is particularly handy because it allows a quick calculation of the steady states (SOC at equilibrium conditions).

3.2 The Introductory Carbon Balance Model (ICBM)

The model consists in a mathematical description of C cycle from the inputs to the transformed and stabilized organic carbon and is based on some basic assumptions (Andrén, 1997):

i. Two pools, young (Y) and old (O), of soil carbon are sufficient (Figure 3.2)
ii. Outflows from the pools follow first-order kinetics (k_1, k_2). This means that the rate of C outflux from the system is not constant over time (zero order), but is instead a function of the remaining C itself which varies over time.
iii. External (mainly climatic, but also edaphic) factors can be condensed into one parameter, r_e, which affects the decomposition rates of Y and O equally
iv. Mean annual carbon input to the soil are described by one parameter (i).

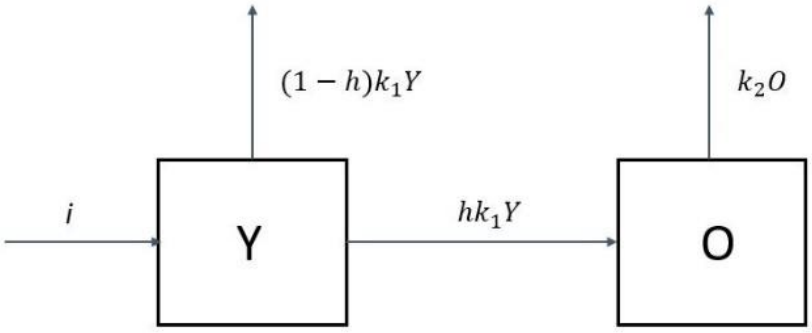

Figure 3.2 – Representation of pools and fluxes involved in the ICBM model definition (from Andrén et al., 1997).

In figure 3.2 is reported a basic scheme of the model with the two pools of carbon that are connected by the flux of the Y pool that through the humification process enters in the O pool. The inputs (i) correspond to the carbon matter that annually enters the young carbon pool. The other two fluxes that exit the carbon pools indicate the rate of carbon losses with the soil respiration process.
It is important to highlight that the parameter r_e does not affect the ''humification coefficient'' (h) and consequently it does not affect the fraction of the annual flux from the young to the old carbon pool (Andrén, 1997), but only the SOC decay kinetics (k_1, k_2).

The model represented in figure 3.2 is mathematically composed by a series of differential equation which describe the variation of Y and O carbon pools throw the time (generally express as years), considering all the fluxes reported in the schematic representation of the model. The model could be defined by two main equations (Kätterer and Andrén, 2001):

$$\frac{\partial Y}{\partial t} = i - k_1 r_e Y \qquad (Eq.1)$$

$$\frac{\partial O}{\partial t} = h k_1 r_e Y - k_2 r_e O \qquad (Eq.2)$$

The variation of the young C pool (Eq. 1) at time *t* is defined by the inputs (*i*) minus the fraction of *Y* lasted from the time *t-1*. The same applies for the old C pool (Eq. 2) but here the inputs correspond to the fraction of carbon that outflows from *Y* through the humification process and enters in the *O* pool.

Once the system is defined in this way, the fluxes from the soil as respiration are given by:

$$\frac{\partial R}{\partial t} = (1 - h) k_1 r_e Y + k_2 r_e O \qquad (Eq.3)$$

The fraction of carbon loss by the system with the respiration (Eq. 3) is given by the sum of respired carbon in the young and old pools at time *t*.

However, it is necessary to consider that the total amount of carbon input to *Y* is determined by plant shoots, roots and root exudates that account for different weight in the total input size and that have different properties, in particular different humification coefficients (Kätterer et al., 2011). Moreover, in agronomic systems, the fraction of carbon derived by the use of compost, green manure or other amendments has to be considered as well. Therefore, shoots (Y_s), roots (Y_r), root exudates (Y_e) and amendments (Y_a) inputs to the Y carbon pool have to be mathematically defined as separated pools:

$$\frac{\partial Y_s}{\partial t} = i_s - k_1 r_e Y_s \qquad (Eq.4)$$

$$\frac{\partial Y_r}{\partial t} = i_r - k_1 r_e Y_r \qquad (Eq.5)$$

$$\frac{\partial Y_e}{\partial t} = i_e - k_1 r_e Y_e \qquad (Eq.6)$$

$$\frac{\partial Y_a}{\partial t} = i_a - k_1 r_e Y_a \qquad (Eq.7)$$

Consequently, equation 1 will be modified to account for the sum of the *Y* input considered:

$$\frac{\partial Y}{\partial t} = (i_s + i_r + i_e + i_a) - k_1 r_e (Y_s + Y_r + Y_e + Y_a) \qquad (Eq.8)$$

Usually, the fractions of shoots, roots and root exudates input are calculated with allometric equations from above-ground plant production (Kätterer and Andrén, 2001).

3.2.1 Input estimation and parameters definition

Once the model structure is defined, still inputs and the model parameters (the kinetics constants and the humification coefficients) should be defined.

Starting from the total plant harvested dry matter it is possible to calculate the fraction corresponding to the shoots and to the roots using the shoot to root ratio (S:R) values. Clearly, each plant species is characterized by a different S:R and in the case of this study it has been decided to apply the S:R used in the works of Lazzerini et al. (2014) and Migliorini et al. (2014) as they took in consideration the same long-term experimental site. Those S:R do not consider the type of management adopted but differentiate only for crop and derive from the work of Bolinder et al. (2007) in which S:R values (Table 3.1) have been calculated for different crops starting from approximately 168 data obtained reviewing Canadian agroecosystem studies.

Table 3.2 - Shoot to root ratio mean values and SD error for different crops (from Bolinder et al., 2007).

Crop		Shoot/root ratio
Small-grain cereals	All studies	7.4 ± 3.6
Winter wheat	Eastern Canada	6.0 ± 1.2
	U.S.	1.1 ± 0.1
Barley	Easter Canada	2.0 ± 0.3
	Western Canada	10.7 ± 3.1
Grain-sorghum	U.S.	11.6
Corn	All studies	5.6 ± 2.8
	Easter Canada	9.5 ± 1.4
Soybeans	All studies	5.2 ± 3.1
Grass species	All studies	1.6 ± 1.2
Legume species	All studies	2.2 ± 0.9

The root exudates fraction has been assumed to be the 65% of the root biomass (Bolinder at al., 2007).

For what concerns the kinetic constants values, k_1 and k_2, they could be calculated from litter-bag data (Andrén, 1997) or appropriate values could be found in literature. In the case of this study no litter-bag data was available and therefore it has been chosen to use the values reported in the Andrén study (1997) where to the constants k_1 and k_2 were associated values of 0.8 and 0.00605 respectively.

The humification coefficient (h) describes the fraction of crop residues converted in stabilized soil organic matter (Kätterer et al., 2011). This coefficent depends on the type and quality of the material involved in the humification process but h values could not be measured and so their estimation have to be done indirectly, based on long-term experiment data and modelling assumptions. The calculated result depends on the assumptions. To minimize the dependency on the assumptions and offer a more robust estimate Kätterer et al. (2011) determined various h values for various assumptions thus reporting a range of h values for shoot, root and organic fertilizers. For the site here considered it has been decided to use the h mean value of all the different scenarios proposed by Kätterer et al. (2011), assuming that the amendments have a coefficient h_a of 0.20, shoots have humification coefficient (h_s) of 0.15 and roots humification coefficient (h_r) has a value of 0.27. It remains to define the humification coefficient of the exudates, which is still a debated issue. Root

exudates are composed by organic substrates with low-molecular weight and high bioavailability (Pausch et al., 2018) and, generally, carbohydrates are the most abundant component present in the form of mono- and poli-saccharides (Gunina and Kuzyakov, 2015). Therefore, it could be argued that root exudates have a mean residence time more similar to that of shoot material than of root, and consequently its humification coefficient has to be nearest to the shoot humification coefficient. For those reason in this study a value of 0.20 has been assumed as exudates humification coefficient (h_e). However, it is necessary to underline that the values attributed to the parameters, at first, will represent only the prior probability distributions defined by average values to which an error will be associated. These parameters will then be combined with the information contained in the data in order to identify the values distribution that best represent the kinetics and the humification within the model considered.

3.2.2 Considering climatic interactions

The ICBM model has been developed and calibrated originally on Swedish soil and climate condition and therefore it needs to be updated with a climatic factor when the area of study change (Andrén et al., 2004). As previously explained, climatic condition and soil type (mainly texture) have a great impact on soil C dynamics and so the estimation of the climatic factor is an essential starting point to obtain an accurate response of the model. In our case soil texture is quite similar in Montepaldi and Ultuna (approximately 22%-23% sand and 30%-37% clay respectively), where the original ICBM was calibrated, and we therefore did not consider it focusing instead on climate.

The needed data for the climatic calibration are daily mean air temperature, precipitation, solar radiation, wind speed and humidity; if there are data missing (i.e. humidity data are on monthly instead of daily base) it is possible to apply an interpolation function to fill the gaps. Moreover, data concerning soil bulk density and soil texture composition are needed for estimating the soil hydraulic parameters and calculating the soil water balance.

These data would be used for determining climatic condition effects on soil decomposition processes. First, the soil hydraulic parameters have to be calculated from soil sand, clay and SOC content (Kätterer et al., 2006). Soil evapotranspiration has been then calculated with a function that depend on weather data of humidity, wind speed, air temperature and solar radiation (Kätterer et al, 2006). Soil water balance is calculated daily and depends on topsoil thickness, evapotranspiration, precipitation, soil wilting point and field capacity. The relative water content (θ_r) is given by the

volumetric water content (θ), the minimum water content ($\alpha\theta_{wilt}$) and the water content at field capacity (θ_{fc}) (Andrén et al., 2004):

$$\theta_r = \frac{(\theta - \alpha\theta_{wilt})}{(\theta_{fc} - \alpha\theta_{wilt})} \qquad (Eq.10)$$

where α is the minimum water store which is a fraction of that at wilting point.

The dependence of the decomposition process on soil water content is given by:

$$r_w = \theta_r^{\gamma} \qquad (Eq.11)$$

where γ = 1.3, a constant chosen to approximate the water response function.

Soil temperature has been calculated as function of topsoil thickness (mm), mean air temperature and crops (Kätterer et al, 2006). The effect of soil temperature on the decomposition rates is (Andrén et al., 2004):

$$r_T = \frac{(T_{soil} - T_{min})^2}{(30 - T_{min})^2} \qquad (Eq.12)$$

where T_{min} is the lower T limit for decomposer activity (here set to -3.8°C).

In the model, the annual climatic condition effects on decomposition are summarize in the r_e factor (see equation 1-8), which is given by:

$$r_e = (r_w * r_T) \qquad (Eq.13)$$

The r_e have to be determined for each day and then the annual value, utilized by the SOC model, is given by the calculation of the mean.

3.2.3 Model calibration

For this study the model has been calibrated on the Montepaldi data within a Bayesian statistical framework (Menichetti et al., 2016). The central idea on which this approach is based is to represent the prior probability distribution of model parameters and data with large numbers of parameter sets, and to calculate the posterior probability distributions of the results (once the new information from the data is added by comparing simulation results with measurements) bringing forward all the parameter sets. This creates discrete distributions, where each element of the population is

represented by one parameter set, which due to the large number of sets approximate a continuous probability distribution. The parameters and data uncertainty is defined with distributions expressing the error of each term (in some cases measured, in some cases estimated). By sampling the multidimensional space (where each uncertain term is one dimension defining the space) the approach combines all sources of uncertainty. This approach can be used to update our previous knowledge (represented by the prior probability distributions of the parameters before considering also the new measured data) with new knowledge (deriving from the probability distributions of the measured data), given a certain model structure.

For the optimization of the model it has been used the sampling algorithm in JAGS, which is a standard Metropolis-Hastings, based on a Gaussian shaped likelihood function (Plummer, 2003). Every calibration has been run in two independent Markov chains, each of which was calibrated with 10000 search runs. At the end the chains showed reasonable convergence.

Priors for both *k* (kinetic constants) and *h* (humification coefficients) have been considered as normally distributed, with mean values correspondent to those reported in chapter 3.2.1 (Parameters definition). To those mean values has been associated an error as reported in table 3.3. The errors relative to the humification coefficients were calculated as the standard deviation of the *h* values for the different scenarios reported in Kätterer et al. (2011), which are the same data used for the calculation of the *h* mean values. For the error of the humification coefficient of root exudates (h_e) it was used the higher error reported for the other *h* values in order to consider a possible high variation for this parameter which error is unknown.

Table 3.3 - Priors for the parameters considered in the model and their relative error.

Parameter	Mean value	Error value
k_1	0.8	0.5
k_2	0.00605	0.62
h_s	0.15	0.013
h_r	0.27	0.097
h_a	0.20	0.08
h_e	0.20	0.097

The error associated to the k_1 kinetic constant has been defined arbitrarily but in a precautionary way as it is more than half of the constant value. The parameter k_2 is referred to the decomposition kinetics and the turnover of the old carbon pool (i.e. SOC). For the estimation of its associated error it has been used the standard deviation of the reciprocal of the mean residence time for the stable C pool estimated by Barré et al. (2010). Specifically, in the work by Barré et al. (2010) some long-term bare fallow experiments have been considered for the determination of decay of more stable fractions of soil C. Long-term bare fallow experiments are characterized by the absence of C input with the aim to consider only stable C pool in the soil. In this way it is possible to estimate empirically the more stable C decay by assuming first order kinetics (Barré et al., 2010). In this study, Barré et al. (2010) used a mathematical model composed by two pools, one decaying according to first order kinetics and one inert representing a small fraction of total remaining SOC. The kinetic of the single decaying pool in absence of inputs becomes quite similar to the old pool in ICBM, and so it could be argued that the kinetics of the two models were roughly comparable. The kinetic constant k_2 could be assumed as the reciprocal of the intermediate turnover time calculated by Barré et al. (2010) and in this way the standard deviation of the intermediate turnover time reciprocal values could be used as error for the k_2 value.

Finally, the model initial state was described by a term expressing the proportion between the old and all the young pols. The prior for this term has been assumed as uniform and varying between 95% and 100%, quite similar to those used for the Swedish experiments model (Menichetti, personal communication). In this way it has been used a precautionary approach without defining a fixed value for the initialization.

For each run of the model, the calibration takes into account one different value for every parameter considered thus exploring all the possible values that each parameter could assume within this set of data and in consideration of the priors previously defined. The final output of the calibration, the posterior probability distributions, represent the values of the parameters identified by the calibration as the most probable for the model within the set of data used.

In figure 3.3 has been reported the density distribution of the probability for both priors (in grey) and posteriors (colored) values relative to all the parameters considered in the model. The density distribution of both priors and posteriors resulted from the unification of all the Gaussian distribution calculated for each parameter value and is determined by kernel density estimation (Karatzoglou et al., 2004).

Since we had only one single data point per year, the error in the data has been estimated as the root mean squared error of a linear regression.

Observing figure 3.3 it has to be highlighted that for the h_s parameter the posteriors have a higher uncertainty compared with the priors and this could be due to the high variance and low contrasts in the set of data considered. Instead, considering the distribution of the h_r posteriors it is possible to deduce that the calibration found more suitable to describe these data higher h_r values than those assumed with the priors.

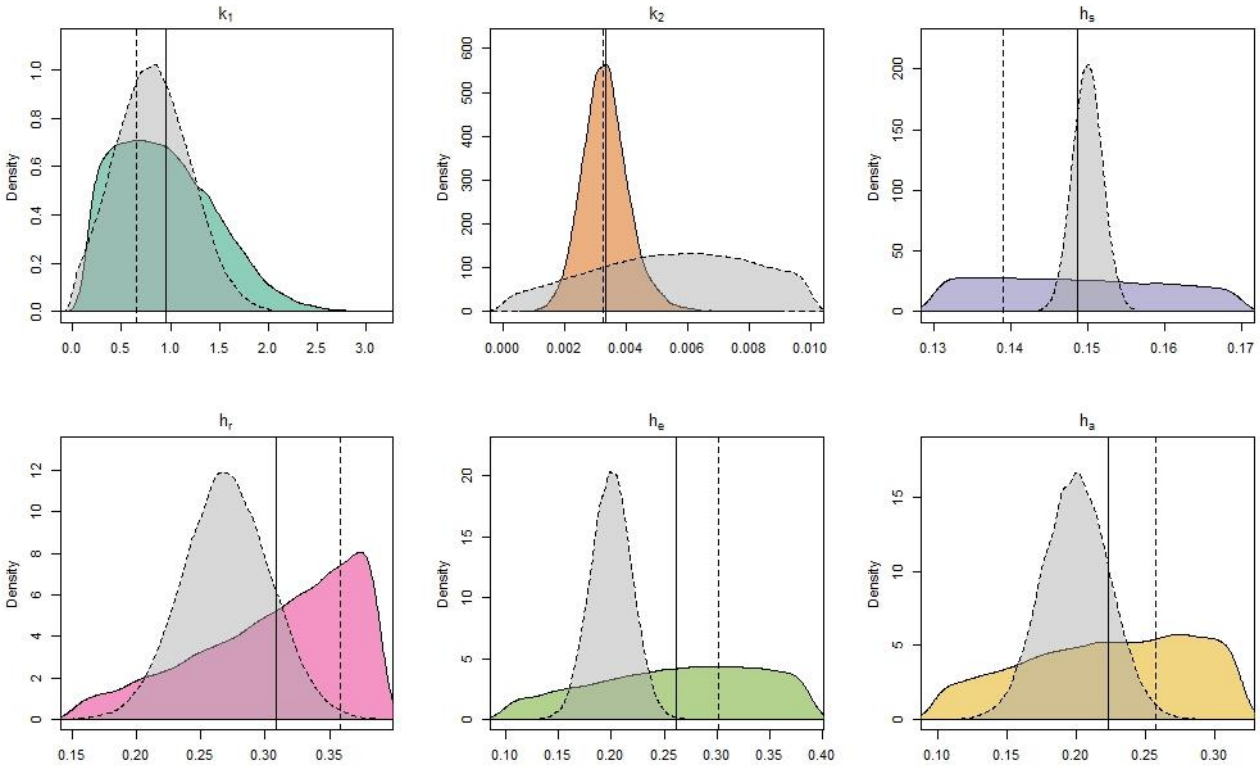

Figure 3.3 - Graphical representation of the probability density distribution of the priors (in grey) and the posteriors (colored) relative to the parameters considered in the model. Soil line represent mean of the posteriors distribution while dashed line represent the mode of the posteriors distribution.

3.3 Model results and comparison with enzyme activities

The data relative to the Montepaldi Long Term Experimental site (MoLTE) were referred to the period 1992-2017 which correspond with the beginning of the experimentation until the year in which soil samples have been collected for the study of the extracellular enzyme activities (see chapter 2). Data relative to crop rotation, crops harvested dry matter and SOC content before the 2017 have been kindly supplied by professor G.C. Pacini (Department of Agrifood Production and Environmental Sciences, University of Florence), who is the site manager, and his research group (Lazzerini et al., 2014). Instead, climatic data have been supplied by Consorzio LAMMA - Laboratory for Meteorology and Environmental Modelling. Summarizing, in the experimental site there is a comparison between two agronomic management organic and conventional. The "Conventional" management (CONV) is spread out on 2.3 ha that are divided in two sub-areas of 1.15 ha each one. These areas are cultivated with barley and sunflower in a biennial rotation since 2015. Before 2015, has been applied a biennial rotation with barley/wheat and sunflower/corn (Migliorini et al., 2014). The "Organic" management (ORG) is spread out on 5.2 ha that are divided in four sub-areas of 1.3 ha each one. These areas are cultivated with barley, leguminous crop and sunflower in a four-year rotation since 2015. Before 2015, has been applied a four-year rotation with barley/wheat, sunflower and clover/leguminous crop; moreover, between 1999 and 2011 organic fertilizer or green manure has been added (Migliorini et al., 2014).

The overall carbon inputs between 1992 and 2017 are reported in figure 3.4 and they were approximatively the same for organic and conventional management system (27.6 and 28.2 ton ha^{-1} respectively). The two systems differ for the source of carbon entering in the soil, indeed the organic once had got a fraction of C from the amendments and the conventional once present a higher fraction of C deriving from shoots. Therefore, considering that SOC sequestration processes are input driven it is expected to observe only slightly difference between the two management in the model results.

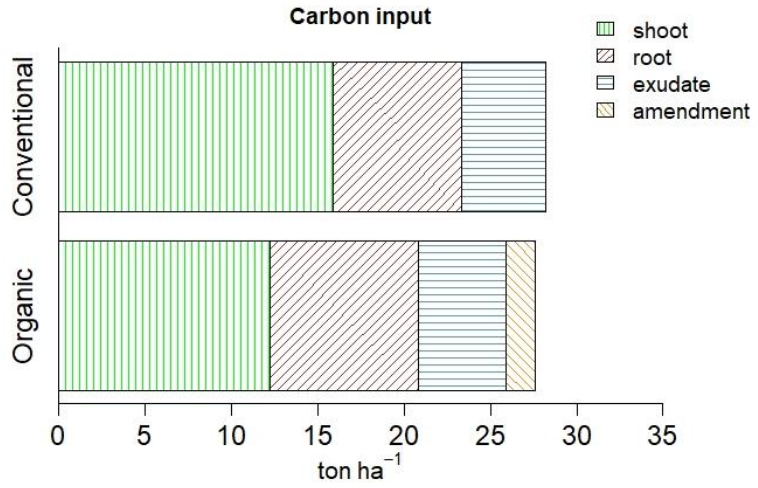

Figure 3.4 – Total carbon input from 1992 until 2017 expressed as ton per hectare.

Model simulations for those 25 years of experimentation are reported in figure 3.5. For both the systems the carbon pools considered (*young* and *old*) and the total SOC are represented. The lines indicate the model that correspond to the mean of the distribution of all possible models, and in some extent it could be considered as the model with the higher probability to fit with parameters distribution and measured data. The light shaded colored areas represent all the other possible models that depend on the error associated to the parameters and on measured data. It is possible to observe that model simulation for the young and old carbon pools have a high uncertainty, which means that the calibration has found many acceptable solutions given the priors for those pools. The wide range of these possible solutions could be due to the high variability of the measured data.

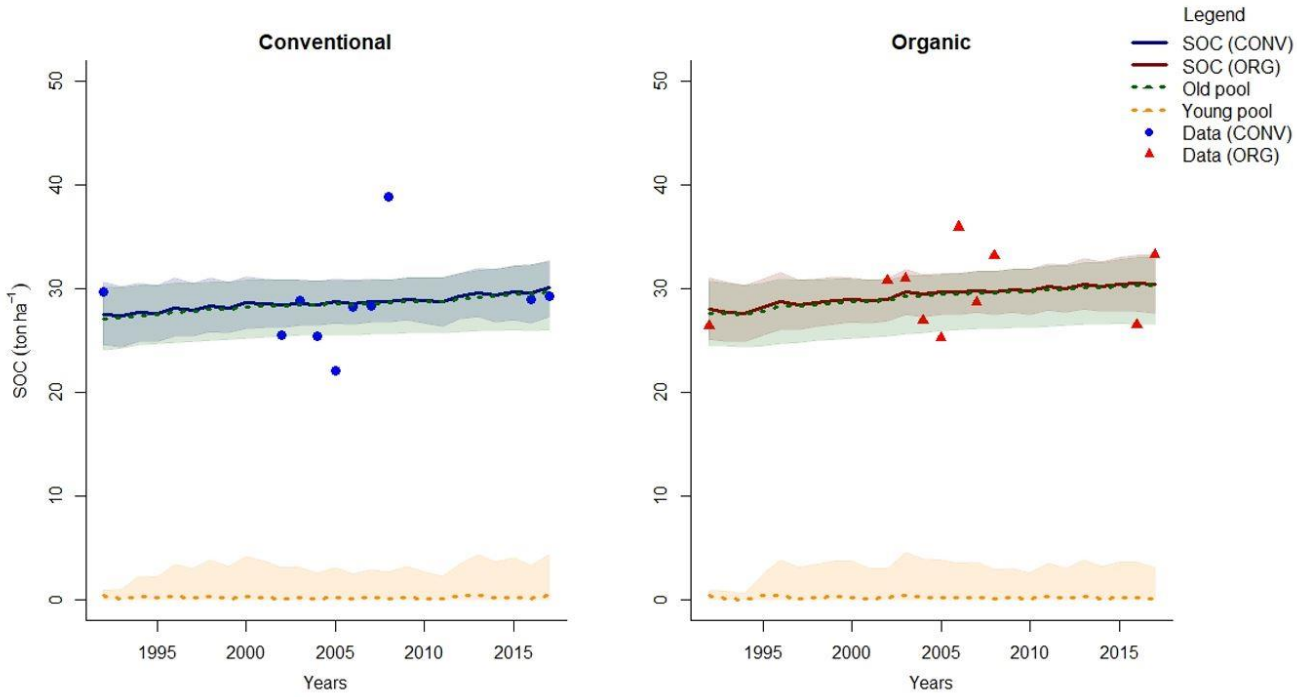

Figure 3.5 - Simulation of SOC pools as described by model structure together with the measured data, for the two systems (conventional and organic) considered.

In general, the model highlights that both the systems were not losing carbon. Comparing SOC modeled data for the two systems (Figure 3.6) it is possible to observe that from the SOC starting point defined by the model the increase in the two systems has been approximatively the same, indeed it has been calculated a yearly mean increase of 0.104 and of 0.097 (ton ha^{-1} $year^{-1}$) for conventional and organic respectively. This clearly express the dependency of the model from the measured C input. Indeed, during those 25 years of experimentation the organic managed plot sequestered 2.43 ton ha^{-1} of SOC and the conventional one sequestered 2.60 ton ha^{-1}. The yearly rolling mean of modeled SOC content has been calculated ('rollmean' R function; Zeileis and Grothendieck, 2005) with a rolling window of 5 years which can be considered a coherent choice with respect to twenty-five years of experimentation as it represents a period of time sufficiently large to calculate a rolling mean but not excessive considering the years taken into account. In figure 3.6 the graph of the rolling mean has been reported and highlights that with the organic management the capacity to store soil organic carbon was higher than those of the conventional one during the first 10 years of experimentation.

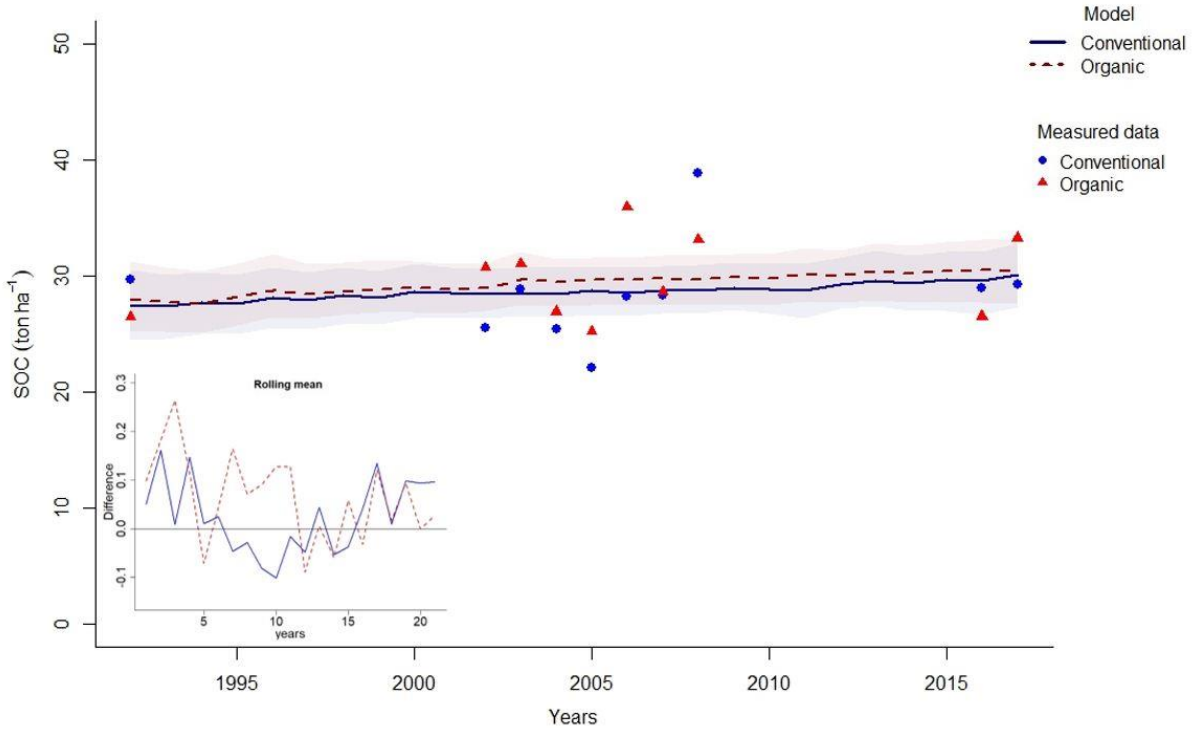

Figure 3.6 - Simulation of SOC content together with measured data for conventional (blue) and organic (red) system. The smaller graph represents the rolling mean of the modeled SOC content, with a window setted at 5 years.

In chapter 2 the same long-term experiment has been studied to assess management and tillage effects on soil enzymatic activities, and their ratio with carbon content (microbial or organic) could be considered a simple index of soil quality (Gil-Sotres et al., 2005; Kandeler and Eder, 1993; Trasar-Cepeda et al., 2008). For the oxidative enzyme activities (laccase, tyrosinase) and the hydrolytic enzyme activities involved in C cycle (glucosidase, xylosidase and cellobiosidase) have been calculated the specific enzyme activity in relation to the organic carbon content (Table 3.4) with the aim to observe if these indexes of soil quality measured in 2017 could fit with the model simulation.

Data related to SOC did not show significant differences between the applied management practices, nevertheless they seem suggest that to the organic management could correspond the higher SOC content. The ratio between C and N labile pool was higher with the organic management and this could be due both to an increase in C input with the organic management and to the N fertilization carried out with the conventional management.

Table 3.4 - Marginal means of microbial biomass carbon (MBC, mg kg^{-1}), soil organic carbon (SOC, g kg^{-1}), extractable C and N ratio (C/N) and the specific enzyme activities of laccase (Lac_{SOC}, $\mu mol_{ABTS}\ g_C^{-1}\ min^{-1}$), tyrosinase ($Tyr_{SOC}$, $nmol_{DOPA}\ g_C^{-1}\ min^{-1}$), β-Glucosidase (β-$Glu_{SOC}$, $mmol_{MUF}\ g_C^{-1}\ h^{-1}$), α-Glucosidase (α-$Glu_{SOC}$, $mmol_{MUF}\ g_C^{-1}\ h^{-1}$), β-Xylosidase (β-$Xyl_{SOC}$, $mmol_{MUF}\ g_C^{-1}\ h^{-1}$), β-Cellobiosidase (β-$Cel_{SOC}$, $mmol_{MUF}\ g_C^{-1}\ h^{-1}$).

System	MBC	SOC	C/N	Lac_{SOC}	Tyr_{SOC}	β-Glu_{SOC}	α-Glu_{SOC}	β-Xyl_{SOC}	β-Cel_{SOC}
Organic	81.5^a	11.1	8.78	17.9	0.261^a	20.2^b	0.817^b	2.75^b	3.21^b
Conventional	64.0^b	9.76	5.26	20.2	0.175^b	24.5^a	0.960^a	3.32^a	4.19^a
p-value	*0.050*	*0.254*	*0.094*	*0.229*	*0.012*	*0.046*	*0.007*	*0.008*	*0.009*

Even if the microbial biomass carbon was higher with the organic management, the specific hydrolytic enzyme activities showed higher values in correspondence of the conventional management thus indicating relatively low SOC and high enzymatic activities levels. Higher specific hydrolytic activities with the conventional management are likely due to nitrogen or phosphate based mineral fertilizers addition that induce the increase of C-cycling enzyme activity (Bowles et al., 2014; Zhang et al., 2019). This could be interpreted as that when P and/or N became more available a disequilibrium in the microbial stoichiometry balance occur and thus microorganisms need to increase C acquisition (Bowels et al., 2014), for example enhancing the activity of those enzymes involved in C recover (Chen et al., 2018; Zhang et al., 2019). This mechanism would be coherent also with the measured C/N ratio. The same trend could be observed also for the specific laccase activity suggesting that in the conventional system also the recalcitrant C pool was involved in the enhanced soil organic matter decomposition process. These results agree with previous studies (Trasar-Cepeda et al., 2008; Zhang et al., 2019) where it has been observed higher specific enzyme activities in cropland than in forested land. Regarding the specific tyrosinase activity its behavior is quite unexpected as it increased in correspondence of the organic managed plot. This particular response could be related to tyrosynase activity positive correlation with both labile and microbial C pools (see chapter 2). Indeed, the other enzymatic activities considered did not correlate with those C pools and the hydrolytic once showed a negative correlation with SOC content thus leading the hypothesis that tyrosinase activity is driven by different mechanisms. Therefore, the overall results obtained in the 2017 suggest that with the organic management a reduction in soil C turnover rate was occurring and that this reduction was driven by soil extracellular enzyme activities (Zhang et al., 2019).

Comparing the model and the data reported in table 3.4 it is only possible to confirm that the SOC content in 2017 was slightly higher with the organic than with the conventional management. Having

more or more informative data or a longer time series at disposal, we could include possible different enzymatic and microbial effects on the decomposition processes as hypothesis in the model, representing it roughly with different humification coefficients for each treatment. This difference would reflect different microbial efficiency.

3.4 Future projection

The ICBM model, as all the mathematical models, is able to estimate future SOC sequestration thus giving the opportunity to test hypotheses on how different management strategy or climate change could impact on carbon stock.

Within the Montepaldi long-term experiment, two different scenarios have been hypothesized and characterized by different cultural rotations until the year 2100. The choice of a long period of time has been done with the aim to get close to the steady state condition for soil C sequestration, when SOC stocks will reach a dynamic equilibrium and their variation will become zero. This state represents the maximum SOC sequestration potential attainable of a certain system and is mathematically defined by the first order theory of SOC decay.

Scenario 1:

In the first scenario the rotation carried out between 1992 and 2017 has been replicated until 2100 thus giving a future perspective of the situation previously studied. With this scenario, in approximatively one hundred years, it has been calculated a cumulative input of carbon of 125 and 123 ton ha^{-1} for the organic and conventional management respectively (Figure 3.7).

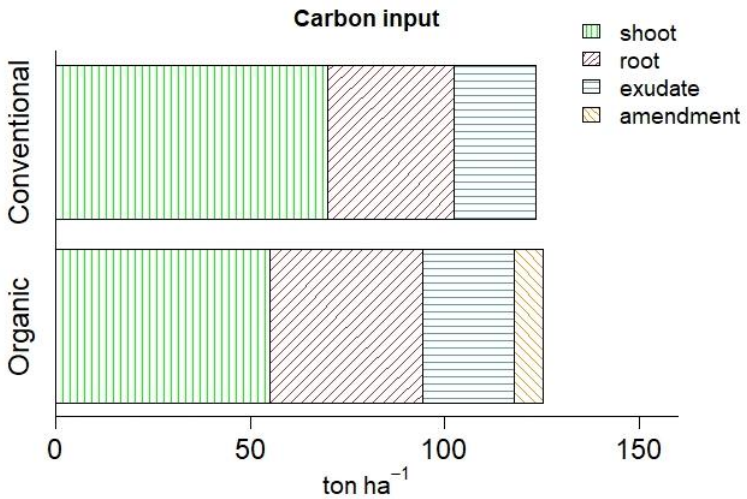

Figure 3.7 - Total carbon input from 1992 until 2100 expressed as ton per hectare and relative to the first scenario.

Again, only with the organic management has been applied a fraction of C that derives from the amendments and, comparing the managements, while the conventional presents a higher fraction of C deriving from shoots the organic once presents higher roots and exudates contribution.

Model results (Figure 3.8) highlight that the organic management will have higher capacity to sequester soil organic C than the conventional. Indeed, from 2018 to 2100, has been calculated a mean rate of C sequestration that is 13% higher with the organic management (0.106 and 0.092 ton ha^{-1} $year^{-1}$ for organic and conventional management respectively).

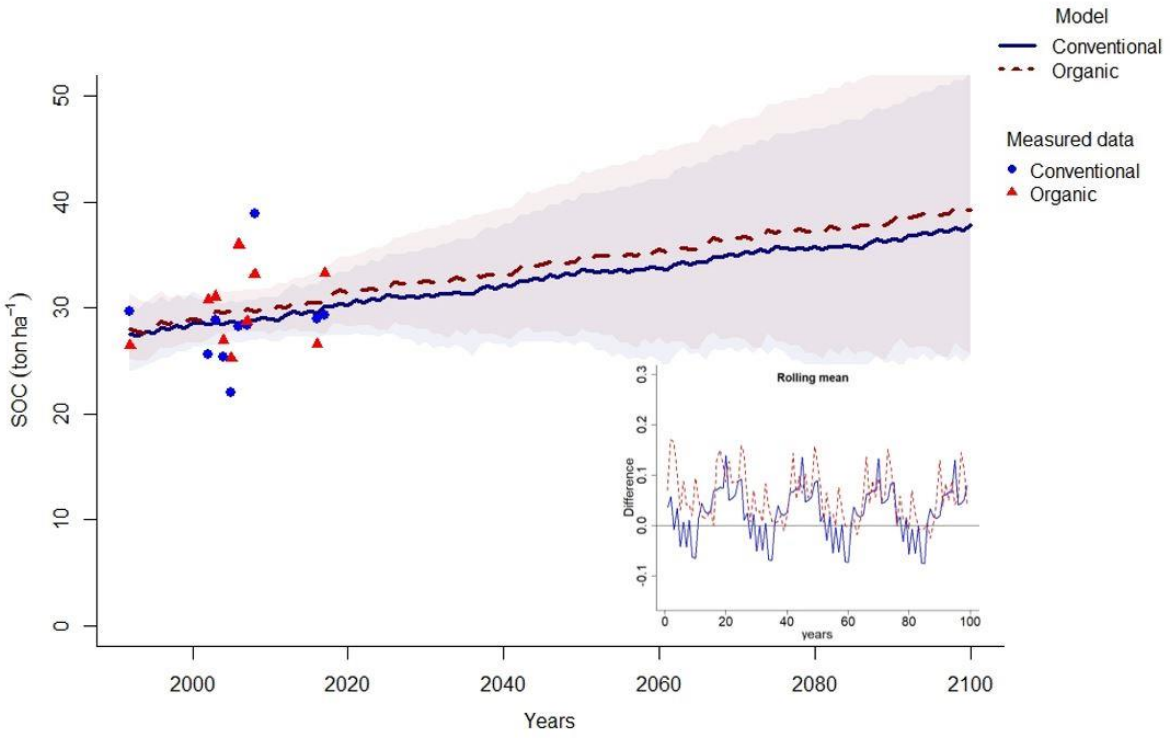

Figure 3.8 - Simulation of SOC content together with measured data for conventional (blue) and organic (red) system. The smaller graph represents the rolling mean of the modeled SOC content, with a window setted at 10 years.

Comparing these results with those for the period 1992-2017, has been calculated a reduction of the mean yearly sequestration rate (-12%) for the conventional management and an increase of the rate (+9%) for the organic one. Therefore, it appears that carbon sequestration rate with the organic management would be in general higher, while the conventional start to reduce its C sequestration rate capacity earlier.

Scenario 2:

The second scenario wants to be an "exasperation" of the two management systems considered with a triennial rotation plan which tries to take into account the ideological differences between the managements. Therefore, within the conventional management has been considered a rotation between barley, sunflower and wheat in order to simulate a management focused on higher production that, in some extent could mean higher economic income. On the other hand, the organic could be interpreted as a management that take into account environmental aspects such as carbon sequestration and therefore it has been chose a rotation between sunflower, barley and clover with amendments addition (every 6 years) in correspondence of barley.

Data relative to crops and amendments has been calculated as the mean of the corresponding data measured between 1992 and 2017.

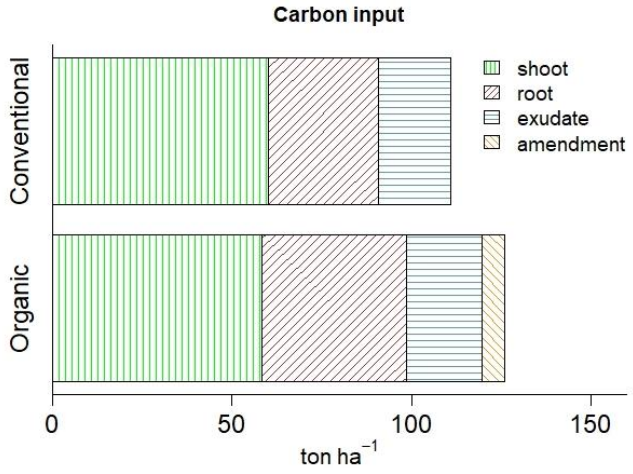

Figure 3.9 - Total carbon input from 1992 until 2100 expressed as ton per hectare and relative to the second scenario.

In this case the total amount of carbon input (Figure 3.9) is of 126 and 111 ton ha^{-1} with the organic and conventional management respectively. It has to be highlight that with the organic management the higher contribution of roots significantly accounts for the total increase of carbon input in this system.

Model results (Figure 3.10) reflect the difference in carbon input between the two management with an increase of 8.31 and 11.2 ton ha^{-1} from 1992 to 2100 respectively for conventional and organic management.

Significant is the difference in the yearly carbon sequestration rate that is 26% higher with the organic (0.104 ton ha^{-1} year^{-1}) than the conventional (0.077 ton ha^{-1} year^{-1}) management. Moreover, comparing the first 25 years (1992-2017) with the period of the prediction (2018-2100) it comes out

that the mean yearly sequestration rate significantly decreases (-34%) in the conventional management and slightly increase (+9%) with the organic management.

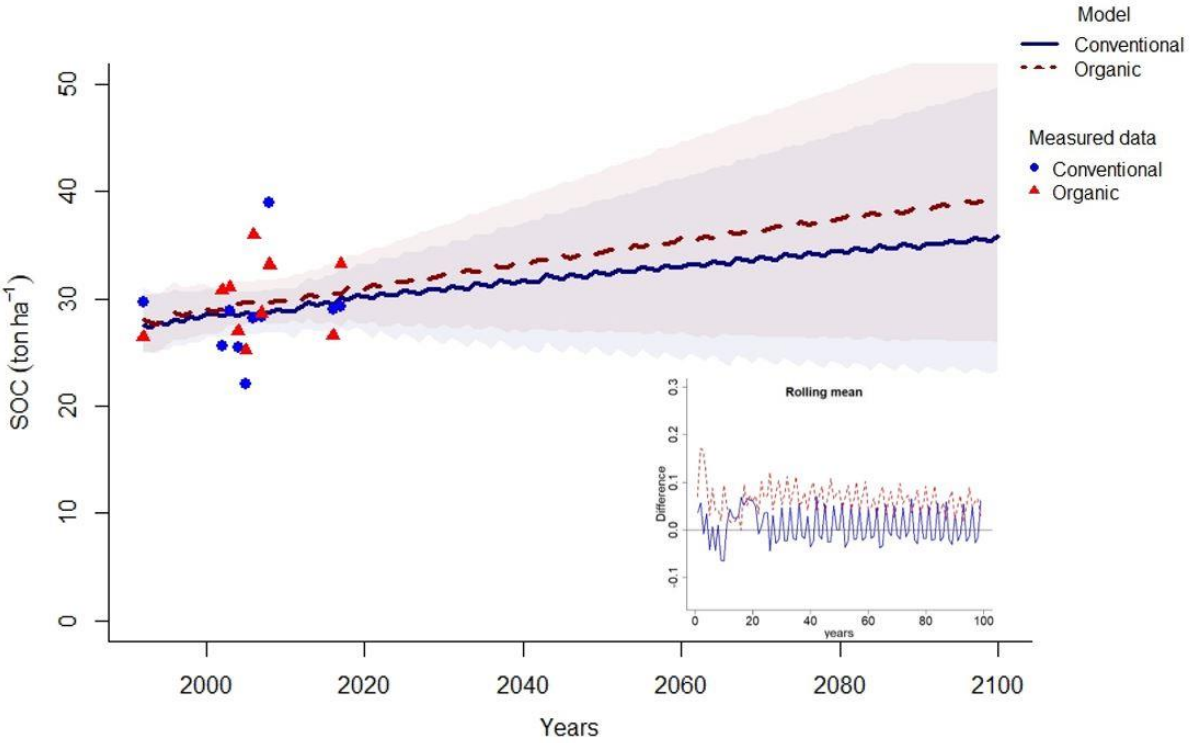

Figure 3.10 - Simulation of SOC content together with measured data for conventional (blue) and organic (red) system. The smaller graph represents the rolling mean of the modeled SOC content, with a window setted at 10 years.

Therefore, both the scenario presented showed that with the organic management the sequestration rate will remain constant over time while that of the conventional management will tend to zero earlier (especially with the second scenario). In both the prediction, SOC content in 2100 is quite similar between the management (in the organic it is 4-9% higher than in the conventional) and this is imputable to the differences in carbon inputs. Instead, differences in the C sequestration rate could be associated to differences in the source of carbon. Indeed, to the organic management correspond a higher fraction of C deriving from the belowground biomass (roots and exudates) which probably promote C storage processes.

3.5 Conclusion

The ICBM model has proved to be an effective and relatively simple tool and its characteristic to be easily solvable analytically permits a manageable adaptation on site characteristics and measured data. The utilization of Bayesian statistic for model calibration permit to include in the model the errors deriving from previous parameters estimation and data. Indeed, parameters definition results to be the most complex process because there are no many references in literature to estimate the errors and the selection of values associated to each parameter, to some extent, implies sometimes a modeler's choice. Thus, using the probability distribution of the parameters instead of fixed values, the model results to be more objective.

The data relative to measured SOC content in the experimental site showed high variability over time and this impacts on the model calibration, which showed a high uncertainty. This, together with the low contrasts in the management and the rather flat curves of SOC over time, reduced the resolving power of the calibration and the model found only small differences between the two management considered. On the other hand, biochemical parameters measured in 2017 suggest that the organic management would be a better agronomical choice in terms of soil quality and processes that could enhance carbon sequestration. To represent this difference in terms of processes within the model we could introduce different humification coefficients for organic and conventional management, thus trying to make explicit the differences in the biochemical processes between the managements. To do so we would need, though, more data either on the SOC kinetics, for calibrating indirectly the parameters with the needed definition, or direct and precise data about the difference in humification (for example on a mid/long term incubation) to drive the model.

Nevertheless, the use of the model for future predictions can be considered a practical tool to evaluate possible consequences on SOC sequestration deriving from the agronomic choice. Clearly, this use of the model gives only some indications and strictly depend on which information are given to the model (for example climatic variations, crops rotation, yield, and external input).

In conclusion, from obtained results it is possible to state that mathematical models are practical tools for making assessments over time regarding carbon sequestration and it would be important in the future to try to explicit, within the model, the biochemical processes related to microbial activity.

CHAPTER 4

QUABIO PROJECT: THE AGRO-ECOLOGY STRATEGY TO MAINTAIN SOIL QUALITY

4.1 State of the art

As seen in chapter 2, agriculture is moving from the conventionally managed systems to type of management that are environmentally more sustainable. Indeed, due to the environmental effects of chemical technology (Altieri, 1989) and intensive tillage (Garini et al., 2017), agricultural evolution finds in the "sustainability" the new keyword towards which directs all the agricultural methods (Tilman et al., 2002). The concept of agricultural sustainability includes both the maintenance of high productivity and the adoption of agricultural practices with acceptable environmental impacts (Tilman et al., 2002). Edwards et al. (1993) reported a complete definition of sustainable agriculture constructed by Edwards himself in 1987. He defined the sustainable agriculture as *"Integrated systems of agricultural production, with minimum dependence upon high inputs of energy, in the form of synthetic chemicals and cultivation, that substitute cultural and biological techniques for these inputs. They should maintain, or only slightly decrease, overall productivity and maintain or increase the net income for the farmer on a sustainable basis. They must protect the environment in terms of soil and food contamination, maintain ecological diversity and the long-term structure, fertility, and productivity of soils. Finally, they must meet the social needs of farmers and their families and strengthen rural communities in a sustainable manner."* (Edwards et al., 1993).

Organic agriculture could represent the right way to achieve this purpose and is defined as ''*a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects.*'' (IFOAM, 2012).

Indeed, nowadays the major challenge is to identify the right way to produce food reducing the negative impacts of agriculture on the environment (Dupré et al., 2017) and going through the sustainability view of the agriculture it is possible to identify a kind of strategy that could be considered another step forward with this agricultural change: it is the agro-ecology.

4.1.1 The agro-ecology

Before explaining on what agro-ecology consists, there is the need to define the subjects of the agro-ecology that are the agroecosystems. Hence, agroecosystems are communities of plants and animals interacting each other and with the surrounding environment that have been modified to produce food, fuel and other products for human consumption (Altieri, 2011). Therefore, we are talking about

systems heavily modified by humans that nowadays are no longer able to be resilient, productive, biologically diversified and natural.

Indeed, the concept of sustainability and then of agro-ecology have been introduced with the emerging awareness regarding the decrease of precious resources for agricultural production and for humanity as fossil fuels, fresh water and phosphorus (Francis and Wezel, 2015). The decrease of these resources is strictly connected with the ecosystem degradation induced by many agricultural practices which lead to soil erosion, water contamination, deforestation, desertification and, consequently, loss of productivity (Edwards et al., 1993). Another factor that mostly from the last years is impacting on agroecosystems is the increased climate variability with less frequent but more intense rainfall and major disruptive storm events (Francis et al., 2015).

Therefore, agro-ecology and the concept of sustainability has been promoted by the increased need to adjust the conventional agriculture model to agricultural strategies more environmentally, socially and economically viable (Altieri, 2011; Porter and Francis, 2016).

Going specifically to agro-ecology, this could be interpreted as the "*marriage of agriculture and ecology*" (Francis et al., 2015), meaning that it takes in consideration the ecological processes that operate in the agricultural production systems (Porter et al., 2016). Agro-ecology could be also defined as the application of ecological concepts and principles to design and manage sustainable production agroecosystems (Altieri, 1989; Altieri, 2011; Francis et al., 2003). Thus, the agro-ecological management aims to improve biological efficiency, biodiversity, soil fertility and conservation working on the use of low-input technologies and natural pest regulation (Altieri, 1989; Altieri, 2011). This kind of approach tries to deal with weeds control, soil quality maintenance and soil erosion reduction for example using the crop rotation with cover crops, which also represent a source of nutrients, limiting the soil tillage (Canali et al., 2013; Francis and Wezel, 2015) and reducing the impacts of synthetic inputs (Dupré et al., 2017).

In this context, soil quality and fertility are a central point as if for the agronomist they imply productivity and mineral nutrition, for the ecologist they determine the biological equilibrium and regulation of the agroecosystems (Lemanceau et al., 2014). Indeed, the soil is an open and dynamic system which represents the junction between all the environmental compartment interacting with atmosphere, minerals, water, and living organisms (Voroney, 2007) and this capacity gives to the soil the ability to support food production thus representing a fundamental support to natural and human activities (Rao and Gianfreda, 2014). Moreover, the maintenance of soil quality and fertility could

impact on soil microorganisms (and consequently on soil enzymatic activities) which are involved in soil biogeochemical cycles, thus contributing to soil nutritional capacity for plants. Therefore, the agro-ecology strategy could be a key choice for favoring soil microbial biodiversity and subsequently for the maintenance of soil productivity and stability (Lemanceau et al., 2014).

Nevertheless, in literature has been found references mainly regarding the concept of agro-ecology and it seems that not many practical study exists. Specifically, only in the work of Aparicio et al. (2018) soil organic matter (SOM) and carbon (SOC) have been taken into account. The above-mentioned study made a comparison between field in an agro-ecological transition and field with an agricultural industrialized system founding that the agro-ecological management increased the surface SOM content thus inducing an improvement in soil conditions. Therefore, a complete study of soil chemical and biochemical properties under the agro-ecology strategy is missing.

4.1.2 Aim of the work

Considering the lack of studies on soil properties with this innovative agricultural strategy and the great opportunities that organic management strategy and reduced tillage give in term of sustainability and soil fertility (Drinkwater et al., 1995; Laudicina et al., 2011; Luo et al., 2019; Roldán et al., 2005; Zuber and Villamil, 2016) it has been supposed that with the adoption of the agro-ecology those positive results could be even higher.

Therefore, in this study has been taken in consideration a long-term experiment in a Mediterranean area (Marche region, Italy) in which conventional, organic and agro-ecology management have been tasted. The aim of the work was to asses if, effectively, the agro-ecology management could be considered a good choice in term of soil fertility and functionality.

Specifically, the hypothesis was to observe (i) higher SOC content in correspondence of the agro-ecology management; (ii) lower hydrolytic enzyme activities with the agro-ecology management compared both to conventional and organic management; (iii) a decrease in the oxidative enzyme activities in correspondence of the agro-ecological management.

4.2 Experimental site description

4.2.1 Area of study

The site here considered is part of the MOnsampolo VEgetable (MOVE) organic long-term experiment started in 2001 at the experimental fields of the Vegetable Research Unit of the Research Council for Agriculture (CRA-ORA). The site is located in Monsampolo del Tronto (AP), Marche region, central Italy (42.53° N, 13.48°E, and 40 m a.s.l.). The experimentation (Figure 4.1) consist in a comparison between conventional, organic and agro-ecologic management (Campanelli and Canali, 2012). Each management covers an area of approximately 0.22 ha and is divided into four plots according with the rotational system adopted.

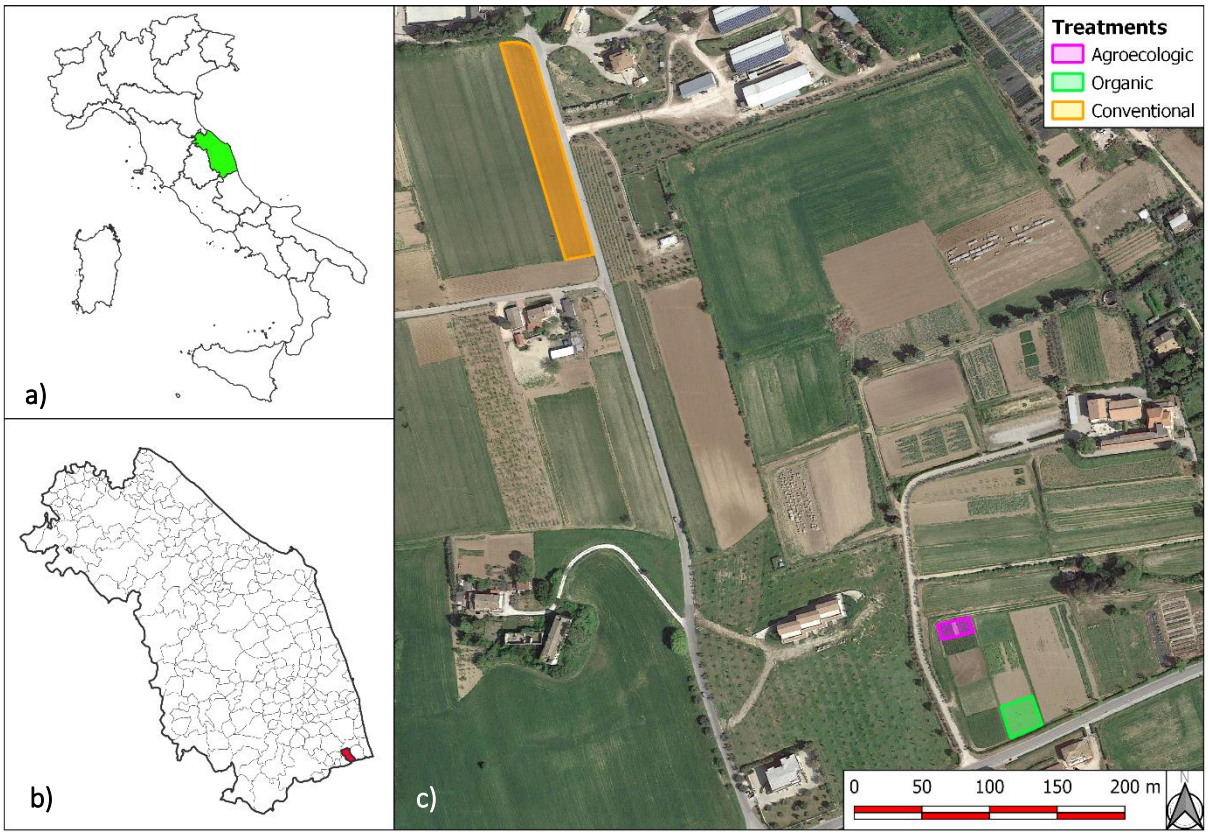

Figure 4.1 - a) Italian map including Marche region colored in green; b) experimental site location indicated by the red area; c) Detail of the experimental site plots (Google orthophoto).

The climate of the area is classified as thermo-mediterranean, characterized by mild-cool winters and hot summers and with cumulative annual precipitation and mean annual temperature referred to 2018 that are 789 mm and 15.7 °C respectively (Figure 4.2).

The soil of the area is classified as Typic Calcixerepts fine-loamy, mixed thermic (USDA, 1996) with a pH of 7.8 at the beginning of the experimentation in 2001 (Campanelli and Canali, 2012).

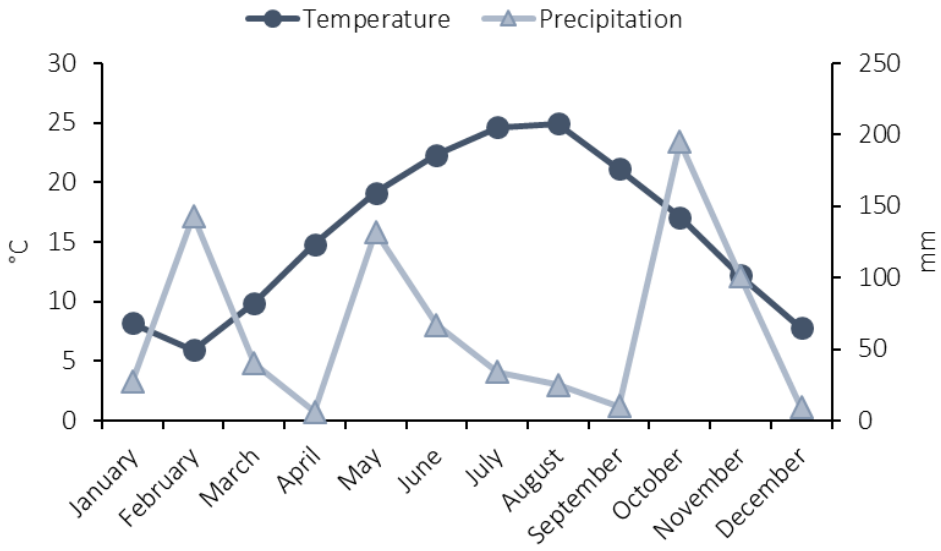

Figure 4.2 - Temperature (°C) and precipitation (mm) data referred to the year 2018. Data has been taken from the accuweather website.

4.2.2 The experimental design

The experimental site consists on a long-term comparison between conventional and organic management in which, in the organic management has been introduced the roller-crimper technique as agro-ecological practice (Figure 4.1).

Conventional agriculture

In the conventional plot soil was ploughed to a depth of 0.4 m and mineral NPK fertilizer were applied. The irrigation was carried out with the drop system (Burgio et al., 2015) and weeds were controlled by synthetic herbicides (Campanelli and Canali, 2012).

Organic agriculture

In the organic plots soil was tilled by rotary spader to a depth of 0.2 m and organic fertilizer selected in accordance to the European regulation were applied. The irrigation was carried out with the drop system (Burgio et al., 2015).

Agro-ecologic agriculture

The novelty that differentiate the agro-ecology management from the organic one has been the application of the roller-crimper technique to lie down the green manure crop in stripes. The machinery is a roller crimper with installed vertical disk or chisel that allows to flatten the cover crops, obtaining an adequate transplanting furrow without upsetting the mulch layer, which lasting on soil surface is able to protect the soil and control the weeds (Canali et al., 2013).

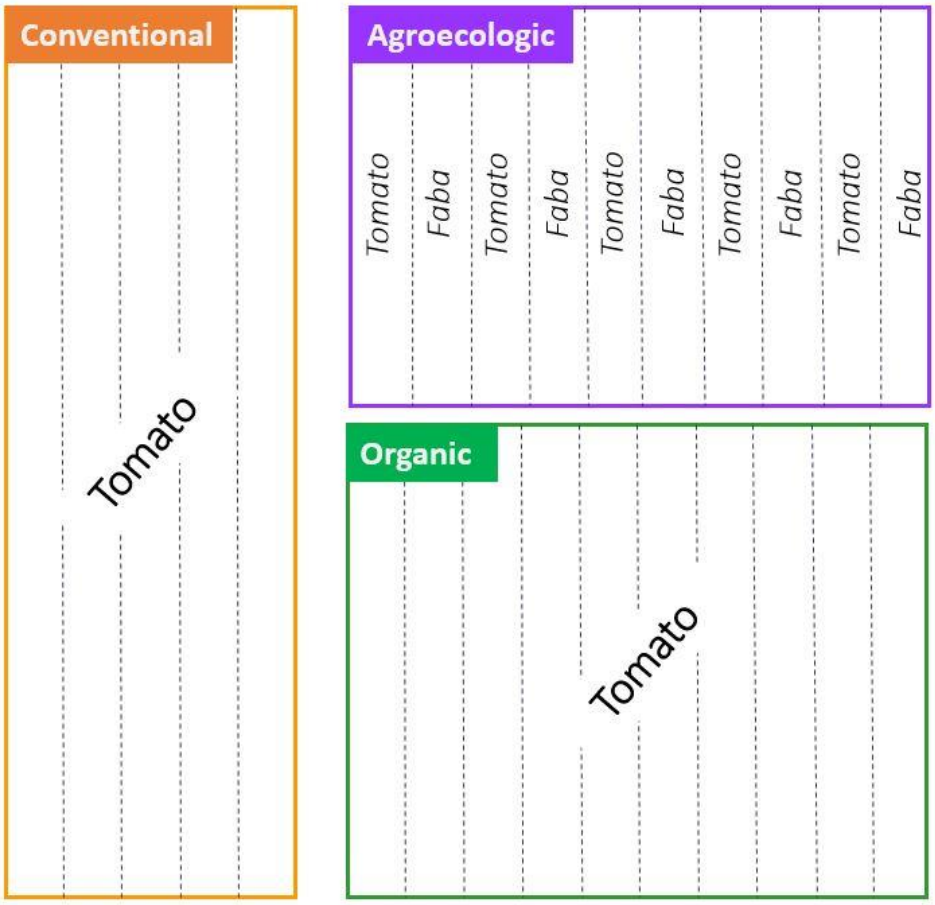

Figure 4.3 - Scheme of the experimental design.

In all the three management considered a four-year crop rotation has been established and it involved the following crops: tomato (*Lycopersicon esculentum* Mill.), melon (*Cucumis melo* L.), fennel (*Foeniculum vulgare* M. var. *azoricum*), lettuce (*Lactuca sativa* L.), cauliflower (*Brassica olaracea* L. var. *botrytis*), and bean (*Phaseolus vulgaris* L.) (Campanelli and Canali 2012).

In the organic and agro-ecology management have been included three different green manures: hairy vetch (*Vicia villosa* R.) or faba bean (*Vicia faba*) before the tomato, barley (*Hordeum vulgare* L.) before the melon and radish (*Raphanus sativus* L.) before the lettuce (Campanelli and Canali 2012).

The plots considered during the sampling were cultivated with tomato and in the agro-ecology management there were both tomato and faba bean arranged in alternated stripes with tomato that has been transplanted on lied down faba (Figure 4.3).

This intercropping with faba and tomato in the agro-ecology plot represent, with the roller-crimper technique, an innovative cultivation method. Indeed, in literature, has not been found reference to experimental site where two crops stand together, at the same time, in the field. Therefore, this kind of cultivation approach could be considered an innovation and one step closer toward the above mentioned agricultural sustainability.

4.2.3 Soil sampling

In June 2018, during tomato growing season, soil samples from the top 20 cm soil profile were taken along the tomato rows for a total of 24 soil samples. In each field the external rows were not sampled to avoid border effect due to neighboring crops. Fresh samples were kept in a cooler for transportation to the laboratory where they were sieved through to 2 mm; roots and plant residues were carefully removed by forceps. Samples were then homogenized and divided into three aliquots: one was air-dried, one was stored in plastic bags at 4 °C and the third was frozen.

The analysis of variance (ANOVA) has been performed as a one-way ANOVA with the management as factor and at the significance level of $P \leq 0.05$.

4.3 Results

4.3.1 Soil chemical and biochemical properties

Soil reaction was significantly affected by management with higher values in correspondence of the agro-ecologic plots (Table 4.1). No significant differences have been measured for the soil basal respiration even if the higher respiration rate has been registered in correspondence of the organic management (Table 4.1).

The ratio between extractable C and N highlight a significant deficiency of N with the agro-ecologic management (Table 4.1) indeed to this strategy correspond the higher extractable C:N ratio.

For which regard the available P content (P_{Olsen}) no significant differences has been measured although the lower values corresponded to the conventional managed plots (Table 4.1).

Table 4.1 - Marginal means of soil active reaction (pH), extractable carbon to nitrogen ratio ($C:N_{ext}$), microbial carbon to nitrogen ratio ($C:N_{mic}$), and soil basal respiration (SBR, mg $C\text{-}CO_2$ kg_{ds}^{-1} h^{-1}). Differences between treatments has been considered significant for $P<0.05$ and has been indicated with different lowercase letters.

	Management			
	AgroEc	Org	Conv	*p-value*
pH	8.49^{a}	8.37^{b}	8.34^{b}	*0.003*
$C:N_{ext}$	5.43^{a}	3.73^{b}	4.48^{ab}	*0.021*
$C:N_{MB}$	7.24	7.42	6.42	*0.320*
SBR	0.280	0.375	0.309	*0.087*
P_{Olsen}	21.5	21.5	16.8	*0.175*

Both organic and conventional management promoted extractable C and N (+16% and +42% respectively) compared with the agro-ecologic management (Figure 4.4). On the other hand, microbial biomass carbon content result enhanced by the agro-ecologic management with the lower content that correspond to the conventional management (-26% respect to agro-ecologic and organic management). The same trend has been observed also for the microbial biomass nitrogen (-15% respect to agro-ecologic and organic management) even if in this case differences are not statistically significant (Figure 4.4).

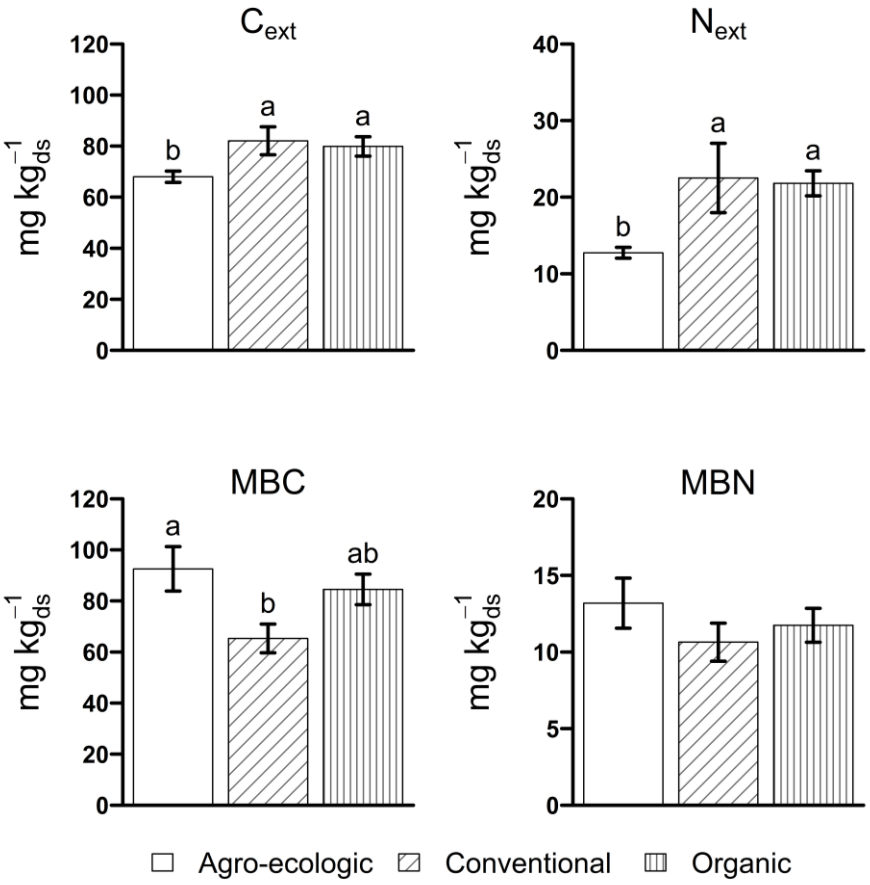

Figure 4.4 - Marginal means of extractable C (C_{ext}), extractable N (N_{ext}), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN). Error bars represent the standard error and different lowercase letters represent significant differences ($P< 0.05$).

Total organic carbon and total nitrogen content (Figure 4.5) showed the same trend with values that increase from conventional to agro-ecologic (+26% and +30% for total C and N respectively) to organic management (+37% for both total C and N).

Significant differences have been measured also for the $\delta^{13}C$, which showed values less negative with both agro-ecologic and organic management compared to the conventional one. Moreover, to the conventional management correspond the lower $\delta^{15}N$ content (-40%) compared to the other two agricultural strategies (Figure 4.5).

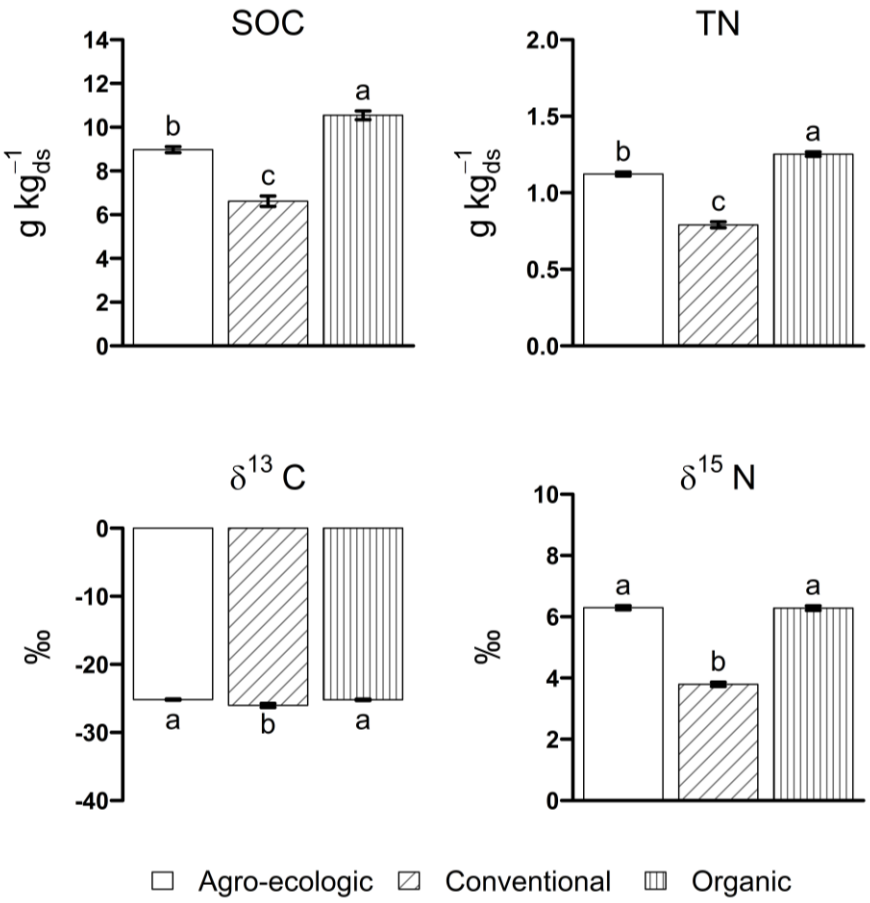

Figure 4.5 - Marginal means of total organic carbon (TOC), total nitrogen (TN), stable ^{13}C isotope ($\delta^{13}C$) and stable ^{15}N isotope ($\delta^{15}N$). Error bars represent the standard error and different lowercase letters represent significant differences ($P< 0.05$).

4.3.2 Soil enzyme activities

In figure 4.6 are reported the three oxidoreductase enzymatic activities measured. Dehydrogenase and laccase showed the lower activity in correspondence of the conventional management while the higher activity corresponded to the agro-ecologic once (+49% and +34% of dehydrogenase and laccase activities with the agro-ecologic compared to the mean of the organic and conventional management). On the other hand, tyrosinase activity was higher with the conventional management (+23% compared to the mean of the organic and agro-ecologic management) and decrease with the agro-ecologic and the organic.

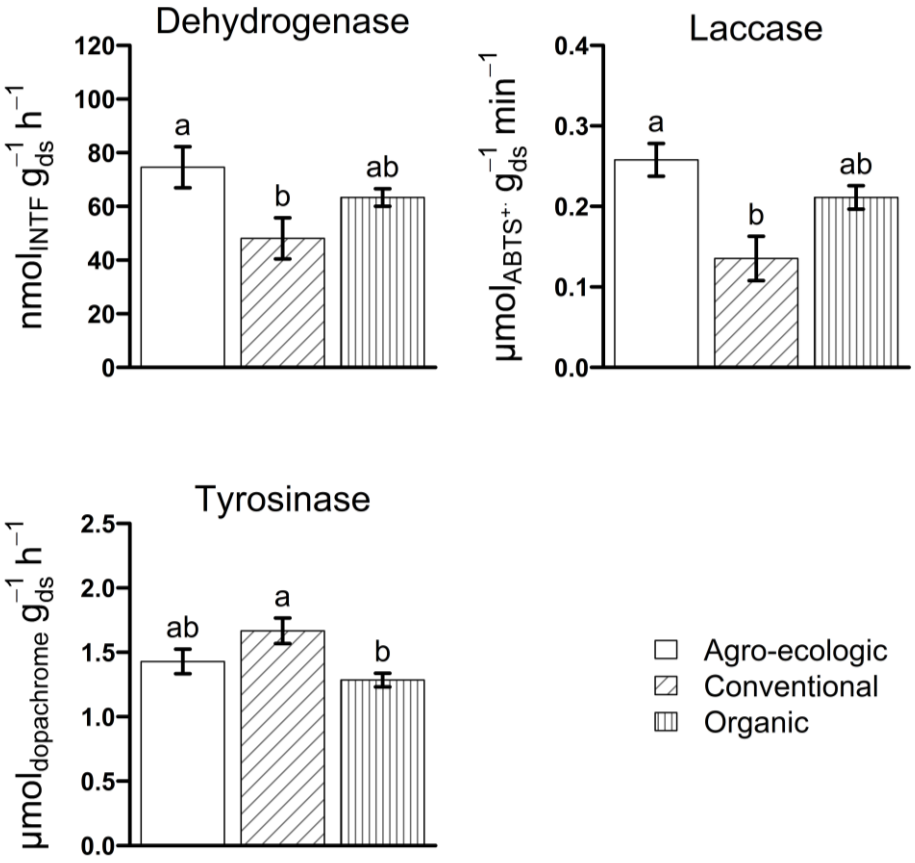

Figure 4.6 - Marginal means of dehydrogenase, laccase and tyrosinase activities. Error bars represent the standard error and different lowercase letters represent significant differences (P< 0.05).

In figure 4.7 has been reported the measured hydrolytic enzyme activities. The activities of β-glucosidase, β-cellobiosidase and N-acetyl- β-glucosaminidase resulted significantly affected by the management with higher values in correspondence of the organic one (+35%, +57% and +33% respectively). The same trend has been observed also for α-glucosidase activity (+15%) even if differences were not statistically significant. β-xylosidase showed the higher activity in correspondence of the organic management while it significantly decreased with conventional (-24%) and agro-ecologic (-32%) management. The phosphomonoesterase activity resulted significantly increased by both the organic and the agro-ecologic management, while the arylsulfatase activity decreased from the organic to the agro-ecologic management with the lower values measured in correspondence of the conventional management.

Finally, the lipase activity resulted to be the only hydrolytic activity enhanced by the conventional management (+73% compared to the other two management) with values that decrease from the organic to the agro-ecologic management.

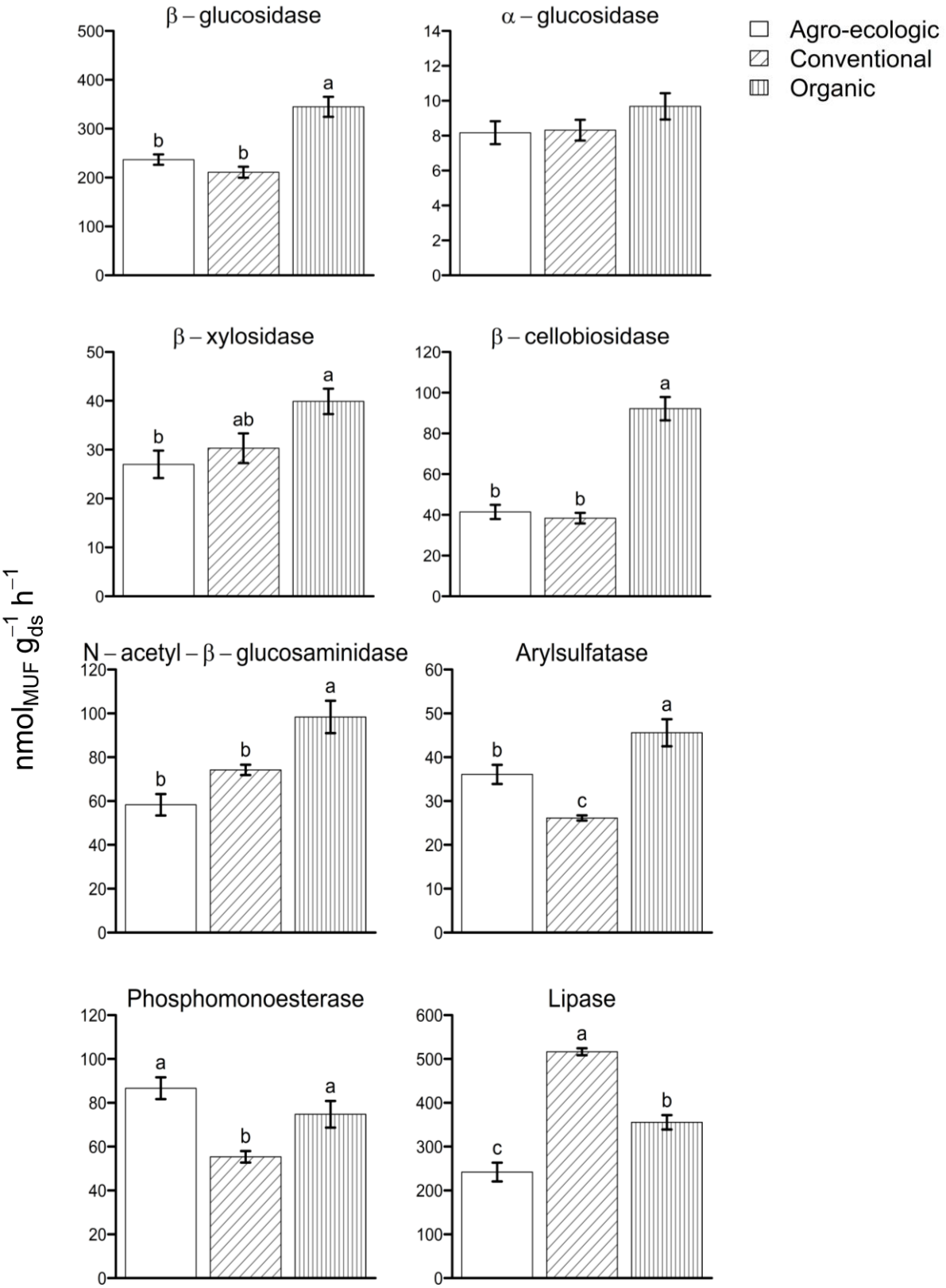

Figure 4.7 - Marginal means of β-glucosidase, α-glucosidase, β-xylosidase, β-cellobiosidase, N-acetyl-β-glucosaminidase, arylsuphatase, phosphomonoesterase and lipase activities. Error bars represent the standard error and different lowercase letters represent significant differences (P< 0.05).

4.3.3 Soil biochemical indexes

Of the measured biochemical indexes (Figure 4.8) only the metabolic index (MI) showed significant differences with the higher value corresponding to the agro-ecologic management (+60% compared to the mean of the organic and conventional management). However, the metabolic quotient (qCO_2) showed interesting results that are comparable to those of the MI with the lower qCO_2 measured in correspondence of the agro-ecologic managed samples (-33% respect to the other two management). The calculated values of the microbial quotient did not highlight significant differences between the management.

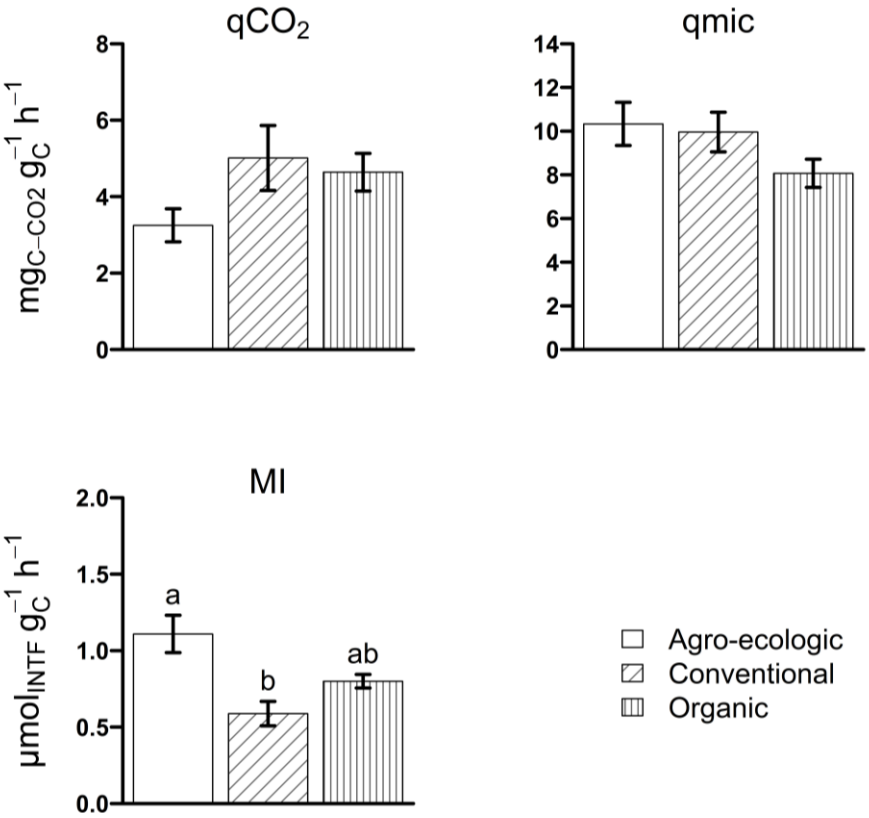

Figure 4.8 - Marginal means of the metabolic quotient (qCO_2), the microbial quotient (qmic) and the metabolic index (MI). Error bars represent the standard error and different lowercase letters represent significant differences ($P< 0.05$).

For which concern specific enzyme activities (Table 4.2), within the oxidative activities only the specific tyrosinase activity showed significant differences between management with higher values in correspondence of the conventional management (+65%). For what concern the specific hydrolytic activities, lower values have been calculated in correspondence of the agro-ecologic management with slightly differences between the organic and the conventional. Only specific lipase and α-

glucosidase activities showed higher values with the conventional instead of the organic management. Finally, the specific activities of dehydrogenase, lipase and phosphomonoesterase did not show differences that were statistically significant but the higher activities have been measured in correspondence of the agro-ecologic management.

Table 4.2 - Marginal means of dehydrogenase ($Dehy_{MBC}$, $\mu mol_{INTF}\ mg_C^{-1}h^{-1}$), laccase ($Lac_{MBC}$, $\mu mol_{ABTS}\ mg_C^{-1}\ min^{-1}$), tirosinase ($Tyr_{MBC}$, $\mu mol_{DOPA}\ mg_C^{-1}\ h^{-1}$), β-glucosidase (β-$Glu_{MBC}$, $\mu mol_{MUF}\ mg_C^{-1}h^{-1}$), α-glucosidase (α-$Glu_{MBC}$, $\mu mol_{MUF}\ mg_C^{-1}h^{-1}$), β-xylosidase (β-$Xyl_{MBC}$, $\mu mol_{MUF}\ mg_C^{-1}h^{-1}$), β-cellobiosidase (β-$Cel_{MBC}$, $\mu mol_{MUF}\ mg_C^{-1}h^{-1}$), N-acetyl-β-glucosaminidase (NAG_{MBC}, $\mu mol_{MUF}\ mg_C^{-1}h^{-1}$), arylsuphatase ($AS_{MBC}$, $\mu mol_{MUF}\ mg_C^{-1}h^{-1}$), phosphomonoesterase ($PME_{MBC}$, $\mu mol_{MUF}\ mg_C^{-1}h^{-1}$) and lipase ($LIP_{MBC}$, $\mu mol_{MUF}\ mg_C^{-1}h^{-1}$) specific activities. Error bars represent the standard error and differences between treatments has been considered significant for $P<0.05$ and has been indicated with different lowercase letters.

	Management			
	AgroEc	Org	Conv	*p-value*
$Dehy_{MBC}$	0.820	0.781	0.790	*0.963*
Lac_{MBC}	2.86	2.57	2.13	*0.271*
Tyr_{MBC}	16.1^{b}	16.1^{b}	26.5^{a}	*< 0.001*
β-Glu_{MBC}	2.67^{b}	4.24^{a}	3.42^{ab}	*0.015*
α-Glu_{MBC}	0.090^{b}	0.117^{ab}	0.134^{a}	*0.029*
NAG_{MBC}	0.644^{b}	1.19^{a}	1.20^{a}	*< 0.001*
β-Xyl_{MBC}	0.303^{b}	0.485^{a}	0.482^{a}	*0.012*
β-Cel_{MBC}	0.462^{b}	1.13^{a}	0.628^{b}	*< 0.001*
AS_{MBC}	0.407^{b}	0.552^{a}	0.419^{b}	*0.020*
PME_{MBC}	0.971	0.901	0.876	*0.572*
LIP_{MBC}	2.79^{b}	4.45^{b}	8.28^{a}	*< 0.001*

4.3.4 Correlation between measured parameters

A correlation plot including all the considered soil quality indicators is reported in Figure 4.9. Soil reaction is positively correlated with the microbial biomass and with laccase, dehydrogenase and phosphomonoesterase activities. For which concern the biochemical indexes, the microbial quotient resulted negatively correlated with the metabolic quotient and with β-glucosidase, N-acetyl-β-glucosaminidase, β-xylosidase and β-cellobiosidase activities, instead it is positively correlated with the microbial biomass, the metabolic index and the laccase activity. The metabolic quotient resulted negatively correlated with microbial biomass C and N and presented positive correlation only with N-

acetyl-β-glucosaminidase and lipase activities. Finally, the metabolic index showed a positive correlation with the activities of laccase, dehydrogenase and phosphomonoesterase.

The oxidative enzymes showed a different behavior indeed, while the laccase activity resulted positively correlated with microbial biomass, soil organic carbon, available phosphorus and nitrogen content, the tyrosinase activity showed negative or no correlation with these parameters. These two activities showed a different behavior also in relation to the hydrolytic enzyme activities.

With the exception of lipase, the other hydrolytic enzyme activities resulted positively correlated with both soil organic C content and total N content. Moreover, the activities of β-glucosidase, α-glucosidase and β-cellobiosidase showed no correlation with the extractable and microbial C and N pools. Instead, N-acetyl-β-glucosaminidase, β-xylosidase and phosphomonoesterase resulted to be positively correlated with both C_{ext} and N_{ext}, but only the phosphomonoesterase was positively correlated also with MBC, MBN and P_{Olsen}.

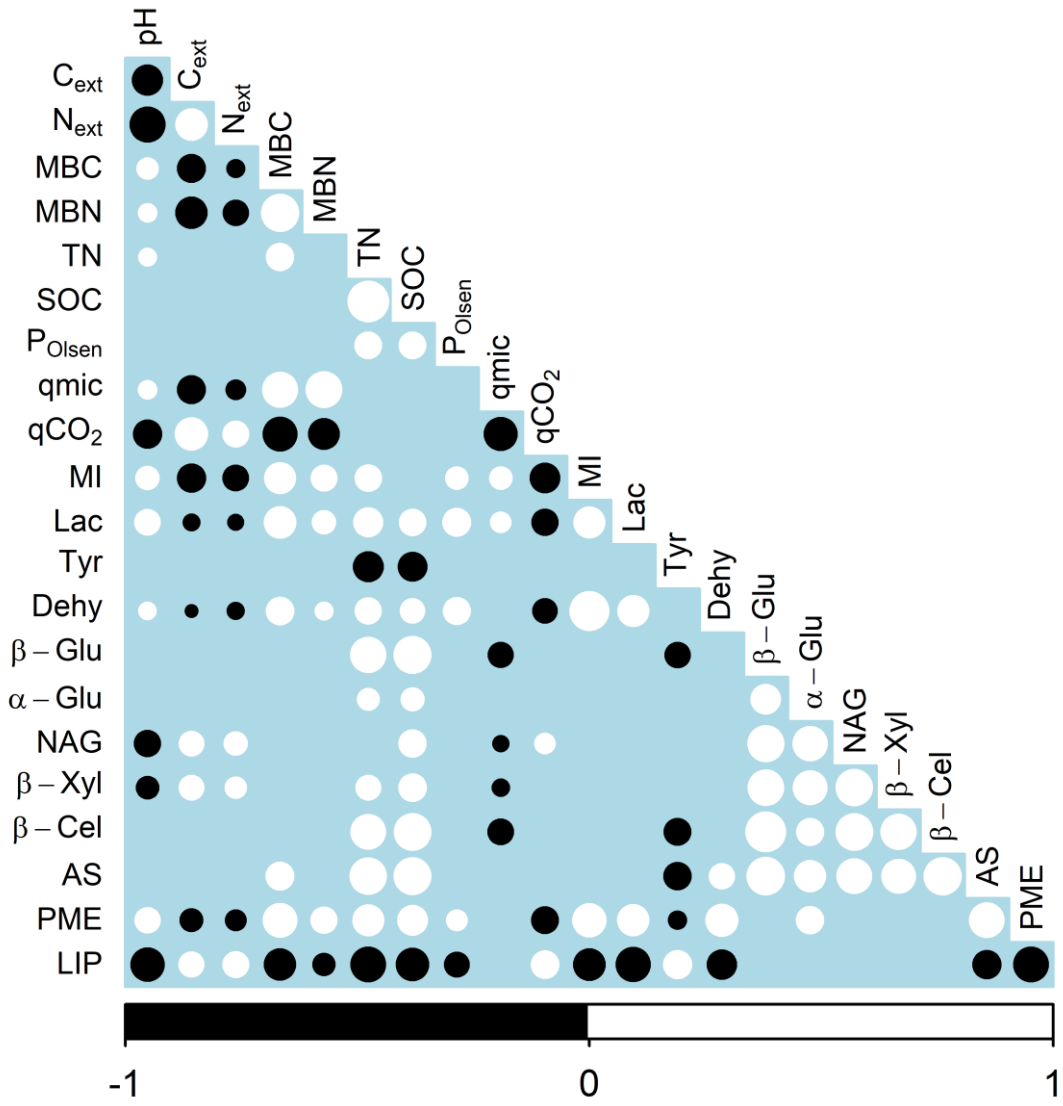

Figure 4.9 - Correlation plot including all the considered soil properties.

4.3.5 Principal Component Analysis

A principal component analysis (PCA) was carried out with C and N pools, available P, and the enzyme activities (Figure 4.10) for the management considered. The first two principal components accounted for the 39% and 22% of the total variance, respectively.

Conventional, organic and agro-ecologic management clearly separate and resulted characterized by different parameters indeed, while the majority of the hydrolytic activities defined the organic management with SOC and TN, the microbial biomass with the activities of dehydrogenase, tyrosinase and phosphomonoesterase characterized the agro-ecologic management. Lipase and laccase activities resulted to be the only two parameters that significantly account for the conventional management.

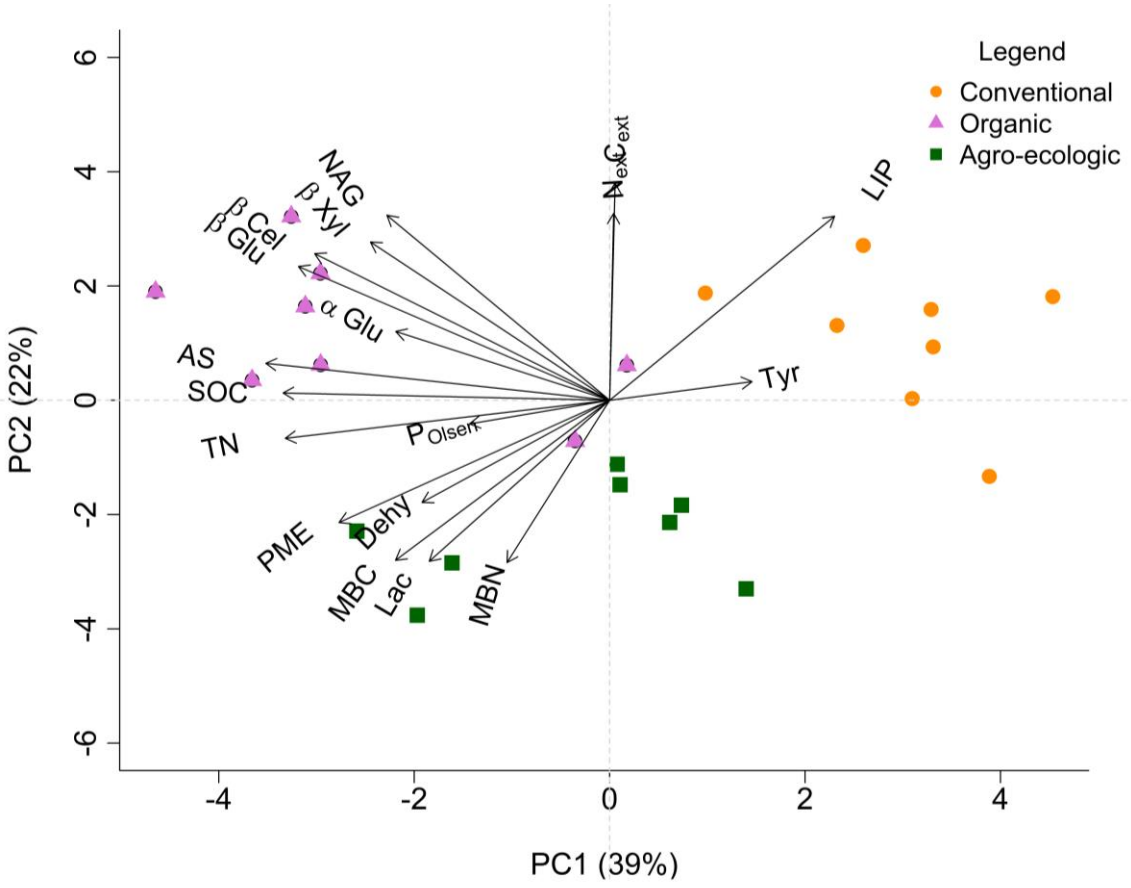

Figure 4.10 - PCA plot with C and N pools, available P, and the enzyme activities for the management considered.

Table 4.3 - Correlation between the two principal component of the PCA and the parameters included in the PCA analysis.

	PC1	PC2
C_{ext}	0.02	0.76***
N_{ext}	0.01	0.65***
MBC	-0.59**	-0.56**
MBN	-0.30	-0.57**
SOC	-0.88***	0.02
TN	-0.88***	-0.14
P_{Olsen}	-0.38	-0.08
Lac	-0.47*	-0.55**
Tyr	0.41*	0.08
Dehy	-0.49**	-0.35
β-Glu	-0.85***	0.46**
α-Glu	-0.58**	0.23
NAG	-0.63***	0.63***
β-Xyl	-0.65***	0.55**
β-Cel	-0.81***	0.50**
AS	-0.94***	0.12
PME	-0.73***	-0.43*
LIP	0.60**	0.64***

Significant correlations between PC1 and the original variables were observed for SOC, TN and the activities of tyrosinase, dehydrogenase, α-glucosidase and arylsulfatase indicating that these variables contributed more to the separation of the samples along this component (Table 4.3). Similarly, for the PC2 significant correlation has been observed for soil extractable C and N and microbial biomass N. The other parameters considered significantly correlated with both PC1 and PC2.

4.3.6 Hierarchical clustering

The hierarchical clustering has been carried out taking into account the values of C and N pools, available P and the enzyme activities. From the clustering analysis (Figure 4.11) emerged a clear separation of the agro-ecologic management which seems to be closer to the organic management. This latter present two samples that resulted to be similar to those of the conventional management thus not permitting a clear and net separation between organic and conventional management.

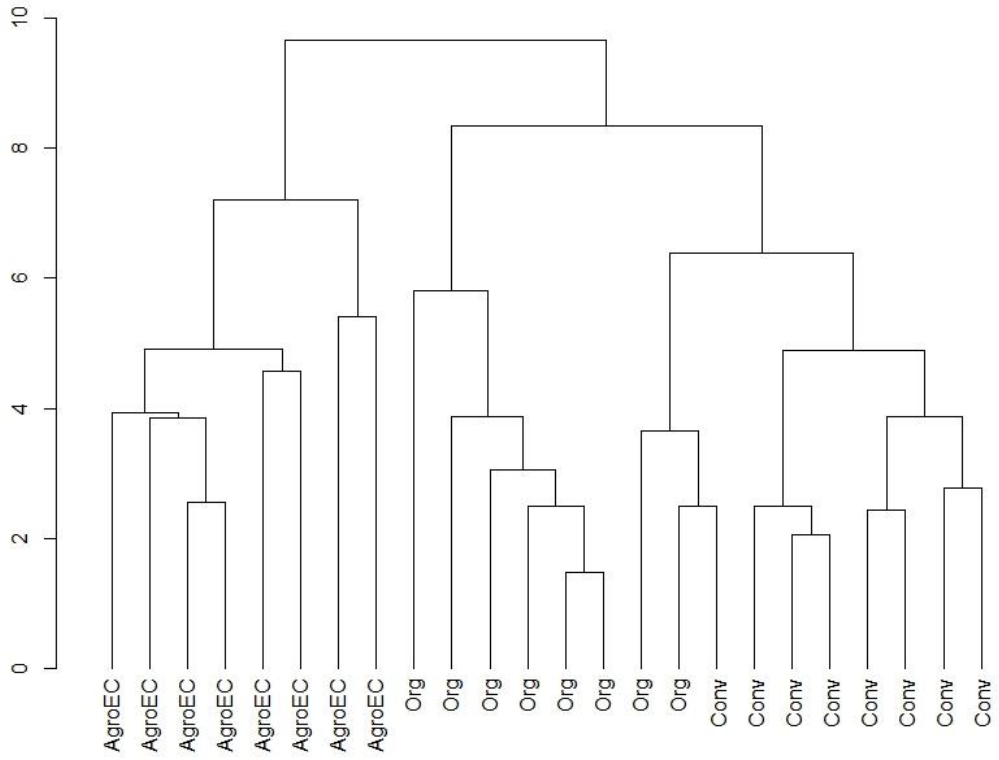

Figure 4.11 – Dendrogram of the hierarchical clustering made on the base of C and N pools values, and hydrolytic and oxidative enzyme activities.

4.4 Discussion

The organic and mineral fertilizer applied in the organic and conventional managed plots, as expected, lead to an increase of the available C and N content (Figure 4.4) which resulted to be the same with the two management. Instead, the other C and N pools considered showed differences also between organic and conventional management. Indeed, as previously observed in chapter 2, also at this experimental site the organic management lead to higher values of SOC and microbial biomass compared to the conventional one (Figures 4.4 and 4.5).

On the other hand, the agro-ecology management favored the microbial C and N content even more then the organic management (Figure 4.4), thus confirming that the adoption of the agro-ecology could effectively be a positive solution for soil microbial community (Lemanceau et al., 2014). Moreover, despite the low content of the $C_{ext,}$ the agro-ecology strategy positively favored SOC storage that showed values (Figure 4.5) higher than those measured in correspondence of the conventional management.

It is possible to suppose that the observed differences in SOC content between organic and agro-ecologic management could be linked to the input of C entering the soil. Indeed, while the organic management involves the addition of organic fertilizer to the soil, the agro-ecology management only relies on the intercropping with the faba and probably this agronomic difference could be the source of the different SOC content with this two type of management. Consequently, following this reasoning and considering the equally high content in C_{ext} in correspondence of the conventional management, one would suppose that even with conventional management a greater SOC content should be measured with respect to agro-ecological management. Instead, the lower SOC content has been measured in the conventionally managed plot and this could be explained observing the biochemical indexes values (Figure 4.8). Indeed, the increase of the qCO_2 index and the decrease of the MI index in correspondence of the conventional management, let suppose that the microbial community was in a stressed (Balota et al., 2004; Laudicina et al., 2011) and less efficient (Masciandaro et al., 1998) condition. On the other hand, the increasing value observed for the SOC content in correspondence of the agro-ecology management, despite of the scarce C input to the soil, could again be justify with the biochemical indexes (Figure 4.8). Indeed, within the agro-ecological management has been calculated the lower qCO_2 value and the higher MI value thus indicating that this type of agronomical management led to better soil condition for the microbial community activity.

Observing the values on available C and N content, it was expected to measure similar hydrolytic enzymes activities for both organic and conventional managed plots with major differences for the agro-ecology management. Instead, the activities of the enzymes involved in C and N cycles (β-glucosidase, α-glucosidase, β-xylosidase, β-cellobiosidase and N-acetyl-β-glucosaminidase) resulted to be higher in correspondence of the organic managed plot and significantly different in comparison to those of the conventional and agro-ecological managements. However, it is worth considering that in correspondence of the conventional management has been measured the lower soil microbial biomass content and thus it could be more appropriate to consider the specific hydrolytic enzyme activities (Table 4.2). Indeed, the ratio between soil enzyme activities and MBC could provide more information on microbial activity (Gil-Sotres et al., 2005) as, for example, increasing ratio could indicate increased enzymes production or increased release by microorganisms or by clay/humic colloids (Gil-Sotres et al., 2005; Kandeler and Eder, 1993). Within this experimental site, soil specific hydrolytic enzyme activities involved in C and N cycles highlight that both conventional and organic management were inducing the activation of the immobilized enzymes or the enzymes production by the microbial community. Moreover, it has been confirmed for those enzyme activities that the lower activities corresponded to the agro-ecology strategy thus indicating one more time that this type of management was favoring soil microbial community. Continuing with the hydrolytic enzyme activities, arylsuphatase and phosphomonoesterase showed the lower activities in correspondence of the conventional management with slightly or no differences between organic and agro-ecological management. These results (confirmed also by the specific enzymes activities, table 4.2) strictly reflect the mineral fertilization applied with the conventional management that probably covered soil microbial biomass need in P and S.

Concerning oxidative enzyme activities, it resulted that while dehydrogenase and laccase activities showed the same trend across management with higher values in correspondence of the agro-ecological once, tyrosinase activity resulted to be higher with the conventional management. Differently from what observed in chapter 2 and in the study of Mazzon et al. (2018), in this case tyrosinase instead of laccase activity is the one that showed no correlation with the most of the measured parameters. Particularly interesting resulted to be laccase activity as it is the third enzymatic activity that presented higher values in correspondence of the agro-ecological management. Therefore, analyzing all the measured and calculated parameters under the agro-ecological management, where there is the higher content of microbial biomass, it seems that the

microbial community is in a situation of "well-being" (qCO_2 and MI) and that it is highly specialized in the production/activation of phosphomonoesterase (PME), dehydrogenase (Dehy) and laccase (Lac) activities. The PME is the only hydrolytic activity that resulted higher with the agro-ecologic management and considering that has been measured similar values of P_{Olsen} content within the management, it is possible to assess that with the agro-ecological (and organic) management P was a limiting factor (Acosta-Martínez and Tabatabai, 2011). At this point it is already possible to deduce that within the agro-ecological management the microbial activity is principally focused on the oxidative processes. Moreover, it seems convenient and with low energy consumption for microorganisms produce or activate laccases than other enzymes and this could be related with the low content of N_{ext} measured in the agro-ecological managed plot. Indeed, Sinsabaugh (2010) sustained that one of the factors controlling phenol oxidazing enzyme activities is N availability.

Furthermore, as previously observed (Saiya-Cork et al., 2002; Sinsabaugh et al., 2002; Sinsabaugh, 2010; Zeglin et al., 2007), hydrolytic and oxidative enzymes showed different responses to the applied management and in the PCA they are clearly separated. Moreover, from the PCA resulted a sharp differentiation also between laccase and tyrosinase activities which also resulted to be negatively correlated each other.

Finally, both the PCA (Figure 4.11) and the dendrogram (Figure 4.12) display a clear separation between the three agronomic management considered thus indicating that their effects on soil biochemical properties are effectively different. Moreover, it is possible to observe from both the figures that the organic management stay in a middle position between conventional and agro-ecological.

4.5 Conclusion

At the end it is possible to conclude that, as for the organic management, the agro-ecological management could be considered a good choice in term of soil fertility and functionality.

It has been hypothesized to find the higher SOC content in correspondence of the agro-ecological management instead it has been measured in correspondence of the organic management. This result could be explained by the lower C input entering the soil with the agro-ecological management and by the higher laccase activity measured in this plot. Indeed, it is known that laccases generally degrade the recalcitrant organic matter or the stabilized organic C (Bach et al., 2013; Sinsabaugh, 2010). However, even if the obtained results were not perfectly in accord with this hypothesis, it is to underline that the lower SOC content was relative to the conventional management and therefore the agro-ecologic one could still be considered a good agronomic choice from this point of view.

Moreover, it has been supposed to find the lower enzymatic activities (both hydrolytic and oxidative) in correspondence of the agro-ecological managed plot. This supposition was principally true for the hydrolytic activities but not for the oxidative once. Indeed, it seems that the maintained soil functionality under the agro-ecological and the good SOC content suppressed the hydrolytic activities as expected, while the particularly low N_{ext} content probably could be considered the drivers for the increased laccase activity.

Concluding, it is possible to assert that also the agro-ecological management could be considered a valid type of agronomic management for the maintenance and the improvement of soil functionality and fertility. Clearly, other experimental field are needed to deeper the knowledge on this topic. Another important point for future research could be include in the study also the yields so that it could be possible to have a complete study of sustainability of the agronomic management.

CHAPTER 5

NITROGEN DEPOSITION IN A EUTROPHIC BEECH FOREST: PRELIMINARY RESULTS OF THE EFFECTS ON SOIL BIOCHEMICAL INDEXES AND SOIL FUNCTIONALITY

5.1 State of the art

Human impact not only directly undermines agroecosystems but unfortunately it could also have a direct and/or indirect impact on forest ecosystems.

Indeed, nitrogen (N) input in terrestrial ecosystems is a relevant topic, especially from an ecological point of view, as it could produce an increase in atmospheric nitrous oxide concentration which contribute to the global warming (Vitousek et al., 1997). During the last decades, anthropogenic activities such as industry, vehicular traffic, combustion and agricultural fertilization (Bobbink et al., 2010), were the main causes of the emission into the atmosphere of nitrogenous compounds such as NO, N_2O and NH_3 (Frey et al., 2004; Vitousek et al., 1997). Moreover, the oxidation of NO produce nitric acid which is the main component of acid rain and NH_3, as well, is an acid agent in the atmosphere influencing the pH of aerosol and rainfall (Vitousek et al., 1997). It was estimated (Manning, 2012) that total NH_y and NO_x depositions worldwide increased from 34 Tg N yr^{-1} in 1860 to 100 Tg N yr^{-1} in 1995; and it is expected that in 2050 these depositions will increase up to 200 Tg N yr^{-1}.

Specifically, increasing N depositions significantly damage the vegetation through the decline of species richness, toxicity, changes in soil-plant feedback, and soil acidification favoring alterations in microbial communities and reducing radical colonization (Bobbink et al. 2010; Hess et al., 2018). Already in 1992, Bergkvist and Folkeson wrote, "*During the last decades the situation has changed, and atmospheric N deposition now contributes N in quantities well above the demand of forests in much of Europe*." and observed that atmospheric N deposition had as consequence a lack of biomass productivity response to N supply. Considering this and Manning (2012) predictions, it is possible to suppose that nowadays there is a situation of "N excess" in many European forest soils.

These changes negatively affect all the ecosystem services related to soil-plant system (Manning, 2012), because N impacts plants and soil microbes, both involved in the primary production and decomposition processes (Carreiro et al., 2000).

Many studies have been done on mixed forest ecosystems with the aim to observe soil activity response to increasing N deposition (Carreiro et al. 2000; Compton et al., 2004; DeForest et al. 2004; Fatemi et al., 2016; Frey et al. 2004; Magill et al., 2004; Saiya-Cork et al., 2002; Waldrop et al., 2004; Wallenstein et al., 2006). Results highlight that prolonged N excess increases soil acidity that could has an impact on soil element availability (Bergkvist et al. 1992) and on microbial community, inducing changes in carbon (C) and N cycling processes (i.e. lignin degradation, decomposition processes, nitrification and organic matter mineralization) (Compton et al., 2004). In 1988, Fog studied the implication of N enrichment in

forest ecosystems and found that N effects on soil decomposition processes depend on litter lignin content, and in presence of recalcitrant plant litter, N addition could reduce the decomposition processes. This reduction could be explained by the decrease of extracellular enzymes efficiency or the inhibition of ligninolytic enzymes (Carreiro et al. 2000; DeForest et al. 2004; Frey et al. 2004; Fog, 1998; Wallenstein et al. 2006). Therefore, depending on litter and site characteristics, N surplus could induce a modification in the soil microbial community and in the production and activity of cellulolytic, ligninolytic and phenol oxidizing enzymes (Carreiro et al. 2000; DeForest et al. 2004; Saiya-Cork et al. 2002).

Consequently, the increase of N could affect in different ways every stage of the decomposition process because, as suggested by Fog (1988), N supply could increase the early stages (decomposition of labile material) and repress the later ones when recalcitrant material (e.g. lignin) degradation occur. These changes have consequence on the formation and stabilization of soil organic matter (SOM) (Carreiro et al. 2000; Frey et al. 2014) and thus also on soil organic C (SOC) storage (Fatemi et al., 2016). Specifically, it was found that long-term N supply or high level of N atmospheric deposition lead an increase of soil C storage (Frey et al., 2014; Magnani et al., 2007).

Soil processes related to SOM decomposition, SOC cycling and N mineralization are strictly dependent on microbial community composition (Finn et al., 2016) and, consequently, on enzymatic activities (Herold et al., 2014), which mediate all these processes. Therefore, to observe N effects on soil dynamics is relevant to study microbial activity through the determination of a wide group of extracellular enzymatic activities, considering enzymes involved in earlier decomposition stages and others involved in the later ones (Fog, 1988). This consideration appear to be of particular importance especially in the case of forest ecosystems because plant diversity and, consequently, litter properties, with substantial N deposition, drive the dynamics between litter decomposition and enzymes production and activity (Waldrop and Sinsabaugh 2004).

5.1.1 Aim of the work

With the purposes to observe N deposition effects on forest ecosystems through enzymatic activities study, it has been chosen a deciduous temperate forest of beech (*Fagus sylvatica L.*) located in North Italy as object of study. This could be considered a novelty as the previous studies focused on forests located at latitudes much northern than that of this experimental site or on mixed forests. Moreover, attention has to be paid to the size of N depositions relative to the experimental site considered. Indeed, Marchetto et al. (2014) reported that, in this area, total N deposition, comprising nitrates, ammonium and organic N, reaches values of 16.6 kg N ha^{-1} yr^{-1} under the canopy and along the trunk, and of 20.5 kg N ha^{-1} yr^{-1} in the open. These are quite high values, essentially linked to the heavy rainfall of the site, which far exceed beech critical load, which is placed around 10-15 kg N ha^{-1} yr^{-1} (Bobbink et al., 2010). Therefore, the aim of the work is to evaluate soil functionality through the study of soil enzymatic activities and soil biochemical indexes after N supply in a context characterized by a eutrophic pure beech forest. Specifically, it has been hypothesized that N addition would induce (i) a decrease in the microbial biomass C content, (ii) a reduction of both hydrolytic and oxidative enzymatic activities and (iii) an increase in SOC content.

5.2 Experimental site description

5.2.1 Area of study

The experimental site (Figure 5.1) is located in a beech forest at Pian del Cansiglio (Veneto region, Italy), in the south-west sector of Cansiglio upland (46.06° N, 12.38° E, and 1130 m above sea level). This upland has a particular "basin" shape due to its different altitude of the bottom (1000 m) in respect to the peak all around (1400 m). The "basin" shape has some implication on the climate of the area; indeed, a thermal inversion occurs with condition that is more continental in the plain but mainly oceanic on the slopes.

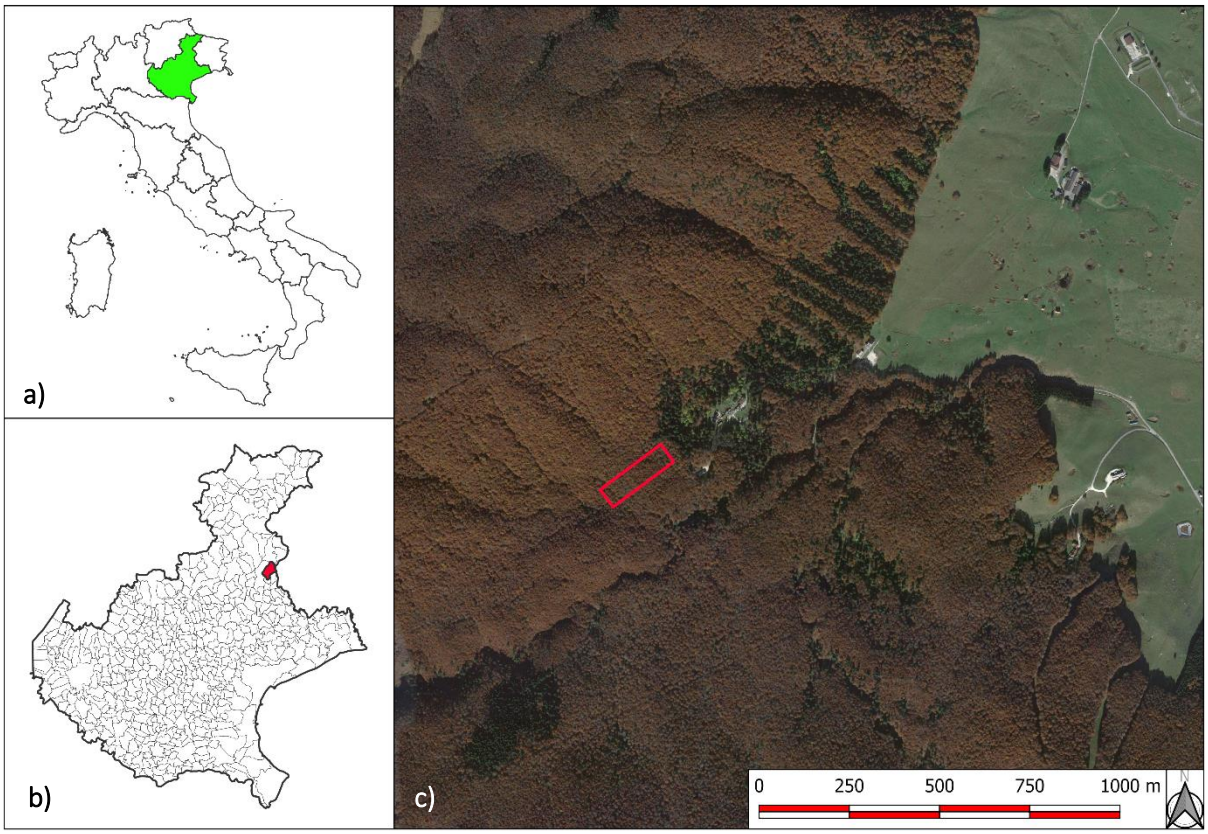

Figure 5.1 – a) Italian map including Veneto region colored in green; b) the beech forest site indicated by the red area; c) Detail of the area (surrounded by a red square) in which the experimental site is loacated (Google orthophoto).

Mean annual precipitation and temperature (Figure 5.2) are 1900 mm and 5 °C respectively, with the minimum temperature in January (-3.8°C) and the maximum in July (15.4°C). Therefore, the climate of the area is classified as continental with cold winters and cool summers.

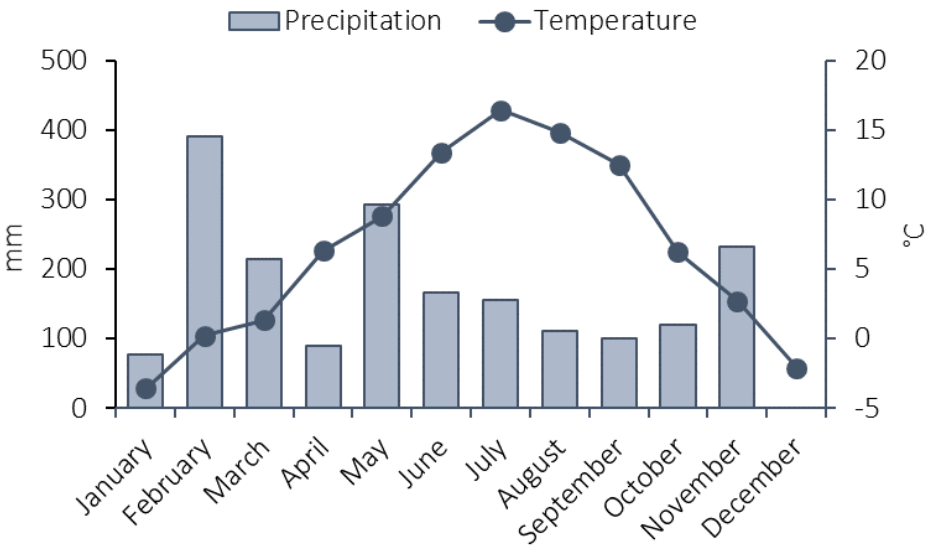

Figure 5.2 - Temperature (°C) and precipitation (mm) data referred to the year 2016.

The soil is classified as Halpic Luvisols (FAO), with accumulation of clay and a simple, normal horizon sequence (O, A, AE, B1t, B2t) and with a pedogenetic substrate consisting of sedimentary rocks of limestone. The soil is acid in surface and more neutral in depth; Marchetto et al., (2014) reported that in 2009 the pH values ranged between 5.4 and 5.8 for the surface horizons (0 – 0.40 m).

Concerning the biocenosis, the dominant vegetation is represented by beeches belonging to the species *Fagus sylvatica L.*, which cover more than 95% of the entire area, constituting a timber forest (approximately 120-145 years). Surely, this massive beech development has been linked to human management of the forest. Nevertheless, the predominance of this species could be also related to its remarkable ecological plasticity, thus showing a surprising grow and expansion regardless of substrate nature. Indeed, the species has no particular need for nutrients, as well as shows wide tolerance to pH.

5.2.2 The experimental design

Within this site, have been established nine 30 x 30 m experimental plots with a complete randomized design (Figure 5.3). Three plots serve as control (0 kg N $ha^{-1}yr^{-1}$, only natural deposition), other three plots received ambient N deposition plus 30 kg N $ha^{-1}yr^{-1}$ (N30) and the last three plots received ambient N deposition plus 60 kg N $ha^{-1}yr^{-1}$ (N60). Since 2015, NH_4NO_3 solution has been sprayed on soil surface three times per year in June, July and August.

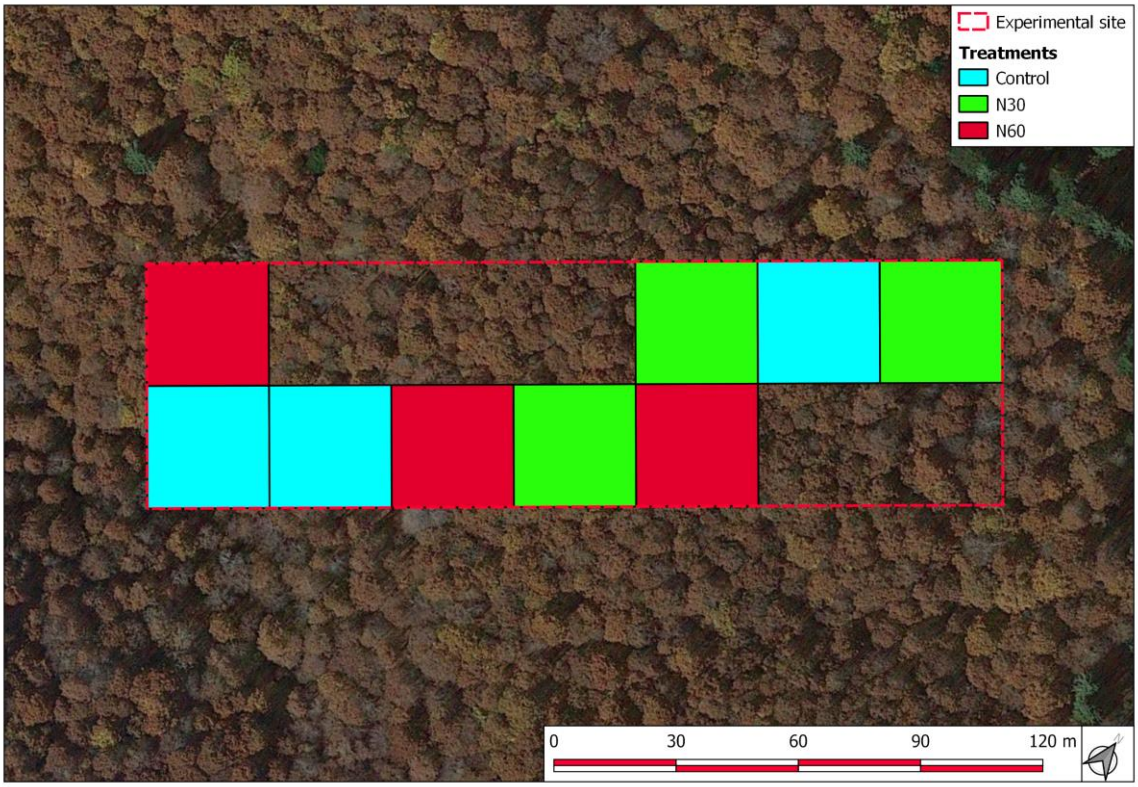

Figure 5.3 - Scheme of the experimental design (complete randomized design) with the applied treatments.

5.2.3 Soil sampling

In June 2016, before the first treatment of the year, soil samples from the top 0.25 m soil profile were taken within an area of 15 x 15 m from each plot (six sub-samples for every plot) using a soil core sampler with diameter of 55 mm and length of 0.50 m. Each sample was divided into two subsamples (approximately 300 g of soil each one): the first was representative of the 0-0.10 m of soil (A horizon) and the second of the 0.10-0.22 m of soil (AE horizon). In total, 18 soil composite samples were collected. Fresh samples were kept in a cooler for transportation to the laboratory where they were sieved through to 2 mm; roots and plant residues were carefully removed by forceps. Samples were then homogenized and divided into two aliquots: one was air-dried while the other was stored in plastic bags at 4 °C.

The analysis of variance (ANOVA) has been performed for a nested design with the N treatment as the main factor and the horizon as the subsampling factor. The ANOVA has been carried out at the significance level of $P \leq 0.05$.

5.3 Results

5.3.1 Soil chemical and biochemical parameters

Soil pH in water ranged from 4.30 to 4.83, with major differences from A to AE horizon (Table 5.1), indicating that soil acid condition not only concern treated plots but also control once. Clear differences between the two considered soil horizons came to light also for the available phosphorus and for the basal respiration (Table 5.1) with higher values in correspondence of the A horizon. Even if it is not statistically significant it is noticeable the decrease in P_{Olsen} content with the N60 treatment.

Table 5.1 - Marginal means and of soil active reaction (pH), extractable carbon to nitrogen ratio ($C:N_{ext}$), microbial carbon to nitrogen ratio ($C:N_{MB}$), total nitrogen (TN, g kgds-1), soil organic carbon to total nitrogen ratio (C:N), stable carbon isotope ratio ($\delta^{13}C$, ‰), available phosphorus (P_{Olsen}, mg kg_{ds}^{-1}), soil basal respiration (SBR, mg $C\text{-}CO_2$ kg_{ds}^{-1} h^{-1}). Differences between treatments has been considered significant for P<0.05 and has been indicated with different lowercase letters.

Treatments	pH	$C:N_{ext}$	$C:N_{MB}$	TN	C:N	$\delta^{13}C$	P_{Olsen}	SBR
Fertilizations								
Control	4.63	7.94	7.86	2.90	11.0	-26.0	6.27	0.479
N30	4.56	8.18	6.70	2.59	10.7	-26.1	7.09	0.509
N60	4.56	9.51	6.77	2.58	10.9	-26.0	4.79	0.409
p-value	*0.722*	*0.556*	*0.456*	*0.534*	*0.258*	*0.693*	*0.196*	*0.286*
Fertilization x Horizon								
Control (A)	4.50^{ab}	6.38^{c}	7.71	3.75^{a}	11.6^{a}	-26.1^{ab}	9.41^{a}	0.679^{a}
Control (AE)	4.76^{a}	9.49^{b}	8.02	2.06^{b}	10.4^{bc}	-25.9^{ab}	3.13^{b}	0.280^{b}
N30 (A)	4.35^{b}	6.52^{c}	6.31	3.45^{a}	11.3^{ab}	-26.3^{b}	11.3^{a}	0.745^{a}
N30 (AE)	4.76^{a}	9.84^{b}	7.08	1.74^{bc}	10.0^{c}	-25.8^{a}	2.86^{b}	0.274^{b}
N60 (A)	4.30^{b}	7.28^{c}	6.64	3.57^{a}	11.3^{ab}	-26.1^{ab}	7.67^{a}	0.636^{a}
N60 (AE)	4.83^{a}	11.7^{a}	6.91	1.58^{c}	10.4^{bc}	-25.8^{a}	1.90^{b}	0.182^{b}
p-value	*<0.001*	*<0.001*	*0.40*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

The stable nitrogen isotope ratio ($\delta^{15}N$) values confirmed that N treatment effectively reach the mineral soil (Figure 5.4). Indeed, $\delta^{15}N$ showed an increase with both the N30 and the N60 treatment at both the depth considered. Concerning to extractable C (Figure 5.4), in the surface horizon, has been measured a high concentration (400-500 mg kg^{-1}) than the subsurface horizon (200 mg kg^{-1}). Moreover, an increasing concentration (+22%) was observed from the no-treated control to the N60

treatment. The same tendency, in the A horizon, was observed for the extractable N content (+16%) even if in this case differences are not statistically significant (Figure 5.4). Regarding the extractable C and N ratio ($C:N_{ext}$), regardless of the soil horizon, it showed an increase from control to N60 treatment (+11%), which means that N supply enhanced soil extractable C (C_{ext}) more than soil extractable N (N_{ext}) content (Table 5.1). The same tendency was measured also for SOC (Figure 5.4) and TN (Table 5.1). In fact, even if significant differences were due only to the depth factor, it is possible to observe a decrease of content in the treated plots compared with control in both the soil layer sampled. Specifically, regardless of the soil horizon, the measured downturn of SOC and TN was respectively of -13% and -11%, while the only differences on their ratio (C:N) were due to soil depth (Table 5.1).

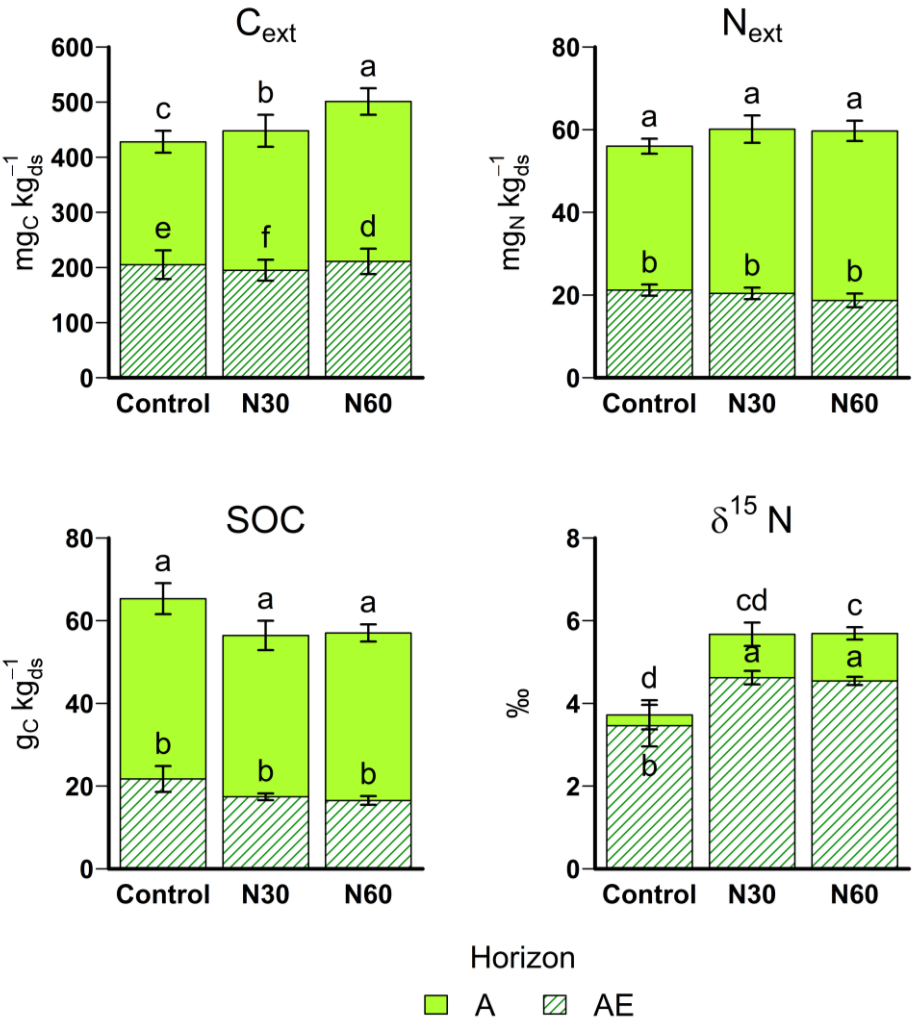

Figure 5.4 - Marginal means of extractable C (C_{ext}), extractable N (N_{ext}), soil organic carbon (SOC) and stable nitrogen isotope ratio ($\delta^{15}N$). Error bars represent the standard error for each soil horizon. Different lowercase letters across N treatments and within soil horizon represent significant differences ($P< 0.05$).

Instead, microbial biomass C (Figure 5.5) showed significant differences only in respect to soil horizons with higher values in the A horizon (600-700 mg kg^{-1}), typical for upper horizon in forest soils. Nevertheless, considering the averages between the two depths for each treatment (Figure 5.5), the observed tendency is a decrease of the microbial C content (-12%) in the treated plots. Likewise, the MBN (Figure 5.5) showed significant differences relative only to soil horizon with higher values in correspondence of the A horizon (80-90 mg kg^{-1}).

Looking at the MBC to MBN ratio ($C:N_{MB}$), the situation changed and appear that treatments promoted a reduction (-14%) of this ratio compared with no-treated control (Table 5.1).

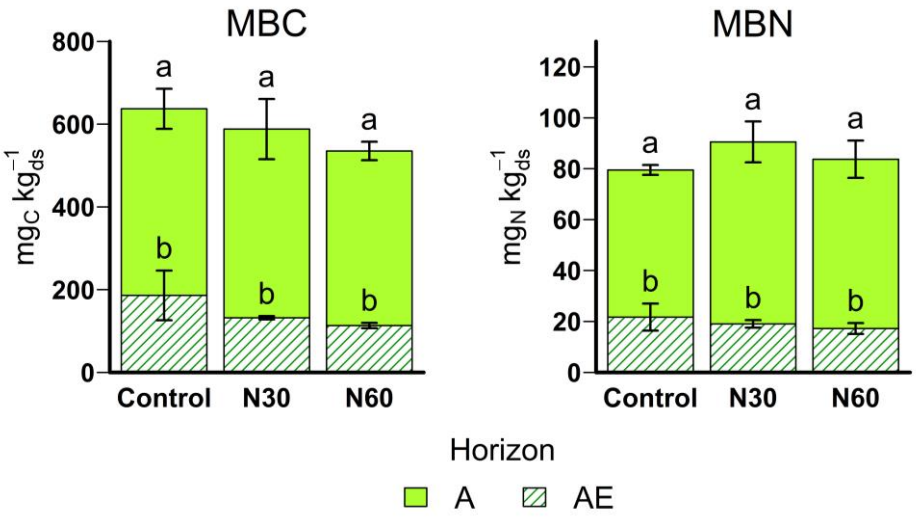

Figure 5.5 - Marginal means of microbial biomass C (MBC) and microbial biomass N (MBN). Error bars represent the standard error for each soil horizon. Different lowercase letters across N treatments and within soil horizon represent significant differences ($P< 0.05$).

5.3.2 Soil enzymatic activities

Considering the oxidative enzymatic activities (Figure 5.6) it comes to light that the major differences were between the soil horizons, with higher values corresponding to the surface horizon.

However, the dehydrogenase activity trend let suppose a decrease, in both soil horizons, in correspondence of the treated plots compared to the control. Instead, the two phenol oxidase activities measured showed an opposite behavior in relation to N supply (Figure 5.6). Specifically, in the A horizon, it is possible to observe an increase of tyrosinase activity (Tyr) (+15%) both for N30 and N60 compare to the no-treated control.

Instead, laccase activity (Lac), which is considered the main responsible for the degradation of recalcitrant compounds such as lignin and stabilized organic substance, seems to decrease (-16%) with the treatments.

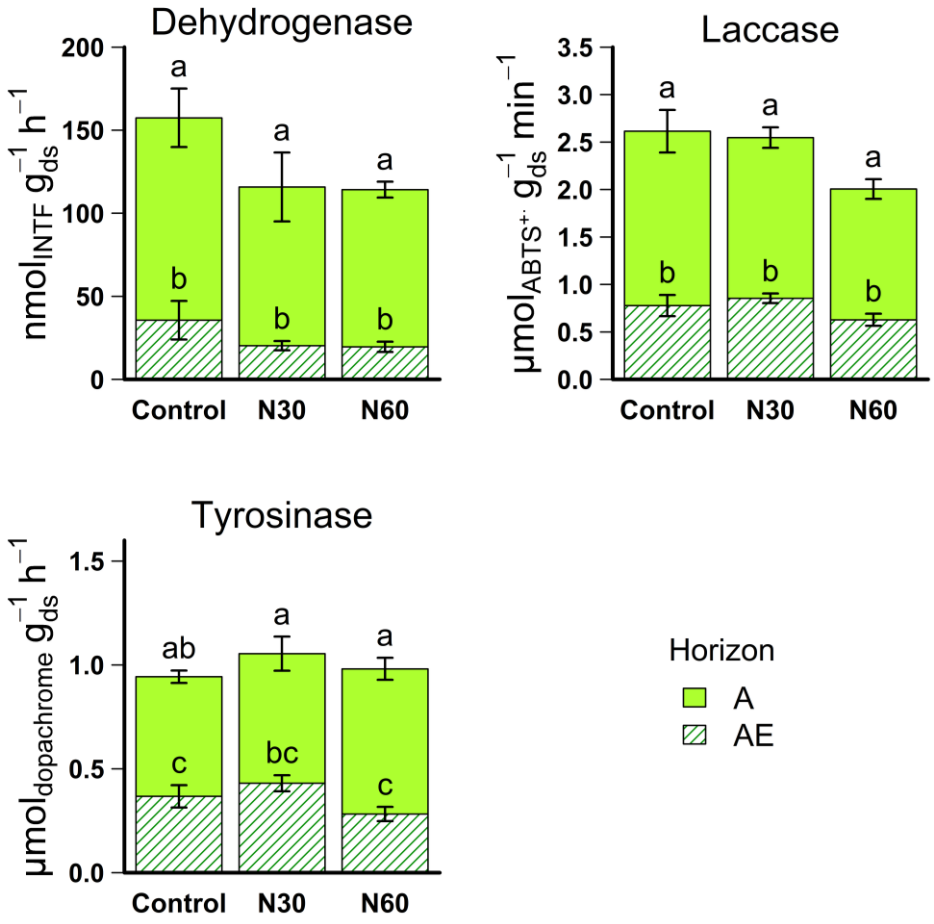

Figure 5.6 - Marginal means of dehydrogenase, laccase and tyrosinase activities. Error bars represent the standard error for each soil horizon. Different lowercase letters across N treatments and within soil horizon represent significant differences (P< 0.05).

Regarding the hydrolytic enzyme activities (Figure 5.7), the most significant differences has been observed for β-glucosidase (β-Glu), N-acetyl-glucosaminidase (NAG) and β-cellobiosidase (β-Cel) with a reduction of the activity in the surface horizon of the treated plots. Specifically, β-Glu decreased by 33%, NAG by 34% and β-Cel by 37%. Moreover, it has been measured a decrease of 15% of the β-xylosidase activity (β-Xyl) even if it is not statistically significant. In general, for these enzymatic activities, the major reduction has been measured with the N30 treatment in the A horizon, while the N60 treatment seems to had a reduced effect. Considering the hydrolytic enzyme activities of α-glucosidase (α-Glu) and phosphomonoesterase (PME), it has been measured higher values in the A

horizon compared with the AE horizon, without differences due to treatments. Finally, the activities of arylsulphatase (AS) and lipase (LIP) showed the most univocal behavior in response to the N application. Indeed, AS showed a decrease of the activity under the N treatments, which is evident also in correspondence of the subsurface horizon. Instead, for what concern the lipase activity it showed considerable difference in the A horizon but with the higher activity that has been measured in correspondence of the N30 treated plots (Figure 5.7), thus highlighting a reaction to the treatment that goes on the opposite direction compared to the other enzymatic activities.

5.3.3 Soil biochemical indexes

The metabolic quotient (qCO_2), an indicator of the microbial biomass efficiency for C utilization (Wardle and Ghani, 1995), resulted not significantly affected by nor the N treatment nor the sampling depth (Figure 5.8), even if higher values have been calculated in correspondence of the AE horizon. For which concern the microbial quotient (qmic), which indicates the organic carbon rate available for the microbial community (Laudicina et al., 2011), it showed (Figure 5.8) a dose-dependent decrease in correspondence of the AE horizon that did not correspond to the trend observed in the surface horizon where the higher value has been calculated for the soil samples treated with the N30 dose. Proceeding towards the measured biochemical parameters, of particular interest is the metabolic index (MI) that is given by the ratio between the dehydrogenase activity, which could represent soil microbiological activity (Masciandaro et al., 1998), and extractable C. The MI (Figure 5.8) showed significant and dose-dependent differences due to the treatments with higher values in correspondence of the control plots.

For which concern the specific enzymatic activities (Table 5.2), the only one that significantly responded to the treatment independently from the horizon has been the specific NAG activity (NAG_{MBC}) which showed the lower value in correspondence of the N30 treatment. With the exception of β-Glu and α-Glu specific activities, the other enzymatic activities resulted significantly affected by the horizons or by the interaction between N treatments and horizons (Table 5.2).

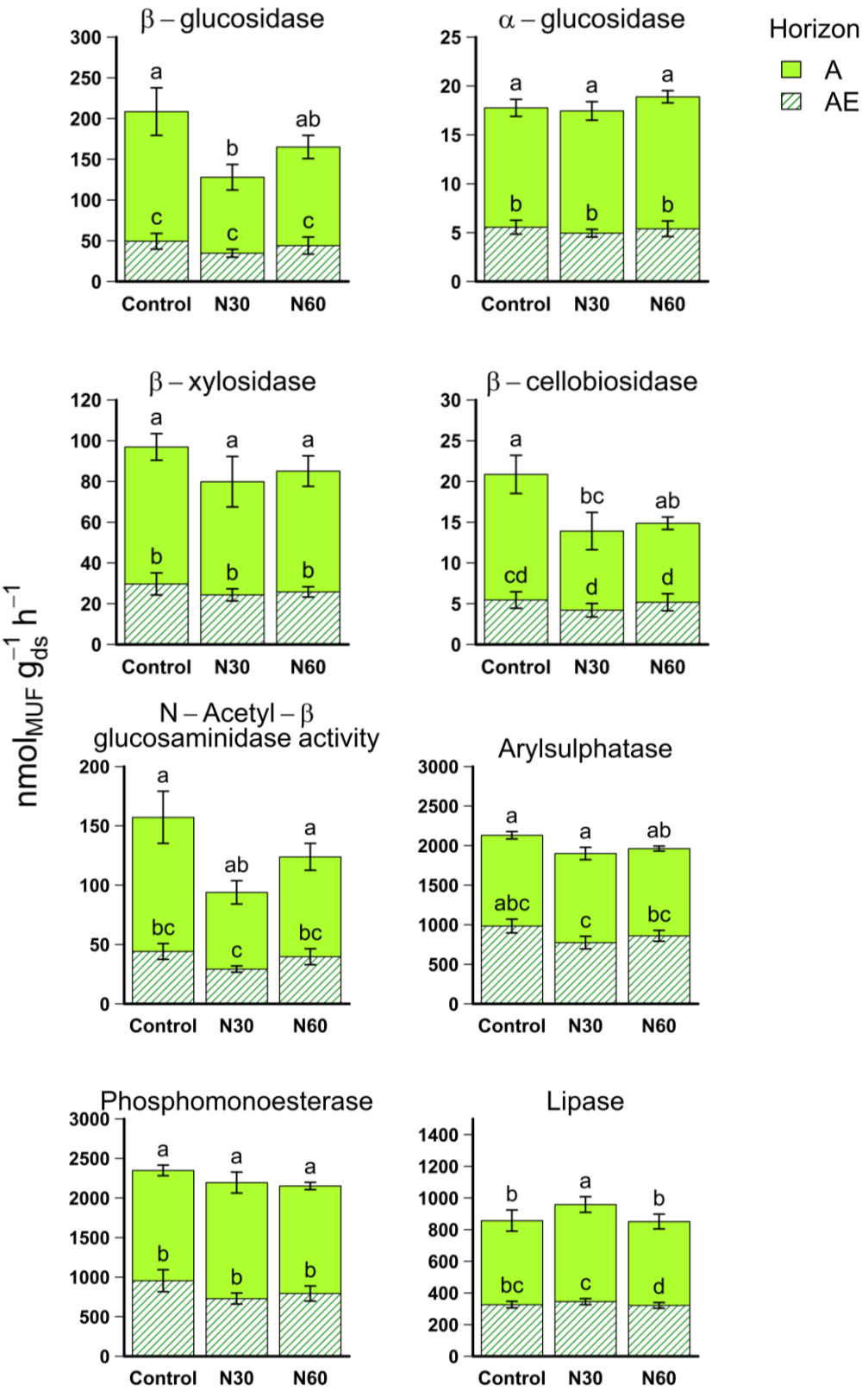

Figure 5.7 - Marginal means of β-glucosidase, α-glucosidase, β-xylosidase, β-cellobiosidase, N-acetyl-β-glucosaminidase, arylsuphatase, phosphomonoesterase and lipase activities. Error bars represent the standard error for each soil horizon. Different lowercase letters across N treatments and within soil horizon represent significant differences ($P < 0.05$).

In particular, the specific activities of PME, AS, LIP and Tyr resulted affected only by the horizons with higher values in correspondence of the AE horizon. Going beyond, specific NAG, β-Cel and β-Xyl activities showed the lower values in correspondence of the N30 treatment in the A horizon and the higher values with the N60 treatment in the AE horizon. Passing to the other two oxidative enzymes, the specific dehydrogenase activity showed a different trend compared to that of the specific hydrolytic activities with the higher value in correspondence of the control in the surface horizon and the lower once in relation to the 30N treatment in the AE horizon. Finally, the downturn of laccase activity previously observed in the A horizon (Figure 5.6) come to light also looking at the specific activity (Lac_{MBC}) which is significantly lower with the N60 treated plots (Table 5.2) always in the A horizon.

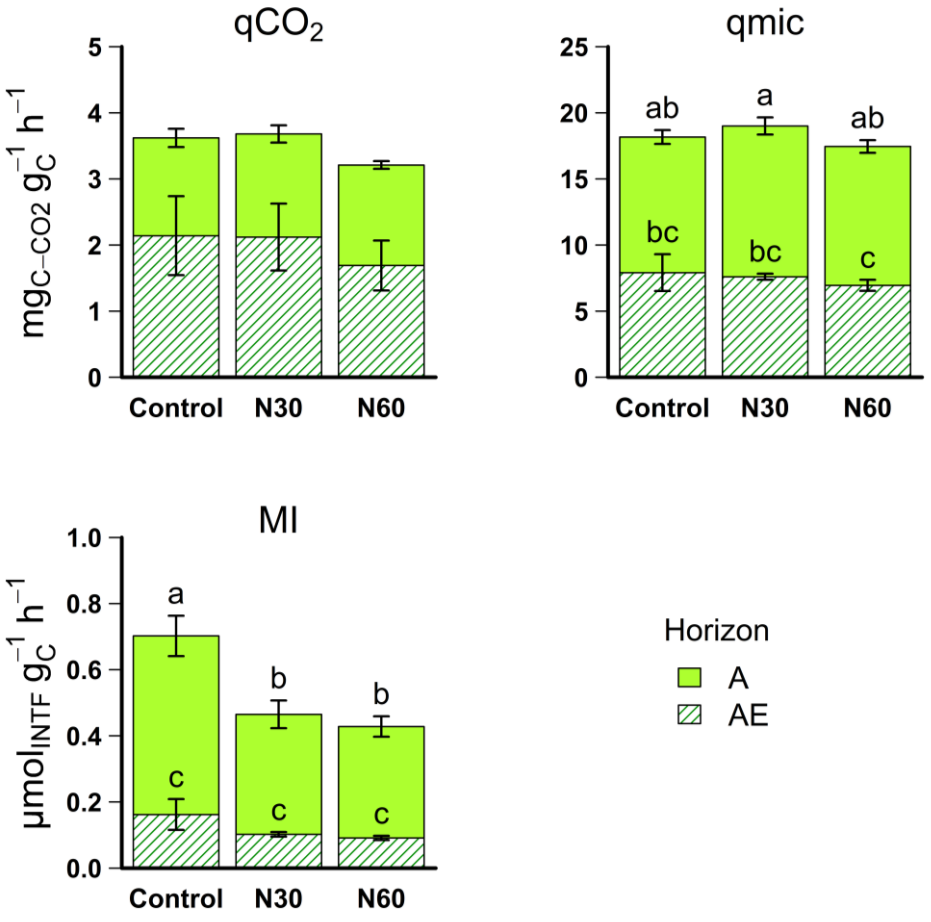

Figure 5.8 - Marginal means of the metabolic quotient (qCO_2), the microbial quotient (qmic) and the metabolic index (MI). Error bars represent the standard error for each soil horizon. Different lowercase letters across N treatments and within soil horizon represent significant differences ($P< 0.05$).

Table 5.5 - Marginal means of β-glucosidase (β-Glu$_{MBC}$, µmol$_{MUF}$ mg$_C^{-1}$h^{-1}), α-glucosidase (α-Glu$_{MBC}$, µmol$_{MUF}$ mg$_C^{-1}$h^{-1}), β-xylosidase (β-Xyl$_{MBC}$, µmol$_{MUF}$ mg$_C^{-1}$h^{-1}), β-cellobiosidase (β-Cel$_{MBC}$, µmol$_{MUF}$ mg$_C^{-1}$h^{-1}), N-acetyl-β-glucosaminidase (NAG$_{MBC}$, µmol$_{MUF}$ mg$_C^{-1}$h^{-1}), arylsuphatase (AS$_{MBC}$, µmol$_{MUF}$ mg$_C^{-1}$h^{-1}), phosphomonoesterase (PME$_{MBC}$, µmol$_{MUF}$ mg$_C^{-1}$h^{-1}), arylsuphatase (AS$_{MBC}$, µmol$_{MUF}$ mg$_C^{-1}$h^{-1}), lipase (LIP$_{MBC}$, µmol$_{MUF}$ mg$_C^{-1}$h^{-1}), dehydrogenase (Dehy$_{MBC}$, µmol$_{INTF}$ mg$_C^{-1}$h^{-1}), tirosinase (Tyr$_{MBC}$, µmol$_{DOPA}$ mg$_C^{-1}$ h^{-1}) and laccase (Lac$_{MBC}$, µmol$_{ABTS}$ mg$_C^{-1}$ min^{-1}) specific activities. Error bars represent the standard error for each soil horizon. Differences between treatments has been considered significant for P<0.05 and has been indicated with different lowercase letters.

	Dehy$_{MBC}$	Lac$_{MBC}$	Tyr$_{MBC}$	β-Glu$_{MBC}$	α-Glu$_{MBC}$	NAG$_{MBC}$	β-Xyl$_{MBC}$	β-Cel$_{MBC}$	PME$_{MBC}$	AS$_{MBC}$	LIP$_{MBC}$
Fertilizations											
Control	0.232	4.80	1.91	0.329	33.8	0.273^{a}	0.173	35.0	4.95	5.02	2.04
N30	0.181	5.26	2.35	0.237	33.2	0.183^{b}	0.153	26.3	4.43	4.23	2.07
N60	0.201	4.50	2.08	0.342	40.5	0.279^{a}	0.186	34.9	5.15	5.21	2.05
p-value	*0.319*	*0.558*	*0.339*	*0.098*	*0.285*	*0.005*	*0.347*	*0.221*	*0.672*	*0.678*	*0.998*
Fertilization x Horizon											
Control (A)	0.264^{a}	4.22^{bc}	1.36^{b}	0.338	28.1	0.243^{ab}	0.151^{ab}	33.4^{ab}	3.26^{b}	2.71^{b}	1.37^{b}
Control (AE)	0.200^{ab}	5.38^{ab}	2.46^{a}	0.320	39.6	0.304^{ab}	0.196^{ab}	36.6^{ab}	6.64^{a}	7.32^{a}	2.71^{a}
N30(A)	0.207^{ab}	4.01^{bc}	1.41^{b}	0.211	29.2	0.143^{b}	0.123^{b}	21.1^{b}	3.35^{b}	2.61^{b}	1.50^{b}
N30 (AE)	0.154^{b}	6.51^{a}	3.29^{a}	0.263	37.3	0.222^{ab}	0.183^{ab}	31.6^{ab}	5.51^{a}	5.85^{a}	2.63^{a}
N60 (A)	0.227^{ab}	3.30^{c}	1.65^{b}	0.286	32.5	0.198^{ab}	0.139^{ab}	22.8^{ab}	3.25^{b}	2.66^{b}	1.26^{b}
N60 (AE)	0.175^{ab}	5.69^{ab}	2.50^{a}	0.399	48.6	0.360^{a}	0.233^{a}	47.0^{a}	7.06^{a}	7.77^{a}	2.84^{a}
p-value	*0.073*	*<0.001*	*<0.001*	*0.464*	*0.079*	*0.053*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

5.3.4 Correlation between measured parameters

A correlation plot including all the principal soil properties is reported in Figure 5.9. Soil reaction is negatively correlated with the extractable and microbial N, and lipase activity. The extractable N (N_{ext}) showed negative correlation with the metabolic quotient (qCO_2), the metabolic index (MI) and the α-glucosidase activity (α-Glu). The available phosphorus (P_{Olsen}) resulted positively correlated only with the laccase activity. For which concern the enzymatic activities, the lipase activity (LIP) is the only one that resulted negativity correlated with almost all the other parameter with the exception of the N_{ext} (Figure 5.9). The other activities, both hydrolytic and oxidative, when significantly correlated to a parameter, showed a positive correlation. The only exception is the α-Glu activity correlation with the N_{ext}. Within the oxidative enzyme activities, the tyrosinase did not correlate with any of the other two activities considered. Finally, while tyrosinase and laccase activities showed a singular behavior, the dehydrogenase activity seems to have a relation with the other biochemical parameters that is similar to those of the hydrolytic enzyme activities.

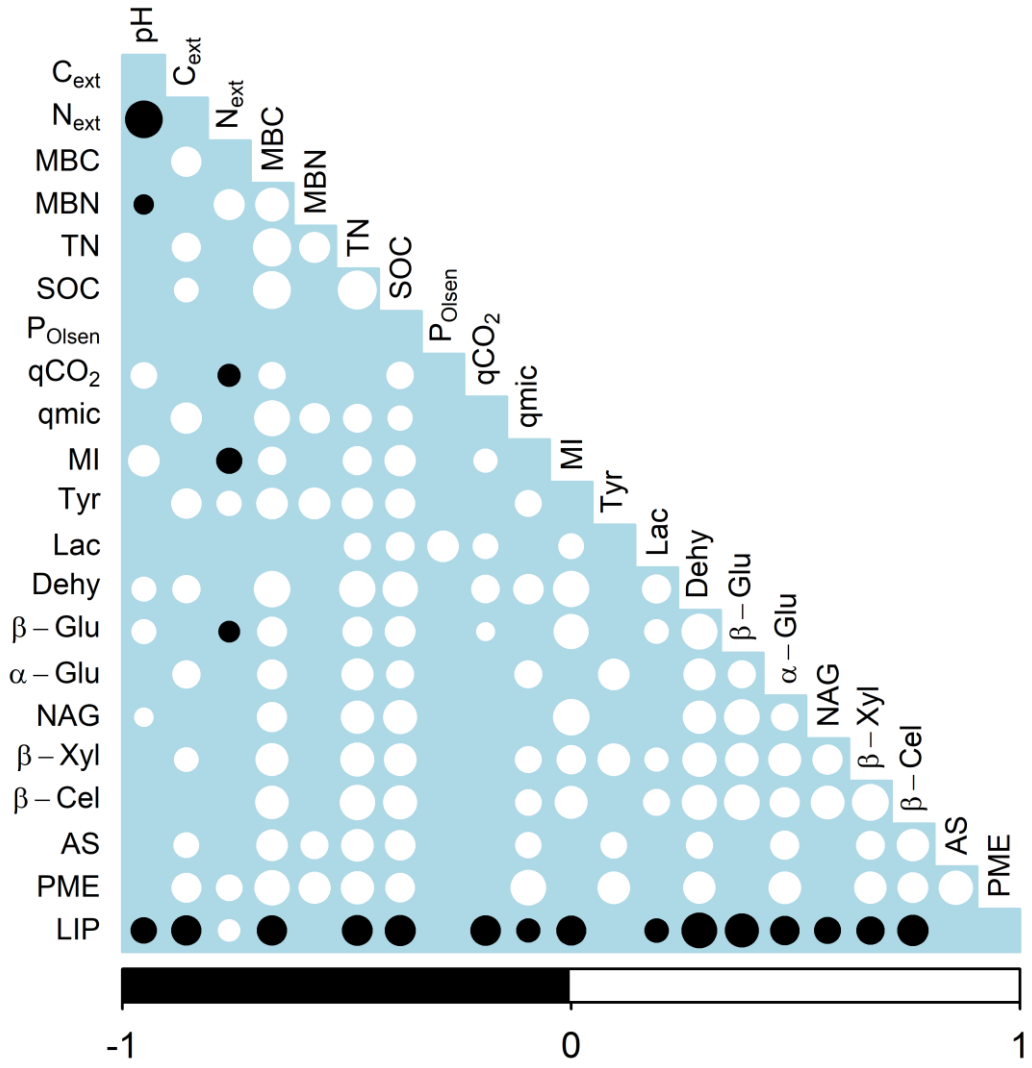

Figure 5.9 - Correlation plot including all the considered soil properties. The correlation has been carried out only for the A horizon values has they were the most informative.

5.3.5 Canonical Correspondence Analysis

In the attempt to have an overall view of the system, a canonical correspondence analysis has been performed (Figure 5.10) taking in consideration soil C and N pools, available P, pH and the specific enzymatic activities in the A horizon. Even if the treatments do not clearly separate in cluster, some indications could be anyway deduced. In fact, it can be seen that while the untreated control seems to be predominantly driven by the MBC (Figure 5.10), the N30 treatment seems fundamentally driven by the N pools (MBN and N_{ext}) and not by specific enzymatic activities (Figure 5.10). Indeed, as noted above, the N30 plots seem to have reached a sort of equilibrium despite being in the N saturating conditions.

Finally, the samples corresponding to the N60 treatment present an ambiguous trend, indeed, some of them stay with the N30 plots while the others are placed alone and seems to be predominantly led by C_{ext}.

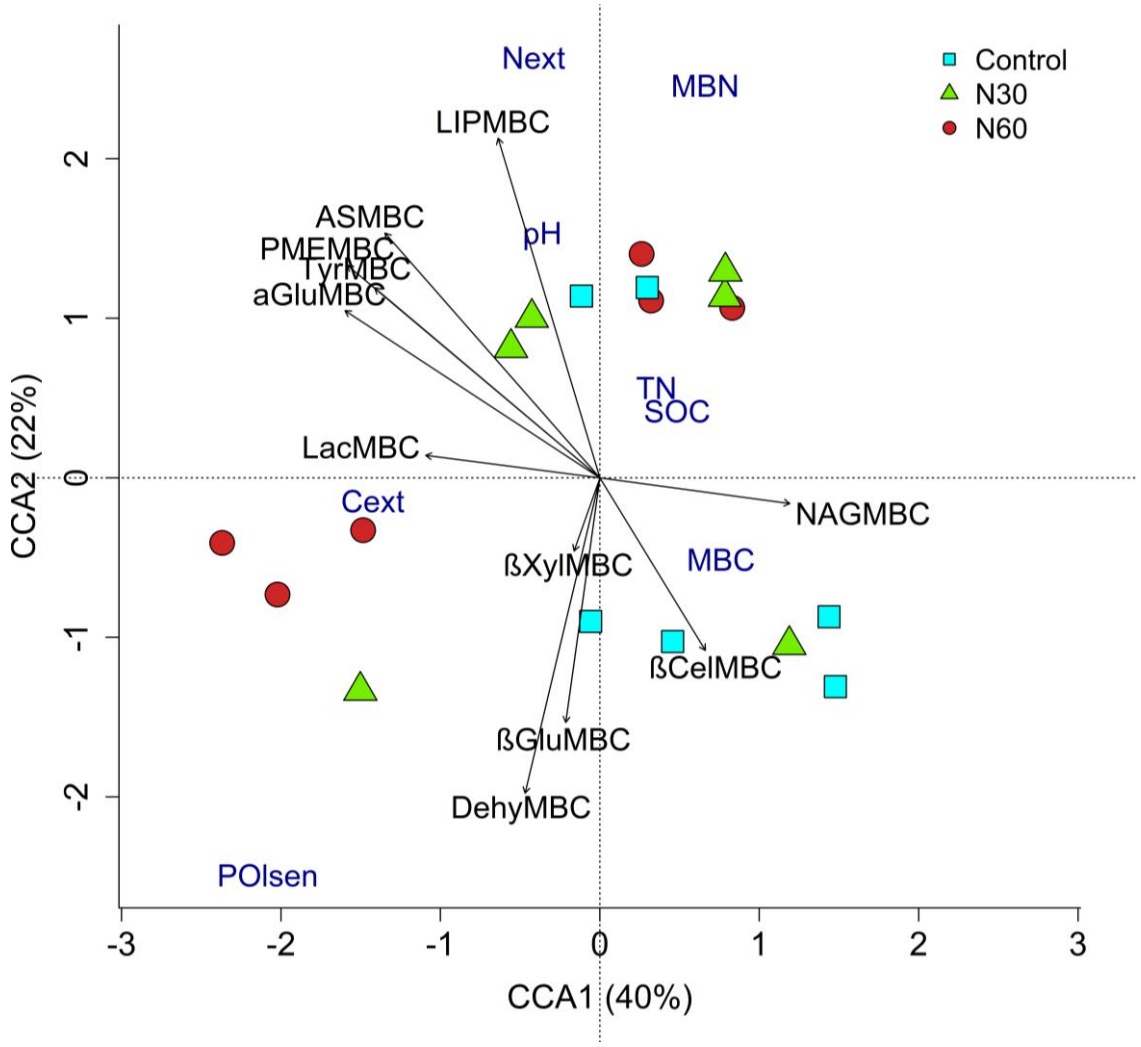

Figure 5.30 – Canonical Correspondence Analysis (CCA) plot of the specific enzyme activities for the three treatments considered (Control, N30, N60) in the A horizon.

5.4 Discussion

Considering the pH values reported in Marchetto et al. (2014) it is possible to notice that, in approximately seven years, soil acidity has decrease of 1 unit independently on N application. This plausible acidification is probably related to the ambient N depositions as stated by previous studies (Bergkvist et al., 1992; Bobbink et al., 2010; Hess et al., 2018) and the small differences between treatments may be due to the short experimentation period (1 year). Therefore, independently from the treatments, the soil of this site is in ~~an~~ acidic condition that influence soil elements mobility and availability, for example, low pH lead to a reduction of N, S, P, K, Ca and Mg availability, and to an increase of Al toxicity (Bobbink et a., 2010). Moreover, low pH values induce in soil of forest ecosystems a slowdown in the decomposition and humification processes by depressing the size and the activity of the soil microbial community (Asakawa et al., 2011).

However, despite the treatments have been carried out with a relatively low concentrations of N (30 kg N $ha^{-1}yr^{-1}$ and 60 kg N $ha^{-1}yr^{-1}$), looking at the data relative to the $\delta^{15}N$ it is possible to assess that the N, superficially applied on the vegetated undergrowth, arrived at least at 0.20 m of depth.

The decrease in MBC content has already been observed in other studies concerning N enrichment effects (Demoling et al., 2008; Frey et al., 2004; Liu and Greaver, 2010), and it is in agreement with the potential effect of acidic condition of soil pH on microbial biomass. Moreover, the downturn of the $C:N_{MB}$ ratio could be an indication that N application would favor bacteria more than fungi. In fact, this hypothetical shift in microbial community composition is in agreement with the soil acidification effect on soil microorganisms (Asakawa et al., 2011). Furthermore, other studies (Carreiro et al., 2000; Frey et al., 2004; Manning, 2012) highlight that a possible response to N enrichment is a shift to bacterial dominance in soil microbial community.

Regarding soil organic carbon content (SOC), it seems that SOC storage is not occurring and it is quite expected after only one year of treatment, even more in a eutrophic forest system. Anyway, N addition and low pH are commonly responsible for the reduction of the microbial decomposition rate which is a key process in soil C sequestration (Chen et al., 2018). Moreover, it has been hypothesized that in forest ecosystem N could stimulates plant growth thus producing an increase in recalcitrant (e.g. woody) litter material with slow turnover that, combined with reduced decomposition rates, enhance C sequestration (Liu and Greaver, 2010; Magnani et al., 2007; Manning, 2012; Nave et al., 2009). In contrast, there are studies that highlight no differences or a reduction in C storage after N addition due to litter quality (i.e.

litter with low lignin) (Knorr et al., 2005), or to the increased labile C to soil that act on soil microbial decomposer as a 'priming effect' (Neff et al., 2002).

Passing to the biochemical indexes, the differences in the qCO_2 values were expected as in the deepest soil horizons there is a lower content of MBC which generally come across a more stressed condition thus reducing the microbial efficiency. Regarding the metabolic index (MI), its downturn with the treatments could indicate that the N addition decrease the efficiency of the microbial metabolism (Masciandaro et al., 1998) and it is confirmed, in the A horizon, by the negative correlation (Figure 5.9) between the N_{ext} and the MI.

Finally, the enzymatic activities most involved in C and N cycles showed a clear decrease in correspondence of the treated plots. On the contrary, previous studies related to the effects of N depositions in forest ecosystems (Carreiro et al., 2000; Chen et al., 2018; Frey et al., 2004) have shown increases in β-Glu and β-Cel activities. Moreover, Frey et al. (2004) found a decrease of 20-30% of the labile C in the N-treated plots and also this is an opposite result to what has been observed in this study (approx. +20% of C_{ext}). Therefore, it seems possible to suppose that the increased availability of the labile C here observed has downturn these enzymatic activities. Following this hypothesis, in the A horizon of the N60 treated plots, where the higher C_{ext} content has been measured, it is expected to observe the lower enzymatic activities. Otherwise, the decrease of β-Glu, β-Cel and NAG activities is not N-dose and C_{ext}-content dependent. In fact, these enzymatic activities did not correlate (Figure 5.9) with the extractable C and only β-Glu showed a negative correlation with extractable N. Even if there are no changes due to the treatments in the MBC content in the A horizon (Figure 5.5), it is possible to observe a reduction of these specific enzymatic activities only in correspondence of the N30 dose thus indicating that the MBC has decrease its enzyme production and/or there is a reduced release of the immobilized enzymes (Gil-Sotres et al., 2005; Kandeler and Eder, 1993). Looking at the CCA plot (Figure 5.10) comes to light this ambiguous behavior under the treated plots which let suppose that, has observed for the hydrolytic enzymatic activities, there is not a linear evolution from the control to the N60 even if the N treatment dose follow a liner increase. No reference to similar enzymatic activities responses to N addition has been found in literature as usually the changes are dose-dependent, probably this disagreement is due to eutrophic condition of site.

Previous studies showed that N enrichment induced a suppression of phenol oxidizing activities and a braking in litter decay in those forest (i.e. oak forest) that are characterized by litter with high lignin content and slow decomposition rates (Carreiro et al., 2000; Sinsabaugh, 2010; Sinsabaugh et al., 2008). Another N effect is related to the decrease in soil fungi content as supported by Carreiro et al. (2000),

which highlighted that increase in N availability could reduce soil white-rot fungi and their ligninolytic enzymes production. This two N-induced effects observed in other studies perfectly match with laccase activity reduction measured in this study. Indeed, Jacob et al. (2010) demonstrated that pure beech litter has high C:N ratio and high lignin content, resulting in a litter with very slow degradation rates. Moreover, it is well known that fungi are the principal agents in lignin degradation (Sinsabaugh, 2010) and that the higher activity of laccases could be related to wood-rotting basidiomycetes and white-rot fungi (Asina et al., 2016; Baldrian, 2006; Dwivedi et al., 2011).

It seems that those groups of fungi are specialized in extracellular laccases production while extracellular tyrosinases appear to be produced by a great number of both bacterial and fungal groups (Asina et al., 2016; Durán et al., 2002; Dwivedi et al., 2011). Even if this difference could explain the different phenol oxidizing activities behavior, it should be noted that previous studies (Chen et al., 2018; De Forest et al., 2004; Frey et al., 2004) highlight decreasing tyrosinase activity after N addition. The main reason that could explain this difference is soil C_{ext} content. Indeed, while De Forest et al. (2004) and Frey et al. (2004) observed that N addition induce no change or a decrease in C_{ext}, in this study the labile C pool increased in the N tested plots. The correlation plot (Figure 5.9), indeed, highlight, for the A horizon, a positive correlation between soil C_{ext} and tyrosinase activity thus leading the hypotesis that the labile C pool is the driven force for tyrosinase activity. As already observed (Mazzon et al., 2018), laccase activity is the enzyme that less correlate with the other measured parameters (Figure 5.9), meaning that its functionality could be driven also from some different biochemical mechanism. On the other hand, laccase activity is positively correlate with the dehydrogenase activity and SOC content, two parameters that have no correlation with tyrosinase activity. This let deduce that even if laccases and tyrosinases are both phenol oxidizing enzymatic activities probably their role, their driving force and the processes in which they are involved are different.

5.5 Conclusion

In general, N addition induce a decrease in the most of the measured parameters. The assumed reduction of litter decomposition rate with the downturn of laccase and hydrolytic enzyme activities let suppose that under N addition SOC storage is occurring. On the other hand, SOC data does not support this hypothesis but it is possible to suppose that the processes that enhance SOC storage are occurring.

Interesting and unexpected is the increase in C_{ext} content and tyrosinase activity in correspondence of the N treated plots. Indeed, these parameters and, probably, the metabolic index, seems to work against the soil C stock. Thus, it seems possible that the labile C is acting on soil microbial decomposer as a 'priming effect' (Neff et al., 2002) and this "effect" has been detected on the tyrosinase activity.

Nevertheless, two points remain still unclear: (i) the increase in C_{ext} content and (ii) the non-dependency of the hydrolytic activities on N dose. For both these two results no indication has been found in literature and therefore, in the future, deeper investigation on this site are needed. Certainly, there is the need to take in consideration that the site exceeds the "N saturating condition" and that it represents a eutrophic system with high microbial biomass content and high enzymatic activities that lead to unusual behavior of the microbial community compared to those of the agronomic systems. Moreover, after one year of experimentation it is possible only to have an indication on how the system could proceed and other sampling are needed to monitor the evolution of this particular environment and to find an explanation of what is still unclear in this moment.

CHAPTER 6

ENZYME ACTIVITIES AND SOIL QUALITY INDEXES THROW DIFFERENT ECOSYSTEMS

6.1 State of the art

Across this study has been considered a wide range of biochemical parameters, which has been measured or calculated for each experimental site considered. Therefore, it has been obtained a great number of biochemical parameters useful in soil quality evaluation for three different ecosystems (two agronomics and one forest), located in different part of Italy. This could give the possibility to make a comparison between ecosystems and applied management using those variables. Clearly, due to the high differences occurring between the experimental site it has to be carefully evaluated which parameters consider and in which way they could be condensed in a reliable way.

6.1.1 Enzyme activities as soil quality indicators

Soil enzyme activities are able to give information on the capacity of soils to carry out biochemical reactions (Nannipieri et al., 2018) as they play a central role in these biogeochemical processes, for example the degradation, transformation and mineralization of soil organic matter (Sinsabaugh, 2010; Xiao et al., 2018). Therefore, the relation between soil enzymes and the environmental factors that could affect their activities covers an important part in ecosystem, and more specifically soil, functionality (Burns, 1982). Indeed, soil enzyme activities are suitable parameters for the analysis and the description of ecosystem, and soil, function and quality (Tabatabai and Dick, 2002).

Thus, considering the wide range of biochemical processes mediated by soil enzymes and their connection with soil microorganisms, it is plausible to consider soil enzyme activities as useful tools for the study of biogeochemical processes, for the evaluation of microbial functional diversity and microbial ecology and, therefore, for the estimation of soil quality (Nannipieri et al., 2002).

Actually, the measurement of several enzyme activities have already been considered a useful indicator of soil nutrient dynamics (Rao and Gianfreda, 2014). However, Nannipieri et al. (2018) suggested to pay attention when interpreting enzyme activities results as they not always effectively represent soil microbial activity as the measured enzyme activity could be determined by several activity deriving from different sources and locations (i.e. extracellular activity, intracellular activity, enzymes adsorbed on humic or mineral compounds, enzymes produced by microorganisms or enzymes derived by cellular lysis). Instead, they could be used as indexes to assess the impacts

deriving from agriculture and forest anthropogenic management or from pollution on soil (Nannipieri et al., 2018).

Hence, the use of soil enzyme activities as indicators of soil quality and fertility is widespread accepted (Kotroczó et al., 2014; Nannipieri et al., 2018, 2002; Piotrowska-Długosz, 2014; Rao and Gianfreda, 2014; Xiao et al., 2018) principally for their capacity to rapidly adapt to changes caused by both anthropogenic and natural factors (Kotroczó et al., 2014), their involvement in soil nutrient cycle (Piotrowska-Długosz, 2014), their sensitiveness to soil pollution and perturbation (Rao and Gianfreda, 2014) and because they are relatively simple and rapid to measure (Kotroczó et al., 2014; Piotrowska-Długosz, 2014).

For example, the activity of the dehydrogenase, which is an intracellular enzyme, is considered an indicator of soil microbial oxidative activity (Dick and Tabatabai, 1992; Gil-Sotres et al., 2005) and it has been observed that in many cases it correlates with soil respiration rate (Nannipieri et al., 2018).

The abovementioned example, however, take in consideration only one enzyme activities and, as mentioned above, to have a useful indication of soil quality more than one enzyme activity is needed. Indeed, if the analysis of each single enzyme activity could give information about a specific process, the combination of several soil enzyme activities is significant for the determination of the overall soil functionality (Nannipieri et al., 2002). Consequently, researchers are trying to condense soil biogeochemical parameters and soil enzyme activities in an index that could easily help in differentiating soils characterize by different quality and functional properties (Rao and Gianfreda, 2014).

6.1.2 Simple and complex indexes

The search of soil quality indicators occurs from many decades and it could involve general biochemical properties (i.e. microbial biomass and soil respiration) and/or specific biochemcial properties (i.e. soil enzymatic activities). According to Gil-Sotres et al. (2005) there could be three ways to estimate soil quality: using an individual soil property, using a “simple index” or using a “complex index”.

Going in order, an individual soil property used as soil quality indicator could be, as seen previously, the dehydrogenase activity. Others biochemical paramiters generally considered as good estimators of soil quality are the microbial biomass C, soil respiration rate, N mineralization capacity and urease

actitivity (Gil-Sotres et al., 2005). Clearly, nowadays, with the advancement of research and the increase of notions and analytical methods related to soil biochemistry, the use of single parameters, although useful, represents only the initial phase of exploration of the biochemical processes of the soil. Therefore, the use of indexes that combine two or more soil biochemical properties is generally preferred and, as stated before, it is increasing the need to find indexes that are able to condense as much parameters as possible (Rao and Gianfreda, 2014). For example, the metabolic index (qCO_2) is a widespread used index. It is calculated as the ratio between soil respiration and microbial biomass C and it represents the quantity of substrate mineralized per unit of microbial biomass, per unit of time (Gil-Sotres et al., 2005). Generally, an increase of this index is associated to alterated ecosystem and could be due to a decrease in microbial community efficiency (Gil-Sotres et al., 2005). Likewise, the microbial quotient (qmic), that is the ratio between MBC and SOC, could be used as indicator of organic matter availability changes (Bastida et al., 2008; Haynes, 1999) and the metabolic index (MI), which is the ratio between dehydrogenase activity and extractable C (Masciandaro et al., 1998), connects the possible C availability for microbial metabolism with the microbial activity generally expressed as dehydrogenase activity (Bastida et al., 2008).

Other simple indexes are the specific enzyme activities which consist in the ratio between the enzymatic activity and the SOC or the MBC (Gil-Sotres et al., 2005; Kandeler and Eder, 1993). In the first case the index better reflect soil quality status, with the second it could be deduced ecological information concerning the microbial activity (Gil-Sotres et al., 2005; Kandeler and Eder, 1993). Within this second case, an increase of the specific activity could be due to increased enzyme production/release by microorganisms or increased enzyme release from clay or humic colloids (Kandeler and Eder, 1993).

One complex index of soil quality based on enzyme activities is the Geometric Mean enzyme activities index (GMea) which is calculated as the geometric mean of the enzymes tested (García-Ruiz et al., 2008; Piotrowska-Długosz, 2014):

$$GMea = \sqrt[n]{ea_1 + ea_2 + ea_3 + .. + ea_n}$$

In fact, has been observed (García-Ruiz et al., 2008) that this GMea index, gathering together all the enzymatic activities into a single value, significantly discriminated between organic and conventionally managed soils.

Another way to gather enzyme activities as been proposed by Sinsabaugh et al. (2008). Using the natural logarithm of some enzyme activities involved in C, N and P cycles they calculated the

ecosystem ratios of C:N, C:P and N:P acquisition activity. Moreover, Sinsabaugh and Shah (2011) proposed the utilization of the ratio between the natural logarithm of β-glucosidase activity and the natural logarithm of phenol oxidizing enzyme activity as an index of recalcitrant C abundance (C:C), indeed they assumed that the ratio is inversely related to recalcitrant C content.

6.1.3 The Soil Quality Index (SQI)

The core of the soil quality index (SQI) is evaluate soil quality using a minimum dataset choose from a larger dataset which could include physical, chemical and biological soil properties (Andrews and Carroll, 2001). Indeed, the SQI index represent a useful tool to assess soil quality as the soil factors included could be different according to the tested sperimentation or to the objective of the work (i.e. crop productivity under different management or the effect of soil management on soil quality). Specifically, the SQI integrate selected soil properties into a single value using a process (Figure 6.1) that include i) indicator selection, ii) indicator scoring and iii) integration of the scores into the index (Andrews et al., 2002, 2004; Askari and Holden, 2015).

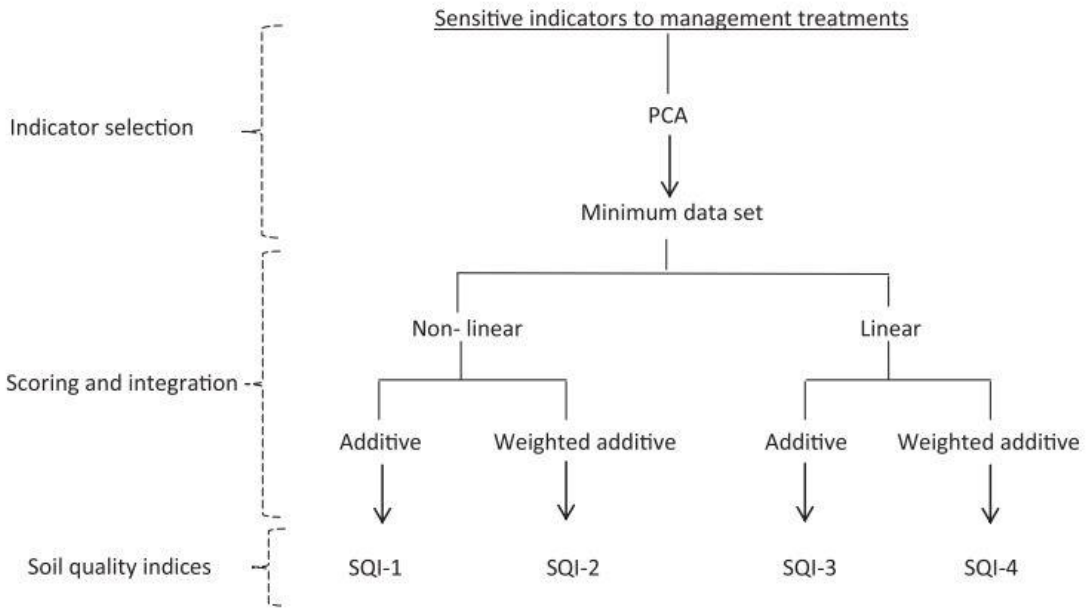

Figure 6.1 - Soil quality index steps. The figure has been taken from Askari and Holden (2015).

Briefly, the process firstly consists in the selection of the minimum dataset (MDS) that is done through a principal component analysis (PCA) from which the principal components (PC) with eigenvalues ≥ 1 and the properties that recived high loadings are assumed to best represent the system (Andrews et

al., 2002, 2004; Andrews and Carroll, 2001; Askari and Holden, 2015). The parameters that could be included in the MDS are those with the higher loadings and those with absolute values within the 10% of the highest weight. At this point a correlation is used to reduce redundancy between parameters and finally only the parameters that do not correlate each other could be included in the MDS (Andrews et al., 2002, 2004; Andrews and Carroll, 2001; Askari and Holden, 2015).

At this point each parameter should be standardized to a value between 0 and 1 (Andrews and Carroll, 2001) using the function "more is better", "less is better" or the "optimum" depending on the variables. Finally, there are two options for the calculation of the SQI (Figure 6.1): the first "additive" (SQI_A) , and the second "weighted additive" (SQI_W). In the first case the index result to be the sum of the scores (S_i) form the MDS, in the second each score in the MDS is weighted using the PCA. Therefore, the "weighted additive" index consist in the sum of the scores of the MDS multiplied by the amount of variation (W_i) explained by the correspondent PC (Andrews et al., 2002, 2004; Askari and Holden, 2015).

$$SQI_A = \sum_{i=1}^{n} S_i \qquad SQI_W = \sum_{i=1}^{n} W_i S_i$$

6.1.4 Aim of the work

The objective of this chapter is therefore to use the simple indexes calculated for each experimental site considered and to calculate the other indexes previously presented for have a general picture of soil quality through the three systems (two agronomics and one forest) examinated in the previous chapters. Moreover, taking into account that several indexes would be considered, the purpose is to evaluate if all the indexes produce the same response so as to make them equivalent for the assessment of soil quality. In this context particular attention would be paied to the use of soil enzymatic activities as indicators of soil quality and functionality.

6.2 Methods

For the determination of GMea, of C:N, C:P, N:P and C:C ecosystem ratios, and of SQI same detail regarding the calculation process and the parameters involved have to be made explicit.

6.2.1 Geometric mean of the enzyme activities

The determination of the geometric mean of the enzyme activities (GMea) has been determined taking into account all the hydrolytic (β-glucosidase, α-glucosidase, N-acetyl-β-glucosaminidase, β-xylosidase, β-cellobiosidase, Arylsulphatase, Phosphomonoesterase and Lipase) and oxidative (Dehydrogenase, Laccase and Tyrosinase) enzymatic activities determined for each experimental site. Therefore, the GMea resulted to be:

$$GMea = \sqrt[11]{Dehy * Lac * Tyr * \ \beta Glu * \ \alpha Glu * NAG * \ \beta Xyl * \ \beta Cel * AS * PME * LIP}$$

6.2.2 Ecosystem ratios

The ecosystem ratios proposed by Sinsabaugh et al. (2008, 2011) have been here calculated utilizing the measured enzyme activities related to C, N and P cycles.
Consequently, they resulted to be determined as follows:

$$C:N = \frac{ln(\beta Glu + \alpha Glu + \beta Xyl + \beta Cel)}{ln(NAG)}$$

$$C:P = \frac{ln(\beta Glu + \alpha Glu + \beta Xyl + \beta Cel)}{ln(PME)}$$

$$N:P = \frac{ln(NAG)}{ln(PME)}$$

$$C:C = \frac{ln(\beta Glu + \alpha Glu + \beta Xyl + \beta Cel)}{ln(Lac + Tyr)}$$

6.2.3 The soil quality index

As regards the choice of the variables to be included in the determination of the soil quality index (SQI), initially it was decided to use the aforementioned biochemical indices. In doing so, however, the problem arises in scoring the enzymatic activities or the indexes based on them. In fact, it would be wrong to associate a "more is better" or "less is better" function to the enzymatic activities, as well as, to date, it is difficult make the choice of an optimum, which would be extremely subjective and "casual". Therefore, it has been decided to consider only the soil properties and indexes that could be scored in a realistic way. Thus, the initial dataset used for the SQI determination was composed by soil pH, extractable C and N (C_{ext}, N_{ext}), microbial C and N (MBC, MBN), soil organic C (SOC), total N (TN), microbial quotient (qmic), metabolic quotient (qCO_2) an metabolic index (MI).

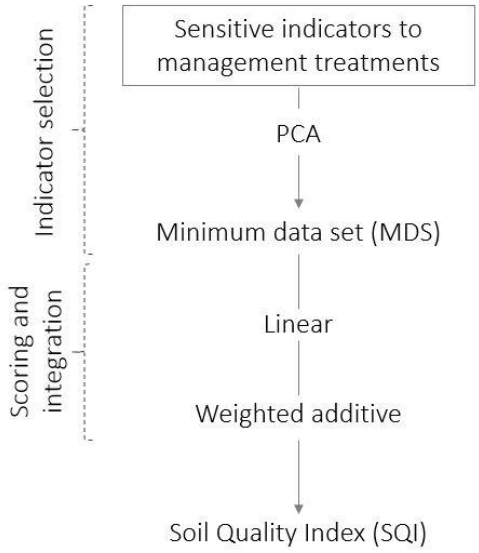

Figure 6.2 - Scheme of the process followed in this study for the determination of the SQI. The scheme has been adapted from that of Askari and Holden (2015).

On this dataset a PCA has been carry out and only the first two principal component (PC) resuted to have an eigenvalue higher than 1. Whithin this two PC, only MBC, qmic and MI resulted to be the parameters with the higher loadings and that are not correlated each other. Therefore, the minimum dataset (MDS) used in this study was composed by MBC, qmic, qCO_2 and MI.

The scoring process has been done with a score from 0 to 1, that has been arranged following the function “more is better” for MBC and MI parameters, “less is better” for the qCO_2 index and the “optimum” (subjectively decided looking at the mean data as 7÷11 = 1; <7 or >11=0) for the qmic index. Than, the explained variance of each PC has been calculated and used as weight in the determination of the weighted additive SQI (see paragraph 6.1.3)

6.3 Results

Considering the high variability between the forest and the agronomic ecosystems for the simple indexes considered has not been possible to carry out the analysis of variance (ANOVA) as the requaired assumptions (variance homogenity and normality of the distribution) were not respected. Therefore, it has been decided to show the variation between ecosystems using boxplots and to give a complete overview with a PCA. For the other indexes there was not this problem and thus one-way ANOVA has been carry out with management as factor and at a significance level of $P \leq 0.05$.
The ecosystems and the treatments or managements applayed, for semplicity, have been indicated with acronyms as reported in table 6.1.

Table 6.1 - Report of the acronyms used for the identification of the experimental sites.

Site	Management	Acronym
Beech Forest	Control	F0
	+ 30 kg N $ha^{-1}yr^{-1}$	F30
	+ 60 kg N $ha^{-1}yr^{-1}$	F60
Montepaldi Long Term Experiment (MoLTE)	Conventional	MC
	Organic	MO
QUABIO project	Agro-ecologic	QA
	Conventional	QC
	Organic	QO

6.3.1 Simple indexes

The microbial biomass C (MBC) and the dehydrogenase activity resulted to be higher in the beech forest site with no particular differences within the management (Figure 6.3). The difference between agronomic and forest ecosystems resulted to be particularly evident for the MBC content.

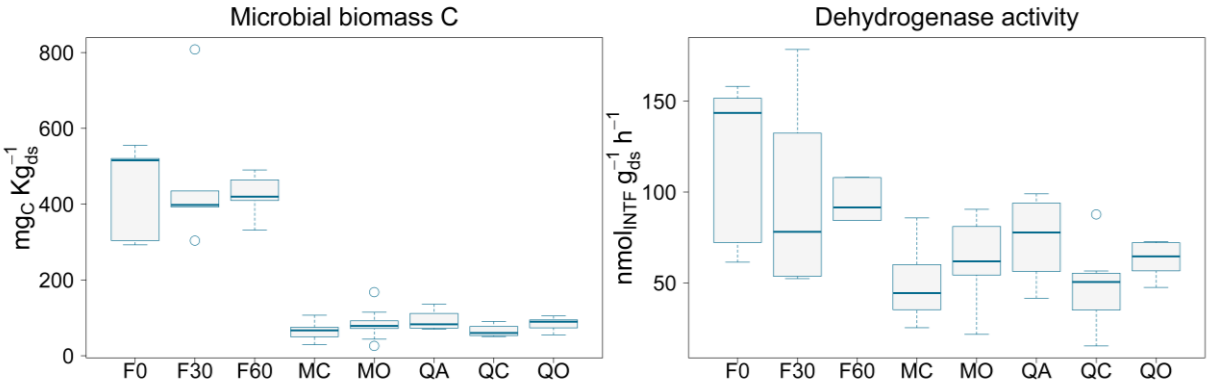

Figure 6.3 - Microbial biomass C and dehydrogenase activity for each experimental site and management considered. The line inside the box represent the median.

The microbial quotient (qmic) approximately ranged from 6 to 11. Looking at the median values it seems higher in correspondence of the beech forest site and lower in MoLTE site where the conventional management has been applied (MC). On the other hand the metabolic quotient (qCO_2) resulted higher in the MoLTE experimental site regardless of the management while the lower values corresponded to the forest site. In this case the discrepancy between agronomic and forest ecosystems resulted to be evident and clear. Finally, the metabolic index (MI) resulted to be the index that showed higher variability through the considered trial. Indeed, looking at the median reported in the boxplots (Figure 6.4) the higher MI value corresponded to the agro-ecology management (QA), followed by the organic management in both the agronomic experimental sites (QO and MO). Moreover, the conventionally managed agronomic plots (MC and QC) and the control plots in the beech forest (F0) seem to have the same values of MI, which decrease in the F30 and F60 beech forest treated plots.

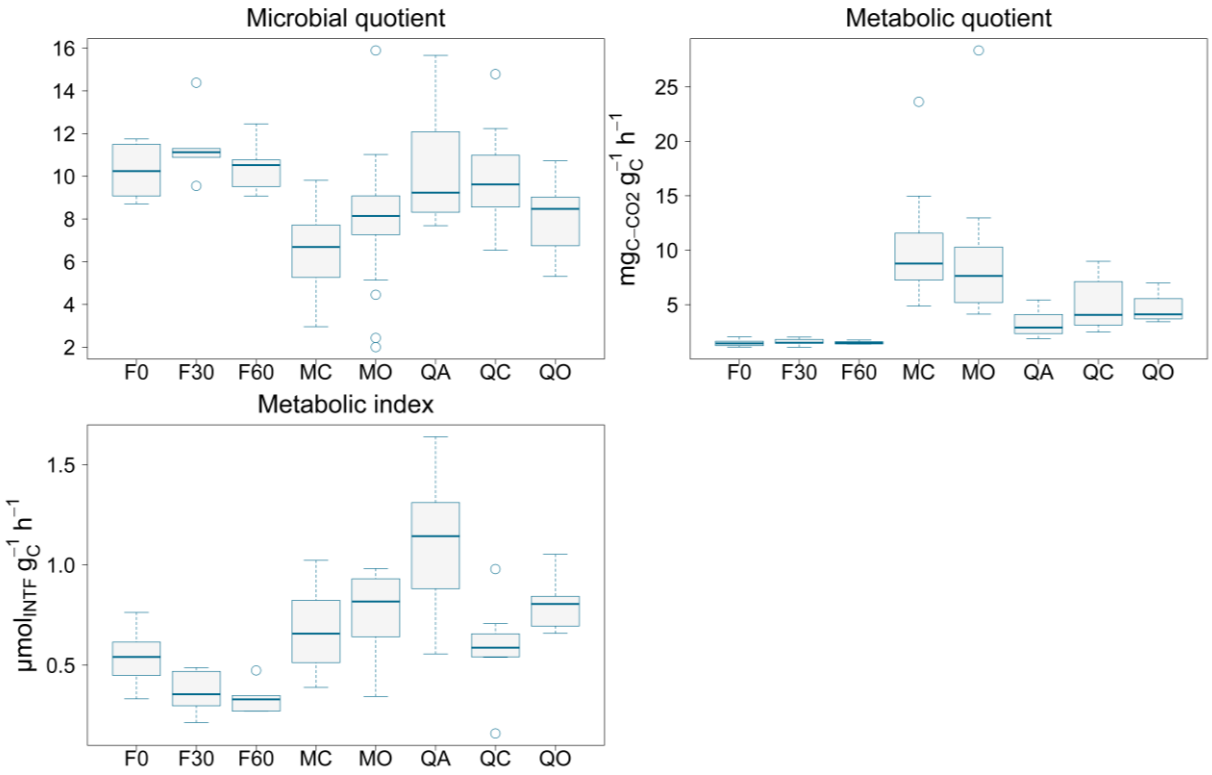

Figure 6.4 – Microbial quotient (qmic), metabolic quotient (qCO_2) and metabolic index (MI) for each experimental site and management considered. The line inside the box represent the median.

The specific enzyme activities have been here calculated as the ratio between the enzymatic activities and the SOC content because, as stated before (paragraph 6.1.2) it better reflects soil quality status (Gil-Sotres et al., 2005; Kandeler and Eder, 1993).

Regarding the specific oxidative activities (Figure 6.5), both dehydrogenase and tyrosinase showed the lower values in correspondence of the forest site, and the dehydrogenase resulted to have the higher specific activity in correspondence of the agro-ecologic management (QA). On the contrary, the specific laccase activity presented the higher activity at the beech forest site with slightly differences among treatments (only F60 seems to have a lower activity compared to F0 and F30). Within the agro-ecosystems, the QA seems to be the one that showed (looking at the median) the highest specific laccase activity.

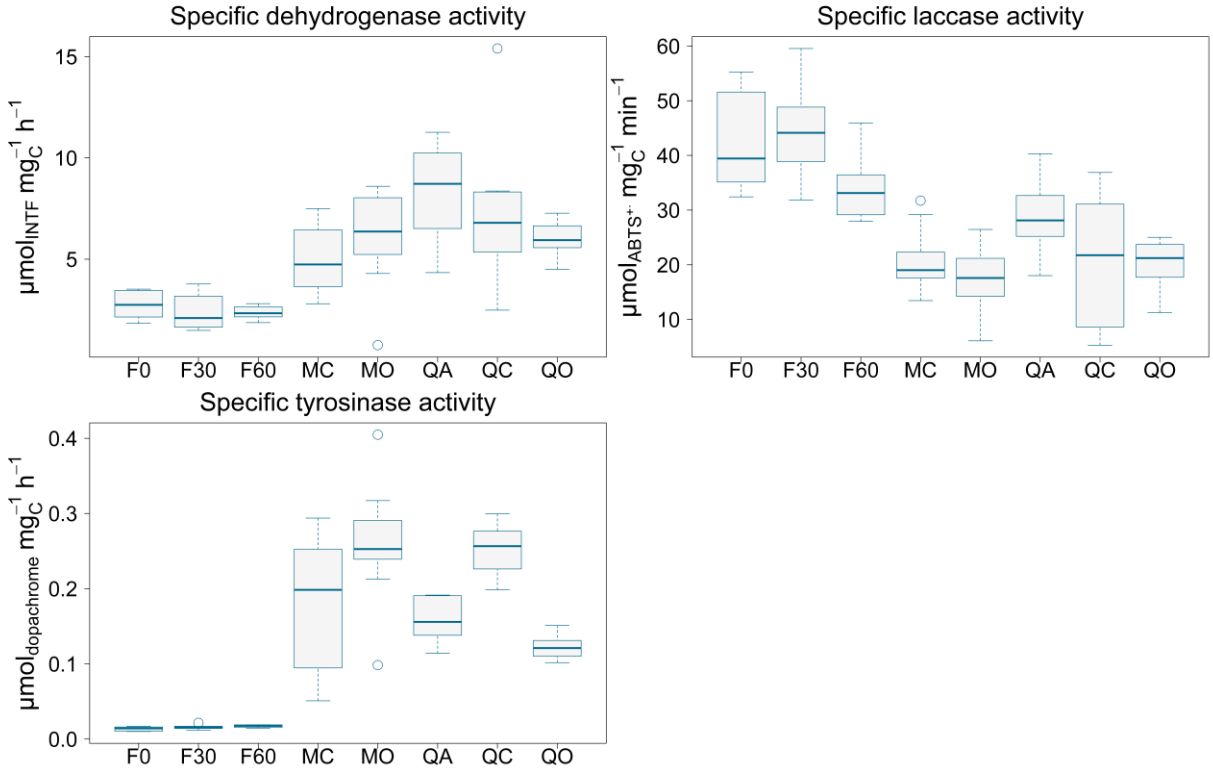

Figure 6.5 – Specific dehydrogenase ($Dehy_{SOC}$), laccase (Lac_{SOC}) and tyrosinase (Tyr_{SOC}) activities for each experimental site and management considered. The line inside the box represent the median.

Going forward, in figure 6.6 have been reported the specific hydrolytic enzyme activities which could be divided in two groups according to their response. The first one is composed by specific β-Glucosidase (βGlu_{SOC}), α-Glucosidase (αGlu_{SOC}), β-Xylosidase (βXyl_{SOC}), β-Cellobiosidase ($\beta Cell_{SOC}$) and N-acetyl-β-glucosaminidase (NAG_{SOC}) which showed the lowest specific activities in correspondence of the beech forest site. Within this group, some differences occurred between the agro-ecosystems and the more striking are the higher specific α-Glucosidase and N-acetyl-β-glucosaminidase activities in correspondence of the QC site and the higher specific β-Cellobiosidase activity with the QO site.

The second group is composed by arylsulphatase (AS_{SOC}) and phosphomonoesterase (PME_{SOC}) specific activities, which resulted to be higher in the forest plots with no differences among treatments or within the agronomic experimental sites.

Finally, the specific lipase activity (LIP_{SOC}) resulted to be enhanced only in the QC system with slightly differences between the other sites.

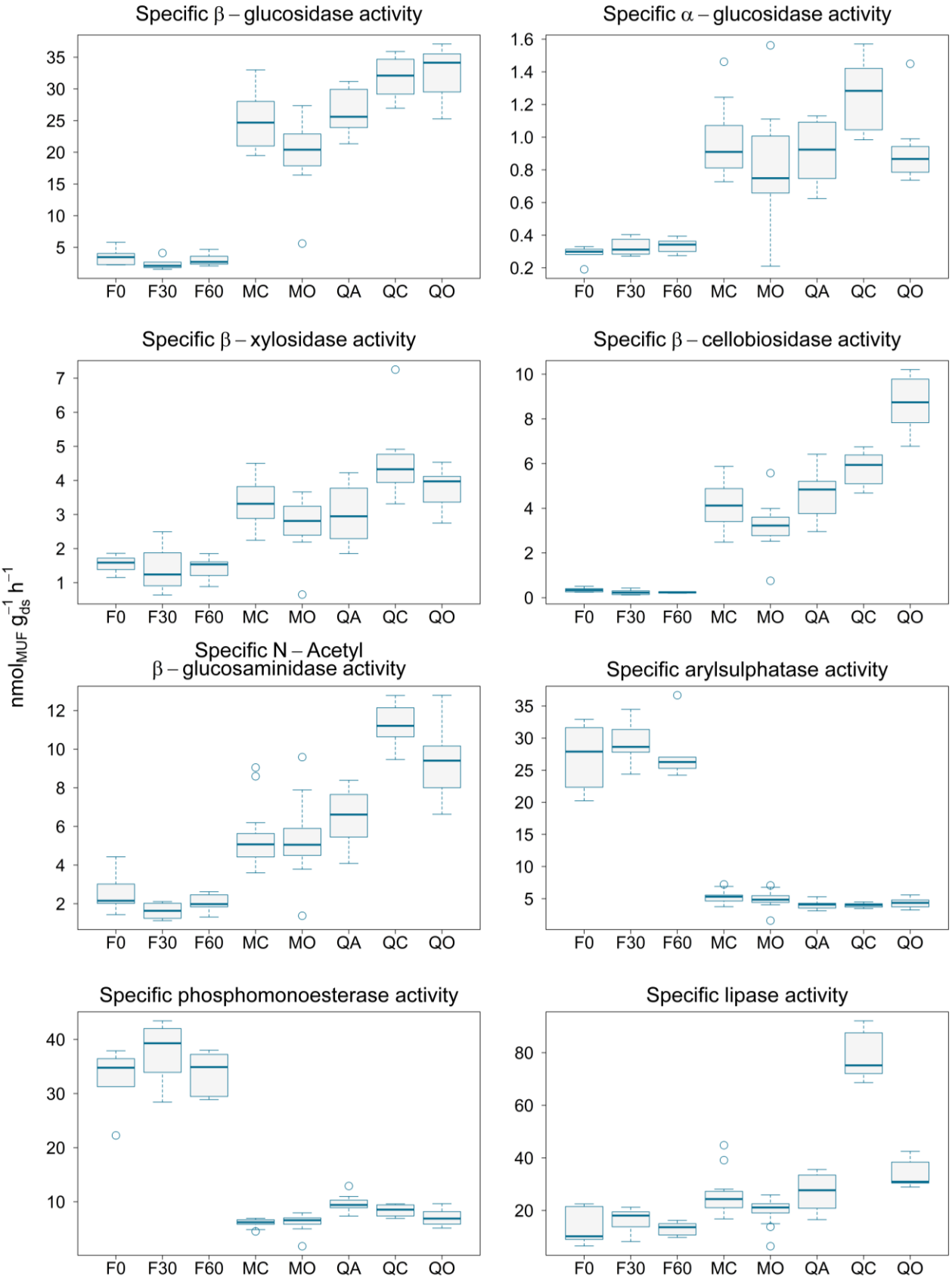

Figure 6.6 - Specific hydrolytic enzyme activities for each experimental site and management considered. The line inside the box represent the median.

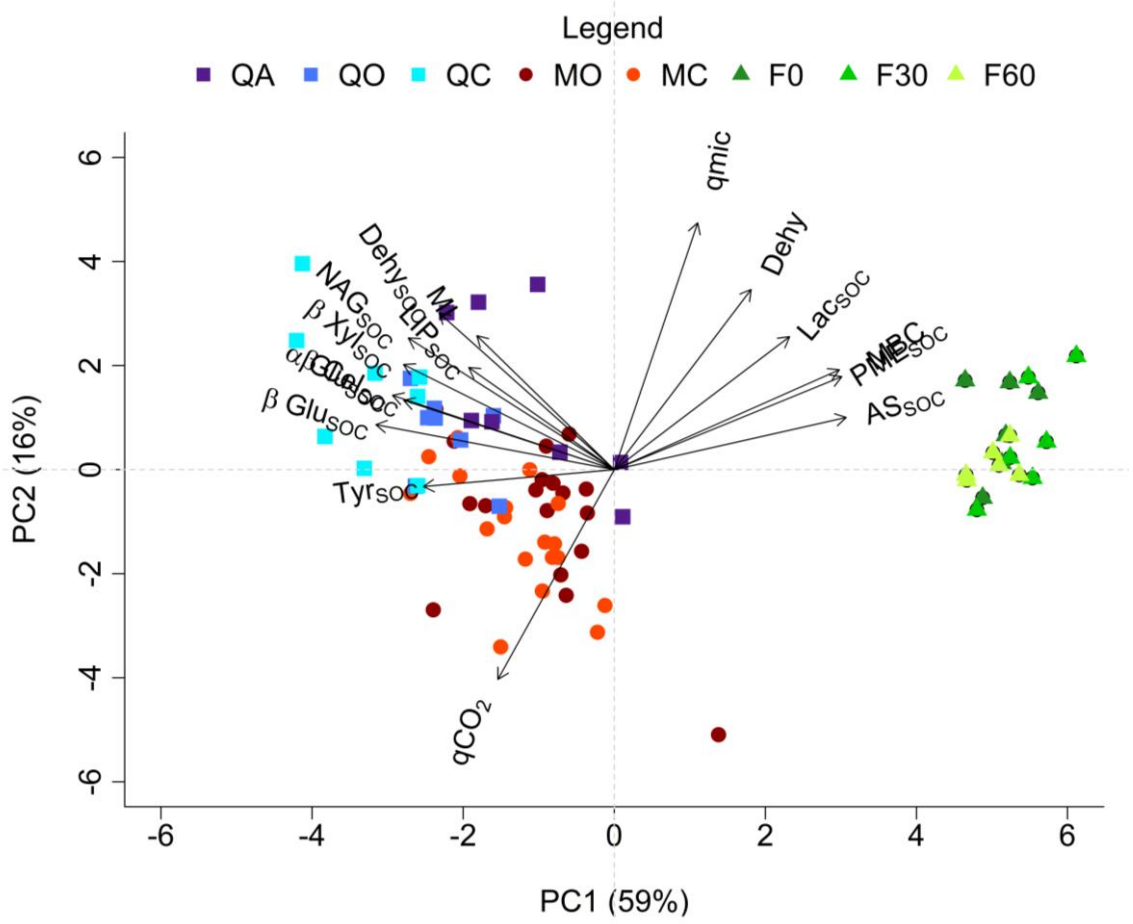

Figure 6.7 - PCA analysis in which are included all the simple indexes previously considered (MBC, Dehy, qmic, qCO_2, MI and all the specific enzyme activities.

Ultimately, all these simple indexes have been analyzed with the principal component analysis (PCA, Figure 6.7) which showed a clear segregation between forest and agronomic systems (PC1) with MBC, specific laccase, arylsufatase and phosphomonoesterase activities that characterized the forest system. Unfortunately, those parameters were not able to clearly separate the management within each macro-group (agronomic and forest). Anyway, it seems that the qCO_2 was the parameter that predominantly identify the MoLTE experimental site thus producing a soft separation between the two agronomic sites considered (MoLTE experimental site in red and QUABIO experimental site in blue).

6.3.2 Complex indexes

The geometric mean of the enzyme activities (GMea) showed the higher values in correspondence of the forest site thus indicating that the higher amount of enzymatic activity has been measured at this experimental site (Figure 6.8). Therefore, this index highlight differences between agronomic and forest systems but not between the management adopted within each system.

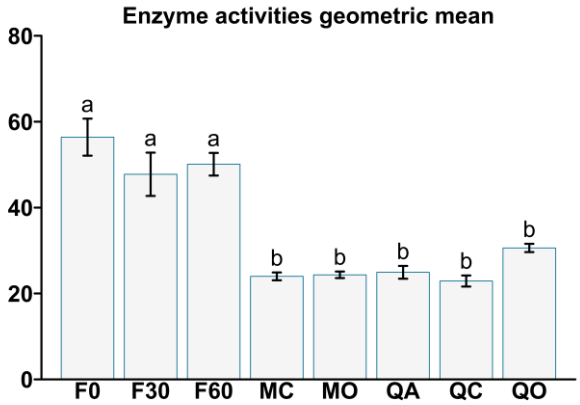

Figure 6.8 – *Marginal means of the geometric mean of the enzyme activities (GMea) for each experimental site and management considered. Error bars represent the standard error and different lowercase letters represent significant differences (P< 0.05).*

For what concern the ecosystem ratios, the C:N, C:P and N:P ratios (Figure 6.9) highlight significant differences not only between agronomic and forest sites but also between the agronomic management. However, even if also in this case differences were statistically significant, they were as well less striking. In general, the forest site resulted to have the lowest ecosystem ratios compared to those of the agronomic sites. Within the agronomic sites, conventional and organic management at MoLTE (MC and MO) and the agro-ecologic (QA) showed the same values of both C:N and N:P ratios that were in the first case the highest and in the second the lowest values of these two ecosystem ratios. Instead, regarding the organic and conventional management at the QUABIO site (QO and QC) it was possible to observe the higher C:P and N:P ratios.

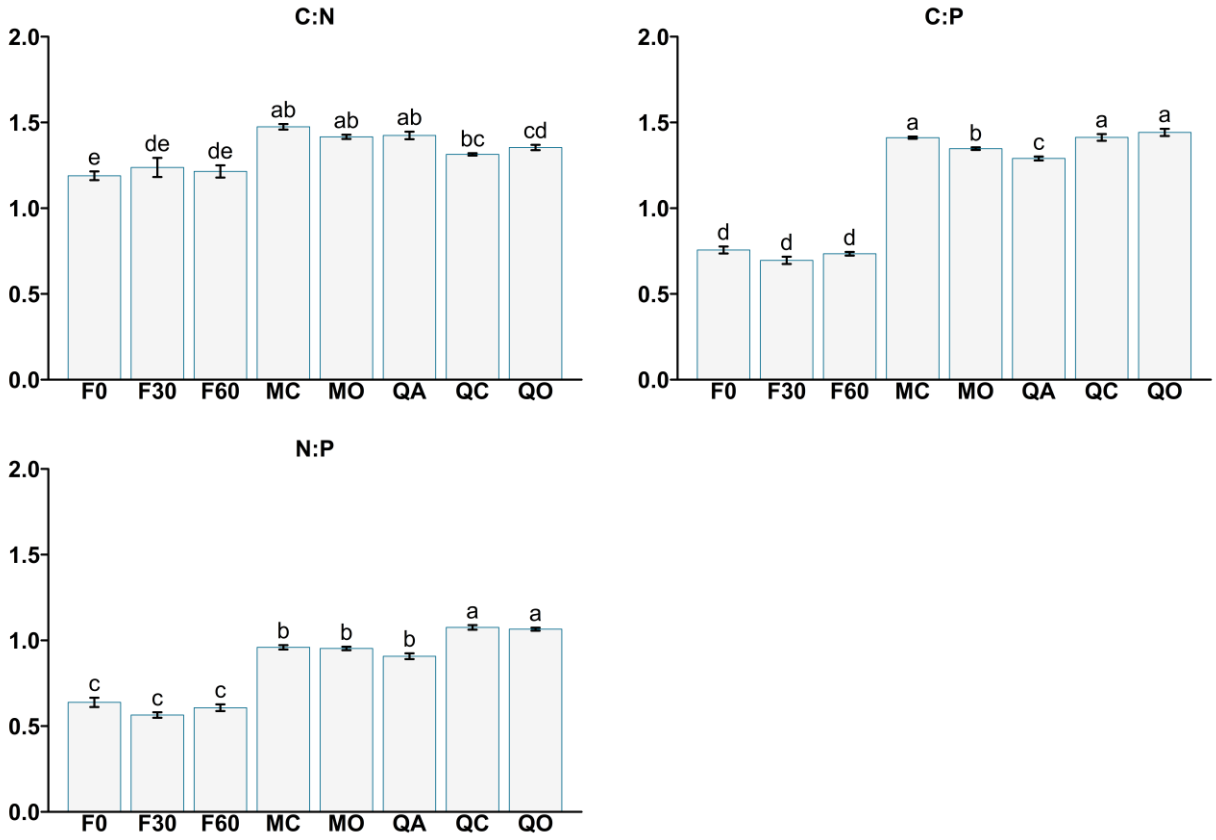

Figure 6.9 - Marginal means of the ecosystem ratios for each experimental site and management considered. Error bars represent the standard error and different lowercase letters represent significant differences (P< 0.05).

The fourth ecosystem ratio considered is the C:C, here graphically reported in relation to the SOC content (Figure 6.10). Indeed, as it has been considered (Sinsabaugh and Shah, 2011) an index of recalcitrant C abundance it was supposed to be appropriate observe its variation in relation to SOC content. Also in this case, results reported in figure 6.10 showed a clear separation between the forested and the agronomic sites and, specifically, in the forest experimental site, where the SOC content is higher, has been calculated low values of C:C. On the contrary, for the agronomic systems which have a great lower content of SOC, the C:C ratio showed values lower than what expected. Specifically, according to Sinsabaugh and Shah (2011) the C:C should to be inversely related to recalcitrant C content. Therefore, this has been true for the forested site but not always for the agronomic sites where actually, only the MC system resulted to has a higher C:C ratio.

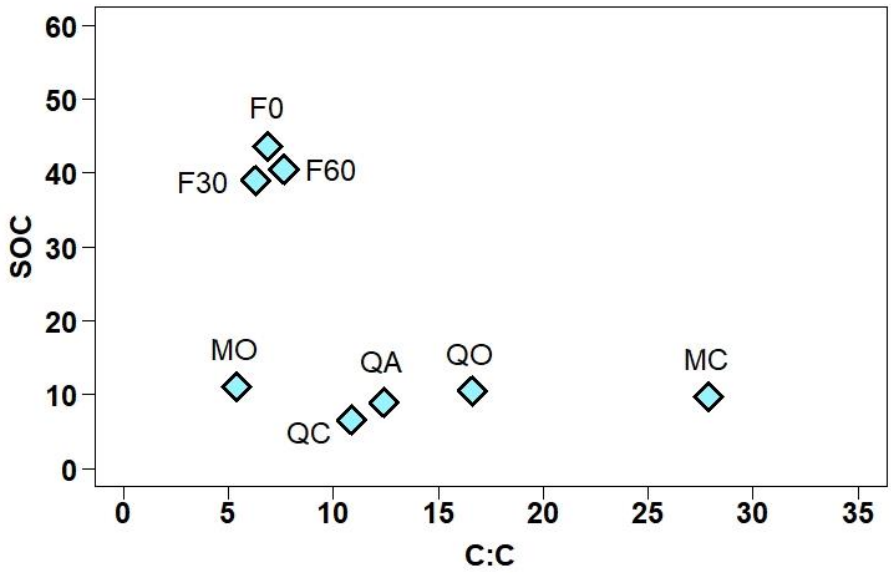

Figure 6.5 - Scatterplot of the variation of the C:C ratio with SOC content for each experimental system considered.

6.3.3 The soil quality index (SQI)

The first PCA performed for the determination of the minimum dataset then used for the calculation of the SQI (Figure 6.11), showed that in many cases the parameters considered were autocorrelated and thus some variables resulted to be close each other thus characterizing the same group of data (i.e. MBC with MBN, SOC with TN, C_{ext} and N_{ext}). Nevertheless, also in this case, with a PCA done on a restricted group of soil chemical and biochemcial parameters, the only separation clearly observable was that between the forest and the agronomic sites along the first PC that showed an explained variance of 71%.

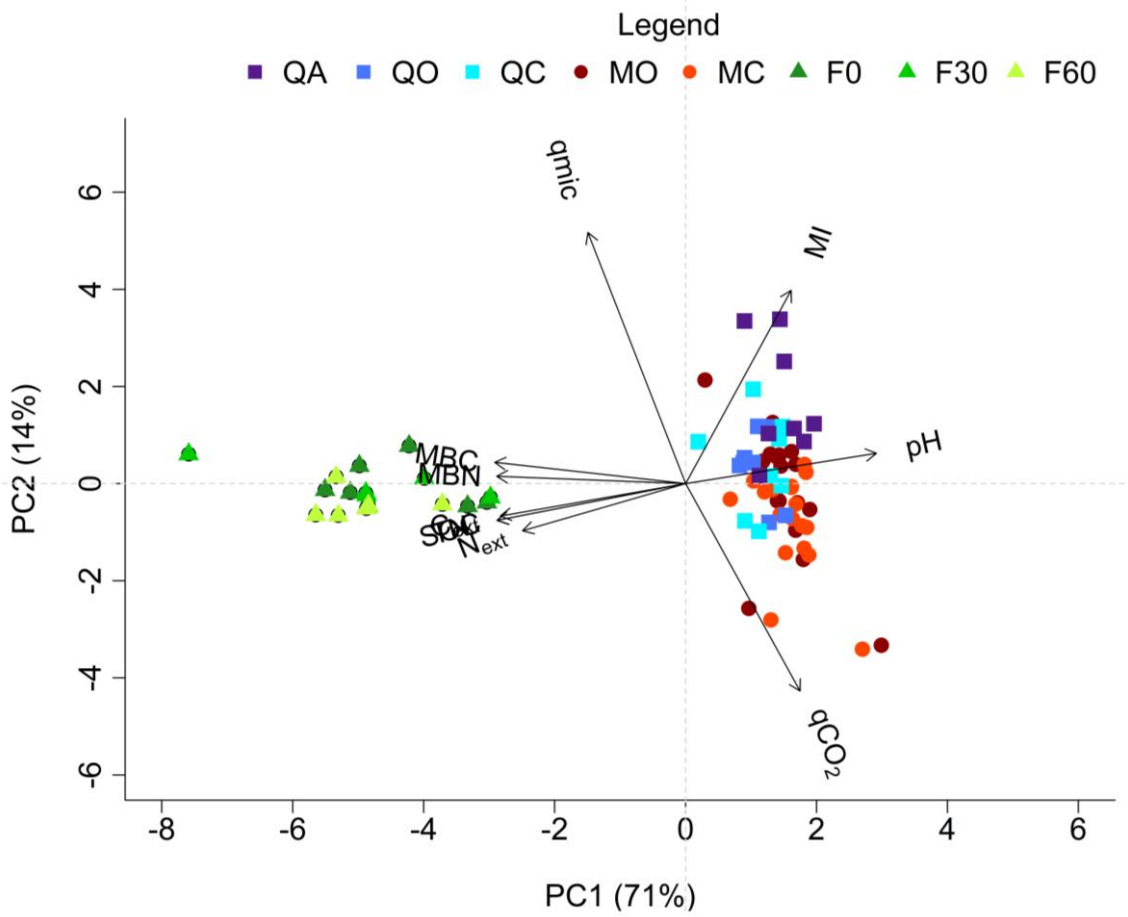

Figure 6.11 - PCA performed with all the chemical and biochemical parameters considered (pH, MBC, MBN, C_{ext}, N_{ext}, SOC, TN, qmic, qCO_2 and MI) for the determination of the SQI.

After the selection of the minimum dataset (MDS), that in this case has been composed only by four of the ten parameters initially considered (MBC, qmic, qCO_2 and MI), a new PCA based on the MDS has been performed (Figure 6.12). With this second PCA it is still possible to observe the main separation between forest and agronomic sites along the first PC even if with the utilization of the MDS the explained variance decreased to 56%. However, with this reduced dataset the explained variance along the second PC doubled leading to a rough separation also between the two agronomic sites (MoLTE represented in red and QUABIO represented in blue).

With the choice of this MDS it is possible also to confirm what previously observed with the simple indexes (Figure 6.3 and 6.4). Indeed, in that case emerged that the forest site was characterized by a higher MBC content and qmic. The least resulted to be higher also in correspondence of the agro-ecologic management where has been registered also great values of MI, and finally the highest level of qCO_2 has been observed in correspondence of the MoLTE site. Therefore, looking at the PCA

reported in figure 6.12 it is possible to observe that sites and management resulted characterized exactly from the abovementioned biochemical parameters.

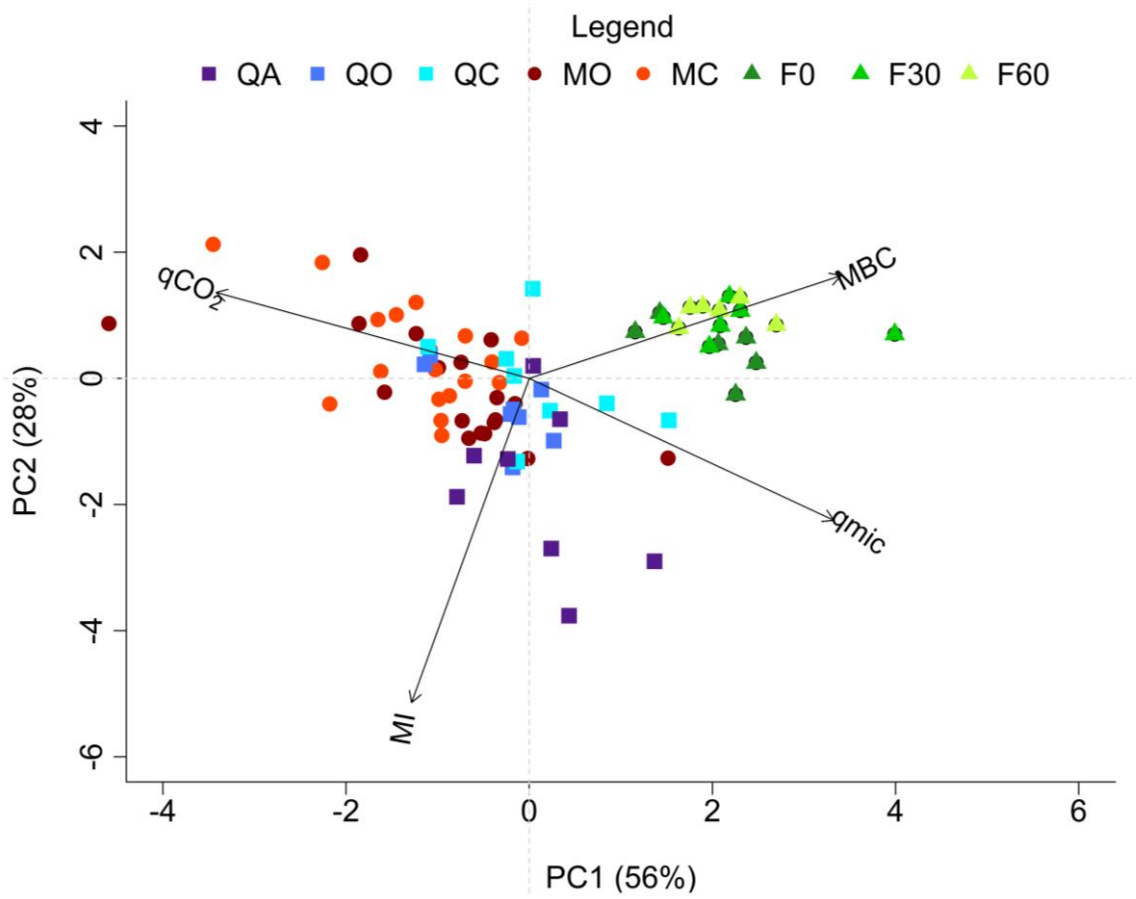

Figure 6.12 - PCA performed with the parameters included in the minimum dataset.

The final result of the SQI, obtained with a weighted additive procedure, has been reported in figure 6.13. The SQI resulted to be significantly higher in correspondence of the beech forest site (F0, F30 and F60) and where the agro-ecologic management has been applied (QA). On the other hand, the lowest SQI value resulted to correspond to the conventionally managed system at MoLTE experimental site (MO), while the organically managed system of both MoLTE and QUABIO (MO and QO) and the conventionally managed plots of the QUABIO site (QC) showed intermediate values of SQI.

It could be interesting also evaluating the SQI within each experimental site as to verify if the here obtained results correspond to those obtained in the study of each single experimentation.

Among the forest site, the F30 treatment resulted to have the lower SQI value even if without statistically significant differences. In the context of the MoLTE experimental site, a significant difference between conventional and organic management came out also with the SQI which presented higher values in correspondence of the organic once. Finally, considering the QUABIO project, also in this case significant differences have been highlighted from the SQI with higher values with the agro-ecological management and with no statistically significant differences between the organic and the conventional management.

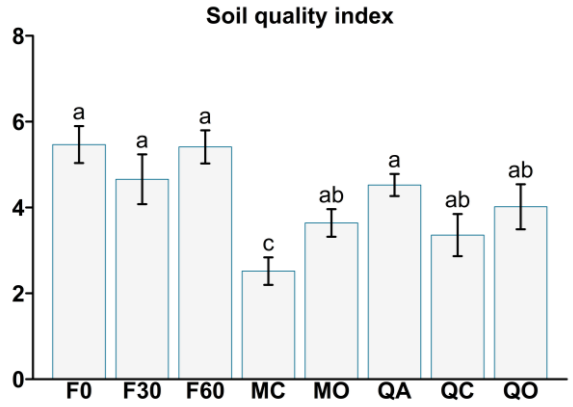

Figure 6.13- Marginal means of the SQI for each experimental site and management considered. Error bars represent the standard error.

6.4 Discussion

The simple indexes presented clearly distinguish between forest and agronomic ecosystems with the forest one that is characterized by higher MBC content, dehydrogenase activity, microbial quotient and lower metabolic quotient. Therefore, these results highlight that the forest soil, even if enriched in N, remain a favorable environment for microbial biomass wellness and activity. Within these simple indexes, only the metabolic index (MI, figure 6.4) showed noticeable differences between the management considered. Indeed, the agro-ecology management (QA) resulted to have the highest MI value thus indicating that this management was able to increase microbial efficiency in using the available C substrates (Bastida et al., 2008; Masciandaro et al., 1998). On the contrary, the lowest values of the MI has been calculated in correspondence of the treated forest plots (F30 and F60). Therefore, even if the microbial biomass content was high (MBC) and no indication of stressed conditon for the microbial activity have been highlitgh from the other indexes (i.e. qCO_2 values), it seems that the N addition lead to a striking reduction of the microbial metabolic efficency.

For which concern the specific enzymatic activities, $Dehy_{SOC}$ and Tyr_{SOC} showed similar trend in relation to the two macro-ecosystems considered with higher values in correspondence of the agronomic one. Moreover, within the agronomic sites differences came out and the trend of the two specific oxidative activities have not be the same indeed the $Dehy_{SOC}$ was higher at the QA while the Tyr_{SOC} was higher in correspondence of the MO and QC systems. This different response could probably be connected with the characteristics of the available substrates as, for example, the Tyr activity is generally related with great content of phenolic compounds. Particularly explicative has been the Lac_{SOC} specific activity, which being involve in the degradation processes of recalcitrant compounds as for example lignin, resulted to be enhanced as expected at the forest site. Anyway, also the QA system showed a considerable Lac_{SOC} specific activity and this could be related to the presence on the soil surface of faba residues which degradation process probably require the activation of laccases.

The hydrolytic specific activities involved in C and N cycles resulted, as attended, downturned in correspondence of the forest site. Indeed, the forest soil has resulted to be rich in both C and N pools however, with the consequence that an imbalance has been created in the stoichiometric equilibria and the content of P and S resulted to be no longer sufficient inducing an increase of PME_{SOC} and AS_{SOC} activities. Also in the case of the specific hydrolytic activities some differences between the agronomic management have been observed with the QC that registered the higher αGlu_{SOC}, βXyl_{SOC},

NAG_{SOC} and LIP_{SOC} specific activities, the QO showed an increase of $\beta Cell_{SOC}$ activity and the MO showed lower specific activities of the enzymes involved in C and N cycles. Again, these responses could presumably be related to the type of C and N source available in the soil of each system.

Among the simple indexes considered the MBC resulted to be the one that has been able to distinguish only between forest and agronomic sites. On the contrary, the other indexes highlighted differences also between management thus indicating their higher sensitivity to the applied treatment and/or management.

Proceeding, the calculated complex indexes has been able to discriminate only between the forest site and the agronomic sites without bring out any differences among the treatments. The GMea index used by García-Ruiz et al. (2008) was able to distinguish organic and conventional management, but it is not the case of this study. Probably, this could be related by the relevant discrepancy here presented between the forest and the agronomic systems that in some way hide the possible differences existing among the agronomic management. Within the ecosystem ratios, only the C:C ratio put in relation to SOC content highlight a clear separation also of the MC system from the other agronomic management. The result obtained with the C:C ratio and the SOC content reflect those obtained by Sinsabaugh and Shah (2011), confirming that the ratio is inversely related to recalcitrant C content and therefore could effectively be considered an index of recalcitrant C abundance. Consequently, in summary, these complex indexes appear to be less sensitive leading to the supposition that combining analytically many variables has caused a reduction of the information available and therefore the use of these complex indexes alone should be take into account carefully.

The last index tested was the SQI which gave interesting results showing the lowest value of soil quality in correspondence of the MC system and higher values at the forest site and with the application of the agro-ecological management. The lower SQI calculated for the MC could be related with the high C:C ecosystem ratio measured within the same system, indeed the SQI confirmed that high levels of phenol oxidizing activity in respect to the hydrolytic activities involved in C cycle, with low content of SOC induce a reduction of soil quality. Moreover, observing the SQI values within each experimental site highlight that the treatment or management that downturned the SQI (F30, MC and QC respectively in the forest site, in MoLTE and in QUABIO) perfectly match with what has been observed in each experimental site (see chapter 2, 4 and 5) where those systems resulted to be the once that less promote soil quality, functionality and sustainability.

It has to be notice that some problems occurred in the determination of the SQI, first of all enzyme activities could not be used in this index as there is not the possibility to score them in a realistic way. Specifically, judgments on enzyme activities levels have been always done comparing soils under different management, treatment, crops and vegetation, and therefore associate a function "more/less is better" or an "optimum" to an enzymatic activity is a pure subjective action that could result in unrealistic values. Moreover, for the variables that have to be considered with an "optimum" there is no references on which is this optimum and consequently the value has to be decided subjectively or in consideration of the mean value of the parameter considered. Therefore, with the objective to make results comparable, should be considered to create a list of soil chemical and biochemical parameters that have to be initially examined for the determination of the SQI. Within this list the MDS more appropriate for the data will be defined by the PCA. Finally, for each parameter included in the list it should be explicit the associate function and if needed the optimum value.

6.5 Conclusion

The use of simple and complex indexes to asses soil quality in different ecosystems turned out to be powerful for discriminate between forest and agronomically managed sites. However, it was greatly expected to obtain indications of higher soil quality in correspondece of the forest system. Instead, the considered indexes have not been able to discriminate at all between the two agronomic sites.

Results obtained with these indexes were not always clear and not always at the same level of detail in decribing soil quality and soil biochemical processes. However, in general it could be assessed that most of them highlight, as stated before, an higher level of soil quality in correspondence of the forest site followed by the agro-ecological management. Moreover, the combination site-management that predominatly resulted to has the lowest soil quality appeared to be the conventionally managed plot at the MoLTE site.

Unfortunately, the use of soil specific anzyme activities with the others biochemical indexes resulted to be really difficult as they refer to different levels of detail, indeed enzymatic activities contain information not only relative to the general concept of soil quality but also to the specific processes that were occurring in the soil. Therefore, a detailed explanation of enzyme activities behavior under each site-management combination was not possible as there were too much variation. However, thinking at the two macro-groups considered (forest and agronomic) differences came out.

Certainly, the use of both enzymatic activities and biochemical indexes is fundamental in the evaluation of soil quality and functionality, and within a certain level it is possible to asses that generally they are in accordance in the evaluation of the status of a soil. However, many efforts have to still be done for the identification of an all-encompassing index. Until that point, and considering the results here obtained, it would be better to consider as much indexes as possible being careful about the detail degree that would be explored.

GENERAL CONCLUSION

The obtained results certainly highlight the importance to consider biochemical parameters and enzymatic activities in the evaluation of soil quality changes after environmental alteration caused by human activities. Indeed, these parameters demonstrated to be strictly related to soil microbial biomass and to be particularly sensitive both in short and long-term to agricultural management, tillage and N addition to soil.

Within the agricultural management and tillage considered and studied in this work, it is possible to assert that in both the experimental sites the conventional management has been the one that significantly downturn the quality and the functionality of the soil leading to reduced microbial biomass and organic C content. Moreover, in correspondence of this management has been observed a decrease in microbial metabolic efficiency thus indicating that, probably, the addition of mineral fertilizers and agro-chemicals significantly alter soil microbial wellness. Similarly, also plowing negatively affected soil microbial biomass, activity and efficiency thus indicating that this kind of intensive tillage significantly alter soil functionality. On the contrary the reduced tillage appeared to be less impacting but slightly differences between the two minimum tillage has been observed and therefore it is possible to suppose that the application of reduced tillage practices could be anyway a valid alternative independently from which minimum tillage has been choose.

Finally, in the perspective to increase agriculture sustainability both organic and agro-ecologic management showed reassuring results that highlighted higher SOC and MBC content with more suitable metabolic efficiency. Despite this interesting results, there will be the need to verify the yields under both the organic and the agro-ecological management. Indeed, even if these practices significantly maintain soil quality and functionality, agronomically speaking there is also the demand for high productivity and to declare one agronomic practice as “sustainable”, this aspect could not be ignored.

For which concern the use of biochemical indexes and soil enzymatic activity in the forest site to study soil quality changes after N addition, it is again possible to confirm their sensitivity to environmental changes even if in this case the experimentation was started just one year before. Unfortunately, even if differences in enzymatic activities and biochemical indexes has been measured, conclusion on how N increase could act on SOC sequestration cannot yet be drawn as probably the system, which is very stable, needs more than one year to show the effects of this alteration.

It has to be highlight that all the soil samples analyzed derived from field trial and this clearly produce, despite from the replicates considered, a higher variability in the measured parameters thus making results interpretation more difficult.

The ICBM model has proved to be a tool particularly useful in the evaluation of SOC storage in the long-term period and for making prediction. It would be really interesting, in the feature, to implement the model including in it also the microbial component and the enzymatic activities.

Finally, the tentative to condense all the information in a unique and reliable index revealed that probably this is still not possible. Specifically, testing many different indicators of soil quality emerged that all of them were in accordance to the fact that the forest soil was in a condition of higher soil quality compared to those of the two agronomic sites. Some of the indicators point out that, within the agronomic management considered, the agro-ecological was the nearest to the forest site in terms of soil quality and functionality. However, the calculated soil quality indicators appear to be difficult to compare as they measure the soil quality at different scale and thus imply that the degree of information change from one index to one other. With all these considerations it appears better to consider more than one index if the aim of the work is to evaluate the overall quality and functionality of a soil.

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