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***Effects of organic amendment on soil quality as  
assessed by biological indicators***

***Ph.D. Dissertation***  
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*“Who hath created seven universes one above another: Thou seest not, in the creation of the All-merciful any incongruity, Return thy gaze, seest thou any rifts. Then Return thy gaze, again and again. Thy gaze, Comes back to thee dazzled, aweary”*

*Al-Quran,  
(Chapter 67: Verse 3-4)*

This, in effect, is the faith of all scientists; the deeper we seek, the more is our wonder excited, the more is the dazzlement for our gaze



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## **Acknowledgment**





# Chapter 1

## Introduction

### 1.1 Soil and its importance

Relevance of soils as a resource in terrestrial ecosystems has been pointed out on 30 May 1972 by the Committee of Ministers in the Council of Europe that has written the European Soil Charter, declaring:

- 1. Soil is one of humanity's most precious assets. It allows plants, animals and humans to live on the earth's surface*
- 2. Soil is a limited resource which is easily destroyed*
- 3. Industrial society uses land for agriculture as well as for industrial and other purposes. A regional planning system must be conceived in terms of the properties of the soil and the needs of today's and tomorrow's society*
- 4. Farmers and foresters must implement methods that preserve the quality of the soil*
- 5. Soil must be protected against erosion*
- 6. Soil must be protected against contamination*
- 7. Urban development must be planned so that it causes as little harm as possible to adjoining areas*
- 8. In civil engineering projects, the effects on adjacent land must be assessed during planning, so that adequate protective measures can be reckoned in the cost*
- 9. An inventory of land resources is indispensable*
- 10. Further research and interdisciplinary collaboration are required to ensure wise use and conservation of the soil*
- 11. Soil conservation must be taught at all levels and be kept to an ever-increasing extent in the public eye*

12. Governments and those in command must purposefully design and administer soil resources

Soil is linked to everything around us and performs many vital roles in sustaining life on Earth. Moreover, the European Commission Communication on the Thematic Strategy have been identified and underlined the key role of soil in ecosystems as living and powerful element which supports plant and animal life, as a source of food and raw materials. It is a fundamental part of the biosphere that, together with vegetation and climate, helps to regulate the circulation and affects the quality of water (Blum, 2005; EC, 2006).

Additionally, soil has filtering, buffering and transformation capabilities influencing the water cycle and the gas exchange between terrestrial and atmospheric systems and protecting the environment against the contamination of ground water and the food chain (Fig. 1.1).

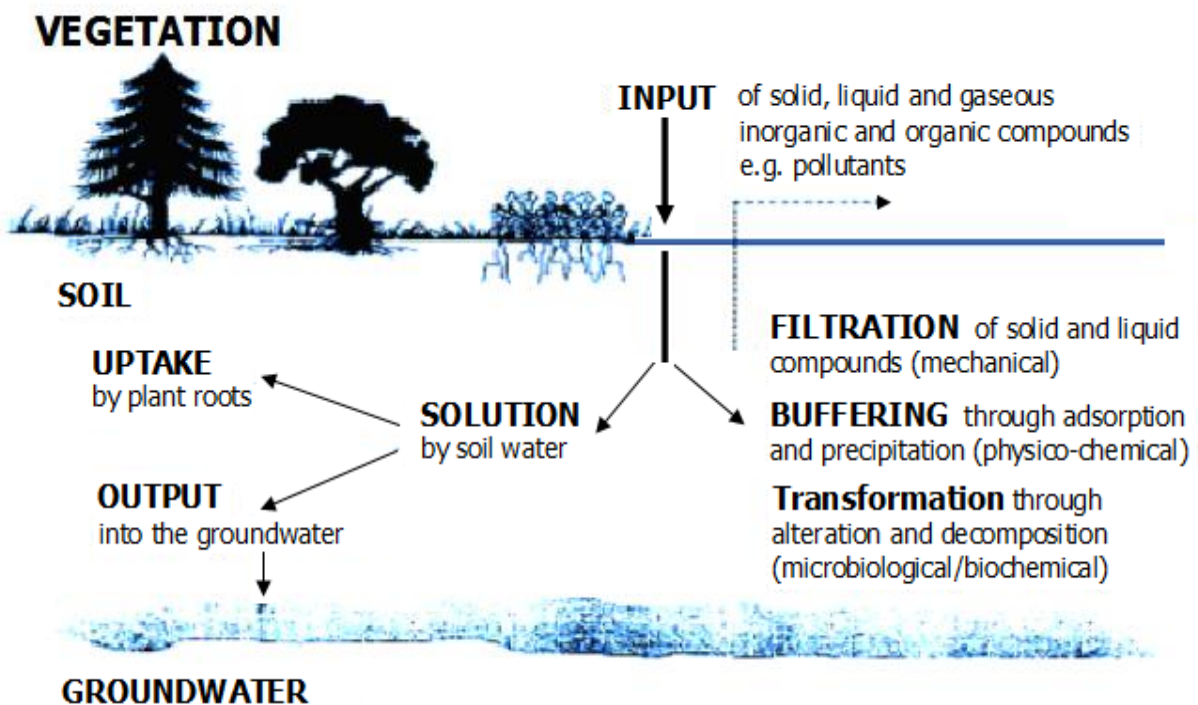


Fig. 1.1 Filtering, buffering and transformation activities of soil (Blum, 2005).

As long as this filtering, buffering and transformation capacities are maintained, there is no danger for the ground water or the food chain (Blum 2005). Furthermore, soil has other fundamental functions in the ecosystems, being the basis for biomass production, controlling and regulating biogeochemical cycles, storing carbon, providing habitats and sustaining biodiversity, supplying raw material, preserving cultural and archaeological heritage. All the same, soil is also a vulnerable resource because it is a thin layer covering part of the earth's surface that develops slowly by physical, chemical and biological processes, but it can be immediately destroyed by careless action. Therefore, soil use must be planned rationally, considering immediate needs but also ensuring long-term conservation of the soil in order to increase or, at least, maintain its quality.

The European Soil Charter points out the fundamental role of research in the preservation of this essential but limited resource: *“on it depends the perfecting of conservation techniques in agriculture and forestry, the elaboration of standards for the use of chemical fertilizers, the development of substitutes for toxic pesticides, and methods of suppressing pollution. Scientific research is essential to prevent the consequences of the wrong use of the soil in any human activity”*, underlining that researches on soil and its use *“must be supported to the full”* and *“must form part of the work of multidisciplinary centres”*, considering the complexity of the problems involved.

## **1.2 Agricultural use of soils**

### *1.2.1 Intensive agricultural management and its impact*

The key role of agriculture now and in the future is the distribution of safe food at reasonable prices. Since the middle of the 20<sup>th</sup> century, global agricultural production has been expanded to meet the soaring demand for cheap food for ever rapidly growing population. From 1965 to 2005, world population has increased by

111% whereas, crop production rose by 162% (Burney et al., 2010; Fig. 1.2). This dynamic increase in productivity have been possible through extensive use of land resources, fertilizers and pesticides, modern machineries and advanced techniques. Additionally, demand for biofuels, booming population growth in developing countries, globalization trends and economic considerations in developed countries have put increasing demand on food supplies that can only be satisfied by intensification and industrialization of farming practices.

On the other hand, the extent of intensive agricultural management is causing great pressure on the environment by degradation and depletion of the natural resource, like soil, water, natural plant and animal resources (Burney et al., 2010; Moeskops, et al., 2010; OECD, 2001; Tilman et al., 2002).

Agricultural intensification is a prime driver of global biodiversity, loss often at a rapid pace, both locally and globally, by massive degradation of habitat and extinction of species (Lupwayi et al., 2001; Novacek and Cleland, 2001; Oehl et al., 2004), a worrying question considering that a high biodiversity is essential for maintaining ecosystem services. According to the Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA, 2003), habitat loss and fragmentation due to modern intensive farming represent the greatest threat to natural genetic variation, reflecting the degradation or destruction of a whole ecosystem. In particular, habitat loss is identified as a main threat to 85% of all species described in the IUCN Red List (IUCN, 2000). On the other hand, the introduction of thousands of new and foreign genes or genetic stocks is a prime threat to biodiversity. They are introduced with trees, shrubs, herbs, microbes, and higher and lower animals each year (Sukopp & Sukopp, 1993). Many of these new species survive and, after many years of adaptation, become invasive (Starfinger et al., 1998).

Genetic diversity reduces with the introduction of new commercial varieties. Terrestrial and aquatic biodiversity have also declined rapidly due to excessive use of fertilizers, pesticides, tillage and even crop rotation (Tilman et al., 2002; Tilman et al., 2006).

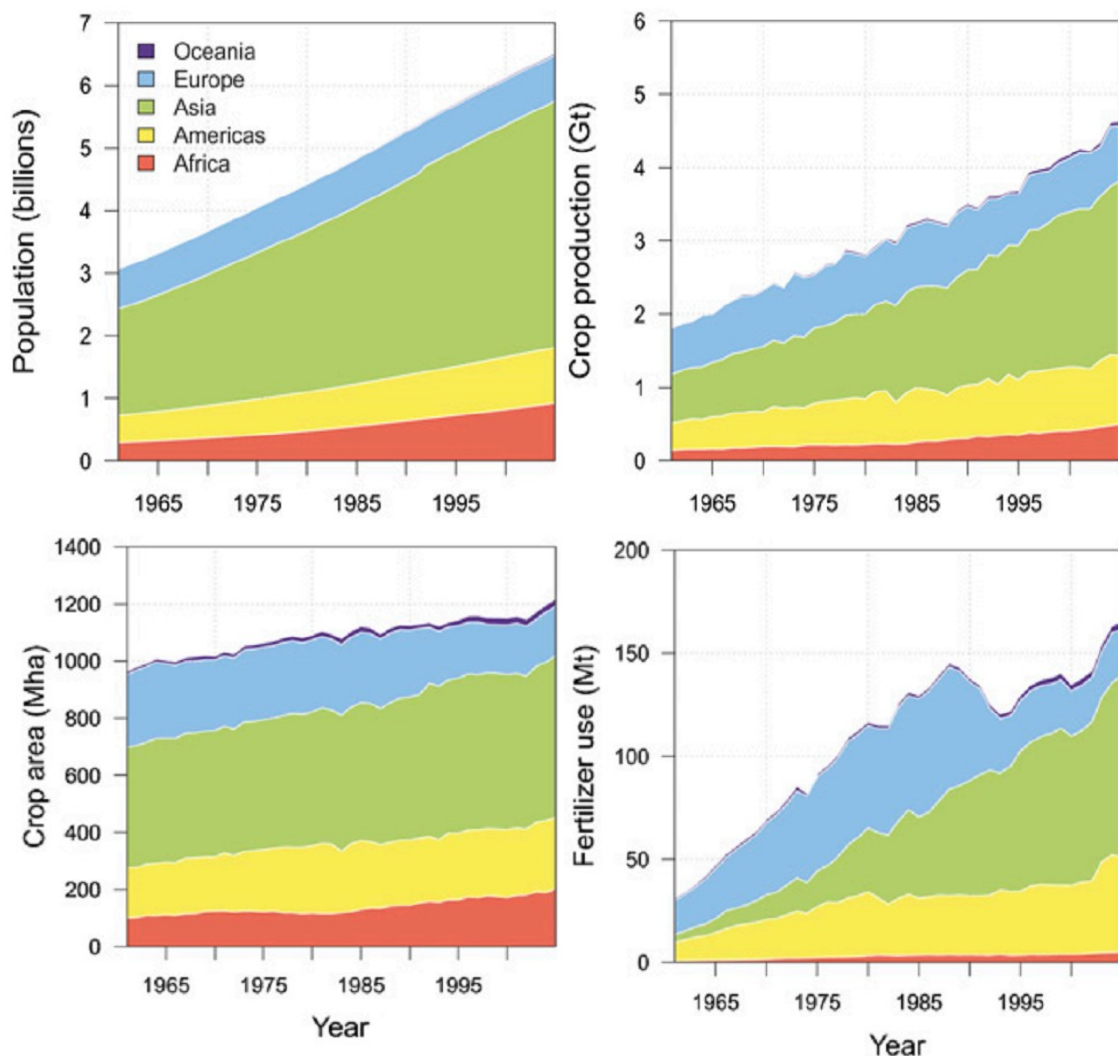


Figure 1.2. Regional and global trends in population (upper left), crop production (upper right), crop area (lower left), and fertilizer use (lower right), 1961–2005 (Burney et al., 2010).

One of the predominant effects of intensive agricultural management is degradation of soil, also due to rapid depletion of soil organic matter that affects, in turn, soil physical, chemical and biological properties. The declining trend in soil quality is posing a serious threat to sustainability of intensive agriculture, because

intensive farming relies on the extensive use of synthetic fertilizers, pesticide applications and energy inputs that all together have an enormous impact on soil and water, causing degradation in form of erosion, deforestation, alkalinity and salinity, acidification, micronutrient deficiency and water logging that ultimately affect soil quality and productivity (Lopez et al., 2011). Moreover, the widespread use of mineral fertilizers and the optimal water content and temperature in the greenhouses, promoting mineralization processes in soil, together with the crop removal and the systematic elimination of crop residues to limit plant diseases (Bonanomi et al., 2007), cause loss of organic matter in agricultural soils with a negative feedback on soil microbial populations (Su et al., 2006; Lou et al., 2011). Soil erosion is another most obvious form of soil degradation; it is estimated that one-sixth of the world's soils has already been degraded by water and wind erosion (Oldeman et al., 1991). The rate of erosion is highest when soil is not covered with a protective layer of plants or decaying organic matter. Industrial farmland is particularly vulnerable to erosion due to intensive tillage (plowing), which eliminates protective ground cover from the soil surface and destroys root systems that help holding the soil together. In extreme cases, erosion can lead to desertification.

Excessive use of fertilizer and pesticide is responsible for accumulation of toxic compounds in the soil. Leaching loss of nitrate cause eutrophication of surface waters and contamination of ground water (Vitousek et al., 1997). Additionally, when pesticides fail to reach the target, affect adjacent ecosystems via leaching or aerial drift, where it can have significant impacts on the diversity and abundance of non target species and can have complex effects on ecosystem processes and trophic interactions (Pimentel & Edwards, 1982). The chemicals used may leave the field as runoff, eventually ending up in rivers and lakes, alter their biology or may drain into groundwater aquifers which is increasingly raising environmental and public health concerns (Horrigan et al., 2002). Some environmentalists attributed the dead or hypoxic zone in the Gulf of Mexico as being encouraged by

nitrogen fertilization of the algae bloom (Beck, 2008). Moreover, nitrogen-contaminated groundwater is harmful to humans, particularly to vulnerable populations such as children, the elderly, and people who have suppressed immune systems. Infants who drink water contaminated with nitrates can suffer from methemoglobinemia, or blue baby syndrome, a condition that can cause brain damage or death, according to US EPA (2002).

Soil is the primary natural habitat that determines the long-term wealth of nations. At the same time, as intensive agricultural management has brought substantial economic and social development, it has also contributed to environmental degradation via increased greenhouse gas emissions, biodiversity loss, and the reduced delivery of many ecosystem services including soil and water conservation (Kirschenmann, 2010; Millennium Ecosystem Assessment, 2005). However, most declines in civilizations throughout history have been largely caused by the mismanagement and subsequent degradation of the land (Carter & Dale, 1974; Hyams, 1952). So it is necessary to imply successful management of resources for agriculture to satisfy changing human needs, maintaining high productivity per unit area on a continuous basis, minimizing the magnitude and rate of soil degradation as well as maintaining the environmental quality and conserving natural resources.

### *1.2.2 Sustainable agricultural management*

The issue of sustainability is an important goal for modern agriculture arisen from the increased awareness that human population is growing at a rate that the finite natural resources available may not be able to support (La1 and Pierce, 1991). World population is increased from 3.08 to 6.51 billion in 1961 to 2005, as shown in figure 1.2, and needs about a 60-70% increase in food production (La1 and Pierce, 1991); however, 88% of the soil resources possess one or more constraints to sustainable production (Oldeman, 1994). Simultaneously, La1 and Pierce (1991)

cautioned that this could lead to increased human-induced land degradation if sustainable agricultural management strategies are not adopted.

Sustainable agricultural management is defined as the use of land to meet the changing human needs, while ensuring long-term socio-economic and ecological functions of the lands, for the benefit of present and future generations, and provides for:

- ❖ *using land resources on a long-term basis*
- ❖ *meeting present needs without jeopardizing future potential*
- ❖ *enhancing per capita productivity*
- ❖ *protecting the potential of natural resources and prevent degradation of soil and water quality and*
- ❖ *restoring productivity and degraded and impoverished ecosystems.*

(Damanski et al, 1993; Lal and Miller, 1993; Tilman et al., 2002).

The goal of sustainable agriculture is to maximize the net benefits that society receives from agricultural production of food and fibre and ecosystem services. This will require increased crop yields, increased efficiency of nitrogen, phosphorus and water use, ecologically based management practices, judicious use of pesticides and antibiotics, and sweeping changes in some livestock production practices.

Advances in the fundamental understanding of agroecology, biogeochemistry and biotechnology that are linked directly to breeding programmes can contribute immensely to sustainability (Cassman, 2002; Tilman et al., 2002). For this reason, sustainable agriculture is now our definitive way for an environmentally sound, productive, economically viable, and socially desirable agriculture.



### 1.3 Soil quality

Soil lies at the heart of Earth's 'critical zone'- the thin veneer extending from the top of the tree canopy to the bottom of our aquifers. Quality of this resource depends in part on its natural composition, also on the changes caused by human use and management (Gianfreda et al., 2005). Human factors influencing the environment of the soil can be divided into two categories: those resulting in soil pollution and those devoted to improving the productivity of soil (Gianfreda and Bollag, 1996; Gianfreda et al., 2005).

Recently the concept of 'soil health' and 'soil quality' have been received considerable attention (Ashard and Martin, 2002; Karlen et al., 2003), because of their fundamental role in the preservation of ecological functions for future generations (Kibblewhite et al., 2008). Although both of this concept are interchangeable (Karlen et al., 2003), it is indispensable to distinguish that soil quality is related to soil functions (Karlen et al., 2003; Letey et al., 2003), whereas soil health is related to non-renewable and dynamic living resource (Doran and Zeiss, 2000).

*"Soil health is defined as the continued capacity of soil to function as a vital living system"*, by recognizing that it contains biological elements that are key to ecosystem function within land-use boundaries (Doran and Zeiss, 2000; Karlen et al., 2001). These functions are able to sustain biological productivity of soil, maintain the quality of the surrounding air and water environments, as well as promote plant, animal, and human health (Doran et al., 1994). In other words, a healthy soil is a stable soil with resilience to stress, high biological diversity, and high levels of internal cycling of nutrients. As a medium for food and fibre production and sustaining ecosystem services, the soil state ultimately affects human health and influencing the quality of our air and water (Janvier et al., 2007). However, Soil Science Society of America (Karlen et al., 1997) defines soil quality *"the capacity of soil to function to sustain plant and animal productivities, to*

*maintain or enhance water and air quality and to support human health and habitation*”. Valuable soil functions (or ecosystem services) include water flow and retention, solute transport and retention, physical stability and support, retention and cycling of nutrients, buffering and filtering of potentially toxic materials and maintenance of biodiversity and habitat (Daily et al., 1997).

On account of the complexity of the soil system, emerging definitions of soil quality have been also suggested by several scientists (Acton and Gregorich, 1995; Larson and Pierce, 1994). All these definitions had in a common goal to underline the capacity of the soil to function effectively at the present and in the future. Doran and Parkin (1994) reviewed several proposed definitions of soil quality and defined it as *“the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health”*.

An important feature of soil quality is the delineation between inherent and dynamic soil properties (Karlen et al., 1997; USDA-NRCS, 2001). Inherent soil quality (Figure.1.3.a) refers to the soil’s natural ability to function, which is related to the five soil forming factors (Jenny, 1961). Dynamic soil quality (Figure.1.3.b) refers to the effects of human use and management on soil functions (Seybold et al., 1999) which is related to soil properties (Karlen et al., 1997). Although some misconceptions exist, the recent emphasis on dynamic soil quality is not intended to detract from the importance of inherent soil properties.

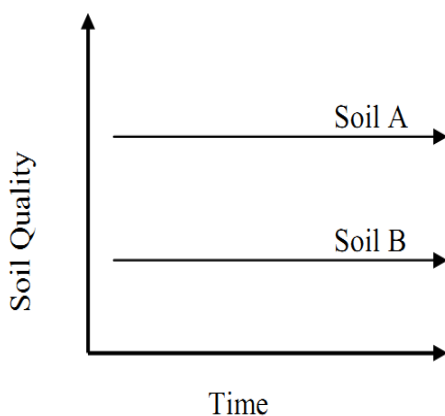


Figure 1.3.a. Inherent soil quality

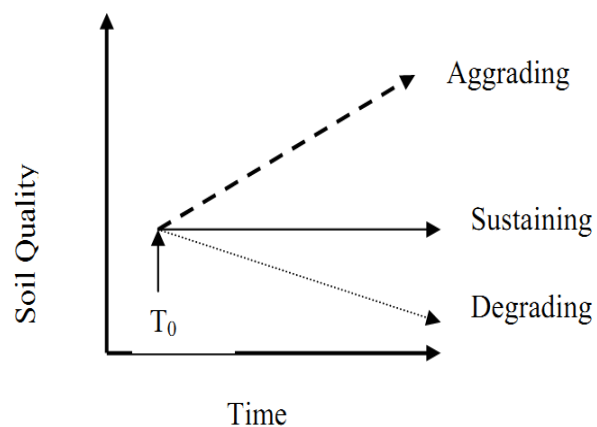


Figure 1.3.b. Dynamic soil quality

The specific definition of soil quality for a particular soil is depend on its inherent capabilities, intended land use, management goals and their interactions. For instance, optimum levels of organic matter (and other soil properties) will differ depending on the condition under which the soils formed, leading to variation in potential functioning.

To adopt a long-term approach to land resource use, the additional view of soil quality, as measured by soil performance and productivity, is now considered inadequate, because not only soil quality expresses the inherent attributes of a soil, but also expresses the ability of the soil to interact with applied inputs (Larson and Pierce, 1994).

Building and maintaining soil quality is the basis for any harmonious and successful farming. The link among soil quality, farming practices, long-term soil productivity, sustainable land management, agriculture and environmental quality is now widely acknowledged (figure.1.4), as it represents the importance of conserving soil as a resource for future generations instead of ‘soil fertility’, which has usually been associated with crop yield only (Gregorich et al., 2001; Siegrist et al., 1998).



Figure.1.4. Relationship among soil quality, environmental quality and agricultural sustainability

Soil quality is the end product of soil degradation or conservation processes and is controlled by chemical, physical, and biological components of a soil and their interactions (Gianfreda et al., 2005). Thus, inappropriate management can directly drive to deleterious changes in soil function. For instance, in Asia, adverse effects on soil health and soil quality arise from nutrient imbalance in soil, excessive fertilization, soil pollution and soil loss processes (Hedlund et al., 2003). For this reason, protection of soil quality under intensive land use is a considerable challenge for sustainable resource use in the developing world (Doran et al., 1994). On account of this, tools and methods to assessing and monitoring of soil health and soil quality are necessary to evaluating the degradation status and changing trends following different land uses and agricultural management interventions (Doran and Jones, 1996; Lal and Stewart, 1995), to develop rigorous forecasting methods to quantify and best utilize soil's natural capital, to appraise options for maintaining or extending it, and to determine how declines can be reversed.

Measuring soil quality is an exercise in identifying soil properties that are responsive to management, affected or correlated with environmental outcomes, and are capable to be precisely measured within certain technicals and economic constraints. For this reason, assessing soil quality will require collaboration among all disciplines of science to examine and interpret their results in the context of land management strategies, interactions, and trade-offs.

### *1.3.1 Qualitative approach in defining soil quality*

A qualitative approach depends on farmers experience and indigenous knowledge to evaluate the descriptive properties such as how the soil looks, feels, and smells as well as its resistance to tillage, the presence of worms, etc. (Acton and Gregorich, 1995; Romig et al., 1995). This approach has much to offer scientists interested in soil quality evaluation (Harris and Bezdicek, 1994). However, others

strongly recommended that qualitative (descriptive) information should be an essential part of quality monitoring programs (Harris and Bezdicek, 1994).

### *1.3.2 Quantitative approaches in defining soil quality*

Quantitative approaches to soil quality evaluation involve sophisticated analytical procedures aimed at generating data. Several approaches to quantitative assessment of soil quality such as the dynamic assessment approach (Larson and Pierce, 1994), the performance-based approach (Doran and Parkin, 1994), and the multi-scale approach (Karlen et al, 1997) has been proposed. One common feature of all these different approaches is that soil quality is assessed with respect to species and functions of the soil. The dynamic assessment approach proposed by Larson and Pierce (1994) measures selected soil quality indicators over time, using statistical quality control procedures, to assess the performance of a given management system rather than comparing it to other systems.

The advantage of this approach is that it pushes the researcher to focus attention on the attributes that contribute to the behaviour of the system. Doran and Parkin (1994) described a performance-based index, which can be used to evaluate soil function with regards to vital issues of sustainable production, environmental quality, and human and animal health. In addition to food and fiber production, erosivity, ground water quality, surface water quality, air quality and food quality also included. In the multi-scale approach presented by Karlen et al. (1997), point- and plot-scale evaluations are aimed at understanding processes that act on soil quality whereas, the higher scales (field - international) of study are used for monitoring soil quality.

Quantitative or qualitative assessment of soil quality requires the use of indicators. The complex nature of soil quality does not allow the use of a single measure (Acton and Gregorich, 1995) and therefore, a range of indicators is used. Because of the wide range over which soil properties vary in magnitude, importance, time,

and space (Karlen and Scott, 1994; Larson and Pierce, 1991), indicators used to measure soil quality must be clearly defined and selected.

## **1.4 Soil quality indicators**

Indicators are measurable properties that provide clues about how well the soil can function (Andrews et al., 2004; Liu et al., 2006). Indicators can be physical, chemical, and biological properties, processes, or characteristics of soils (Paz-Ferreiro et al., 2009). Good indicators are relevant, sound and cost-effective. A relevant indicator is directly related to the most notable aspects of the goal, is self-explanatory, is sufficiently sensitive for its purpose, and can be used to monitor actions. A sound indicator is acceptable to experts in the field, regardless of their backgrounds, thus, it is science-based and sufficiently accurate, precise and robust for its intended purpose. For an indicator to be cost-effective means that the value of its information must be greater than its cost. In general, this means that required data is readily available and computation is relatively easy. Indicators should interact with one another, and thus the value of one is affected by one or more of the other selected parameters.

Soil quality indicators are useful to policy makers to: monitor the long-term effects of farm management practices on soil quality, assess the economic impact of alternative management practices designed to improve soil quality (such as, cover crops and minimum tillage practices), examine the effectiveness of policies addressing the agricultural soil quality issue and improve policy analysis of soil quality issues by including not only environmental values but also taking into account economic and social factors. Many potential parameters of soil quality measurable at various scales of assessment, have been proposed (Table 1.1).

Most of the European countries, USA, Canada and so on developed their own parameters to evaluate soil quality. Since the early 1990, countries within the

European Union have made considerable efforts to develop agro-environmental indicators and the United States has developed soil ratings based on measured soil properties for the comparison of land management systems (Karlen et al., 2001).

Table 1.1 Potential physical, chemical, and biological indicator of soil quality, measurable at various scales of assessment, as proposed by Karlen et al. (2001).

<b>Biological</b>	<b>Chemical</b>	<b>Physical</b>
<b>Point scale indicator</b>		
Microbial biomass	pH	Aggregate stability
Potential N mineralization	Organic carbon and nitrogen	Aggregate dust distribution
Particulate organic matter	Extractable macronutrients	Bulk density
Respiration	Electrical conductivity	Porosity
Earth warm	Micronutrient concentrations	Penetration resistances
Microbial communities	CEC and cation ratios	Water filled pore space
Soil enzymes	Cesium 137 distribution	Profile depth
Fatty acid profiles	Xenobiotic loadings	Crust formation and strength
		Infiltration
<b>Field or farm scale indicators</b>		
Micorrhiza populations	SOM change	Top soil thickness and color
Crop yield	Nutrient loading or mining	Compaction or ease of tillage
Weed infestation	Heavy metal accumulation	Pounding and infiltration
Disease presence	Changes in salinity	Rill and gully erosion
Nutrient deficiencies	Leaching or run off	Surface residue cover
<b>Regional-national-international scale indicators</b>		
Growth characteristics	Acidification	Desertification
Productivity (yield stability)	Salinization	Loss of vegetative cover
Species richness, diversity	Water quality changes	Wind and water erosion
Keystone species and Ecosystem engineers	Air quality changes (dust and chemical transport)	Siltation of the river and lakes
Biomass density and abundance		

### *1.4.1 Soil physical and chemical indicators*

Soil physical and chemical indicators are of paramount importance in soil quality assessment (Bastida et al., 2008). Physical indicators are related to the arrangement of solid particles and pores. Examples include topsoil depth, bulk density, porosity, aggregate stability, texture, crusting and compaction (table 1.1). Physical indicators primarily reflect limitations to root growth, seedling emergence, infiltration or movement of water within the soil profile, indicate how well water and chemicals are retained and transported and provide an estimate of soil erosion and variability. They also indicate productivity potential, even out landscape and geographic variability, describe the potential for leaching, and erosion.

Chemical indicators include measurements of pH, salinity, organic matter, phosphorus concentrations, cation-exchange capacity, nutrient cycling, and concentrations of elements that may be potential contaminants (heavy metals, radioactive compounds, etc.) or those that are needed for plant growth and development. The soil chemical condition affects soil-plant relations, water quality, buffering capacities, availability of nutrients and water to plants and other organisms, mobility of contaminants, and some physical conditions, such as the tendency for crust to form.

### *Water holding capacity and water content*

The water holding capacity that is primarily controlled by soil texture and organic matter of a soil, is immensely influential agronomic characteristic. Soils that hold generous amounts of water are less subject to leaching losses of nutrients or applied pesticides. In addition, microbes show their highest activity when there is a balance between air- and water-filled pore space that is about 50-60% of water holding capacity (Troeh and Thompson, 2005). The finer the soil texture, the



higher its ability to hold or retain water for plant use (Lavelle and Spain, 2001; Troeh and Thompson, 2005).

### *Soil organic carbon*

Soil organic matter (SOC) plays a crucial role in the functioning of agricultural ecosystems, ecosystem productivity and the global C cycle (Weil and Magdoff, 2004). SOC is a key and particularly sensitive indicator of overall quality, because it plays a fundamental role in many of the ecosystem processes facilitated by soil (Lal, 2010). It has a large influence over many soil properties that are critical for soil quality including soil aggregation, soil water availability, cation exchange capacity and nutrient availability, microbial biomass C and pH buffering (Weil and Magdoff, 2004). Integrated crop management (Glover et al., 2010), organic management, reduced tillage (Badalucco et al., 2010) and retention of crop residues (Karlen et al., 1994) are all management strategies that have heavily influence on SOC.

### *Total nitrogen and C/N ratio*

Nitrogen is one the most vital nutrient that is essential for the growth and development of all organisms and most often deficient for crop production in arable soil. For this reason, nitrogen has been applied to soil for many years to enhance agricultural production. However, the loss of excess nitrogen from agricultural soils is of serious environmental concern, either via leaching (usually nitrate) leading to water quality problems or via gaseous emissions (ammonia, nitric and nitrous oxides) which can have knock-on effects on atmospheric pollution and the greenhouse effect. In nitrogen-poor semi-natural ecosystems the use of soil nitrogen is elevated. The carbon nitrogen ratio in soil is related to patterns nitrogen immobilization and mineralization during organic matter decomposition by microorganisms (swift et al., 1979). In natural ecosystems, the C/N ratio of SOM falls within well defined limit, usually from about 10 to 12.

Depending on soil C/N ratio, the interactions of C and N are particularly noteworthy, being N the most commonly limiting nutrient for plant and microbial growth and soluble C the main energy source for microorganisms (Moscatelli et al., 2005). A high C/N ratio will result in a scanty mineral nitrogen availability in the soil being locked up in the soil biomass by soil micro-organisms rather than being available for plants, potentially resulting in crop failure due to lack of available nitrogen. A low C/N ratio may mean there is an excess of available nitrogen in the soil which can be leached to water courses or emitted as nitrous oxide thus affecting the wider environment.

#### *1.4.2 Biological indicators*

It is well established that microorganisms appear to be excellent indicators of soil health because they respond quickly to changes in the soil ecosystem and have intimate relations with their surroundings due to their high surface to volume ratio. Understanding soil quality by biological parameters is reported as critically important by several authors (Doran and Parkin, 1994; Abawi and Widmer, 2000). Soil quality is strongly influenced by microbiologically mediated processes as an integral part of the formation of soil structure and nutrient cycles, and microbial activity is highly dependent on the soil water status, temperature, food supply, pH and other factors that determine what lives in soil and when they are active. Therefore, microbial parameters give an integrated measure of soil quality, an aspect that cannot be obtained with physical/chemical measures alone which integrate short-, middle- and long term changes in soil quality.

Since soil quality is strongly influenced by microbe-mediated processes, and function can be related to diversity, it is likely that microbial community structure will have the potential to serve as an early indication of soil degradation or soil improvement. Therefore, there is growing evidence that soil microbiological and

biological parameters may possess potential as an early and sensitive indicators for soil ecological stress or compensation (Dick, 1994; Gianfreda et al., 2005; Trasar-Cepeda et al., 2000), as is the case of soil enzyme activities, soil microbial biomass, composition of soil micro flora, that were used as potential biochemical/biological indicators of soil quality (Gianfreda et al., 2005; Trasar-Cepeda et al., 2000). Although microbial biomass only forms a small fraction of SOM, it greatly contributes to agricultural sustainability because its high turnover rate is responsible for nutrient release and therefore, promotes plant uptake (Smith et al., 2008). For example, the soil biomass (25 cm top soil layer) is known to process over 100,000 kg of fresh organic material each year per hectare in many agricultural systems. This processing includes the decomposition of dead organic matter by the microbes as well as the consumption and production rates in the soil community food web (Mario, 2006). Thus, information on microbial biomass, activity and nutrient status, combined with indices related to microbial community, such as microbial quotient ( $q_{mic}$ ), coefficient of endogenous mineralization (CEM) and metabolic quotient ( $qCO_2$ ) provide indications on soil quality or sustainability changes (Dinesh et al., 2004). Moreover, Islam and Weil (2000) concluded that total microbial biomass, active microbial biomass and basal respiration per unit of microbial biomass showed the most promise for inclusion in an index of soil quality. However, for correct assessment of appropriate functioning of soil, it is necessary to integrate all soil physical, chemical and biological characteristics, because a proper evaluation of soil quality requires the determination of a large number of parameters (Bloem et al., 2006; Marzaioli et al., 2010).

### *Microbial biomass*

Soil microbial biomass is the active component of soil organic pool (Henrot and Robert, 1994). It plays a crucial role in organic matter decomposition as well as in nutrient transformation and consequently influences ecosystem productivity (Franzluebbers et al., 1999). According to Insam (2001), microbial biomass is an

important indicator of soil productivity and its evaluation is invaluable in soil ecological studies. Studies have shown that soil microbial biomass is often influenced by soil depth, seasonal fluctuations, pH, heavy metal pollution and land management practices (Calbrix et al., 2007; Dai et al., 2004). High concentrations of heavy metals are known to affect the morphology, metabolism and growth of microorganisms in soils (Giller et al., 1998), as they disrupt the integrity of their cell membranes and cause protein denaturation (Leita et al., 1995). Furthermore, microbial biomass has been reported to correlate positively with yield in organic farming compared to conventional farming systems (Tu et al., 2006).

### *Respiration*

Soil respiration involves the oxidation of organic matter and the production of carbon-dioxide ( $\text{CO}_2$ ) and water as end products. The oxidation process is mediated by soil aerobic microorganisms, which makes use of oxygen as electron acceptor. Thus, the metabolic activities of soil microbial communities can be quantified by measuring the amount of carbon-dioxide produced or oxygen ( $\text{O}_2$ ) consumed in a given soil (Nannipieri et al., 1990). Soil respiration can be subdivided into basal respiration and substrate-induced respiration. Basal respiration refers to respiration that occurs without the addition of organic substrate to the soil (Vanhala et al., 2005), while substrate-induced respiration refers to the respiration that occurs in the presence of added substrate (Ritz and Wheatly, 1989). The measurement of soil respiration rates has been used in the assessment of the side effects of heavy metals and pesticide accumulation and various amendments such as, addition of sewage sludge or other forms of substrates in the soil (Fernandes et al., 2005; Ritz and Wheatley, 1989).

*Biodiversity*

Biodiversity is often associated with soil resilience to endure disturbance and increase in the soil microbial community diversity has been reported to increase soil resilience capacity. A huge number of methods exist to measure biodiversity of soil organisms. Some methods directly count the number of species and individuals present in a sample to calculate diversity, while others are based on a community approach estimating the activity of soil organisms or of specific functional groups. In the past few years, considerable efforts have been made towards the standardization of some methods. The genetic diversity of microorganisms (including bacteria, fungi, but also protists) can be estimated through either of two approaches: cellular cultures and molecular biology methods. Cellular cultures are used to encourage the controlled growth of microorganisms under laboratory conditions (e.g. in incubators or flasks containing appropriate growth medium). The main drawback of this method is that it is a selective protocol favoring the growth of some species compared to others. However, the proportion of cells that can currently be cultured is estimated to be only between 0.1% and 10% of the total populations in a given soil sample. As a consequence, cellular cultures only reveal a subset of the original soil microbial community. On the other hand, several methods based on molecular biology have been developed to characterize the genetic information contained in the DNA and RNA of microbes or other soil organisms. The main disadvantage is that there is no standardized DNA extraction procedure and the efficiency may vary depending on the nature of soil sample. One of the most important method to assess functional diversity is the substrate induced respiration (SIR) method, useful to determine the catabolic response profiles of soil microbial community, a method developed by Degens and Harris in 1997. This method is one of the simplest techniques with the most rapid outcome, avoiding the problem of the culturability of soil microbial populations under artificial conditions by adding the individual substrates directly to soil and measuring the resulting respiration response.

Measurement of microbial functional diversity by SIR approach has been used to monitor land management (Asgharipour and Rafiei, 2011; Degens and Vojvodic-Vukovic, 1999; Graham and Haynes, 2005; Romaniuk et al., 2011), cropping intensity (Sparling et al., 2008), soil organic carbon status (Degens et al., 2000), successional sequences (Schipper et al., 2001), stress or disturbance to the soil (D'Ascoli et al., 2005; Degens et al., 2001; Duponnois, et al., 2005; Frey, et al., 2008; Marchante, 2007; Ravit, et al., 2006; Schipper and lee, 2004;), development stages of volcano soil (Shillam, 2008), impact on herbicide (Valiolahpor, et al., 2011).

#### *1.4.3 Soil microbial indices*

Microbial indices have been considered as potential indicators of soil biological properties and processes thus soil quality (Doran and parkin, 1994; Sparling, 1997; Anderson and Domsch, 1989).

#### *Metabolic quotient ( $qCO_2$ )*

The metabolic quotient ( $qCO_2$ ) is the community respiration per biomass unit, usually expressed as ( $mg\ C_{CO_2}\ mg^{-1}\ C_{mic}\ h^{-1}$ ) has been widely used as a sensitive indicator of soil development and response to stress (Wardle and Ghani, 1995; Dilly and Munch, 1998; Anderson, 2003). Odum's theory on "*The Strategy of Ecosystem Development*" states that in a young developing ecosystem there is less competition for energy and less incentive for efficient use, whereas, during a succession, there is a growing competition for energy and selective pressure towards efficient use based on available resources (Insam and Haselwandter, 1989). So, in the case of edaphic communities, during a succession, respiration rate per unit of biomass tends to decrease and then, in a mature ecosystem, we will find a greater amount of microbial carbon and a lower rate of respiration. Anderson,

(2003) affirms that values with higher metabolic quotient more than  $2 \text{ mg mg C}_{\text{CO}_2} \text{ mg}^{-1} \text{ C}_{\text{mic}} \text{ h}^{-1}$  indicate an energetically less efficient microbial community and poor health condition.

Although its reliability as a disturbance or ecosystem development has been recently criticized by some authors, it is recognized to have valuable application as a relative measure of the same critical value (Moscatelli et al., 2005).

#### *Coefficient of Endogenous Mineralization (CEM)*

Coefficient of endogenous mineralization (CEM) represents the fraction of organic carbon mineralized to  $\text{CO}_2$  and usually is expressed as  $\text{mg C}_{\text{CO}_2} \text{ g}^{-1} \text{ C}_{\text{org}} \text{ h}^{-1}$ . It provides important information on organic matter mineralization and soil potential to accumulate or lose organic carbon. CEM value increases in soil under stress such as fire, crop rotation (Gijsman et al., 1997; Rutigliano et al., 2002) and decrease with plant succession (de Marco et al., 2005; Rutigliano et al., 2004).

#### *Microbial Quotient ( $C_{\text{mic}}/C_{\text{org}}$ ratio)*

The microbial quotient ( $C_{\text{mic}}/C_{\text{org}}$ ) reflects the contribution of microbial biomass carbon to soil organic carbon (Anderson and Domsch, 1989; Sparling, 1992). The ratio has been proved to be a sensitive indicator of quantitative changes in SOM due to the changing of management conditions and climate (Anderson and Domsch, 1989; Insam et al., 1989). However, to establish whether the  $C_{\text{mic}}/C_{\text{org}}$  ratio of a soil is in equilibrium, thus whether a soil has achieved equilibrium in organic matter status, it will be necessary to establish a baseline or reference values for each soil and a set of conditions to which the tested soil can be compared (Sparling, 1992). One problem associated with the  $C_{\text{mic}}/C_{\text{org}}$  ratio is that both components have a common origin, and are dependent each other. Also, changes in organic carbon will impact more on the ratio than changes in microbial biomass since the former is quantitatively much more abundant.

## 1.5. Soil Organic Matter

Intensive agriculture, characterized by heavy usage of machinery, pesticides, phytosanitary measurements and/or chemical fertilizers has increased productivity and efficiency of agricultural systems over past decades, causing, in time, seriously compromised by the severe detrimental effects on soil fertility. In fact, one of the most predominant effects of intensive activities of agricultural land management is deterioration of SOM due principally to crop removal and erosion processes.

Moreover, carbon loss in agricultural soils is also due to the increase in mineralization processes deriving from higher activity of soil microbial community, in consequence of tillage and use of greenhouses. This depletion trend is also enhanced by removal of crop residues and reducing organic matter (OM) supply. However, importance of SOM is not only to maintaining soil fertility but also to sustaining the productivity in time of agro ecosystems (Su et al., 2006; Lou et al., 2011).

Since SOM is derived mainly from plant residues, it contains all of the essential plant nutrients; accumulated OM, therefore, is a storehouse of plant nutrients. Upon decomposition, the nutrients are released in plant available forms (figure 1.5.).

SOM reduces by adsorbing toxicity of pollutants and affects growth, activity and diversity of soil biota because it is food for soil organisms from bacteria to earthworms and these organisms hold on to nutrients and release them in forms available to plant. High organic carbon content in soil improves its structural stability and porosity, moreover compounds such as polysaccharides (sugars) bind mineral particles together into micro aggregates. organic acids (e.g., oxalic acid), commonly released from decomposing organic residues and manures, prevents phosphorus fixation by clay minerals and improve its plant availability, especially in subtropical and tropical soils.



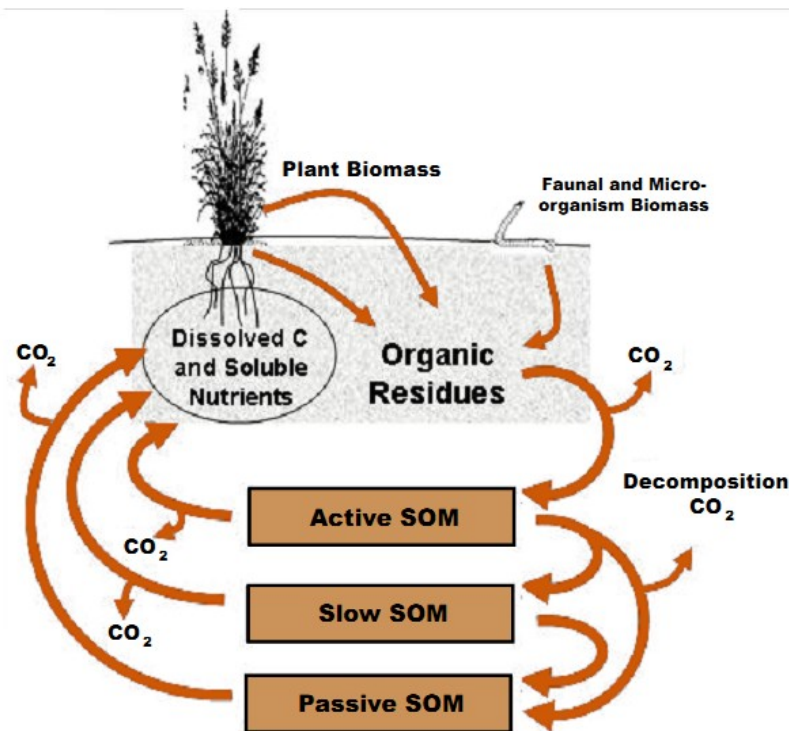


Figure 1.5. Organic matter cycle (modified from Brady and Weil, 1999).

A good content of organic carbon increases water holding capacity (thereby, availability of water for plants, especially in sandy soils), produces a higher resistance to compaction and reduces erosion (reduce crusting, especially in fine-textured soils) because glomalin (substance that account for 20% of soil carbon) glues aggregates together and stabilizes soil structure making soil resistant to erosion but porous enough to allow air, water, and plant roots to move through the soil. It also affects soil physical, chemical and biological properties by enhancing root development (Fernandes et al., 1997).

The input of OM in soils includes passive supply, deriving from catabolic activity of soil biota and dead OM, and active supply, deriving from the activity of microbial community and plant roots (e.g. root exudates). In the natural environment, the input of SOM comes principally from leaf litter fall whereas, the outputs depend on speed of humification and mineralization processes, which, in turn, are affected by physical and chemical properties of the soil and climatic factors, regulating growth and activity of the soil pedofauna and edaphic microflora (figure 1.6)

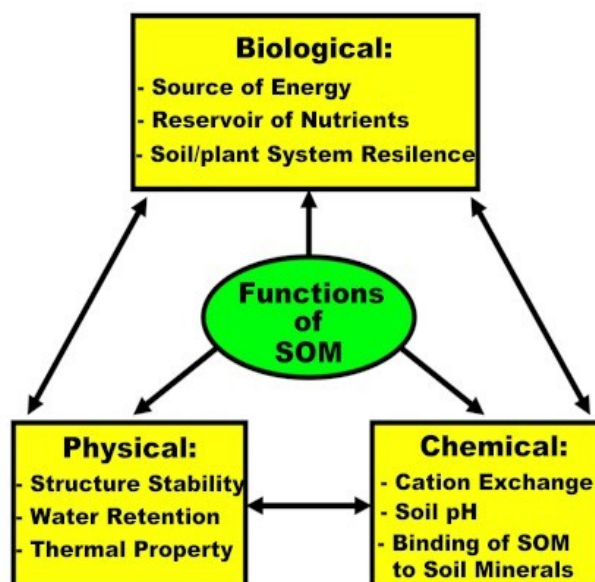


Figure 1.6. Functions of SOM in soils

Transformation processes of OM in soils can affect soil quality and fertility both directly, by the release of macro- and micro-elements and the microbial growth, and indirectly, by determining changes in physical/chemical properties of soil. The rate at which this process occurs depends on a range of factors such as the biochemical composition of the OM, physical factors and the degree to which the OM is protected.

At the current time, much of our knowledge about factors influencing the decomposition process is qualitative. We know what physical factors are decisive, and we know mechanisms that control the rates of OM decomposition. However, we are still not able to quantify the effects of many of mechanisms or understand the interactions between them.

Moreover, increasing the OM content of soils or even maintaining appropriate levels requires a sustained effort that includes returning organic materials to soils and rotations with high-residue crops and deep or dense-rooting crops.

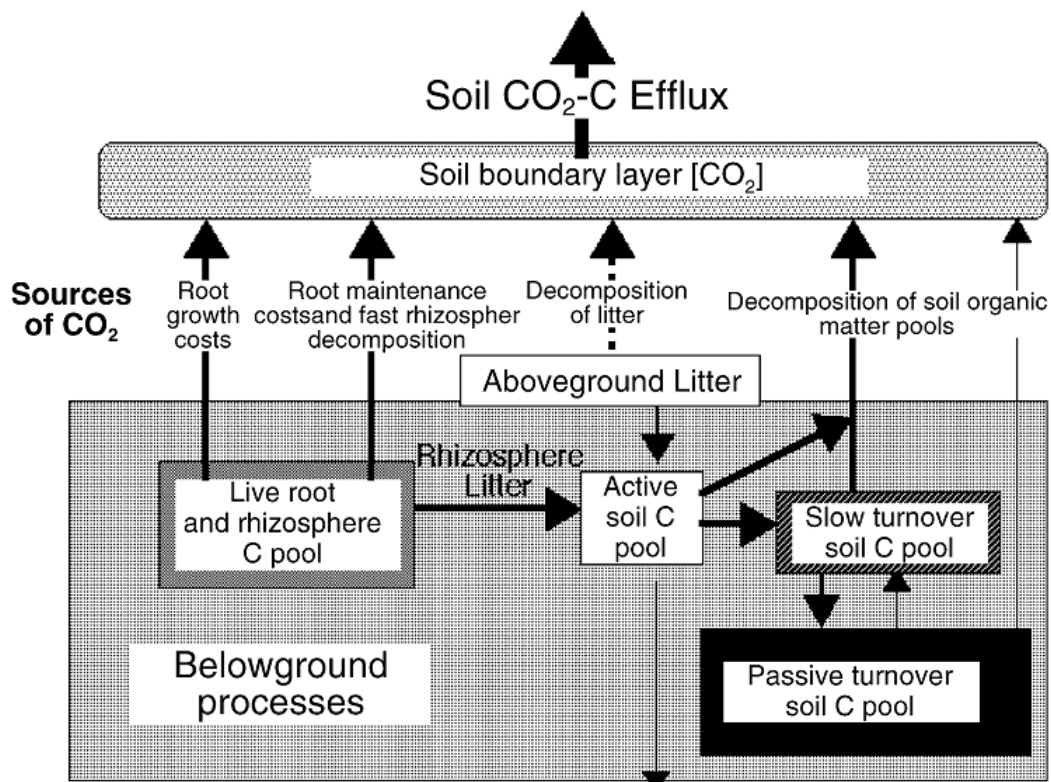


Figure 1.7. Below ground C stocks and fluxes affected by environmental Change (Metcalf, 2006; Pendall et al., 2004). SOM represents simply three main pools: Active, Slow and Passive. The Active pool receives inputs from the rhizosphere and above-ground litter and turns over on relatively rapidly. The Slow pool receives most inputs from the active pool and turns over on decadal to century time scales. The Passive C pool consists of physically or chemically protected organo-mineral complexes, with turnover times of millennia. CO<sub>2</sub> efflux is derived from decomposition of the various C pools, including roots and litter, and varies with soil temperature, moisture, and plant phenology.

The drastic increase in atmospheric CO<sub>2</sub> concentration, mainly due to change of land use since the industrial revolution, necessitates identification of strategies for offsetting the threat of global climate change (Lal, 2010). The soil is composed of a number of distinct fractions which are storing and different quantities of C (figure 1.7), and varying in terms of their sensitivity to environmental change.

For example, soil respiration expels 75-80 billion tons of C annually into the atmosphere (Raich & Potter, 1995) which is more than 11 times of the recent rate

of C production by anthropogenic combustion of fossil fuels (Marland & Boden, 1993). So even a slight fractional change in soil C dynamic could significantly alter atmospheric CO<sub>2</sub> levels, and hence the climate (Metcalf, 2006).

The global soil carbon pool (2500 gigatons [Gt]) is 3.3 times the size of the atmospheric pool (760 Gt) and 4.5 times that of the biotic pool (560 Gt) (Lal et al., 2004). Organic carbon represents approximately 60% of global soil carbon (Six et al., 2006) and at least 50% of this carbon have traditionally been categorized as the chemically resistant component known as humic substances (Otto et al., 2005). Therefore, SOM contains vast amounts of carbon and plays a pivotal role in regulating anthropogenic changes to the global carbon cycle.

It also plays essential roles in soil quality and agricultural productivity (Sollins et al., 2006; Kindler et al., 2009), water quality (Lal et al., 2004), immobilization and transport of nutrients and anthropogenic chemicals, while also concealing exciting opportunities for the discovery of novel compounds for potential use in industry and medicine (Kelleher, et al., 2006). It may also be a precursor for some fossil fuels, especially buried anaerobically as peat soil (Knicker and Lüdemann, 1995).

Recently, an issue of *Science* described SOM as the most complicated biomaterial on the planet and stated that there is mounting evidence that the essential features of soil will emerge when the relevant physical and biochemical approaches are integrated (Spence, 2010; Young and Crawford, 2004). There has been an also immense interest in the potential for agriculture to capture atmospheric CO<sub>2</sub>, through the accumulation of soil carbon.

The capacity for appropriately managed soils to sequester atmospheric carbon is enormous. Soil represents the largest carbon sink over which we have control. When atmospheric carbon is sequestered in topsoil as organic carbon, it brings significant additional benefits to agricultural productivity and the environment (Leirós, et al., 1999).

However, accumulation of atmospheric CO<sub>2</sub> has been operational in the United States since 1972 and extensive international research has already been and

continues to be conducted by scientists and institutions in countries all around the world. Afforestation of agricultural land has been recognized to be an effective tool to mitigate elevated atmospheric CO<sub>2</sub> concentration (IPCC, 2007; Lal, 2010; Laganière et al., 2010). Their findings have confirmed that SOM content is a potential source or sink for atmospheric CO<sub>2</sub> and other greenhouse gases (Kirschbaum, et al., 2008).

SOM is composed of a continuum of materials of varying chemical complexity (Kindler et al., 2009) with huge amounts of C and N, and plays an decisive role in regulating anthropogenic changes to the global C and N biogeochemical cycles (Lal et al., 2004). It is therefore widely accepted that relatively small changes in size and the turnover rates of soil C and N pools may potentially bring about substantial effects on atmospheric concentrations and global C and N cycling at large (Belay-Tedla et al., 2009). Thus, it is no surprise that the dynamics of soil organic C and N stabilization are of immense interest in environmental research. This is especially true for the emission of CO<sub>2</sub> from SOM to the atmosphere as a result of perturbation caused by global warming (Gleixner et al., 2002) and nutrient cycling and soil structure maintenance, an important resource in agricultural productivity (Belay Tedla et al., 2009; Kindler et al., 2009).

Finally humification stabilizes organic carbon additions to soil so that the carbon gained from plant roots does not recycle back to the atmosphere as CO<sub>2</sub>. The process involves soil microbes to transform the carbon additions into stable humic substances which are long term stores of SOC (from decades to centuries).

Therefore, knowledge of how soil carbon and OM aggrades or degrades in soil is integral to any land management plan. Promoting soil health and encouraging the development of SOM has always been central tenets of the sustainable approach.

Application of organic resources leads to the improvement of crop yields as a result of improved soil properties (Scholes et al., 1997). Regular additions of OM are esteemed as food for microorganisms, insects, worms, and other organisms, and as habitat for some larger organisms. Soil organisms degrade potential

pollutants, help control disease and bind soil particles into larger aggregates. However, well-aggregated and crumbly soil allows root penetration, improves water infiltration, makes tillage easier and thus reduces erosion.

Management practices that increase plant growth on a field (cover crops, irrigation, etc.) will increase the amount of roots and residue added to the soil each year. While tillage primarily burns younger OM, older, protected organic compounds can be exposed to decomposition if small aggregates are broken apart. In addition to changing the amount of SOM, tillage practices affect the depth of SOM.

To build OM levels in topsoil, OM must be added that is lost to decomposition and erosion. Like a person trying to lose or gain weight, increasing OM is about changing the balance between how much energy goes in and how much is burned off. Intensive tillage aerates the soil and is like opening the flue or fanning the flames. Decomposition is desirable because it releases nutrients and feeds soil organisms, but if decomposition is faster than the rate at which OM is added, SOM levels will decrease. Reducing decomposition is valuable for SOM build up. OM can be either developed or brought to the field and most OM losses in soil occurred in the first decade or two after the land was cultivated. Native levels of OM may not be possible under agriculture but many farmers can increase the amount of active OM by reducing tillage and increasing organic inputs.

OM does not add any "new" plant nutrients but releases nutrients in a plant available form through the process of decomposition. In order to maintain this nutrient cycling system the rate of addition from crop residues and manure must equal the rate of decomposition. Fertilizer can contribute to the maintenance of this revolving nutrient bank account by increasing crop yields and consequently the amount of residues returned to the soil.

Loss of OM is often identified as one of the main factors contributing to declining soil productivity, but it is misleading to equate a loss in SOM with a loss in soil productivity. SOM contributes to soil productivity in several ways, but there is no direct, quantitative relationship between soil productivity and total SOM.

In fact, SOM, the most influential factors maintaining the quality and fertility of soils (Stevenson, 1994; Reeves, 1997) decline throughout the world (Pulleman et al., 2000; Islam and Weil, 2000). That is resulted in the release of large amounts of plant nutrients, particularly nitrogen. For example, a decrease in SOM of 2% releases about 2,400 (lb/ac) of nitrogen (ref). SOM cannot be increased quickly even when management practices that conserve SOM are adopted. However, improved knowledge of how tillage management regulates the interaction between soil aggregates and microbial community structure and function may be helpful to better understanding mechanisms for increasing soil C sequestration and improving fertility in agricultural ecosystems.

## **1.6. Importance of waste recycling to land**

Rapid industrialization and population explosion in human societies, in many first world societies, generate a large amount of wastes from agro-industry and municipality. These wastes contain different amounts of organic carbon, but the amounts of organic materials from these wastes have increased exponentially and millions tonnes of OM are landfilled or incinerated.

For example, only in European Union more than  $200 \times 10^6$  ton municipal solid waste produced annually (Euro stat, 2000) and 65–90% of that is landfilled. Moreover, the land filling of biodegradable waste is proven to contribute to environmental degradation, global warming and pollute underground water, supplies mainly through the given off highly polluting gases such as CH<sub>4</sub>, CO<sub>2</sub>, NO<sub>2</sub>, SO<sub>2</sub> (CEC, 2007).

CH<sub>4</sub> is one of the most powerful greenhouse gases that is responsible for the global warming, needs to be reduced, in order to tackle climate change under the Kyoto Protocol (UN, 1998). The CH<sub>4</sub> emissions from landfills constitute about 30% of the global anthropogenic emissions of CH<sub>4</sub> to the atmosphere, 20-times more

potent as a greenhouse gas than CO<sub>2</sub> (European Commission, 2003; COM, 2005; Marmo, 2000). Reducing the amount of CH<sub>4</sub> emitted from landfills is considered to have the greatest potential for reducing the overall climate change impacts of waste management. Concurrently, landfills also provide a dumping ground for non-hazardous waste, but these spaces are running out that have been taken global concerns for energy crisis and environmental protection.

On the other hand, the production of solid waste generates around 45% wastewater sludge can be considered as one of the serious environmental threats (Oral et al., 2005; Gallardo et al., 2010) and as a significant taxpayer of discharge of pollutants to the environment (Ramos et al., 2009; Savant et al., 2006) that need to be solved (e.g. over 370 million tons of paper has produced worldwide in each year from the pulp and paper industry, which demand is continuing to increase).

The increasing sensitivity about environmental problems, need to find a sink for the growing amounts of waste and the necessity to reduce the utilization of non-renewable materials (e.g. peats) have markedly increased the use in recent times of organic waste-based fertilizers in modern agriculture (CEC, 2000).

Environmental regulations Europe prohibit the landfilling of organic waste have led to significant reductions in this practice since 1990 (Blanco et al., 2004; Fraser et al., 2009). At the EU level, the Landfill Directive (CEC, 2000) is the main driver for the management of biodegradable waste. It restricts the disposal of biodegradable waste in landfills. The target dates for the reduction of biodegradable urban waste to landfills are as follows: reduction to 75% (by weight) of total biodegradable waste produced in 1995 by 2006, reduction to 50% by 2009 and reduction to 35% by 2016. At the same time Landfill Directive promotes biodegradable waste diversion towards material cost effective recycling and biological treatment. The biological treatment of waste includes composting, anaerobic digestion, or mechanical-biological treatment. According to the European Environmental Agency (EEA) municipal solid waste and agricultural waste are two of the five leading waste streams in the EU. Urban represents about



14% of the total waste generated in the EU, excluding agricultural waste (COM, 2005).

Declining in SOM represents one of the most serious threats facing many arable lands of the world. Crop residues and animal manures have long been documented as soil organic amendments to preserve and enhance SOM pools. Nowadays, there is a growing recognition that the safe and appropriate application of waste materials may contribute to fight plant diseases and reduce soil contamination, erosion and desertification. Although, the safe and appropriate use application of organic amendments requires an in-depth scientific knowledge of their nature and impacts on the soil-plant system, as well as on the surrounding environment, scientific studies have to focus on the use of organic amendments in modern agriculture, and for the restoration of degraded soils, covering physical, chemical, biological, biochemical, agricultural, and environmental aspects.

#### *1.6.1 Composted urban waste as an organic amendment*

Using organic wastes as a compost to restore or to increase soil fertility has been well known form 2000 years ago to present and is continuing to increase. Composting helps to optimize nutrient management and the land application of compost may contribute to combat SOM decline and soil erosion (Van-Camp et al., 2004). Composting is also as a suitable alternative to land filling for the management of biodegradable waste as well as a mean of increasing or preserving SOM. Compost land application completes a circle whereby nutrients and OM that removed in the harvested, produce are replaced (Diener et al., 1993).

In recent decades, the recycling of compost, from different origins (manure, sewage sludge and municipal organic wastes) to land is considered as a way of maintaining or restoring the quality of soil. The application of organic wastes to degraded soils is a globally accepted practice to recover, replenish and preserve OM, fertility and vegetation (Civeira and Lavado, 2008). Compost, as soil

amendment, may favour agricultural sustainability by promoting soil biological communities, through biomass growth and activity (Mandal et al., 2007; Tejada et al., 2008; Tu et al., 2006), as well as influencing soil physical and chemical properties (van Elsas et al., 2002). Furthermore, it may contribute to the carbon sequestration and decrease greenhouse gas emissions and may partially replace peat and fertilizers (Pankhurst et al., 2005).

However, before applying composted materials to soil it is essential to ensure that these materials do not pose any danger to humans, animals or to the environment. On the other hand, the application of compost to soil could raise environmental risks mainly related to excessive or unbalanced supply of nutrients, introduction of heavy metals and organic pollutants and the spreading of pathogens (EC, 2003). Thus, it is essential to ensure the absence of undesired organic and inorganic substances, especially heavy metals and toxic chemicals, and evaluate the potential for the leaching of nitrate nitrogen ( $\text{NO}_3^-$ -N) from soils amended with N-rich biosolids.

Where compost has not matured, seedling damage can be caused by phytotoxic materials formed by microbial activity in the composting process. At present in the EU, in order for a compost to be suitable for agricultural application, it is considered necessary to fulfil the environmental quality classes established in the 2<sup>nd</sup> draft of the working document on biological treatment of biowaste, to contribute to the improvement of soil conditions for crop production (EC, 2001).

Compost application to agricultural land needs to be carried out in a manner to ensure sustainable development. Management systems have to be developed to enable to maximize agronomic benefit, whilst ensuring the protection of environmental quality. The main determinant for efficient agronomic use is nitrogen availability. High nitrogen utilization in agriculture from mineral fertilizers is well established and understood, whereas increasing the nitrogen use efficiency of organic fertilizers requires further investigation (Amlinger et al., 2003; Gutser et al., 2005). Several works, carried out for using biowaste and

vegetable waste compost in agriculture, has shown the low nitrogen fertilizer value of composts (Amlinger et al., 2003; Gutser et al., 2005).

The composted or available N added to the soil is present mostly in organic compounds and it can be mineralized and thus be taken up by the plants, immobilized, denitrified, and/or leached. In different studies, crop nitrogen recovery was found to range between 2% and 15% of the total compost N applied, depending on various factors, including compost properties, climatic conditions, crop types, soil properties and management practices (Neuens & Reheul, 2003; Wolkowski, 2003; Hartl & Erhart, 2005).

Compost nitrogen availability is still poorly understood. Better understanding of the fate of nitrogen from biowaste and vegetable compost application to soil is necessary in order to quantify nitrogen availability to plants and nitrogen losses to water bodies. It is vital to develop integrated approaches to compost use in agriculture, which take into account agronomic benefits and environmental risks, while identifying the financial implications of compost application. Such a holistic approach is critical to promote acceptance of compost use within the public and agricultural sector.

Organic soil amendments including composted or uncomposted plant residues, animal manures and green manure have widely different effects on the balance of soil microflora and plant diseases depending on the nature of the residue and the method of preparation (Abbasi et al., 2002; Craft and Nelson, 1996). The addition of plant residues to soil in general improves soil structure and soil health (Doran et al., 1994; Garbeva et al., 2004).

The development of composting as a useful biotechnology in transforming organic waste into suitable agricultural products has been favoured (Senesi and Brunetti, 1996). It has been calculated (European Environment Agency, 1999) that 30–50% of MSW (municipal solid waste) is composed of biodegradable OM, depending on local conditions, diets, climate and the degree of industrialization.

On the other hand, conventional agro ecosystems have been characterized by high input of chemical fertilizer, instead of organic amendments, leading to deterioration of soil quality due to reductions in SOM. However, several reports have provided insights into fertilization practices by supplementing chemical fertilizer to alleviate nutrient limitation (Mandal et al., 2007), selecting appropriate quantity or type of organic amendments (Acosta-Martinez and Harmel, 2006), altering the application time of organic amendments.

### *1.6.2 Pulp mill sludge as an organic amendment*

Stabilized sludge disposition for forest or agricultural use in degraded soils have increased over the last few years as it improves soil physical (structure, porosity and water holding capacity), chemical (nutrients mineralization, CEC, aluminum toxicity) and biological (microbial and enzymatic activities) properties.

The main contribution of these residues is their readily degradable OM contributing carbon, nitrogen and phosphorus in their available forms, increasing soil productivity and favoring carbon sequestration. The sludge-like energy source supply significant quantities of nutrients to soil biota (Pearce and Boone, 1998) and increases the microbial population and its activities, thereby reactivating the biogeochemical cycles into the soil. The sludge application will increase the soil OM content by occlusion in soil aggregates and adsorption by the active mineral fraction, and can be visualized as a biofertilizer since it can provide organic N and P. Successive sludge applications to degraded soil will increase the nutrient availability for plants and modify microbial growth and activity, thus increasing soil productivity.

Nitrogen may be high or low in pulp and paper solid waste depending on their origin, and this will influence nitrogen availability when applied to soil (Catricala et al., 1996). Potassium, phosphorus, carbon and sulphur can also be available in beneficial amounts (Zibilske et al., 1987). On the other hand, incorporation of

sludge into the soil can increase the bioavailability of P, which will depend on the capacity that the residue possesses to reduce the adsorption of P in the soil, on the contribution of different species of P (Gallardo et al., 2010; Haynes and Mokolobate, 2001; Pypers et al., 2005) and of the microbiological capacity to degrade compounds of P with the subsequent release of phosphate.

There are a number of examples of beneficial effects on soil through land application of pulp and paper wastes (Pearce and Boone, 1998) with little or no adverse impacts on terrestrial organisms (Bostan et al., 2005). However, there are also studies showing detrimental effects in aquatic environments (Ali and Sreekrishnan, 2001; Jones et al., 2001) and in the terrestrial environment (Jordan et al., 2002), suggesting that land application of solid wastes has to be considered on a case by case basis (Bostan et al., 2005).

As the relationships between contaminant bioavailability, treatment technology, the nature of pulp and paper residual solids, and resident organism tolerances has not been explored, investigation is warranted in order to relate measures of biological effect to the levels of compounds present in pulp and paper solid waste.

The solid waste from pulp and paper wastewater treatment is 45% sludge (0.2-1.2 kg MS/kg DBO removed); 25% ash, 15% wood cuttings and 15% other solid waste. The primary sludge produced in these industries is between 5 and 60 kg/ton of pulp and paper produced and, depending on the manufacturing process, the production of secondary sludge is around 15 kg/ton. This sludge can be used as a pH corrector in acid soils (Gallardo et al., 2010) and can help to recover productivity in degraded or eroded soils (Newman et al., 2005).

Newman et al. (2005) investigated the effect of kraft mill sludge (fresh and composted) on total and particulate OM and their relationships with plant available water and mineral nitrogen in a sandy soil. After 4 years of application all the amendments increased the total organic carbon and nitrogen in the soil. Moreover, annual addition of fresh and composted sludge produced sustained increases in

labile soil carbon and nitrogen pools; however, the OM did not translate into short-term nutrient availability in this sandy soil.

Esparza (2004) observed an improvement in the availability of nutrient (N, P), cationic exchange capacity and physical and biological properties in acidic and degraded soils from southern Chile through the application of stabilized biological kraft mill sludge.

## 1.7 The role of biodiversity in soil

Biodiversity refers to the diversity in a gene, species, community or ecosystem. It comprises all living beings from the most primitive forms of viruses to the most sophisticated and highly evolved animals and plants.

Soil biodiversity was defined as *'the variation in soil life, from genes to communities, and the variation in soil habitats, from micro-aggregates to entire landscapes'* according to Rio de Janeiro Convention in 1992. Whilst, this concept represent the vast number of distinct species (richness) and their proportional abundance (evenness) present in a system, but can be extended to cover phenotypic (expressed), functional, structural or tropic diversity.

The soil contains a plentiful numbers of diverse living organisms with complex communities including macro fauna (e.g. beetles, earthworms, badgers, moles, spiders), mesofauna (e.g. nematodes, collembolan, mites), micro fauna (protozoa) and micro flora (bacteria, fungi, algae, mosses). Concurrently, plant roots also considered soil organisms for their capability to make symbiotic relationship and interactions with other soil organisms. These diverse soil organisms form a complex food web (Figure 1.8) in the soil ecosystem by interacting within themselves and with other plants and animals through different mechanisms such as predation, competition (for nutrients and space), symbiosis and commensalism although they are largely unexplored.

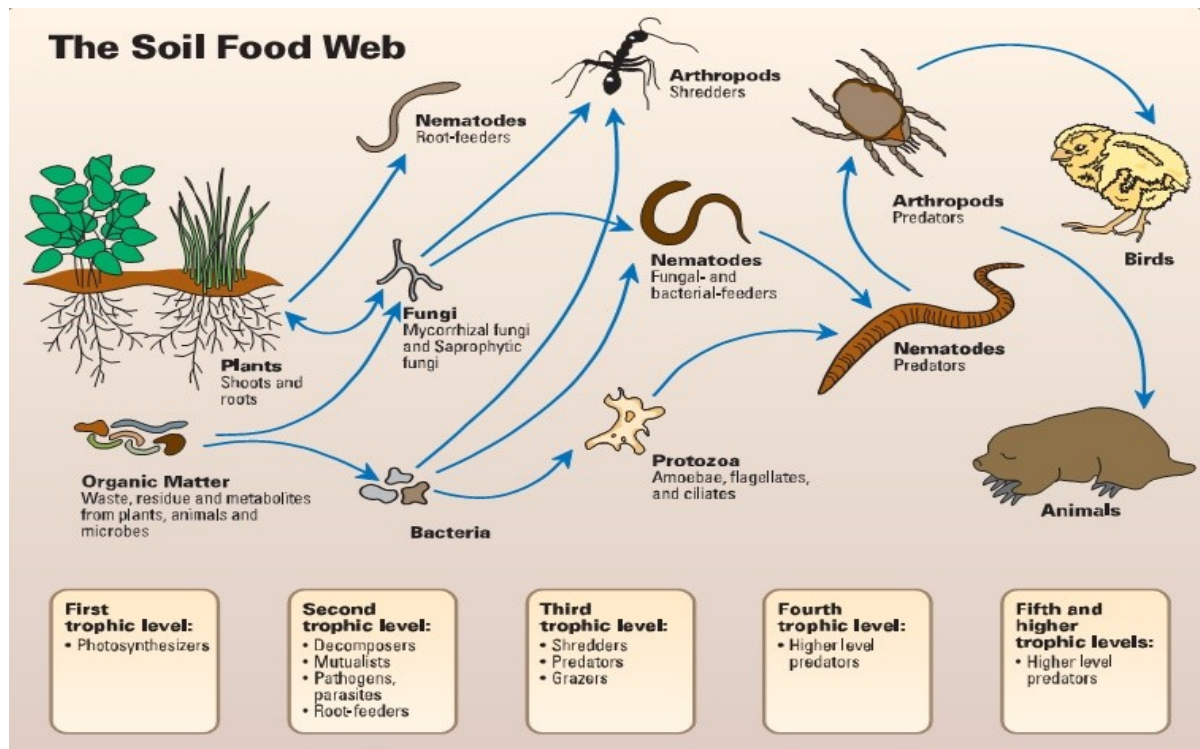


Figure 1.8. Relationships between soil microbes, plants, organic matter, and birds and mammals (Tugel et al, 2000).

As a result of microbial processes of decomposition the essential nutrients present in the biomass of one generation of organisms are available for the next generation. The contribution of soil organisms to nutrient cycling in terrestrial ecosystems is well established and quantified for a number of ecosystems (Nielsen et al., 2011; Swift et al., 2004).

Mainly the soil biota received all the energy from the sun. Plants and other autotrophic microorganisms convert the solar energy and  $\text{CO}_2$  into the simple carbon compounds that are used by other organisms and make nutrients available to plants. Farmers depend on these life cycles for their livelihood. On the other hand, microbes (primarily heterotrophic microorganisms) are also acting as a recycling agent that is responsible for maintaining the biosphere. These agents

develop favorable, thermodynamic, chemical reactions obtaining energy and carbon from dead biomass.

Soil microorganisms also play a crucial role in the bioremediation of toxic organic waste. Bioremediation involves the use of plants and naturally occurring soil microorganisms in processes such as bio-stimulation, bio-augmentation, bio-piling, bio-venting, bioreactors and land farming, to degrade organic waste into less toxic forms (Bento et al., 2005; Marin et al., 2005; Vidali, 2001).

Xenobiotic compounds including petroleum hydrocarbons, nitro-aromatic compounds, aromatic and aliphatic compounds, polychlorinated biphenyls (PCBs), pesticides, and surfactants. These compounds are wide-spread environmental pollutants in the soil, which can be degraded by soil microorganisms and soil microbial processes (Scelza, et al., 2008).

Extracellular enzymes of soil microorganisms help to break down complex polymers of SOM into monomeric units, which are readily available to other microbes that can break it down further into simple compounds (Wolf and Wagner, 2005). The decomposition of SOM such as plant litter, polymers and humic substances release nutrients to the soil, which is essential for the survival of the above ground biomass. This also helps to stabilize the net carbon budget of the whole biosphere (Liski et al., 2003).

Soil organisms in addition play an pivotal role in production and consumption of CO<sub>2</sub>, CH<sub>4</sub> and other greenhouse gases (Panikov, 1999). Under anaerobic conditions, CO<sub>2</sub> is used as an electron acceptor while reduced organic compounds serve as the donor (Fuhrmann, 2005). The anaerobic respiration process enables anaerobic and fermentative bacteria (methanogens) to breakdown complex organic substrates into simple substrates that are subsequently mineralized releasing CH<sub>4</sub> (Tate, 2000).

Soil microorganisms maintain the chemical balance of soil ecosystem by converting the complex organic nutrients into simpler inorganic (mineralization) nutrients and simultaneously absorb the simpler minerals (immobilization) and



prevent them from leaching out. They conserve the essential nutrients in the soil so that when they die and become a part of the organic matter, these essential nutrients are once again mineralized by the microorganisms for plant use. Thus, the soil fertility that is created by the microbes is also conserved by the same.

The soil microbes contribute soil (structure) formation and water regime control (Lavelle and Spain, 2001). Production of extracellular polysaccharides and other cellular debris such as mucilage by microorganisms helps in building and maintaining soil structure, these materials function as the glue that stabilizes soil aggregates. Soil microbes produce lots of gummy substances that help to cement soil aggregates. Fungal filaments called hyphae also stabilize soil structure because these threadlike structures ramify throughout the soil literally surrounding particles and aggregates like a hairnet. The fungi can be thought of as the ‘threads’ of the soil fabric. Microorganisms also affect the water-holding capacity, infiltration rate, crusting, erodibility, and susceptibility to compaction (Winding et al., 2005).

In soil, bacterial communities are closely shaped by the biological alteration of the soil matrix performed by inhabiting macro organisms such as plant roots or macro fauna (Jones et al., 1997; Meysman et al., 2006). By engineering, the soil earthworms generate various soil microsites that differ from the bulk soil in terms of microporosity, moisture, nutrient content or oxygenation (Le Bayon and Binet, 2006). It can be hypothesized that soil bioturbation resulting from earthworm activity may significantly improve soil functioning by increasing the biodiversity within functional groups and the associated functional redundancy.

### *1.7.1 Soil biodiversity and ecosystem functions*

Human societies rely on the vast diversity of benefits provided by nature such as food, fibers, construction materials, clean water, clean air and climate regulation.

All the elements required for these ecosystem services depend on soil and soil biodiversity is the driving force behind their regulation (EC, 2010).

Additionally as soil biodiversity contain complex microbial communities that corresponding to the complex interplay between inter-related trophic levels thus combinations of individual taxa or species in different communities can result in many different communities with different characteristics resulting diverse ecosystem function (figure 1.9).

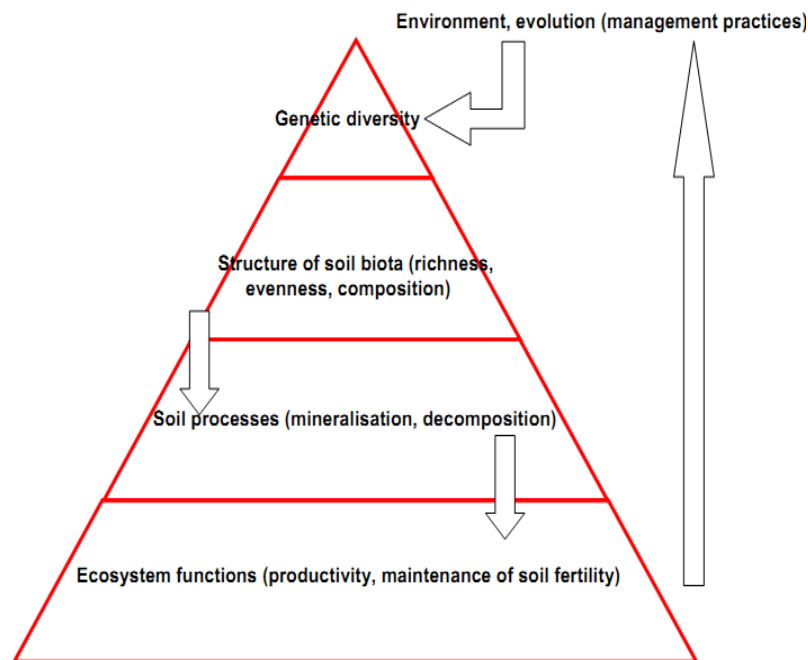


Figure 1.9: Relationships between microbial structure, soil processes and ecosystem functions in soil (Mader et al., 1996).

The Relationships between ecosystem functioning and biodiversity are particularly evident in soil and positive relationships exist between biodiversity and primary (biomass) production and the factors that affect productivity (e.g. Soil fertility, climate, disturbance and herbivores). Although, a certain number or a group of species is necessary to maintain the stability of ecosystems; however, it remains debatable if it is a relatively small number of key species or a larger variety of complementary species that drive ecological processes (Brussaard et al., 2004; Stark, 2005; Tilman et al., 2006; Wardle et al., 2004). It has been proposed that species composition and types (functional groups or species) have greater

influence on ecosystem functioning and stability than species richness (e.g. Bengtsson, 1998; Loreau et al., 2004; Tilman et al., 2006). More research is needed fully understand the relationships between diversity and ecosystem processes.

It is often assumed that diversity is a pre-requisite for the maintenance of soil stability, resistance and resilience of ecosystems (Wall et al., 2004). While the impact of the loss of biodiversity on soil functions seems to be intuitive. It may depend on whether the function is dependent on a few 'specialist' organisms or is performed by many different 'generalist' species. In the latter case, loss of biodiversity may not result any significant loss of function as much of diversity is considered to be redundant.

Several hypothetical relationships between diversity and function have been proposed (Figure 1.10) by Naeem and Wright, 2003). Given the enormous diversity of soil organisms, and a wide range of metabolic processes and functions they are capable of, generalizations are not yet possible. However, it is clear that as the ecological functions of soil depend fundamentally on the soil's biodiversity, loss of biodiversity will potentially undermine one or many inter-related functions. Recent research has been anticipated that species composition and types (functional groups or species) have greater influence on ecosystem functioning and stability than species richness (e.g. Bengtsson, 1998; Loreau et al., 2001). Soil microorganisms take part in 90% of the processes occurring in soil (Nannipieri et al., 2003).

This dependency of terrestrial ecosystem functioning on microorganisms implies that changes in microbial diversity should have a significant impact on the ecosystem performance. Different relationships between diversity and ecosystem function have been proposed so far (Monard et al., 2011; Peterson et al., 1998) as shown in Figure 1.10.

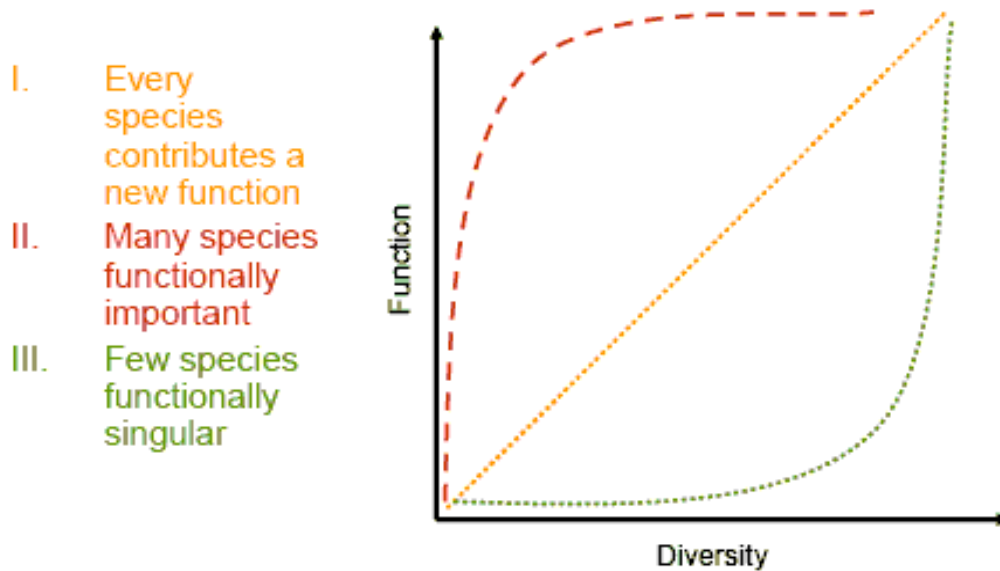


Figure 1.10. Theoretical relationship between diversity and function (Naeem & Wright, 2003).

We acknowledge that understanding the relationships between soil biodiversity and ecosystem function are still progressing in the field of science. However, we consider that now is the time to use the available knowledge to express ecosystem functioning in terms of ecosystem services to society and these to soil biodiversity to the best of our knowledge.

Concurrently, the economic benefits of soil biodiversity clearly shift the debate, from theoretical grounds for conservation and sustainable use, to the practical grounds, of making concrete improvements in current land management practices adequately promote soil biodiversity conservation.

However, links between above- and below-ground communities are neither clear nor consistent but all levels of biodiversity (above-ground fauna and flora, below-ground soil biota, including microorganisms, earthworms and arthropods, etc.) need to be considered (Shepherd et al., 2003). (Brussaard et al., 2004. It is also problematic to make assumptions regarding the role of below-ground diversity for the functioning of the soil system merely based on the knowledge on above-ground biodiversity and its influence on ecosystem stability (Loreau et al., 2001). However, it is widely acknowledged that some aspect of microbial diversity

(species richness, evenness or composition) is vital to sustain soil functioning since the microbial community is responsible for most the ecosystem processes (e.g. organic matter decomposition, nutrient cycling) (Brussaard et al., 2004; Coleman et al., 2008).

During the last decades, a considerable decline in soil biodiversity observed due to a tremendous increase in intensive agricultural practices and over exploitation of natural resources (Tilman, 1996). For instance, human activities have increased the species extinction rates by 100-1000 times (Lawton & Brown, 1994). These deleterious changes in soil biodiversity alter ecosystem processes and change the resilience and resistance of ecosystems to environmental change (Tilman, 1996; Naeem and Wright, 2003; Stark, 2005). This change is continuing due to unscrupulous human behavior and their policy. As a consequence, biodiversity term has often been used to biologists, ecologists and environmentalists for a number of years and is often discussed in the context of sustainability..

### *1.7.2. Soil biodiversity and agricultural sustainability*

Sustainable agriculture involves the successful management of agricultural resources while satisfying human needs, maintaining or enhancing environmental quality and conserving natural resources for future generations. The sustained use of the earth's land and water resources and thereby plant, animal and human health are dependent upon maintaining the health of the living biota that provide critical processes and ecosystem services (FAO, 2005).

Improvement in agricultural sustainability requires, alongside effective water and crop management, the optimal use and it is well known that land management practices alter soil conditions and the soil microbial community. However, the relationship between soil biodiversity and soil functions is less clear, it is well recognized that sustainability of agriculture is highly dependent on a high level of soil biodiversity. The effect of different management regimes and perturbations on

the soil microbial community has been studied in a wide range of soil environments. Most of the researchers reported that intensive farming negatively affect soil biodiversity while organic farming practice increased microbial diversity by enhancing microbial biomass.

Moreover, FAO (2005) considers the issue of soil biodiversity and soil ecosystem management of enormous importance to the achievement of sustainable, resource-efficient and productive agriculture. Soil biodiversity has been identified as an area requiring attention under the programme of work on agricultural biodiversity of the Conference of the Parties (COP) to the Convention on Biological Diversity (CBD). In general, structure of soil communities is largely determined by ecosystem properties and land use systems and prime importance is the contribution to a wide range of essential services that are vital to the sustainable function of all ecosystems. They are acting as the primary driving agents of nutrient cycling, regulating the dynamics of SOM, soil carbon sequestration and greenhouse gas emission, modifying soil physical structure and water regimes, enhancing the amount and efficiency of nutrient acquisition by the vegetation and enhancing plant health.

These services are not only essential to the functioning of natural ecosystems but constitute a valuable resource for agricultural production and food security as well as the sustainable management of agricultural systems (FAO, 2005).

There are several ways for farming management option, where farmers can manage biodiversity, alter the activity of specific groups of organisms through inoculation and/or direct manipulation of soil biota, enhance agricultural production. Inoculation with soil beneficial organisms, such as nitrogen-fixing bacteria, mycorrhiza and earthworms, have been shown to enhance plant nutrient uptake, increase heavy metal tolerance, improve soil structure and porosity and reduce pest damage.

Simultaneously farmers can manage soil biotic processes by manipulating the factors that control biotic activity (habitat structure, microclimate, nutrients and

energy resources) rather than the organisms themselves. Examples of includes most agricultural practices such as the application of organic material to soil (for example through composting), tillage, irrigation, green manuring and liming, as well as cropping system design and management. These must not be conducted independently, but in a holistic fashion, because of the recurrent interactions between different management strategies, hierarchical levels of management and different soil organisms.

Despite recognition of the fundamental role of soil biodiversity in maintaining sustainable and efficient agricultural systems it is still largely neglected in the majority of agricultural development initiatives. However, all can agree that soil is of paramount importance and therefore, strategies for its protection should be found. Soil requires protection and careful management by farmers, the public and policy-makers this is essential if we are to conserve the medium that supports our life, and helps us grow our future.

### *1.7.3. Factors affecting soil biodiversity*

Soil organisms contribute a wide range of essential services to the sustainable functioning of all ecosystems (Coleman, 2008; Hedlund et al., 2004; Six et al., 2006).

On the other hand, the composition and activity of soil microorganisms are directly influenced by changes in soil water content (Bossio and Scow, 1998), pH (Fierer and Jackson, 2006), stress such as fire (D'Ascoli et al., 2005), soil type and field properties (Wu et al., 2008), plant diversity and composition (Carney and Matson, 2006), fertilization regimes (Hatch et al., 2000), herbicide and pesticide application (Johnsen et al., 2001), crop rotations (Campbell et al., 2001), manure applications (Bossio et al., 1998; Girvan et al., 2003), heavy metal contamination (Gianfreda and Rao, 2004), and on different tillage systems (Gianfreda, 2005; Badalucco et al., 2010).

Amongst the researchers investigations were forest soils (Leckie et al., 2004; Liebig et al., 2004), grassland and pasture systems (Grayston et al., 2004; Stevenson et al., 2004), arable soils (Haynes, 1999; Nsabimana et al., 2004), including conventional, low-input and organic systems (Badalucco et al., 2010; Laudiciana et al., 2011). Whereas, results relevant to this project will be reviewed in detail in the respective discussion sections.

Most research suggests that using organic amendment can have a positive, stimulating influence on the soil microbial community by enhancing diversity and improving soil functions like nutrient cycling and antagonistic potential and that soil quality is higher in organically farmed soils (e.g. Bending et al., 2000). In comparison, there is little evidence in the literature of negative effects of conventional production practices, such as use of mineral fertilizers and pesticides, on the SOM, microbial diversity and activity (Belay et al., 2002; Shepherd et al., 2003).

Genetically modified crops may also be considered as a growing source of pollution for soil organisms. Most effects of GMOs are observed on chemical engineers, by altering the structure of bacterial communities, bacterial genetic transfer, and the efficiency of microbial-mediated processes.

Exotic species are called invasive when they become disproportionately abundant. Urbanization, land-use change in general and climate change, open up possibilities for species expansion and suggest that they will become a growing threat to soil biodiversity in the coming years. Invasive species can have outstanding direct and indirect impacts on soil services and native biodiversity.

The lack of awareness of the importance of soil biodiversity in society further enhances the problem of the loss of ecosystem services due to loss of soil biodiversity. While agriculture is expected to affect the diversity and structure of soil microbial communities, the specific responses of various bacterial groups to the changing environment in agricultural soils are not well understood (Buckley and Schmidt, 2001).



Now, up-and-coming management factors are likely to affect agricultural soil biodiversity, especially intensive exploitation of land, soil degradation processes, soil pollution, soil compaction, soil sealing, habitat disruption, organic matter decline, invasive species, use of GMOs, with potential threats that are well known. It is therefore apparent for investigation the various pressures on soil biodiversity. It is needed to allow effective protection for global ecosystem function and services. However, since these practices are commonly linked to organic management systems, it is reasonable to assume that soils cultivated under long-term organic or conventional management show differences in microbial biomass composition and function (Gunapala and Scow 1998; Lundquist et al. 1999).

Understanding the effect of management practices on maintaining fertility and productivity of arable soils is a key to improving sustainability of agro ecosystems. This requires an understanding of the structure and function of soil microbial communities that are affected by farming practices (Beare et al., 1997) in the long time. It might be possible to influence nutrient cycling processes and soil quality by manipulating the microbial community in soils. However, more information is needed on the role of microbial structural and functional diversity in the functioning of the soil ecosystem. The links between microbial growth, activity and soil processes that drive nutrient availability and fertility (Kennedy and Smith 1995).

## **1.8 Methods to assess microbial diversity in soil**

The methods that can be used to describe the microbial community and its functions include measurements of microbial biomass, culture dependent or independent approach, molecular techniques, enzyme activities and respiration assays by SIR, etc.

Only a small proportion (1-10%) of all soil microorganisms are culturable (Insam, 2001; Torsvik, et al., 1998) i.e. traditional methods to determine structural diversity (e.g. soil dilution plating) target only a small fraction of the microorganisms present in the soil. Consequently, accurate identification and determination of functional properties is difficult using these methods and might create an insufficient picture of microbial diversity and its significance in the soil.

The catabolic response profile (short-term substrate-induced respiration), has been used to calculate the diversity (range and evenness) of catabolic functions expressed in situ. Catabolic diversity has been used to investigate the effect of stress and disturbance on the diversity and resilience of soil microbial communities. Degens and Harris (1997) developed a multiple carbon source, substrate induced respiration method (SIR) that measures the response of the whole soil which is both relevance and convenience although it is time consuming.

The application of new, mainly molecular techniques, do not rely on culturing methods to identify soil microorganisms, offers more insights into the functional and structural diversity of soil biota. This can provide information on the relationship between microbial community structure and function and its impact on soil quality, resilience and sustainability (Insam, 2001; O'Donnell et al., 2001) under long time intensive farming system. To minimize bias and obtain more complete information a combined approach using different methods should be employed (Atlas, 2004; Insam, 2001; Widmer et al., 2001).

One of the primary challenges in modern microbial ecology is effectively and accurately assessing total microbial diversity, particularly the present knowledge level of microbial diversity and function in the soil, the link between structural diversity and function of below- and above-ground ecosystems, as well as methods to study plant-microbe-soil interactions are relatively limited. In order to understand the complexity of interacting biological, chemical, and physical factors, botanists, microbiologists, pedologists, and ecologists should cooperate to further the cause of science.

However, it is now well accepted that only a small percentage of the entire profile of microorganisms in environmental samples, such as the soil, can be cultured in the laboratory (Amann et al., 1995; Head et al., 1998). However, actual in-situ diversity of microbial communities in the maximum environmental samples cannot be representable by culture-dependent methods (Amann et al., 1995; Dunbar et al., 2000; Ward et al., 1990). In contrast, culture-independent techniques are able to profile the microbial community with much higher resolution and are more suitable for the analysis of complex microbial communities (Amann et al., 1995; Entry et al., 2007).

Primarily, microbial communities were analyzed using microbial cell fatty acid profiling (Findlay, 1996), but more recently, nucleic acids have become the dominant signature molecule for community analysis (Nakatsu, 2007; O'Callaghan et al., 2006). Now polymerase chain reaction (PCR) amplification is used extensively in microbial community analysis to increase copies of selected target genes for more efficient detection (Nakatsu, 2007). The commonly used genetic fingerprinting techniques are PCR-dependent approaches and including denaturing gradient gel electrophoresis (DGGE; Muyzer et al., 1993), phospholipids fatty acid (PLFA) analysis (Frostegard et al., 1996), amplified rDNA restriction analysis (ARDRA), terminal restriction fragment length polymorphism ((T-RFLP; Liu et al., 1997), single strand conformational polymorphism and automated ribosomal intergenic (Schwieger and Tebbe, 1998) and ribosomal intergenic spacer analysis (RISA; Ranjard et al., 2000). All these molecular methods used to provide information on the species composition, and used to compare common species present in samples. It is well established that, methods leading to a detailed view of a microbial community, such as cloning, sequencing and metagenomics, are expensive, time-consuming and labour-intensive (Nakatsu, 2007; O'Callaghan et al., 2006).

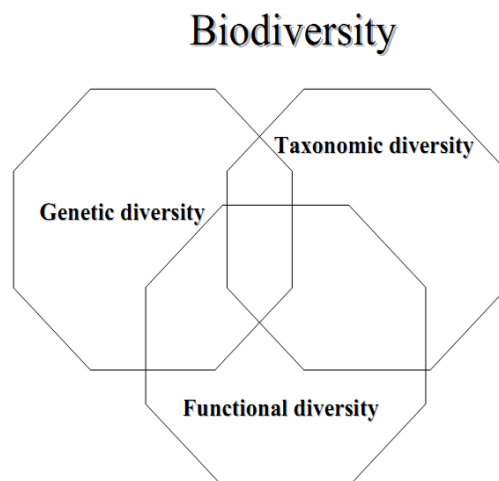


Figure 1.11. Interrelated element of soil biodiversity

In contrast, till now it is not possible to develop any method to establish the complete figure of microbial biodiversity using culture based, molecular and biochemical methods (Zak et al., 1994; Trevors et al., 1998). In fact, biodiversity represents overall diversity including taxonomic, genetic and functional diversity (Figure 1.12). Moreover, community structure does not provide overall information of functional diversity, which is an aspect of the total microbial diversity in soil, and encompasses a range of processes (Degens et al., 2001; Torsvik and Øvreås 2002).

Concurrently, direct measurements of functional diversity of soil microbial communities are likely to provide information more relevant to the functioning of soils than measurements of species diversity (Garland and Mills, 1991; Giller et al., 1997; Graham and Haynes, 2005). A number of functionally inactive microorganisms are often present in soil in resting or dormant stages (White and MacNaughton, 1997), which make it difficult to interpret the functional diversity of soil microbial communities from community structure.

A novel technique to measuring the functional diversity is to examine the number of different C substrates used by the microbial community (Garland and Mills, 1991; Garland, 1996; Zak et al., 1994). The two most commonly used methods of measuring substrate utilization patterns are the Biolog plate method (Garland and

Mills, 1991) and the substrate-induced respiration (SIR) technique (Degens and Harris, 1997).

The Biolog plate method detects catabolism by colour change in an incubated soil suspension-carbon source solution that is relatively easy to use and low cost. This method can assess the diversity of cultivable microbes and targets only the small fraction of the microbial community that can grow within the microtitre plate wells.

Substrate induced respiration (SIR) method uses multiple carbon sources, measuring the respiration response the whole soil by adding the individual substrates directly to the soil. Moreover, it avoids the problem of the culturability of soil microbial populations under artificial conditions. However, the SIR technique is an accurate methodology and sensitive to management practices that can be performed without the technology requires by the biolog method, if it is considered that both techniques are based on the same principle (Graham and Haynes, 2005; Romaniuk et al., 2011; Sparling et al., 2008;).

Among all those techniques, I used PCR-DGGE technique to measure genetic diversity and SIR technique to assess the functional diversity under different land management practices after addition of different type of organic amendment.

### *1.8.1 Assessing bacterial community structure by 16s rDNA-PCR-DGGE*

In recent years, 16S rDNA PCR- DGGE approach have a dedicated development in the study of microbial community (Ercolini D., 2004). This technique is one of the most frequently used techniques to investigate bacterial and fungal community structures in soil samples (Kowalchuk, et al., 2006; Marschner et al., 2002). This technique has been used extensively to monitor differences in microbial community structure associated with farming practices (Garbeva et al., 2003), waste recycling, GM plants (O'Callaghan et al., 2008), and season variations (Smalla et al., 2001), plant growth stages (Marschner et al., 2002). On the basis of

that the use of specific primers to amplified 16S rDNA genes and the following fragmentation on denaturant gradient by DGGE, offers the possibility to monitor structure and dynamics of microbial populations and their temporal variations (Broon et al., 2001).

DGGE allows the separation of the same size but diverse PCR-amplified products in an acrylamide gel composed of linear gradient denaturant chemicals into a profile composed of bands. The separation of the same size PCR products is achieved on the basis of their differing intrinsic stability which depends on the GC content and distribution. As a fragment progresses through the gel and is subjected to increasingly strong denaturing conditions, the double stranded PCR products reach a point where partial strand disassociation occurs. The disassociation results in the physical change of the molecule shape which directly affects its mobility during electrophoresis. Consequently, same size PCR products which differ in sequences are separated on the gel. The profiles from replicate samples can be compared with the treatments to determine the level of similarity in the community structure and to investigate shifts or changes in community composition.

The analysis of PCR-DGGE microbial community profiles was initially restricted to visual interpretation of presence and absence of the bands (Gomes et al., 2001). With the development, of software packages, the analysis of community profiles has significantly improved through more accurate comparison of both the band position and the relative intensity of different bands within gels. After that, statistical analysis of the data could be achieved. However, because of potential PCR biases and influence of signal intensities by gel staining process, some studies only interpret the data based on presence/absence of the bands rather than relative intensity of the bands (O'Callaghan et al., 2008). While, using MEGA ([www.megasoftware.net](http://www.megasoftware.net)) to analyzing data is the new one but relatively easy to understand the relationships among the profiles of DGGE banding. Additionally, the hierarchical cluster analysis was performed using unweighted pair group method with an arithmetic mean (UPGMA) with the software package MEGA 5

and visualized as a dendrogram which construction based on presence-absence bands in DGGE gels with a bootstrap confidence value of 1,000.

The PCR-DGGE technique has a number of advantages over other techniques. Numerous samples can be analyzed on one gel and with correct use of markers and positioning of treatments across lanes it is possible to conduct simultaneous comparison between samples. The technique is also affordable for most laboratories. In addition, individual bands of interest can be excised from the gel for subsequent cloning and sequencing (Nakatsu, 2007; O'Callaghan et al., 2006). However, as with all PCR-based techniques, DGGE profiling relies on the efficiency of nucleic acids extraction from samples and PCR amplification. PCR bias and artifact formation can occur during the amplification process, especially in samples containing multi-templates, as most ecological samples do. PCR bias is caused by differential amplification due to differences in the efficiency of primer binding to templates (Polz and Cavanaugh, 1998), formation of secondary structure of templates and differences in the kinetics of the PCR reaction (Brunk and Eis, 1998). PCR artifacts may arise due to the formation of chimerical or heteroduplex molecules (Wang and Wang, 1997).

As a consequence, many, if not all, PCR-based techniques will not be totally representative of microbial communities, especially on a quantitative level (Farrelly et al., 1995; Ishii and Fukui, 2001). Felske and Akkermans (1998) pointed out that although the most abundant microorganisms are normally represented by the dominant bands on DGGE gels; other important members of the community could be under-represented due to the weaker signals or even absence because of the possible PCR bias and unknown cell lysis efficiencies.

Therefore, O'Callaghan et al., (2006) emphasized the importance of selecting suitable nucleic acids extraction methods for each study and optimization of PCR conditions for each analysed gene sequence. In addition to these PCR-based limitations, the DGGE process itself has some specific disadvantages. DGGE patterns derived from environmental samples, such as rhizosphere soil which

contain a large number of different bacterial populations, might show as smears on the gel (O'Callaghan et al., 2006).

However, this can be avoided by using more specific primers only targeting particular taxonomic or functional groups. Additionally, Kisand and Wikner (2003) stated that the commonly used 16S sequence can contain multiple melting domains which may result in “cloudy bands”. It has also been found that a single band in a DGGE gel may be composed of DNA from several species (Sekiguchi et al., 2001; Yang and Crowley, 2000) and conversely, several bands are sometimes generated from a single species (Nübel et al., 1996). In addition, comparisons between gels must be carried out with caution because of gel variability (Nakatsu, 2007). Inclusion of appropriate DGGE markers on each gel is especially important for comparisons between gels (O'Callaghan et al., 2006). Because of the cumbersome determination of signal intensities of all bands which are heavily affected by staining techniques and processes, DGGE is at best only a semi-quantitative analysis when intensities of bands are included in the analysis (Nocker et al., 2007).

### *1.8.2 Assessing soil functional diversity by substrate induced respiration (SIR)*

Although the relationships between soil microbial diversity, soil function and soil resiliency are difficult to assess since exact microbial diversity is challenging to quantify (Nannipieri et al. 2003). However, it is generally believed that direct measurements of functional diversity of soil microbial communities are likely to provide information more relevant to the functioning of soils than measurements of species diversity (Garlands and Mills, 1991; Giller et al., 1997; Graham and Haynes, 2005). Functional microbial diversity, that is the microbial activity as a whole is primarily related to a soil's capacity to recover from stress and disturbance and soils with higher microbial diversity are more resilient to physical and chemical stress than those of lower microbial diversity (Degens et al. 2001,



Griffiths et al. 2004). Although, the relationships between soil microbial diversity and to interpret the functional diversity of soil microbial communities from community structure as because microorganisms are often present in soil in resting or dormant stages soil function are the subject of much debate, and it is complicated that are functionally inactive (Graham and Haynes,2005). Until recently, unification of community and process level information in the study of soil microbial ecology has been severely hampered by the complexity of soil systems and the inadequacy of available techniques for describing microbial community composition.

The SIR method is relatively effective, non-complex and easy technique that identifies the metabolically active component of the microbial community. This approach directly assesses the functional diversity of microbial communities involved in decomposition activities by adding a range of simple organic substrates directly to soil for measuring the short-term catabolic responses (Degens and Harris, 1997; Degens et al., 2000).

From catabolic response profiles we can assess catabolic evenness, a component of functional diversity (CRPs; Degens and Harris, 1997; Degens et al., 2000). Measurement of microbial diversity SIR approach has been used to monitor land management (Degens and Vojvodic-Vukovic, 1999; Graham and Haynes, 2005), cropping intensity (Sparling et al., 2000), soil organic carbon status (Degens et al., 2000), N fertilization (Frey et al., 2004), successional sequences (Schipper et al., 2001), stress or disturbance to the soil (Degens et al., 2001). Moreover, SIR is one of the most efficient, easy and rapid techniques of all the methods used to estimate microbial biomass in soils (Cheng and Coleman, 1988).

SIR used to measure the maximal respiratory levels of the active microorganisms in soil whereby  $\text{CO}_2$  emanates from the soil surface generated from the metabolic activity of soil microbes (Frank et al., 2005; Lin and Brookes, 1999). The rate at which fixed C substrates are oxidized to  $\text{CO}_2$  (Figure1.12), in a soil sample is proportional to the quantities of organisms mediating the reaction (Tate, 2000).

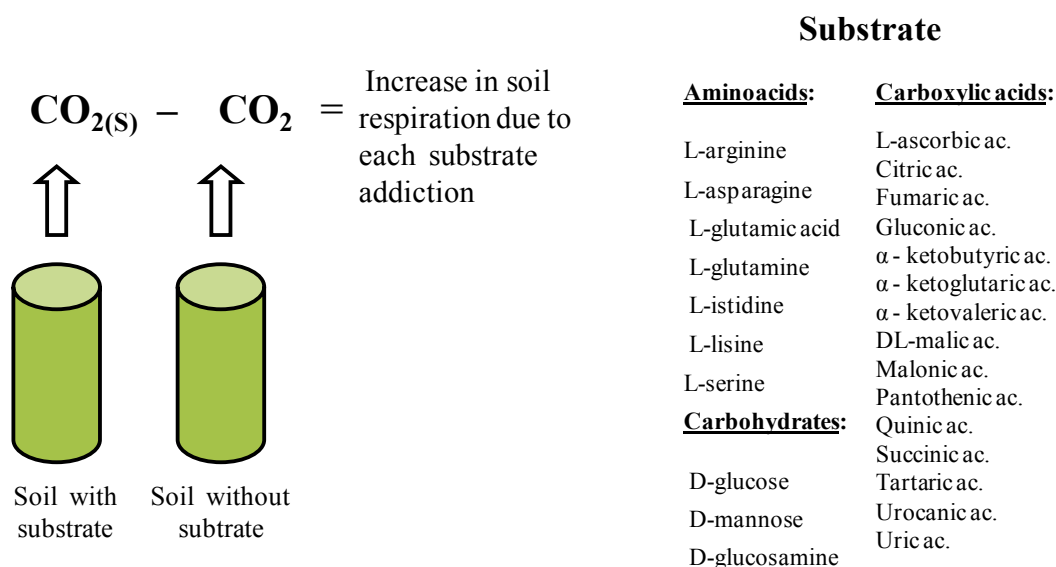


Figure 1.12. General Idea for Substrate induced respiration methods

It reflects the size of the active microbial biomass (Bailey et al., 2002), evaluates the maximum potential activity (Schomberg and Steiner, 1997) not the actual activity, occurring for the residue at the time of sampling. The magnitude of the SIR response of microorganisms over 0-6hrs is characteristic of the initial microbial community in soil before growth of organisms occurs on the added substrates (Degens and Harris, 1997).

Diversity may arguably be defined as the number of groups( richness) and the relative abundance of individuals within each group (evenness) (Magurran,1988; Yan et al., 2000). Diversity groups may be taxonomically based, trophically based or functionally based (functional group diversity). One of the most important components of microbial functional group is the diversity of decomposition functions performed by heterotrophic microorganisms (Beare et al., 1997; Setälä et al., 1998; Yan et al., 2000). Evaluation species and functional diversity is fundamental for the functional capability of soil (Giller et al., 1997; D'Ascoli et al., 2005).

However, it is generally believed that direct measurements of functional diversity of soil microbial communities are likely to provide information more relevant to the functioning of soils than measurements of species diversity (Garland and Mills, 1991; Giller et al., 1997; Graham and Haynes, 2005). Although, it is complicated to interpret the functional diversity of soil microbial communities from community structure as because microorganisms are often present in soil in resting or dormant stages that are functionally inactive (White and MacNaughton, 1997; Graham and Haynes, 2005).

On the other hand by SIR methods we have measured only some catabolic functions in order to calculate catabolic evenness from catabolic response profiles using Simpson-Yule index (Magurran, 1988). In addition, each respiratory response of the soil to the addition of one organic compound can also be considered one specific microbial activity (D'Ascoli et al., 2005) although, it is arguable that SIR technique is time consuming.

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## Chapter 2

### Aim

Food security remains a priority in most of the world in addition to the new challenges of climate changes, natural resource depletions and environmental degradation. The intensive agricultural management, that increases significantly yield per acre, has brought substantial economic and social development, but also contributed to environmental degradation via increased greenhouse gas emissions, biodiversity loss, and the reduced delivery of many ecosystem services including soil and water conservation. In fact, loss of soil quality in areas under intensive farming management is one of the major concerns of the modern agriculture, principally due to the use of mechanical ploughing, chemical fertilizers, plant growth regulators and pesticides, that affects soil physical and chemical properties, causing increases in salinity, heavy metal and xenobiotic contents and reduction in organic matter, affecting in turn soil biological properties and biodiversity. In particular, the reduction in soil organic matter under intensive farming is due principally to crop removal and increase in erosion and mineralization processes.

There is a growing recognition that agricultural management practices based on organic amendment may be a key tool to maintaining soil quality and sustainability in intensive agriculture systems. A soil amendment is any material added to a soil to improve its physical properties, such as water retention, permeability, water infiltration, drainage, aeration and structure. However, organic amendments increase soil organic matter content and, if they contain plant nutrients, offer many benefits, acting also as organic fertilizers. In fact, increases in soil organic matter can affect positively soil quality by reducing compaction, erosion processes and toxicity of pollutants, improving soil structure, porosity, aeration, water infiltration, water holding capacity, nutrient availability and stimulating, in turn, growth and activity of soil biota.

Use in agriculture of biodegradable waste (i.e. municipal solid waste or some by-products of industrial activities) at recommended rates and properly managed, as organic amendments and partial substitutes of mineral fertilizers, could be considered an environmentally friendly use that leads to reduce, at the same time, waste management problems and organic matter depletions in soils, improving soil quality and crop yields. Although it is well established that organic amendments are beneficial for soil quality by improving soil physical properties, effects of repeated applications of organic amendments on chemical properties and growth and activity of soil biota have not been sufficiently evaluated with field trials till now.

The present study was based on the general hypothesis that continual application of organic amendments could improve soil quality, on the long-term, through lasting beneficial effects on chemical and biochemical/biological properties and biodiversity of soil.

For this purpose two different field experiments were carried out:

- 1) the first study, carried out in southern Italy, aimed to assess if use of slow-degradable organic amendments (compost + wood mixtures), as source of organic matter, can improve chemical, biochemical/biological properties and functional diversity of the microbial community in soils affected for long time by intensive agricultural management (under greenhouses).
- 2) the second study, carried out in southern Chile, aimed to test if use of sludge from pulp and paper industry, as organic amendment, can have lasting and positive effects on chemical and biochemical properties and bacterial community structure of the soil.



## **Chapter 3**

# **Use of slow-degradable organic amendments to improve quality of soils under intensive agricultural management**

### **3.1. Introduction**

There is an increasing worry about the long-term productivity of soils as a resource base to provide food for the ever growing world population. Because of population pressure and economic considerations in developed countries, over the past 50 years, agricultural systems have evolved causing loss of biodiversity in the ecosystem, widespread use of intensive managements (Dick, 1992; Harwood, 1990), and, consequently, a gradual decline in soil quality, also due to rapid depletion of organic matter.

The remarkable effects of anthropogenic activities on soil has fuelled efforts to identify and measure factors that affect soil quality. In fact, changes in soil physical and chemical properties, and consequently in microbial growth and activity, influence soil processes, nutrient cycling, and thus soil quality (Gianfreda et al., 2005; Romaniuk et al., 2011; Speeding et al., 2004). However, soil quality cannot be measured directly, but soil quality-related properties (which are sensitive to changes caused by environmental stress or disturbance) may help to monitor changes in sustainability and environmental quality. Change in these indicators can be used to determine whether soil quality is improving, stable, or declining (Brejda et al., 2000; Romaniuk et al., 2011) and this is especially true for the agricultural managements and recovery of soils, and to assist into the establishment of policies for a sustainable land use (Gianfreda et al., 2005). It has to be underlined that soil quality is the outcome of interactions among physical, chemical and biological characteristics, and its proper assessment requires the determination of a large

number of parameters (Bonanomi et al., 2011; Marzaioli et al., 2010), because correct functioning of soil needs interaction of immense number of physical, chemical and biochemical/ biological properties (Gil-Stores et al., 2005). However, in the past, many authors have used principally physical and chemical properties of soil to evaluate changes in its quality (Parr and papendick, 1997; Schloter et al., 2003), but these properties vary extremely slowly and need many years to provide significant results. In contrast, soil biological properties, as microbial biomass (reflecting microbial growth) and soil respiration rate (reflecting total microbial activity) can be considered useful and sensitive indicators of soil quality, as they quickly change in response to stress or disturbance deriving from anthropic activities (Acosta-Martínez et al., 2008; Anderson and Gray, 1990; Doran et al., 1996; Nannipieri et al., 2003; Powlson, 1994). As soil microbial community plays a fundamental role in ecosystem functioning (i.e. in decomposition process, nutrient cycling, maintaining soil structure, suppressing plant pathogens, and providing resistance to stress and disturbance; Bell et al., 2005; Green and Bohannan, 2006; Hu et al., 2011; Morin and McGrady-Steed, 2004; Nannipieri et al., 2003; Wardle and Ghani, 1995), the quantitative description of diversity of soil microbial community has also aroused immense interest in soil quality assessment. Consequently, changes in microbial community structure and functions have been included as feasible biological indicators of soil quality.

Development of a sustainable intensive agriculture is essential for food production, providing also environmental benefits as harmonious delivery of many ecosystem services, including soil and water conservation (Bhardwaj, et al., 2011; Flora, 2010; Gowing and Palmer, 2008; UNDP, 2010; USDS, 2009). Soil management practices including use of organic amendments, as soil ameliorants and partial substitutes of mineral fertilizers, could be a key tool to maintaining in time soil quality and sustainability in intensive agricultural systems (Bulluck et al., 2002; Gunapala and Scow, 1998; Liebig and Doran, 1999; Wander et al., 1994). In fact, soil organic carbon positively affects soil fertility (Happerly et al., 2006; Mader et

al., 2003; Sayre, 2005; Schrader et al., 2006) both directly, by releasing macro and micro elements (Bougnom et al., 2009; Smith, 2009), and indirectly, by determining changes in soil physical (Clapp et al., 2005; Stevenson, 1994; Van-Camp et al., 2004) and chemical properties (Chivenge et al., 2011; Mando and Miedema, 1997), reducing also heavy metal toxicity by adsorbing (D'Ascoli et al., 2005). Moreover, increases in soil organic carbon can also stimulate microbial growth and activity (Chander et al., 1997; Drinkwater et al., 1995; Govaerts et al., 2007; Hansen et al., 2001; Mandal et al., 2007; Shannon et al., 2002; Tejada et al., 2008; Tu et al., 2006), thus preventing depletion in soil quality, although differences in organic matter composition can affect the decomposition process, modifying the availability of substrates, and, consequently, microbial succession and community structure (Marschner et al., 2003).

In the Mediterranean area of southern Italy, protected cultivation under greenhouse is a steadily growing agricultural practice, covering more than 400,000 ha of total land (Enoch and Enoch, 1999). Previous study (Bonanomi et al., 2011) has shown that in this area the intensive agricultural management under permanent plastic tunnels (greenhouse), providing for a large use of fertilizers, negatively affects crop yield and led to a deep degradation of soil, with loss of soil quality. This effect is principally due to the use of plastic tunnels and artificial irrigation, the most common practices in this area, that increase soil salinity and mineralization processes, favouring a more quick decomposition process and thus a decrease in time of organic matter.

In the present study, we have tested the hypothesis that successive applications of slow-degradable organic amendments (compost + wood mixtures), as source of organic matter, can improve on the long term chemical, biochemical/biological properties and functional diversity of the microbial community in soils affected for long time by intensive agricultural management (under greenhouses). For this purpose two soils of the Sele River Plane with different geopedologic characteristics, previously analyzed (Bonanomi et al., 2011), were selected and

different types of mixtures of compost from municipal solid waste and wood from scraps of poplars pruning (in order to have different C/N ratio) were added to soils, with or without an additional mineral fertilizing treatment. The resulting changes in quality of the studied soils were assayed, in the space of 2 years, by using chemical and biological indicators and measuring functional diversity of soil microbial community.

The study was carried out within the framework “*Monitoraggio e recupero della Fertilità dei suoli in sistemi agricoli intensivi*” a research project funded by CCIAA of Salerno (Italy) in collaboration with the research groups of Prof. Astolfo Zoina, Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, and Prof. Maria A. Rao, Dipartimento di Scienze del Suolo, della Pianta, dell’Ambiente e delle Produzioni Animali, Università degli Studi di Napoli Federico II.

## 3.2 Materials and Methods

### 3.2.1. Study area

The present study has been carried out in two farms of the Sele River Plain, located in the Salerno district (Campania region, southern Italy), a highly productive area where intensive agricultural management under greenhouse is predominant (around ~3,500 ha are cultivated under protected permanent plastic tunnels, Bonanomi et al., 2011). The greenhouse structures used in this area are low-cost, unheated polyethylene-covered (height 4-5 m) and with soil-grown crops. The location has a moderate Mediterranean climate with a dry summer (84 mm), and a relatively high mean annual rainfall (988 mm), mainly distributed in winter, spring and fall (354, 217 and 333 mm, respectively); mean monthly temperature range between 23.6 °C, in August, and 9.0 °C, in January (average of 30 years of observation, Bonanomi et al., 2011). Within the selected study area, two farms with different geopedological characteristics have been chosen:

- the farm 1 (named F1) was located in Eboli (Salerno)
- the farm 2 (named F2) was located in Paestum (Salerno).

Physical and chemical properties of soils from F1 and F2 are shown in Table 3.1.

In particular, F1 showed a clay loam soil, classified as *Mollic Haploxeralf*, according to Soil Taxonomy (USDA, 1998; Regione Campania, 2004), with low limestone and electrical conductivity, sub-alkaline pH, and high cation exchange capacity, whereas F2 had a sandy loam soil, classified as *Lithic Haplustolls*, according to Soil Taxonomy (USDA, 1998; Regione Campania, 2004).

F1 showed lower value of soil organic carbon and C/N ratio compared with F2; moreover, considering soil texture, the values of organic carbon were good for F2 (16.19 g kg<sup>-1</sup>), but low for F1 (10.47 g kg<sup>-1</sup>).

Table 3.1. Mean values  $\pm$  standard deviations of the main physical and chemical properties of soils from the two farms of the Campania region of southern Italy.

Properties	Unit	FARM 1	FARM 2
Texture *		Clay loam	Sandy loam
Sand *	%	37 $\pm$ 3	56 $\pm$ 1
Silt *	%	26 $\pm$ 2	27 $\pm$ 1
Clay*	%	37 $\pm$ 2	17 $\pm$ 2
Water holding capacity	%	25.74 $\pm$ 3.01	38.49 $\pm$ 1.84
Bulk density	g cm <sup>3</sup>	1.30 $\pm$ 0.07	1.12 $\pm$ 0.04
pH *		7.74 $\pm$ 0.12	7.65 $\pm$ 0.12
EC, *	dS m <sup>-1</sup>	0.08 $\pm$ 0.02	0.17 $\pm$ 0.03
Limestone*	g kg <sup>-1</sup>	1.55 $\pm$ 1.03	639 $\pm$ 75
Organic C	g kg <sup>-1</sup>	10.47 $\pm$ 0.56	16.19 $\pm$ 0.39
Total N	g kg <sup>-1</sup>	4.13 $\pm$ 0.31	3.90 $\pm$ 0.03
C/N		2.50 $\pm$ 0.20	4.11 $\pm$ 0.15
P <sub>2</sub> O <sub>5</sub> *	mg kg <sup>-1</sup>	162.34 $\pm$ 9.21	174.71 $\pm$ 17.32
CEC*	cmol(+) kg <sup>-1</sup>	21.10 $\pm$ 0.20	13.6 $\pm$ 0.90
Ca <sup>++</sup> *	cmol(+) kg <sup>-1</sup>	15.68	16.48
Mg <sup>++</sup> *	cmol(+) kg <sup>-1</sup>	4.20	1.74
K <sup>+</sup> *	cmol(+) kg <sup>-1</sup>	1.47 $\pm$ 0.06	0.62 $\pm$ 0.20
Na <sup>+</sup> *	cmol(+) kg <sup>-1</sup>	0.74 $\pm$ 0.04	0.40 $\pm$ 0.05

\* data from Scotti, 2010

### 3.2.2. Physical and chemical properties of the tested amendments

In this study for soil treatment were used two different organic amendments:

- compost from municipal solid waste (its chemical properties are reported in Table 3.2)
- wood from scraps of poplars pruning.

Wood from scraps of poplars pruning was used as low mineralization material, having a high C/N ratio (375) and a high content of recalcitrant organic matter (as

lignin). Therefore, compost and wood were mixed together with different doses in order to have two mixtures with different C/N ratio, as reported in the experimental design. Moreover, a commercial chemical fertilizer (N,P,K, 14-7-17) was also used.

Table 3.2. Chemical properties of compost from municipal solid waste.

<b>Parameters</b>	<b>Unit</b>	<b>Amount</b>
Humidity	%	25.00
pH		7.90
Organic carbon	%	28.00
Organic matter*	%	48.27
HAs + FAs	%	14.20
Total N	%	2.10
Organic N	%	2.00
P <sub>2</sub> O <sub>5</sub>	%	0.80
K <sub>2</sub> O	%	1.80
C/N		13.30
Cu	ppm	67.00
Zn	ppm	146.00
Salinity	cmol(+) kg <sup>-1</sup>	53.20

\*Organic matter = Total organic carbon × 1.724 factor

### *3.2.3. Experimental design*

In each farm, six tunnels with a large size (around 160 m<sup>2</sup>) were selected in a greenhouse (Fig. 3.1), that have received the same management practices and cultivation during the last year, and divided in the thirty plots used for the experimental design.

The experimental design included two types of mixtures of compost and wood, in order to have different C/N ratio, supplied at two different doses:

<b><u>abbreviation</u></b>	<b><u>treatment</u></b>
❖ <b>Control</b> =	no treatment
❖ <b>A1L</b> =	Amendment 1 (C/N ratio = 15) Low dose (30 t ha <sup>-1</sup> )
❖ <b>A2L</b> =	Amendment 2 (C/N ratio = 25) Low dose (30 t ha <sup>-1</sup> )
❖ <b>A1H</b> =	Amendment 1 (C/N ratio = 15) High dose (60 t ha <sup>-1</sup> )
❖ <b>A2H</b> =	Amendment 2 (C/N ratio = 25) High dose (60 t ha <sup>-1</sup> )



Figure 3.1 Tunnels of a typical greenhouse in the Sele River Plane sown with lettuce.

Moreover, all treatments were replicated adding also a chemical fertilizer (N,P,K, 200 kg ha<sup>-1</sup>), corresponding to 10 treatments in all (Fig. 3.2). In tables and graphs, abbreviations of plots treated also with mineral fertilizer were followed by –m suffix. The chemical fertilizer was used to test the combining effect of organic amendment and mineral fertilization and also to compare the effect of organic vs chemical fertilizer on quality status of the studied soils.

In both farms, each treatment in field was in triplicate (Fig. 3.2).



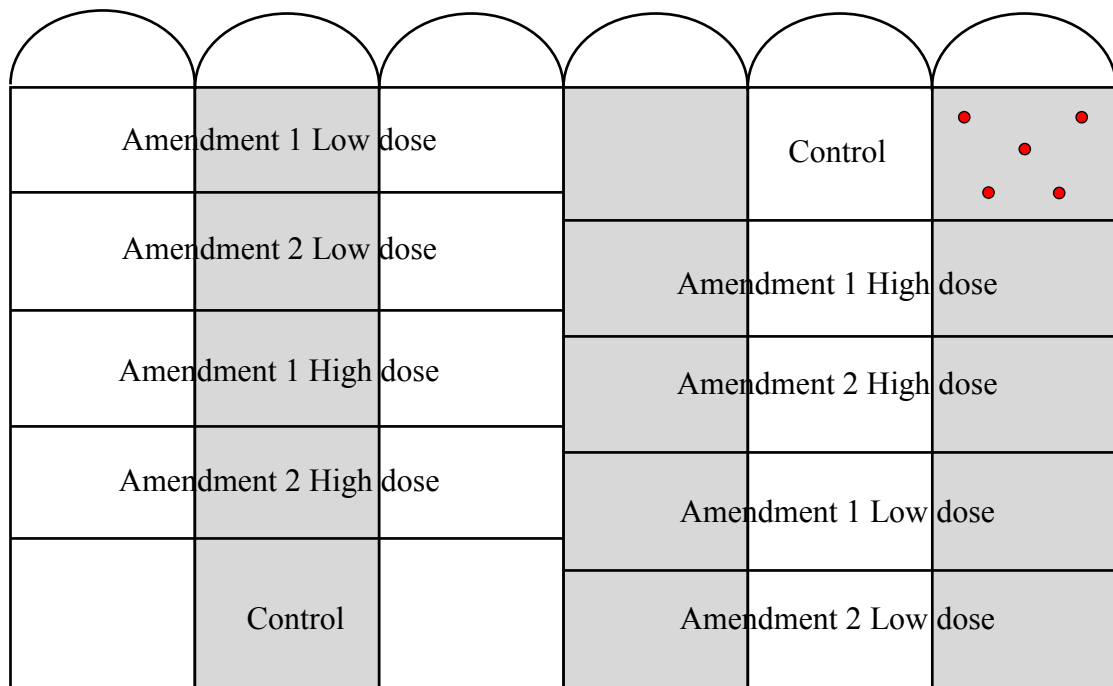


Figure 3.2 In the figure, the six tunnels with the ten treatments are shown. Grey tunnels indicate plots treated also with chemical fertilizer. In the top on the right it has also shown the W scheme used in the soil samplings.

### 3.2.4. Soil amendment and samplings

During the two years of study, two treatments (organic amendments with and without the mineral fertilizer) were carried out, the first one in February and March 2009 (in F1 and F2, respectively) and the second one in February 2010 (for both farms), providing the compost–wood mixtures on the soil surface and mixing with the upper soil layer by ploughing (up to 30 cm of depth).

After amending, an artificial irrigation (the only way to supply water to soil, as plastic tunnels are a hindrance to natural rainfall) was performed and the tunnels were cultivated, and in particular, during the two years of study, in each farm six crop cycles were performed:

#### **Farm 1**

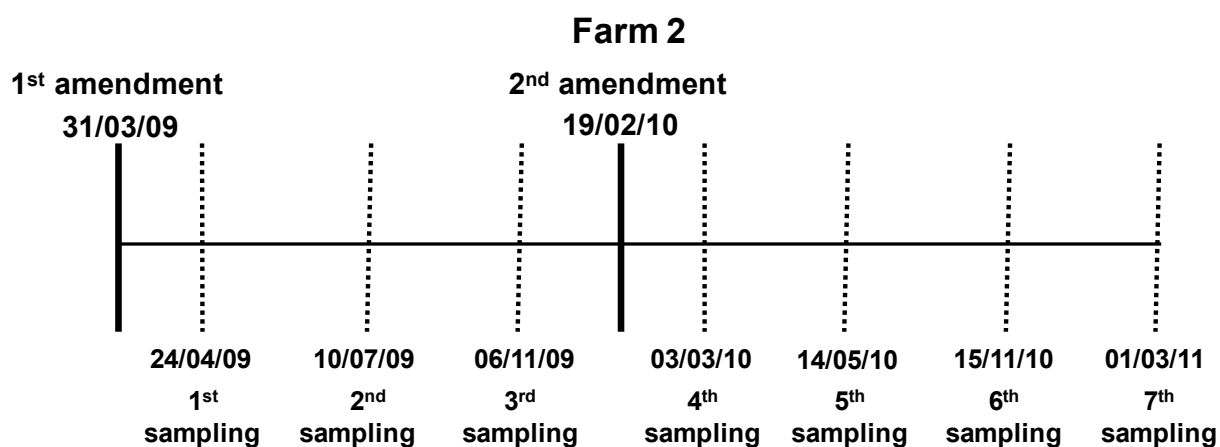
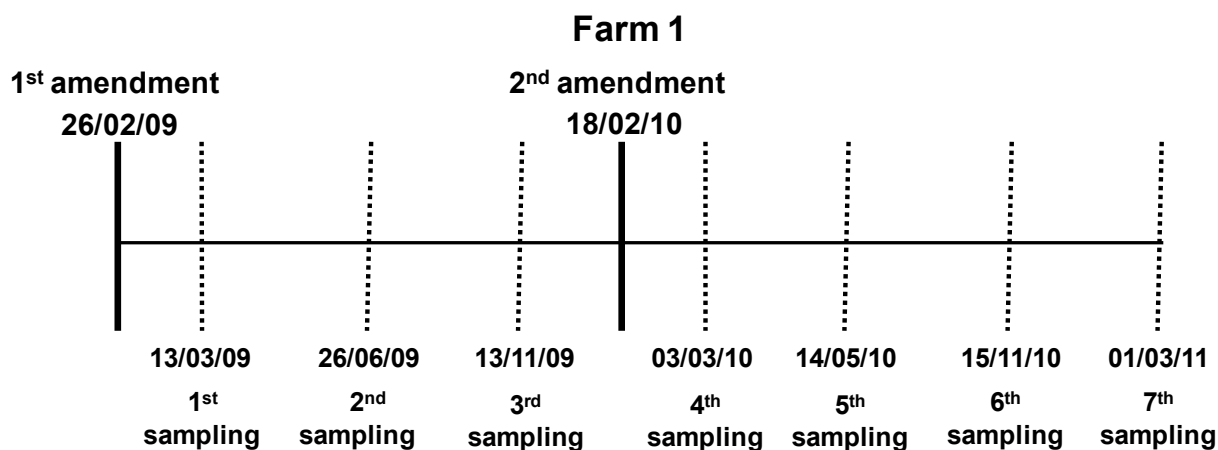
- ❖ Melon in the springtime
- ❖ Two crop cycles of lettuces during the autumn and the winter

- ❖ Melon in the springtime
- ❖ Two crop cycles of lettuces during the autumn and the winter

**Farm 2**

- ❖ Melon in the springtime
- ❖ Kohlrabi, during the autumn and the winter
- ❖ Pepper in the springtime
- ❖ Kohlrabi, during the autumn and the winter

Soil samplings were periodically carried out. In particular, the first sampling was made one month after each addition. Afterwards, further six samplings were carried out, in both farms, at every 4 months, corresponding to 7 samplings in all in the two years of study, according to following schemes:



In each plot, five sub-samples of soil were collected from the topsoil (0-20 cm depth) following a W scheme (Fig. 3.2), and then mixed together and stored in polyethylene bags, in order to have a more representative sample. In laboratory, samples were sieved at 2 mm mesh and then separated in two subsamples, the first one was stored at +4 °C until time of measurements of biochemical and microbiological parameters (at most 10 days from the collection of soil samples), whereas the second subsamples was dried in oven (40 °C until constant weight was reached) and used for chemical analyses.

### *3.2.5 Soil physical and chemical analyses*

Water holding capacity (WHC) and water content (WC) were assayed immediately after soil sampling by gravimetric method (Allen, 1989), the first one after saturation of soil cores (10 cm height) with water, drying soil samples at 105°C and expressing results as percentage on dry soil. Both parameters were fundamental to better standardize incubation conditions for potential respiration and catabolic response profiles. Soil pH was determined by potentiometric method on 1:2.5 soil/water suspensions and available P was assayed by sodium bicarbonate extraction, by the Olsen method (Sparks, 1996). Soil organic carbon was assayed on dried, pulverized and sieved (5 mm) soil samples by chromic acid digestion method (Walkey and Black, 1934). Total N was determined on dry pulverized soil samples by flash combustion with a CNS Elemental Analyzer (Thermo Flash EA 1112). Mineral N, as ammoniacal ( $\text{NH}_4^+$ -N) and nitric N ( $\text{NO}_3^-$ -N) contents, was assayed by using ion-selective electrodes specific for ammonia and nitrate (Castaldi and Agarosa, 2002).

Soil pH and available P were performed by the research group of Prof. Maria A. Rao, Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Università degli Studi di Napoli Federico II (Scotti, 2010).

### 3.2.6. Soil biological analyses

All biological analyses were carried out on fresh soil, stored at 4°C, within 10 days from sample collections. Total microbial biomass carbon ( $C_{mic}$ ) was determined by the chloroform fumigation-extraction method (Vance et al., 1987). The microbial biomass C was calculated, according to Vance et al. (1987), by using the conversion factor 2.64. Total microbial activity was measured as CO<sub>2</sub> release from soil samples by gas chromatographic method. Briefly, fresh soil samples (4g equivalent dry weight) were placed into 30 ml vials and moistened to 55% water holding capacity with distilled water. After two days of incubation (25 °C, at dark), the vials were sealed by butyl rubber septa and washed with standard air from a cylinder using two needles. Afterwards, vials were again incubated for 1 h in the same standard conditions and CO<sub>2</sub> release from soils was determined by sampling, by 5 ml syringe, the air in the headspace of each vial and using a gas chromatograph equipped with ECD (Fisons GC 8000 series: Fisons instrument, Milan, Italy). CO<sub>2</sub> from soils of VII sampling time was measured by a gas chromatograph equipped with TCD (Agilent 6850 Series II Network GC System) in the Dipartimento di Chimica, Università di Salerno, Fisciano, Italy. Triplicates were performed for respiration assay.

### 3.2.7 Soil functional diversity

Functional diversity of soil microbial community was assayed as catabolic fingerprint of soils, determining CO<sub>2</sub> release from soil samples incubated for 4 hours in standard conditions (55% WHC, 25°C, at dark) after addition of 25 simple organic compounds (Table 3.3).

Table 3.3. Substrate used for measurement of catabolic response profiles.

<b>Substrates</b>	<b>F.W.</b>	<b>Concentration (mM)</b>
<b>Amino Acid</b>		
1. L-Serine	105.10	15
2. L-Glutamine	146.10	15
3. L-Arginine	174.20	15
4. L-Asparagine	132.10	15
5. D-Glucosamine	215.60	15
6. L-Histidine	155.20	15
7. L-Glutamic Acid	147.10	15
8. L-Lysine	146.20	15
<b>Carboxylic Acid</b>		
9. L-Ascorbic Acid	176.10	100
10. Citric Acid	192.10	100
11. Fumaric Acid	116.10	100
12. Malonic Acid	104.10	100
13. Pantothenic Acid	238.30	100
14. Quinic Acid	192.20	100
15. Succinic Acid	118.10	100
16. Uric Acid	168.10	100
17. Tartaric Acid	150.10	100
18. Gluconic Acid	196.20	100
19. $\alpha$ -Ketobutyric Acid	102.09	100
20. $\alpha$ -Ketoglutaric Acid	146.10	100
21. $\alpha$ -Ketovaleric Acid	116.10	100
22. DL-Malic Acid	134.10	100
23. Urocanic Acid	138.13	100
<b>Carbohydrate</b>		
24. D-Glucose	180.20	75
25. D-Mannose	180.20	75

After two days of incubation (25 °C, at dark), the 25 substrates (2 ml solution) were separately added to 25 replicates of each sample, then vials were sealed by butyl rubber septa and vigorously shaken by hand. One replicate per each sample

was used as control (no substrate addition). Afterwards, each vial was washed with standard air, again incubated for 4 h and CO<sub>2</sub> concentration in the headspace of each vial was determined similarly with the method reported in 3.2.6 paragraph. The whole of data from substrate induced respiration represents a catabolic fingerprint of each soil sample.

Moreover, catabolic evenness was also calculated by using catabolic response profiles. Starting from functional diversity data, Catabolic Evenness Index was calculated according to Simpson-Yule index:

$$E = 1/\sum p_i^2 \quad (\text{Magurran, 1988})$$

Where 
$$p_i = \frac{\text{individual respiration response}}{\text{total respiration activity (induced by all substrate)}}$$

### 3.2.8 Microbial indices

Microbial quotient, the fraction of soil organic C occurring as microbial biomass (Haynes, 2000), was calculated and expressed as  $\text{mg C}_{\text{mic}} \text{g}^{-1} \text{C}_{\text{org}}$  (Anderson and Domsch 1986).

Metabolic quotient (qCO<sub>2</sub>) was calculated as CO<sub>2</sub>-C evolved per unit of microbial biomass C and expressed as  $\text{mg CO}_2\text{-C g}^{-1} \text{C}_{\text{mic}} \text{h}^{-1}$  (Anderson and Domsch, 1990).

Coefficient of endogenous mineralization was calculated as fraction of organic C evolved as CO<sub>2</sub> and expressed as  $\text{mg CO}_2\text{-C g}^{-1} \text{C}_{\text{org}} \cdot \text{h}^{-1}$ .

### *3.2.9 Statistical analyses*

For each treatment, means and standard deviations were calculated from the three field replicates and reported in graphs and tables. The significance of differences between soil affected by different treatments was tested using one way ANOVA, followed by the Student-Newman-Keuls test ( $P < 0.05$ ; Sigma STAT 3.1). Two-way ANOVA test, followed by the Holm-Sidak test, was used to test the effects of sampling times and treatment on physical, chemical, biochemical and biological parameters ( $P < 0.05$ ;  $n = 210$ ; Sigma STAT 3.1). Pearson correlation coefficients were calculated to determine relationships between chemical, biochemical and biological data ( $P < 0.05$ ;  $n = 210$ ; Sigma STAT 3.1). Principal component analysis (PCA) was performed by JMP 8 (SAS Institute, 2008).

### 3.3 Results

#### 3.3.1. Effect of organic amendment on soil physical and chemical properties

Values of water content in soils from F1 and F2 are shown in tables 3.4 and 3.5 respectively. In general, soil water content in F1 (average mean value 14.60) was lower than in F2 (average mean value 22.46), in spite of the type of texture in two farms, probably due to the different artificial irrigation extent in the studied soils (the only way to supply water to soil, as plastic tunnels are a hindrance to natural rainfall). After treatments, water content showed no significant increase, with the exception of F2 soil that showed an increasing trend of this parameter in amended soils of the 1<sup>st</sup> and 7<sup>th</sup> samplings.

Table 3.4 Mean value ( $\pm$  SD) of water content (% d.w.) in F1 soil after application of organic and mineral fertilizers. Different superscript letters indicated significant differences among treatments for each sampling time (treatments with and without mineral fertilizing were analyzed separately). The -m suffix at the end of abbreviations indicates the soil was also treated with mineral fertilizer

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	16.15 $\pm 1.55$	12.88 $\pm 1.95$	17.08 $\pm 0.31$	16.44 0.51	11.07 3.23	10.64 $\pm 1.06$	15.08 $\pm 1.67$
A1L	13.48 $\pm 2.95$	11.79 $\pm 3.54$	17.59 $\pm 0.90$	16.34 0.19	11.36 $\pm 3.36$	12.84 $\pm 0.79$	17.80 $\pm 0.71$
A1H	12.64 $\pm 2.81$	12.11 $\pm 3.42$	18.74 $\pm 0.90$	16.39 1.35	9.62 $\pm 0.87$	13.31 $\pm 1.02$	19.03 $\pm 0.21$
A2L	16.56 $\pm 2.39$	12.49 $\pm 4.25$	18.57 $\pm 0.76$	17.60 1.01	11.02 $\pm 1.54$	13.95 $\pm 0.89$	16.66 $\pm 0.96$
A2H	16.13 $\pm 0.15$	10.28 $\pm 1.40$	18.01 $\pm 1.19$	18.92 4.54	10.65 $\pm 2.35$	13.20 $\pm 0.58$	16.75 $\pm 1.25$
Control -m	13.25 $\pm 1.94$	11.79 $\pm 0.69$	17.35 $\pm 1.68$	15.90 0.81	11.30 $\pm 0.88$	11.65 $\pm 0.77$	16.18 $\pm 1.26$
A1L-m	12.35 $\pm 2.38$	10.60 $\pm 2.92$	18.17 $\pm 0.40$	16.48 0.72	11.91 $\pm 3.78$	11.97 $\pm 0.42$	17.75 $\pm 1.37$
A1H-m	14.35 $\pm 1.18$	8.78 $\pm 0.77$	18.28 $\pm 1.00$	17.52 0.94	11.97 $\pm 2.46$	17.05 $\pm 6.54$	17.07 $\pm 2.57$
A2L-m	10.56 $\pm 1.71$	12.00 $\pm 3.45$	18.16 $\pm 0.57$	16.54 3.25	14.91 $\pm 3.32$	9.77 $\pm 4.85$	16.93 $\pm 2.53$
A2H-m	14.47 $\pm 2.51$	11.20 $\pm 0.83$	17.91 $\pm 0.77$	16.79 0.24	10.81 $\pm 3.31$	19.78 $\pm 8.57$	17.65 $\pm 1.03$



Table 3.5 Mean value ( $\pm$  SD) of water content (% d.w.) in F2 soil after application of organic and mineral fertilizers. Different superscript letters indicated significant differences among treatments for each sampling time (treatments with and without mineral fertilizing were analyzed separately). The -m suffix at the end of abbreviations indicates the soil was also treated with mineral fertilizer

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	23.04 <sup>a</sup> $\pm 0.58$	16.72 $\pm 1.82$	20.59 $\pm 0.76$	23.21 $\pm 1.16$	21.49 $\pm 0.67$	21.04 $\pm 2.55$	22.73 <sup>a</sup> $\pm 0.64$
A1L	29.85 <sup>b</sup> $\pm 0.46$	16.59 $\pm 4.62$	20.51 $\pm 0.89$	24.66 $\pm 0.93$	21.47 $\pm 1.13$	20.00 $\pm 5.81$	24.86 <sup>ab</sup> $\pm 1.09$
A1H	31.47 <sup>b</sup> $\pm 0.54$	16.45 $\pm 1.39$	20.73 $\pm 0.44$	25.66 $\pm 0.72$	21.57 $\pm 0.52$	21.54 $\pm 0.95$	25.90 <sup>b</sup> $\pm 0.88$
A2L	30.00 <sup>b</sup> $\pm 2.73$	16.25 $\pm 4.16$	19.21 $\pm 1.74$	24.26 $\pm 1.93$	20.08 $\pm 0.14$	22.21 $\pm 1.67$	23.64 <sup>ab</sup> $\pm 1.48$
A2H	30.49 <sup>b</sup> $\pm 0.44$	17.23 $\pm 1.44$	21.16 $\pm 0.19$	24.27 $\pm 1.40$	21.40 $\pm 0.55$	22.13 $\pm 1.42$	24.31 <sup>ab</sup> $\pm 0.12$
Control -m	30.06 $\pm 0.66$	18.24 $\pm 1.98$	20.30 $\pm 0.68$	23.35 $\pm 0.76$	21.73 $\pm 0.40$	18.39 $\pm 2.95$	23.58 <sup>a</sup> $\pm 0.36$
A1L-m	30.73 $\pm 0.60$	16.40 $\pm 2.85$	19.66 $\pm 0.38$	23.37 $\pm 1.03$	21.56 $\pm 1.72$	18.84 $\pm 1.26$	24.05 <sup>ab</sup> $\pm 1.01$
A1H-m	30.26 $\pm 1.93$	15.49 $\pm 0.98$	20.19 $\pm 0.26$	22.71 $\pm 3.66$	21.49 $\pm 1.70$	22.75 $\pm 1.41$	25.45 <sup>b</sup> $\pm 0.71$
A2L-m	29.26 $\pm 0.49$	15.38 $\pm 3.10$	20.15 $\pm 0.98$	24.57 $\pm 0.72$	21.46 $\pm 0.95$	22.09 $\pm 0.56$	23.34 <sup>ab</sup> $\pm 0.53$
A2H-m	29.85 $\pm 4.85$	17.49 $\pm 1.61$	20.91 $\pm 0.15$	23.51 $\pm$ 2.78	22.03 $\pm 1.36$	22.55 $\pm 1.69$	24.25 <sup>ab</sup> $\pm 0.89$

Moreover, the two-way ANOVA showed a significant effect due to sampling times in both farm, but no significant effects were due to the different treatments (table 3.6 and 3.7, respectively).

In table 3.8 and 3.9 water holding capacities of F1 and F2 soils are shown. In the F1 soil, a clay loam soil, the different treatments did not considerably affected WHC of the soil, whereas in F2 soil, a sandy loam soil, a significant increase was found in amended soil at the end of study period (7<sup>th</sup> sampling), that could affect water content in this soil.

In contrast, highly significant differences ( $p < 0.001$ ) were only found, by two way ANOVA, among sampling times (table 3.6 and 3.7, respectively).

Table 3.6 Summarize results of two-way ANOVA for all assessed parameters in F1 soil. Sampling times and organic amendment doses were independent variables.

Parameter	Source	d.f	F	P-value
Water content	Sampling time	6	38.531	<0.001
	Amendment dose	9	1.040	0.412 <sup>NS</sup>
	Interaction	54	1.402	0.060 <sup>NS</sup>
Water holding capacity	Sampling time	6	92.350	<0.001
	Amendment dose	9	1.332	0.226 <sup>NS</sup>
	Interaction	54	0.871	0.714 <sup>NS</sup>
pH	Sampling time	6	295.246	<0.001
	Amendment dose	9	2.090	0.034
	Interaction	54	3.546	<0.001
Phosphorus	Sampling time	6	351.595	<0.001
	Amendment dose	9	3.708	<0.001
	Interaction	54	4.004	<0.001
Total N	Sampling time	6	223.762	<0.001
	Amendment dose	9	6.887	<0.001
	Interaction	54	0.995	0.496
Ammonium-N	Sampling time	6	86.352	<0.001
	Amendment dose	9	4.196	<0.001
	Interaction	54	2.343	<0.001
Nitrate -N	Sampling time	6	519.432	<0.001
	Amendment dose	9	28.551	<0.001
	Interaction	54	17.753	<0.001
Organic-C	Sampling time	6	99.015	<0.001
	Amendment dose	9	59.499	<0.001
	Interaction	54	2.785	<0.001
C/N	Sampling time	6	719.193	<0.001
	Amendment dose	9	1.388	0.199 <sup>NS</sup>
	Interaction	54	1.308	0.108 <sup>NS</sup>
Microbial Biomass	Sampling time	6	10.082	<0.001
	Amendment dose	9	5.151	<0.001
	Interaction	54	2.843	<0.001
Microbial Quotient	Sampling time	6	12.487	<0.001
	Amendment dose	9	2.323	0.018
	Interaction	54	2.719	<0.001
Respiration	Sampling time	6	35.735	<0.001
	Amendment dose	9	6.773	<0.001
	Interaction	54	1.364	0.077 <sup>NS</sup>
CEM	Sampling time	6	32.482	<0.001
	Amendment dose	9	1.841	0.066 <sup>NS</sup>
	Interaction	54	1.282	0.127 <sup>NS</sup>
Metabolic Quotient	Sampling time	6	22.349	<0.001
	Amendment dose	9	0.857	0.549 <sup>NS</sup>
	Interaction	54	1.842	0.002
Catabolic evenness	Sampling time	4	4	21.438
	Amendment dose	9	9	1.486
	Interaction	36	36	9.83

NS = not significant, i.e.  $P \geq 0.05$

Table 3.7 Summarize results of two-way ANOVA for all assessed parameters in F2 soil. Sampling times and organic amendment doses were independent variables.

Parameter	Source	d.f	F	P-value
Water content	Sampling time	6	160.615	<0.001
	Amendment dose	9	2.359	0.016
	Interaction	54	1.450	0.044
Water holding capacity	Sampling time	6	16.313	<0.001
	Amendment dose	9	1.568	0.131 <sup>NS</sup>
	Interaction	54	1.066	0.377 <sup>NS</sup>
pH	Sampling time	6	119.343	<0.001
	Amendment dose	9	5.658	<0.001
	Interaction	54	3.052	<0.001
Phosphorus	Sampling time	6	147.400	<0.001
	Amendment dose	9	3.704	<0.001
	Interaction	54	0.948	0.579 <sup>NS</sup>
Total N	Sampling time	6	90.626	<0.001
	Amendment dose	9	1.752	0.083 <sup>NS</sup>
	Interaction	54	1.603	0.015
Ammonium-N	Sampling time	6	386.149	<0.001
	Amendment dose	9	2.574	0.009
	Interaction	54	2.793	<0.001
Nitrate -N	Sampling time	6	455.693	<0.001
	Amendment dose	9	7.297	<0.001
	Interaction	54	6.363	<0.001
Organic-C	Sampling time	6	34.021	<0.001
	Amendment dose	9	25.005	<0.001
	Interaction	54	2.195	<0.001
C/N	Sampling time	6	106.669	<0.001
	Amendment dose	9	6.779	<0.001
	Interaction	54	1.002	0.484
Microbial Biomass	Sampling time	6	6.306	<0.001
	Amendment dose	9	7.290	<0.001
	Interaction	54	1.945	0.001
Microbial Quotient	Sampling time	6	9.622	<0.001
	Amendment dose	9	4.442	<0.001
	Interaction	54	1.943	0.001
Respiration	Sampling time	6	52.774	<0.001
	Amendment dose	9	3.399	0.001
	Interaction	54	1.689	0.008
CEM	Sampling time	6	52.866	<0.001
	Amendment dose	9	1.176	0.315 <sup>NS</sup>
	Interaction	54	1.086	0.345 <sup>NS</sup>
Metabolic Quotient	Sampling time	6	13.810	<0.001
	Amendment dose	9	3.424	0.001
	Interaction	54	2.017	0.001
Catabolic evenness	Sampling time	4	18.254	<0.001
	Amendment dose	9	4.418	<0.001
	Interaction	36	3.034	<0.001

NS = not significant, i.e.  $P \geq 0.05$

Table 3.8 Mean value ( $\pm$  SD) of water holding capacity (% d.w.) in F1 soil. Different superscript letters indicated significant differences among treatments for each sampling time (treatments with and without mineral fertilizing were analyzed separately). The -m suffix at the end of abbreviations indicates the soil was also treated with mineral fertilizer.

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	41.31 $\pm 2.29$	48.77 <sup>a</sup> $\pm 0.63$	52.88 $\pm 0.49$	52.20 $\pm 1.52$	49.80 $\pm 1.65$	44.72 $\pm 0.73$	43.42 $\pm 0.93$
A1L	45.61 $\pm 3.35$	48.55 <sup>a</sup> $\pm 1.42$	53.96 $\pm 0.69$	51.84 $\pm 2.38$	51.17 $\pm 2.73$	45.65 $\pm 1.10$	42.57 $\pm 1.82$
A1H	45.76 $\pm 0.88$	47.98 <sup>a</sup> $\pm 1.93$	51.99 $\pm 3.23$	51.66 $\pm 2.52$	50.32 $\pm 0.71$	43.70 $\pm 1.04$	43.32 $\pm 1.25$
A2L	41.02 $\pm 3.82$	44.04 <sup>b</sup> $\pm 1.89$	52.44 $\pm 2.62$	52.14 $\pm 4.31$	48.86 $\pm 1.94$	45.08 $\pm 2.17$	43.37 $\pm 3.62$
A2H	44.76 $\pm 4.40$	46.18 <sup>b</sup> $\pm 0.81$	52.21 $\pm 0.50$	54.33 $\pm 4.37$	55.08 $\pm 5.43$	45.59 $\pm 0.91$	43.78 $\pm 4.74$
Control -m	44.19 $\pm 4.02$	48.79 $\pm 0.76$	53.18 $\pm 2.06$	52.28 $\pm 2.43$	50.35 $\pm 0.58$	45.04 $\pm 2.39$	43.43 $\pm 0.78$
A1L-m	41.78 $\pm 3.07$	48.49 $\pm 1.82$	53.54 $\pm 0.73$	53.84 $\pm 0.56$	49.92 $\pm 3.68$	46.82 $\pm 1.25$	43.45 $\pm 2.56$
A1H-m	42.78 $\pm 4.79$	45.70 $\pm 1.12$	52.51 $\pm 1.03$	54.11 $\pm 2.78$	50.35 $\pm 1.39$	44.87 $\pm 1.90$	45.18 $\pm 2.99$
A2L-m	46.16 $\pm 2.12$	46.49 $\pm 2.08$	53.82 $\pm 1.74$	52.52 $\pm 2.21$	49.92 $\pm 0.74$	45.94 $\pm 0.52$	42.84 $\pm 0.47$
A2H-m	44.99 $\pm 4.28$	47.05 $\pm 0.84$	53.65 $\pm 1.58$	52.46 $\pm 0.82$	50.75 $\pm 1.01$	45.23 $\pm 0.71$	44.38 $\pm 3.06$

Table 3.9 Mean value ( $\pm$ SD) of water holding capacity (% d.w.) in F2 soil.

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	56.02 $\pm 1.07$	55.41 $\pm 0.47$	55.46 $\pm 1.04$	53.01 $\pm 1.33$	53.19 $\pm 1.42$	55.99 $\pm 1.35$	48.57 <sup>a</sup> $\pm 1.76$
A1L	56.00 $\pm 0.71$	55.67 $\pm 0.44$	56.13 $\pm 0.91$	52.48 $\pm 1.41$	53.61 $\pm 2.08$	55.47 $\pm 1.03$	52.68 <sup>b</sup> $\pm 1.45$
A1H	55.23 $\pm 1.47$	56.96 $\pm 0.61$	56.04 $\pm 1.36$	51.78 $\pm 0.66$	53.39 $\pm 0.25$	54.24 $\pm 1.23$	53.41 <sup>b</sup> $\pm 1.40$
A2L	55.25 $\pm 2.18$	55.35 $\pm 1.35$	56.69 $\pm 2.89$	52.71 $\pm 0.64$	52.75 $\pm 0.28$	55.15 $\pm 0.28$	53.65 <sup>b</sup> $\pm 1.57$
A2H	56.59 $\pm 0.11$	60.64 $\pm 9.19$	55.65 $\pm 1.07$	52.55 $\pm 1.17$	53.28 $\pm 1.27$	54.94 $\pm 0.61$	55.89 <sup>b</sup> $\pm 0.89$
Control -m	56.26 $\pm 2.31$	55.44 $\pm 0.26$	54.75 $\pm 0.91$	52.18 $\pm 1.35$	52.46 $\pm 1.74$	56.89 $\pm 3.34$	51.37 <sup>a</sup> $\pm 1.62$
A1L-m	55.85 $\pm 1.43$	56.54 $\pm 2.84$	56.32 $\pm 2.50$	53.51 $\pm 0.82$	53.14 $\pm 1.31$	55.25 $\pm 1.96$	54.29 <sup>ab</sup> $\pm 1.31$
A1H-m	55.09 $\pm 1.73$	55.70 $\pm 1.29$	54.84 $\pm 3.16$	52.43 $\pm 1.29$	52.93 $\pm 0.60$	53.37 $\pm 1.57$	56.50 <sup>b</sup> $\pm 1.45$
A2L-m	56.28 $\pm 0.96$	57.02 $\pm 1.46$	55.90 $\pm 1.45$	53.57 $\pm 2.66$	52.83 $\pm 0.54$	55.57 $\pm 1.64$	54.27 <sup>ab</sup> $\pm 2.70$
A2H-m	52.45 $\pm 7.11$	55.02 $\pm 0.95$	55.15 $\pm 1.95$	52.39 $\pm 1.82$	51.70 $\pm 1.76$	56.20 $\pm 1.13$	53.21 <sup>ab</sup> $\pm 1.05$

It has to be emphasized that F1 soil showed significantly lower values of water content and water holding capacity (in spite of its clay nature) compared to F2 soil (that showed a sandy nature) probably due to different content of organic carbon. The addition of organic and mineral fertilizers caused in F1 and F2 soils a slight increase in pH (see table 3.10 and 3.11, 1<sup>st</sup> sampling). At the end of the study period, pH in amended soils differed from that in control soil only in F2 plots treated with mineral fertilizing. However, differences were always extremely limited. Two-ways ANOVA test showed significant differences due to sampling time, amendment dose and interaction between these variables.

Table 3.10 Mean value ( $\pm$  SD) of pH in F1 soil (data from Scotti, 2010). Different superscript letters indicated significant differences among treatments for each sampling time (treatments with and without mineral fertilizing were analyzed separately). The -m suffix at the end of abbreviations indicates the soil was also treated with mineral fertilizer.

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	7.74 $\pm 0.08$	7.13 $\pm 0.00$	8.26 <sup>a</sup> $\pm 0.08$	8.10 $\pm 0.31$	6.95 $\pm 0.23$	7.91 <sup>ab</sup> $\pm 0.05$	8.30 $\pm 0.04$
A1L	8.01 $\pm 0.04$	7.21 $\pm 0.03$	8.22 <sup>a</sup> $\pm 0.02$	7.77 $\pm 0.08$	7.43 $\pm 0.33$	7.98 <sup>a</sup> $\pm 0.08$	8.40 $\pm 0.09$
A1H	7.95 $\pm 0.05$	7.23 $\pm 0.06$	8.38 <sup>b</sup> 0.07	7.82 $\pm 0.13$	7.46 $\pm 0.36$	7.84 <sup>ab</sup> $\pm 0.11$	8.26 $\pm 0.08$
A2L	7.83 $\pm 0.11$	7.25 $\pm 0.08$	8.46 <sup>b</sup> $\pm 0.03$	8.14 $\pm 0.17$	7.45 $\pm$ $\pm 0.33$	7.82 <sup>ab</sup> $\pm 0.05$	8.32 $\pm 0.02$
A2H	7.88 $\pm 0.05$	7.18 $\pm 0.08$	8.34 <sup>a</sup> $\pm 0.02$	7.79 $\pm 0.10$	7.29 $\pm 0.32$	7.73 <sup>b</sup> $\pm 0.10$	8.35 $\pm 0.06$
Control -m	7.50 <sup>a</sup> $\pm 0.16$	7.20 $\pm 0.14$	8.23 <sup>a</sup> $\pm 0.04$	7.87 <sup>a</sup> $\pm 0.10$	7.51 $\pm 0.13$	8.03 <sup>a</sup> $\pm 0.07$	8.19 $\pm 0.19$
A1L-m	8.00 <sup>b</sup> $\pm 0.07$	7.24 $\pm 0.09$	8.21 <sup>a</sup> $\pm 0.04$	7.74 <sup>a</sup> $\pm 0.06$	7.47 $\pm 0.07$	7.89 <sup>b</sup> $\pm 0.05$	8.29 $\pm 0.05$
A1H-m	7.91 <sup>b</sup> $\pm 0.05$	7.22 $\pm 0.05$	8.67 <sup>b</sup> $\pm 0.09$	8.11 <sup>b</sup> $\pm 0.09$	7.19 $\pm 0.38$	7.75 <sup>c</sup> $\pm 0.01$	8.30 $\pm 0.07$
A2L-m	7.95 <sup>b</sup> $\pm 0.05$	7.16 $\pm 0.02$	8.44 <sup>c</sup> $\pm 0.04$	7.85 <sup>a</sup> $\pm 0.07$	7.44 $\pm 0.34$	7.77 <sup>c</sup> $\pm 0.01$	8.32 $\pm 0.05$
A2H-m	7.90 <sup>b</sup> $\pm 0.06$	7.27 $\pm 0.12$	8.30 <sup>ac</sup> $\pm 0.03$	7.78 <sup>a</sup> $\pm 0.05$	7.88 $\pm 0.13$	7.75 <sup>c</sup> $\pm 0.01$	8.30 $\pm 0.12$

Table 3.11 Mean value ( $\pm$  SD) of pH in F2 soil (data from Scotti, 2010). Different superscript letters indicated significant differences among treatments for each sampling time (treatments with and without mineral fertilizing were analyzed separately). The -m suffix at the end of abbreviations indicates the soil was also treated with mineral fertilizer.

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	7.65 <sup>a</sup> $\pm 0.07$	7.82 <sup>a</sup> $\pm 0.02$	8.10 $\pm 0.14$	8.13 $\pm 0.15$	7.74 <sup>a</sup> $\pm 0.11$	8.14 $\pm 0.26$	8.46 $\pm 0.05$
A1L	7.52 <sup>a</sup> $\pm 0.25$	7.54 <sup>b</sup> $\pm 0.14$	8.03 $\pm 0.04$	7.90 $\pm 0.06$	7.91 <sup>b</sup> $\pm 0.01$	8.41 $\pm 0.09$	8.39 $\pm 0.14$
A1H	7.87 <sup>b</sup> $\pm 0.11$	7.55 <sup>b</sup> $\pm 0.03$	8.15 $\pm 0.07$	8.09 $\pm 0.10$	7.98 <sup>b</sup> $\pm 0.05$	8.28 $\pm 0.07$	8.33 $\pm 0.08$
A2L	7.87 <sup>b</sup> $\pm 0.14$	7.69 <sup>a</sup> $\pm 0.14$	7.99 $\pm 0.04$	8.14 $\pm 0.10$	7.98 <sup>b</sup> $\pm 0.03$	8.32 $\pm 0.03$	8.51 $\pm 0.02$
A2H	8.04 <sup>b</sup> $\pm 0.12$	7.76 <sup>a</sup> $\pm 0.08$	8.08 $\pm 0.04$	8.06 $\pm 0.08$	8.01 <sup>b</sup> $\pm 0.03$	8.36 $\pm 0.05$	8.34 $\pm 0.06$
Control -m	7.83 $\pm 0.17$	7.71 <sup>a</sup> $\pm 0.14$	7.94 <sup>a</sup> $\pm 0.04$	7.86 $\pm 0.11$	7.85 <sup>a</sup> $\pm 0.04$	8.29 $\pm 0.06$	8.53 <sup>a</sup> $\pm 0.07$
A1L-m	7.68 0.07	7.36 <sup>b</sup> $\pm 0.21$	8.06 <sup>a</sup> $\pm 0.04$	8.14 $\pm 0.09$	7.90 <sup>ab</sup> $\pm 0.02$	8.30 $\pm 0.07$	8.36 <sup>b</sup> $\pm 0.05$
A1H-m	7.53 $\pm 0.25$	7.56 <sup>ab</sup> $\pm 0.07$	8.22 <sup>b</sup> $\pm 0.14$	8.23 $\pm 0.21$	8.02 <sup>b</sup> $\pm 0.04$	8.32 $\pm 0.05$	8.35 <sup>b</sup> $\pm 0.04$
A2L-m	7.84 $\pm 0.13$	7.54 <sup>ab</sup> $\pm 0.06$	7.95 <sup>a</sup> $\pm 0.06$	8.13 $\pm 0.09$	7.93 <sup>ab</sup> $\pm 0.01$	8.29 $\pm 0.13$	8.49 <sup>a</sup> $\pm 0.03$
A2H-m	7.83 $\pm 0.18$	7.84 <sup>a</sup> $\pm 0.05$	8.02 <sup>a</sup> $\pm 0.05$	8.24 $\pm 0.18$	7.92 <sup>ab</sup> $\pm 0.09$	8.31 $\pm 0.04$	8.34 <sup>b</sup> $\pm 0.06$

In tables 3.12 and 3.13 the available phosphorus content in soils from F1 and F2 is shown. Although no significant differences were found among plots treated with different doses of organic amendment, a slight decreasing trend in available P was found in the 1<sup>st</sup> sampling for both farms, whereas, in the final sampling, available P values measured in amended plots did not differ from that measured in control plots, except for F1 soil that showed an increase in available P in amended plots treated also with mineral fertilizing. Moreover, the initial values of available P were almost similar ( $\sim 160$  mg kg<sup>-1</sup>) in both soils. In contrast, two way ANOVA test showed significant differences due to sampling time, amendment dose and interaction between these variables.

Table 3.12 Mean value ( $\pm$  SD) of available phosphorus ( $\text{mg kg}^{-1}$ ) in F1 soil (data from Scotti, 2010). Different superscript letters indicated significant differences among treatments for each sampling time (treatments with and without mineral fertilizing were analyzed separately). The -m suffix at the end of abbreviations indicates the soil was also treated with mineral fertilizer.

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	162.34 $\pm 9.47$	124.41 $\pm 0.26$	190.29 $\pm 9.62$	209.11 $\pm 2.81$	274.18 <sup>ab</sup> $\pm 7.66$	189.37 <sup>a</sup> $\pm 4.96$	206.38 $\pm 30.81$
A1L	166.59 $\pm 9.03$	148.90 $\pm 13.44$	196.00 $\pm 10.65$	223.76 $\pm 14.26$	314.84 <sup>a</sup> $\pm 13.47$	196.21 <sup>a</sup> $\pm 5.32$	237.95 $\pm 18.47$
A1H	148.81 $\pm 19.55$	138.54 $\pm 1.29$	179.50 $\pm 13.65$	225.61 $\pm 5.12$	296.65 <sup>ab</sup> $\pm 24.53$	217.69 <sup>b</sup> $\pm 2.25$	232.09 $\pm 13.30$
A2L	144.78 $\pm 14.63$	135.70 $\pm 11.98$	207.41 $\pm 22.72$	223.45 $\pm 12.98$	264.06 <sup>b</sup> $\pm 28.89$	210.96 <sup>b</sup> $\pm 6.41$	233.99 $\pm 21.35$
A2H	152.17 $\pm 12.95$	141.67 $\pm 8.19$	189.52 $\pm 7.96$	230.23 $\pm 13.46$	248.33 <sup>b</sup> $\pm 18.86$	206.54 <sup>b</sup> $\pm 9.27$	206.23 $\pm 35.88$
Control -m	167.46 $\pm 7.86$	135.99 $\pm 9.03$	197.85 $\pm 16.71$	213.73 $\pm 3.34$	279.68 <sup>a</sup> $\pm 12.39$	185.26 $\pm 10.19$	212.45 <sup>a</sup> $\pm 8.46$
A1L-m	137.69 $\pm 19.68$	142.60 $\pm 10.22$	176.72 $\pm 15.48$	226.84 $\pm 3.77$	315.87 <sup>b</sup> $\pm 2.34$	207.41 $\pm 6.68$	234.14 <sup>b</sup> $\pm 11.34$
A1H-m	154.52 $\pm 21.98$	143.85 $\pm 7.81$	179.96 $\pm 2.12$	225.45 $\pm 5.75$	284.00 <sup>a</sup> $\pm 12.89$	207.15 $\pm 6.40$	250.64 <sup>b</sup> $\pm 8.43$
A2L-m	138.35 $\pm 2.78$	136.95 $\pm 6.74$	217.74 $\pm 22.65$	226.84 $\pm 5.60$	236.20 <sup>c</sup> $\pm 5.80$	207.31 $\pm 11.35$	249.77 <sup>b</sup> $\pm 9.57$
A2H-m	134.47 $\pm 4.48$	138.84 $\pm 6.94$	195.38 7.52	232.39 $\pm 7.69$	263.60 <sup>a</sup> $\pm 25.73$	224.68 $\pm 24.08$	178.06 <sup>c</sup> $\pm 12.27$

Table 3.13 Mean value ( $\pm$  SD) of available phosphorus ( $\text{mg kg}^{-1}$ ) in F2 soil (Scotti, 2010).

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	174.71 <sup>a</sup> $\pm 17.46$	128.12 $\pm 7.64$	178.57 $\pm 10.08$	176.57 $\pm 17.08$	182.89 <sup>ab</sup> $\pm 27.22$	160.28 $\pm 19.06$	188.29 $\pm 35.89$
A1L	145.52 <sup>ab</sup> $\pm 17.71$	156.08 $\pm 13.52$	180.12 $\pm 35.06$	184.90 $\pm 27.89$	223.14 <sup>a</sup> $\pm 25.41$	174.98 $\pm 16.52$	211.27 $\pm 42.55$
A1H	145.88 <sup>ab</sup> $\pm 6.23$	145.09 $\pm 14.76$	182.43 $\pm 3.77$	182.74 $\pm 26.49$	206.02 <sup>ab</sup> $\pm 21.09$	181.71 $\pm 12.52$	200.52 $\pm 37.44$
A2L	132.64 <sup>ab</sup> $\pm 18.57$	152.00 $\pm 23.46$	136.94 $\pm 28.56$	171.94 $\pm 32.37$	146.55 <sup>ab</sup> $\pm 28.44$	143.83 $\pm 20.77$	175.54 $\pm 37.72$
A2H	123.06 <sup>b</sup> $\pm 13.44$	146.37 $\pm 14.51$	158.53 $\pm 9.62$	178.42 $\pm 17.25$	128.87 <sup>b</sup> $\pm 62.70$	156.32 $\pm 7.88$	184.28 $\pm 59.41$
Control -m	163.22 $\pm 18.01$	147.34 $\pm 6.62$	184.90 $\pm 5.56$	171.79 $\pm 29.51$	219.95 $\pm 43.76$	165.88 $\pm 22.49$	176.52 $\pm 26.52$
A1L-m	129.35 $\pm 28.50$	140.49 $\pm 23.58$	159.61 $\pm 21.80$	163.31 $\pm 27.69$	203.04 $\pm 50.94$	152.51 $\pm 45.63$	167.32 $\pm 10.11$
A1H-m	128.40 $\pm$ $\pm 5.75$	141.86 $\pm 1.77$	142.64 $\pm 13.96$	163.31 $\pm 22.18$	165.83 $\pm 56.72$	159.61 $\pm 23.72$	201.45 $\pm 24.20$
A2L-m	147.35 $\pm$ $\pm 34.66$	130.86 $\pm 21.38$	155.29 $\pm 25.38$	156.68 $\pm 20.97$	144.60 $\pm 54.49$	169.94 $\pm 27.17$	186.08 $\pm 11.72$
A2H-m	139.59 $\pm$ $\pm 3.63$	132.15 $\pm 9.32$	176.26 $\pm 7.70$	183.82 $\pm 22.33$	198.98 $\pm 4.46$	163.36 $\pm 4.92$	190.76 $\pm 28.41$

The graphs in figures 3.3 and 3.5 show effect of organic amendment (without and with mineral fertilizer) on total N content in F1 and F2 soils, whereas figures 3.4 and 3.6 show percentage variation of this parameter in each treated plot of F1 and F2 compared to control.

During the two years of study, an increasing trend of total N was found in both farms. In particular, soil from F1 showed a significant increase in N content starting from 4<sup>th</sup> sampling (in A2H plot), with more marked effects in the 6<sup>th</sup> and 7<sup>th</sup> samplings, whereas soil from F2 showed a significant increase in A1H-m plot (5<sup>th</sup> sampling) and in A1L, A2L and A2H plots (7<sup>th</sup> sampling). However, at the end of 2 year the percentage increase, compared to control, reached 52% and 38% in F1 and F2, respectively. However, no marked effect was due to the mineral fertilizing treatment.

In tables 3.14 and 3.15 mean values ( $\pm$  standard deviation) of ammoniacal nitrogen ( $\text{NH}_4^+$ -N) in soils of F1 and F2 were shown. No clear trend or increase in ammoniacal N content was found, but in F1 soil increases were found only in A2L-m (1<sup>st</sup> sampling), A1H-m (2<sup>nd</sup> sampling) and A2H-m plots (in 4<sup>th</sup> sampling) and in A1H plot (in 5<sup>th</sup> and 6<sup>th</sup> samplings), whereas a decrease was found in A2L-m (final sampling). In F2 soil an increase in this parameter was only found in A1H-m and A2L-m plots (3<sup>rd</sup> sampling), and A1L and A1L-m plots (4<sup>th</sup> sampling), whereas a general decreasing trend was found in 2<sup>nd</sup> and 6<sup>th</sup> samplings, but no differences in final sampling.

In tables 3.16 and 3.17 mean values ( $\pm$  standard deviation) of nitric nitrogen ( $\text{NO}_3^-$ -N) in soil of F1 and F2 are reported. A clear and significant increase was found in this parameter at the end of the study period in both farm, when all treated plots of F1 soil (without and with mineral fertilizing treatment) showed values ranging from 2 (A1L) up to 10 times (A2H-m) higher than control plots, and, in F2 soil, A1H, A2H, A1H-m and A2H-m plots showed values ranging from 2 up to 3 times higher than control plots, with more marked effects due to the high dose.



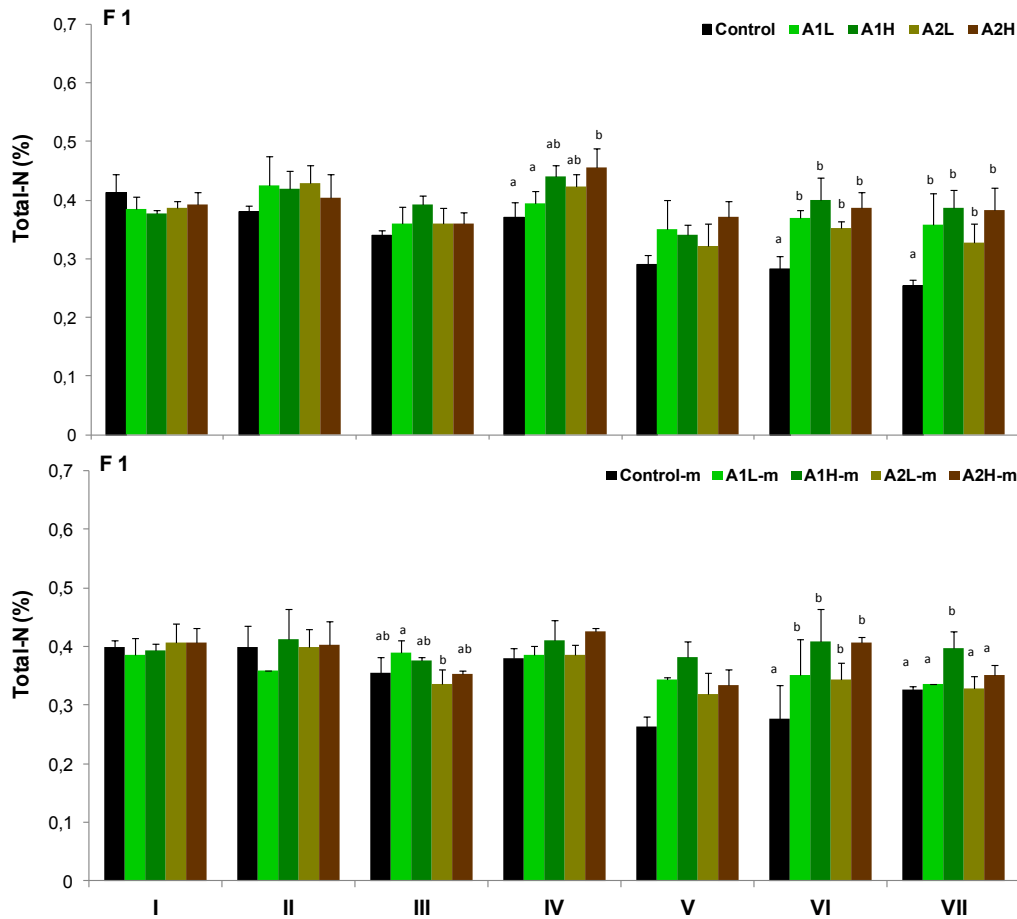


Figure 3.3 Effect of organic amendment on total N content in F1 soil.

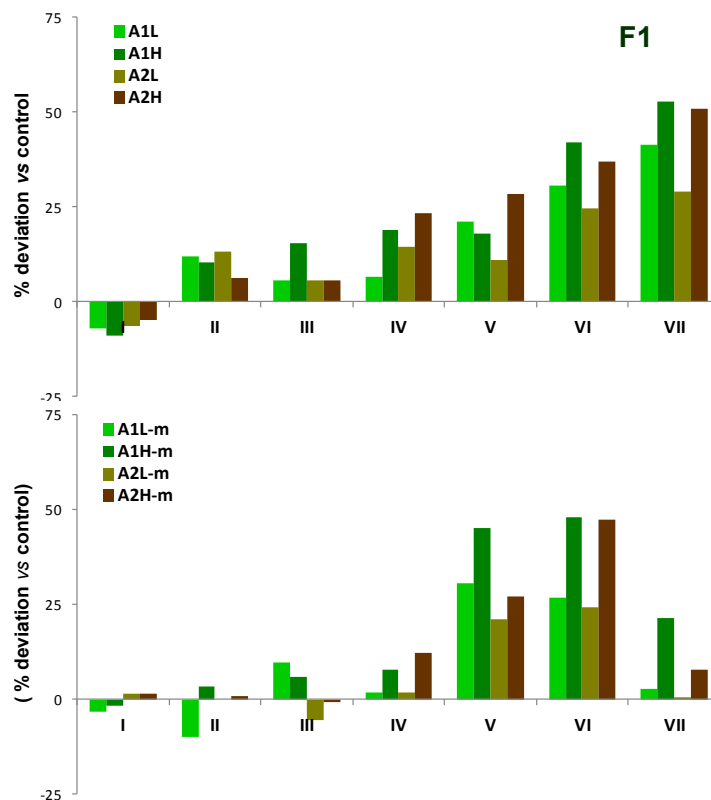


Figure 3.4 Percentage variations compared to control for total N in F1.

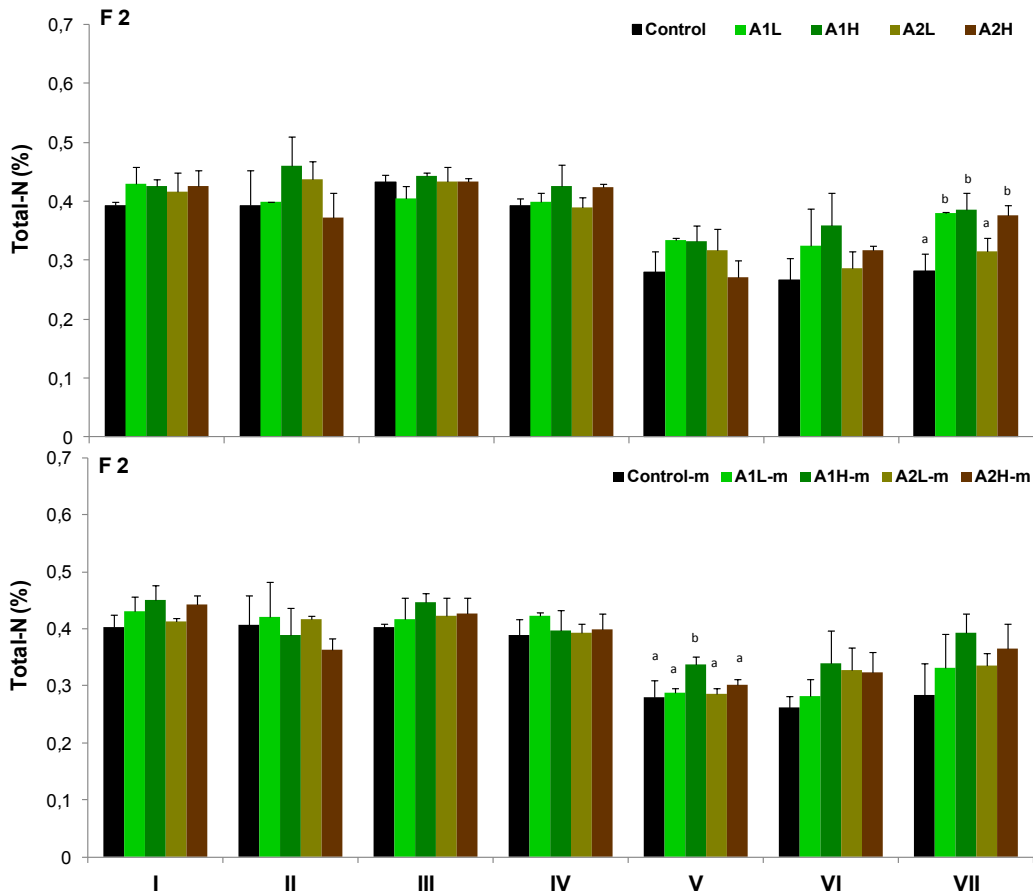


Figure 3.5 Effect of organic amendment on total N content in F2 soil

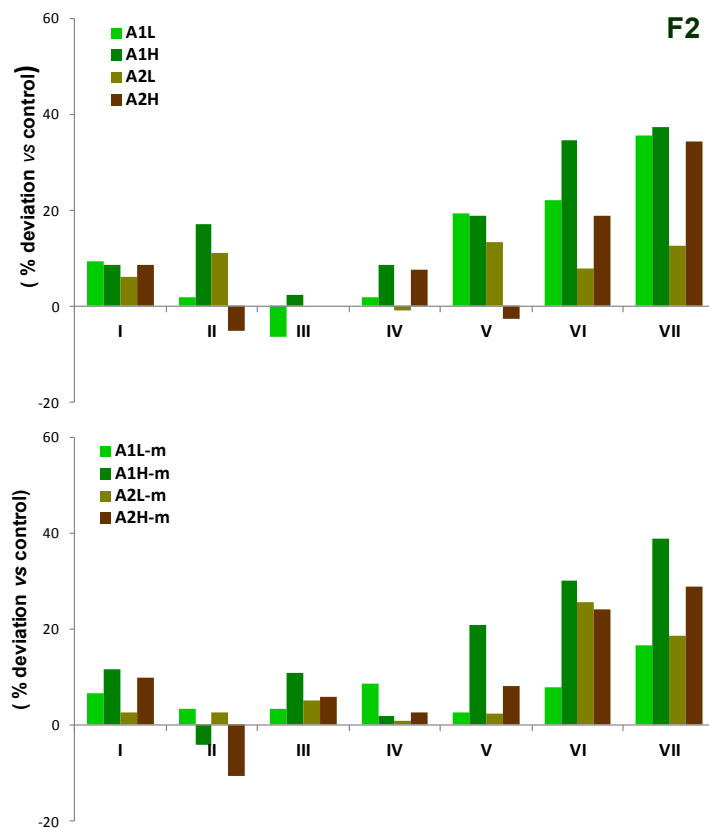


Figure 3.6 Percentage variations compared to control for total N in F2.

Table 3.14 Mean value ( $\pm$  SD) of  $\text{NH}_4^+$ -N content ( $\mu\text{g g}^{-1}$ ) in F1 soil. Different superscript letters indicated significant differences among treatments for each sampling time (treatments with and without mineral fertilizing were analyzed separately). The -m suffix at the end of abbreviations indicates the soil was also treated with mineral fertilizer.

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	1.71 $\pm 0.52$	3.25 $\pm 1.22$	3.99 $\pm 0.18$	1.25 $\pm 0.11$	10.16 <sup>ab</sup> $\pm 3.27$	0.78 <sup>a</sup> $\pm 0.18$	4.60 $\pm 0.34$
A1L	1.88 $\pm 0.93$	4.47 $\pm 2.07$	2.51 $\pm 1.09$	0.98 $\pm 0.06$	9.09 <sup>a</sup> $\pm 1.67$	0.66 <sup>a</sup> $\pm 0.34$	2.62 $\pm 1.65$
A1H	2.79 $\pm 1.81$	8.11 $\pm 3.49$	2.84 $\pm 0.73$	1.27 $\pm 0.16$	19.21 <sup>b</sup> $\pm 3.92$	1.24 <sup>b</sup> $\pm 0.32$	4.16 $\pm 0.15$
A2L	1.32 $\pm 0.73$	6.02 $\pm 2.81$	2.57 $\pm 0.34$	1.29 $\pm 0.34$	9.87 <sup>a</sup> $\pm 3.50$	0.39 <sup>a</sup> $\pm 0.07$	3.75 $\pm 0.41$
A2H	2.17 $\pm 2.42$	6.32 $\pm 4.49$	2.48 $\pm 0.10$	2.04 $\pm 0.73$	12.49 <sup>ab</sup> $\pm 5.66$	0.54 <sup>a</sup> $\pm 0.19$	3.22 $\pm 0.32$
Control -m	1.75 <sup>ab</sup> $\pm 1.24$	3.02 <sup>a</sup> $\pm 0.55$	1.85 $\pm 0.63$	1.13 <sup>a</sup> $\pm 0.21$	13.77 $\pm 0.24$	0.57 $\pm 0.12$	4.95 <sup>a</sup> $\pm 0.38$
A1L-m	1.41 <sup>a</sup> $\pm 0.33$	6.63 <sup>ab</sup> $\pm 1.88$	1.95 $\pm 0.27$	1.01 <sup>a</sup> $\pm 0.07$	14.56 $\pm 5.20$	1.26 $\pm 0.84$	4.81 <sup>a</sup> $\pm 0.11$
A1H-m	2.33 <sup>ab</sup> $\pm 1.27$	10.91 <sup>b</sup> $\pm 5.91$	2.82 $\pm 1.09$	1.14 <sup>a</sup> $\pm 0.08$	23.30 $\pm 4.39$	0.71 $\pm 0.35$	4.16 <sup>a</sup> $\pm 0.52$
A2L-m	3.96 <sup>b</sup> $\pm 0.50$	3.36 <sup>a</sup> $\pm 1.29$	2.32 $\pm 0.50$	1.06 <sup>a</sup> $\pm 0.05$	8.36 $\pm 6.49$	0.44 $\pm 0.24$	4.00 <sup>b</sup> $\pm 0.18$
A2H-m	1.81 <sup>ab</sup> $\pm 0.72$	3.94 <sup>a</sup> $\pm 0.41$	2.45 $\pm 0.44$	2.28 <sup>b</sup> $\pm 0.93$	17.15 $\pm 8.34$	0.60 $\pm 0.23$	3.27 <sup>b</sup> $\pm 0.39$

Table 3.15 Mean value ( $\pm$  SD) of  $\text{NH}_4^+$ -N content ( $\mu\text{g g}^{-1}$ ) in F2 soil

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	9.68 $\pm 1.60$	4.20 <sup>a</sup> $\pm 0.45$	2.14 <sup>a</sup> $\pm 0.27$	0.71 <sup>a</sup> $\pm 0.06$	1.17 $\pm 0.31$	0.63 <sup>a</sup> $\pm 0.24$	5.30 $\pm 0.65$
A1L	8.10 $\pm 1.99$	3.60 <sup>a</sup> $\pm 0.59$	1.20 <sup>b</sup> $\pm 0.17$	2.51 <sup>b</sup> $\pm 0.50$	0.89 $\pm 0.19$	0.25 <sup>b</sup> $\pm 0.11$	5.87 $\pm 0.97$
A1H	7.70 $\pm 1.01$	2.41 <sup>b</sup> $\pm 0.32$	3.21 <sup>a</sup> $\pm 0.69$	1.12 <sup>a</sup> $\pm 0.41$	1.54 $\pm 0.59$	0.31 <sup>b</sup> $\pm 0.12$	5.41 $\pm 0.04$
A2L	7.53 $\pm 1.16$	1.70 <sup>b</sup> $\pm 0.13$	3.20 <sup>a</sup> $\pm 0.44$	1.06 <sup>a</sup> $\pm 0.21$	1.27 $\pm 0.19$	0.27 <sup>b</sup> $\pm 0.09$	6.29 $\pm 1.10$
A2H	9.43 $\pm 0.87$	2.88 <sup>ab</sup> $\pm 0.65$	2.93 <sup>a</sup> $\pm 0.86$	1.00 <sup>a</sup> $\pm 0.23$	1.52 $\pm 0.59$	0.24 <sup>b</sup> $\pm 0.07$	5.72 $\pm 0.35$
Control -m	9.24 $\pm 2.50$	4.28 <sup>a</sup> $\pm 0.43$	1.66 <sup>a</sup> $\pm 0.07$	0.75 <sup>a</sup> $\pm 0.02$	1.76 $\pm 0.79$	0.46 $\pm 0.31$	5.81 $\pm 0.12$
A1L-m	8.01 $\pm 0.75$	3.65 <sup>a</sup> $\pm 0.41$	1.24 <sup>a</sup> $\pm 0.29$	3.56 <sup>b</sup> $\pm 1.88$	1.63 $\pm 0.05$	0.40 $\pm 0.23$	5.72 $\pm 0.73$
A1H-m	6.91 $\pm 0.79$	1.87 <sup>b</sup> $\pm 0.30$	1.32 <sup>a</sup> $\pm 0.13$	1.27 <sup>a</sup> $\pm 0.27$	1.03 $\pm 0.26$	0.37 $\pm 0.07$	5.92 $\pm 0.46$
A2L-m	7.77 $\pm 1.01$	2.31 <sup>b</sup> $\pm 0.74$	2.40 <sup>b</sup> $\pm 0.20$	1.23 <sup>a</sup> $\pm 0.35$	1.01 $\pm 0.02$	0.23 $\pm 0.08$	5.86 $\pm 0.81$
A2H-m	7.00 $\pm 0.92$	2.70 <sup>b</sup> $\pm 0.69$	3.40 <sup>c</sup> $\pm 0.17$	1.47 <sup>a</sup> $\pm 1.01$	2.38 $\pm 0.99$	0.23 $\pm 0.04$	5.16 $\pm 0.25$

Table 3.16 Mean value ( $\pm$  SD) of  $\text{NO}_3^-$ -N ( $\mu\text{g g}^{-1}$ ) content in F1 soil. Different superscript letters indicated significant differences among treatments for each sampling time (treatments with and without mineral fertilizing were analyzed separately). The -m suffix at the end of abbreviations indicates the soil was also treated with mineral fertilizer.

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	28.31 $\pm 11.35$	44.80 <sup>a</sup> $\pm 7.98$	11.03 $\pm 4.85$	8.15 $\pm 0.92$	130.57 $\pm 38.99$	138.77 <sup>a</sup> $\pm 24.76$	120.02 <sup>a</sup> $\pm 47.38$
A1L	14.82 $\pm 5.90$	87.39 <sup>a</sup> $\pm 26.72$	13.04 $\pm 6.52$	9.67 $\pm 4.61$	128.85 $\pm 12.90$	216.03 <sup>b</sup> $\pm 17.80$	284.44 <sup>b</sup> $\pm 65.37$
A1H	20.85 $\pm 4.72$	127.52 <sup>b</sup> $\pm 38.48$	8.55 $\pm 0.00$	7.90 $\pm 1.87$	128.82 $\pm 22.94$	277.46 <sup>b</sup> $\pm 35.59$	349.58 <sup>b</sup> $\pm 36.36$
A2L	12.16 $\pm 3.87$	74.41 <sup>a</sup> $\pm 13.85$	8.44 $\pm 3.96$	6.89 $\pm 1.21$	152.12 $\pm 12.30$	228.60 <sup>b</sup> $\pm 43.36$	314.32 <sup>b</sup> $\pm 70.99$
A2H	17.44 $\pm 6.85$	89.85 <sup>a</sup> $\pm 37.20$	10.99 $\pm 0.77$	8.75 $\pm 2.16$	151.08 $\pm 40.29$	227.38 <sup>b</sup> $\pm 25.60$	588.03 <sup>c</sup> $\pm 36.41$
Control -m	59.95 <sup>a</sup> $\pm 5.06$	64.70 <sup>a</sup> $\pm 28.94$	7.07 <sup>a</sup> $\pm 1.31$	7.12 $\pm 1.82$	182.31 $\pm 49.85$	84.28 <sup>a</sup> $\pm 10.13$	61.56 <sup>a</sup> $\pm 12.43$
A1L-m	19.17 <sup>b</sup> $\pm 10.14$	134.36 <sup>ab</sup> $\pm 30.01$	9.44 <sup>b</sup> $\pm 1.68$	7.23 $\pm 3.73$	157.00 $\pm 41.59$	173.45 <sup>b</sup> $\pm 17.33$	228.67 <sup>a</sup> $\pm 69.06$
A1H-m	11.40 <sup>b</sup> $\pm 15.30$	177.33 <sup>b</sup> $\pm 36.10$	6.21 <sup>a</sup> $\pm 1.56$	8.18 $\pm 2.94$	151.36 $\pm 15.71$	247.00 <sup>c</sup> $\pm 58.29$	374.46 <sup>b</sup> $\pm 28.75$
A2L-m	21.57 <sup>b</sup> $\pm 8.36$	109.20 <sup>ab</sup> $\pm 31.90$	7.65 <sup>a</sup> $\pm 1.58$	7.49 $\pm 1.56$	205.77 $\pm 52.12$	175.69 <sup>b</sup> $\pm 29.18$	328.02 <sup>b</sup> $\pm 49.67$
A2H-m	13.36 <sup>b</sup> $\pm 3.27$	75.85 <sup>a</sup> $\pm 18.61$	12.80 <sup>b</sup> $\pm 3.25$	7.27 $\pm 1.28$	176.09 $\pm 9.45$	294.81 <sup>c</sup> $\pm 54.20$	592.47 <sup>c</sup> $\pm 39.07$

Table 3.17 Mean value ( $\pm$  SD) of  $\text{NO}_3^-$ -N ( $\mu\text{g g}^{-1}$ ) content in F2 soil.

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	62.26 $\pm 7.91$	83.32 $\pm 7.19$	18.68 $\pm 13.31$	11.43 <sup>a</sup> $\pm 4.24$	213.52 $\pm 53.53$	193.33 $\pm 18.85$	275.44 $\pm 47.29$ <sup>a</sup>
A1L	64.22 $\pm 18.04$	98.51 $\pm 7.84$	13.82 $\pm 1.50$	38.54 <sup>b</sup> $\pm 13.99$	220.64 $\pm 19.54$	265.71 $\pm 54.51$	421.98 $\pm 87.66$ <sup>b</sup>
A1H	66.42 $\pm 26.11$	117.63 $\pm 17.95$	15.56 $\pm 5.15$	40.71 <sup>b</sup> $\pm 12.32$	235.18 $\pm 19.27$	307.15 $\pm 91.20$	523.25 $\pm 134.1$ <sup>c</sup>
A2L	43.77 $\pm 8.59$	94.11 $\pm 19.27$	6.99 $\pm 4.95$	23.57 <sup>ab</sup> $\pm 7.00$	213.72 $\pm 52.15$	300.45 $\pm 53.68$	411.57 $\pm 15.73$ <sup>b</sup>
A2H	39.15 $\pm 13.33$	95.60 $\pm 7.61$	9.09 $\pm 2.76$	23.17 <sup>ab</sup> $\pm 3.35$	241.80 $\pm 29.23$	324.58 $\pm 36.98$	624.25 <sup>c</sup> $\pm 52.80$
Control -m	164.77 <sup>a</sup> $\pm 32.78$	108.79 $\pm 25.00$	12.17 <sup>a</sup> $\pm 4.07$	25.95 $\pm 0.52$	271.75 $\pm 84.69$	165.28 <sup>a</sup> $\pm 38.06$	235.23 $\pm 6.49$ <sup>a</sup>
A1L-m	142.50 <sup>a</sup> $\pm 38.21$	124.09 $\pm 20.01$	28.26 <sup>b</sup> $\pm 8.41$	23.00 $\pm 11.55$	221.33 $\pm 9.10$	193.88 <sup>ab</sup> $\pm 24.31$	408.31 $\pm 24.79$ <sup>b</sup>
A1H-m	70.05 <sup>b</sup> $\pm 3.88$	133.88 $\pm 37.80$	12.85 <sup>a</sup> $\pm 0.87$	30.08 $\pm 15.17$	273.16 $\pm 38.60$	276.98 <sup>ab</sup> $\pm 40.10$	427.10 $\pm 56.67$ <sup>b</sup>
A2L-m	65.68 <sup>b</sup> $\pm 22.06$	99.33 $\pm 8.49$	9.31 <sup>a</sup> $\pm 6.83$	11.97 $\pm 0.53$	239.20 $\pm 32.68$	280.99 <sup>b</sup> $\pm 71.41$	411.04 $\pm 49.57$ <sup>b</sup>
A2H-m	42.84 <sup>b</sup> $\pm 1.35$	75.38 $\pm 1.95$	10.88 <sup>a</sup> $\pm 0.64$	28.74 $\pm 16.05$	313.60 $\pm 29.46$	252.87 <sup>ab</sup> $\pm 38.91$	630.68 $\pm 30.59$ <sup>c</sup>

However, a significant increase in nitric N content was also found in F1 soil in A1H, A1L-m and A1H-m plots of the 2<sup>nd</sup> and 6<sup>th</sup> samplings and in F2 soil in A1H-m plot of 2<sup>nd</sup> sampling and in A1L-m plot of 3<sup>rd</sup> sampling. Finally, two way ANOVA showed significant differences due to sampling time, amendment dose and interaction between these variables for ammoniacal and nitric N (in both farms) and total N (in F1 farm).

The organic C content ( $C_{org}$ ) in F1 and F2 soils is reported in figures 3.7 and 3.9, whereas figures 3.8 and 3.10 show percentage variation of this parameter in each treated plot of F1 and F2, compared to control. Organic carbon content showed generally a prompt and lasting increase in all amended plots of F1 and F2 soils, with more marked effect after second addition (i.e. starting from 4<sup>th</sup> sampling included). However, the increase of this parameter after the first amendment was not always significant. The percentage variation, compared to control, ranged from 10 up to 125% in F1 soil and from 10 to 75% in F2 soil. No marked differences were found due to type or amount of organic amendment or to mineral fertilization. Finally, two ways ANOVA showed significant differences due to sampling time, amendment dose and interaction between these variables in both farms.

In tables 3.18 and 3.19 C/N ratios in F1 and F2 soils are reported. As expected, C/N ratio showed an increasing trend in amended plots of both farms, with more marked effect in F1 than in F2 soil. In particular, in F1 soil increased values of C/N ratio were found in all treated plots of 1<sup>st</sup>, 3<sup>rd</sup> and 6<sup>th</sup> samplings, in plots without mineral fertilizing of the 4<sup>th</sup> sampling and in plots with mineral fertilizing of the 2<sup>nd</sup> and 7<sup>th</sup> samplings, whereas in F2 soil increased values of C/N ratio were only found in mineral fertilized plots of the 1<sup>st</sup> and 3<sup>rd</sup> samplings and in plots amended without mineral fertilizing of the 4<sup>th</sup> sampling. However, no considerable effect was due to mineral fertilizer addition or different doses of amendment. Although the used compost mixtures were characterized by a C/N ratio of 15 and 25 (A1 and A2, respectively), no obvious effect was even found due to these differences.

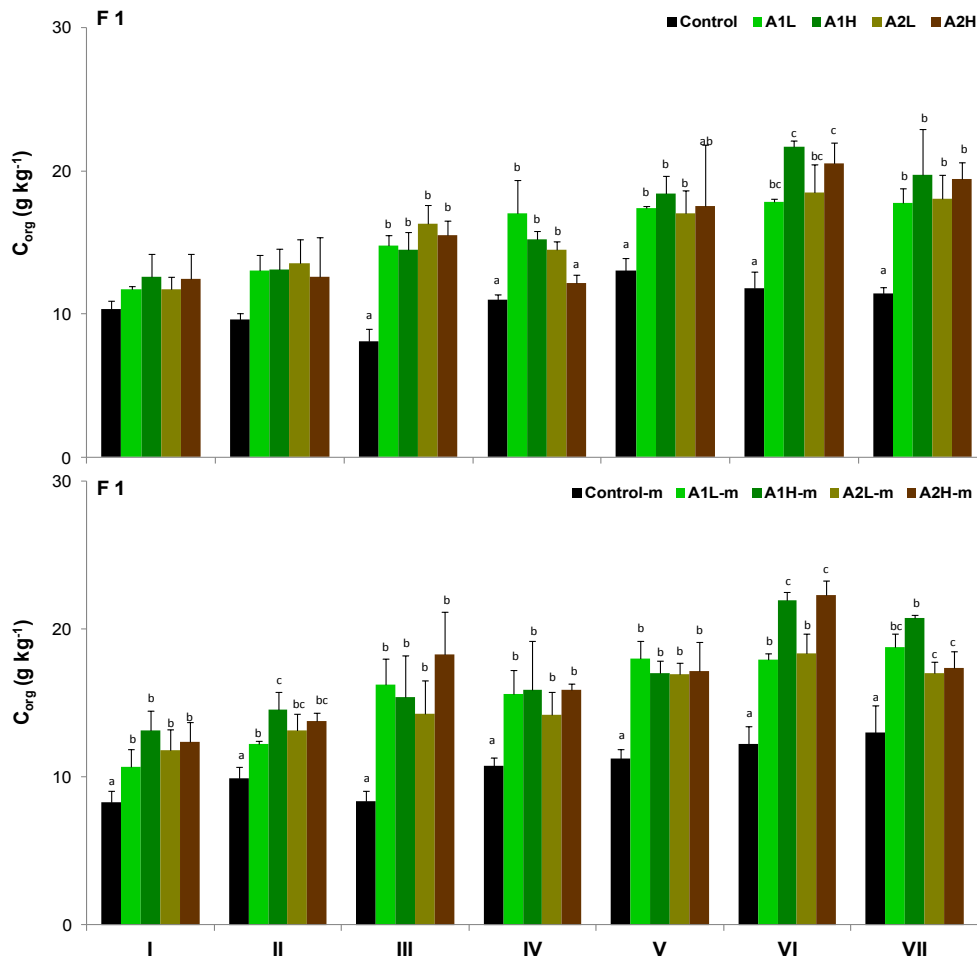


Figure 3.7 Effect of organic amendment on organic C content in F1 soil.

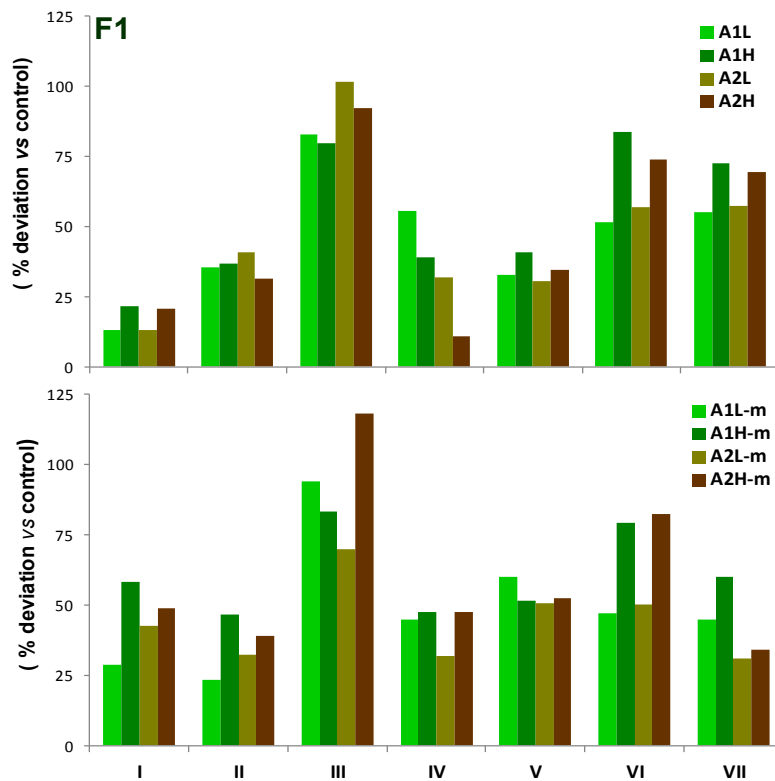


Figure 3.8 Percentage variations compared to control of  $C_{org}$  in F1 soil.

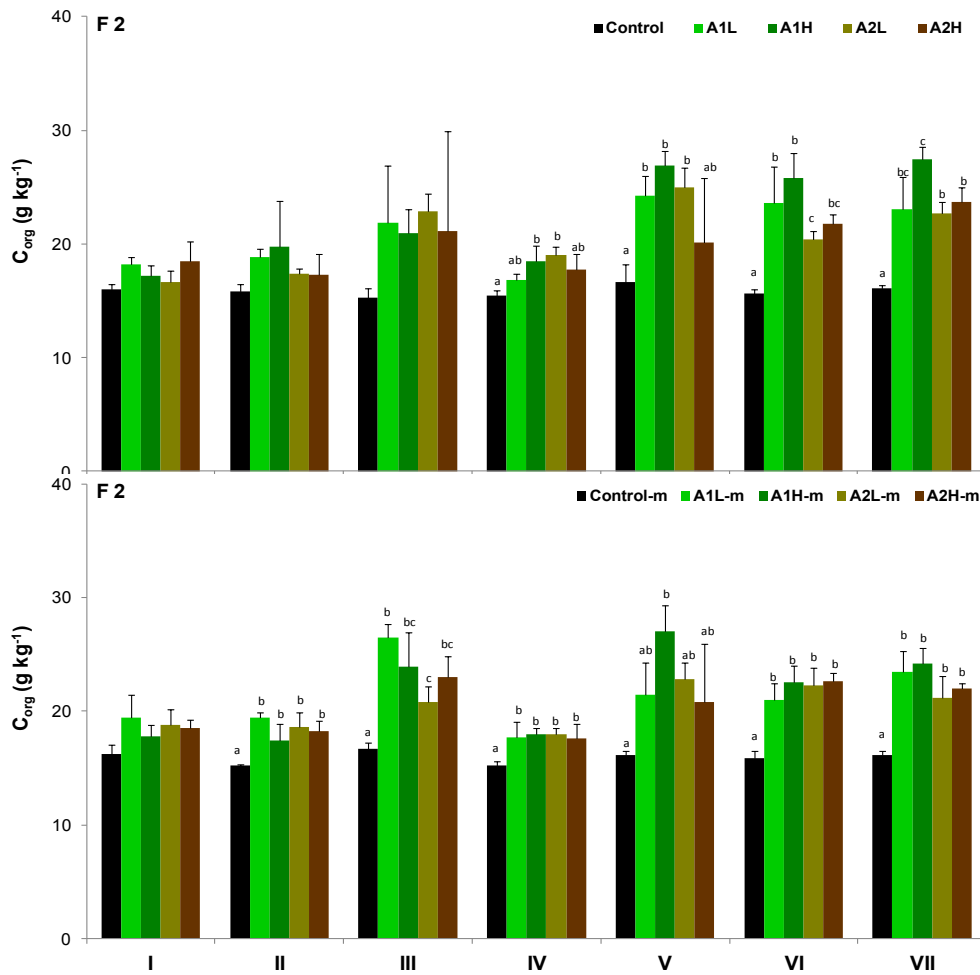


Figure 3.9 Effect of organic amendment on organic C content in F2 soil.

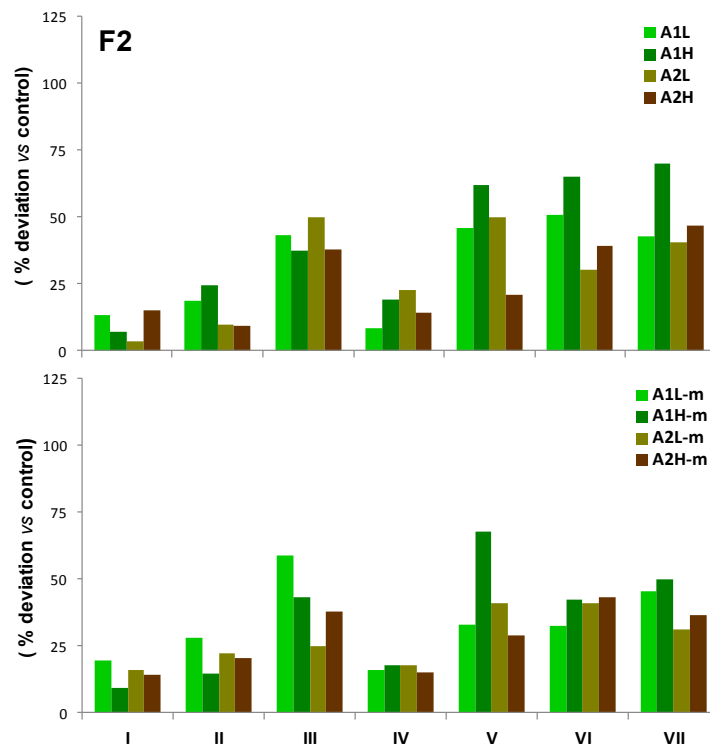


Figure 3.10 Percentage variations compared to control of  $C_{org}$  in F2 soil.

Table 3.18 Mean value ( $\pm$  SD) of C/N ratio in F1 soil. Different superscript letters indicated significant differences among treatments for each sampling time (treatments with and without mineral fertilizing were analyzed separately). The -m suffix at the end of abbreviations indicates the soil was also treated with mineral fertilizer.

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	2.51 <sup>a</sup> $\pm 0.20$	2.53 $\pm 0.18$	2.37 <sup>a</sup> $\pm 0.20$	2.98 <sup>a</sup> $\pm 0.31$	4.51 $\pm 0.10$	4.18 <sup>a</sup> $\pm 0.32$	4.53 $\pm 0.11$
A1L	3.01 <sup>b</sup> $\pm 0.17$	3.17 $\pm 0.69$	4.21 <sup>b</sup> $\pm 0.18$	4.62 <sup>b</sup> $\pm 0.10$	4.99 $\pm 0.78$	4.82 <sup>a</sup> $\pm 0.12$	5.15 $\pm 1.09$
A1H	3.35 <sup>b</sup> $\pm 0.44$	3.13 $\pm 0.44$	3.70 <sup>a</sup> $\pm 0.38$	3.47 <sup>c</sup> $\pm 0.17$	5.38 $\pm 0.44$	5.43 <sup>b</sup> $\pm 0.46$	5.08 $\pm 0.41$
A2L	3.03 <sup>b</sup> $\pm 0.29$	3.14 $\pm 0.26$	4.52 <sup>b</sup> $\pm 0.17$	3.42 <sup>c</sup> $\pm 0.22$	5.31 $\pm 0.14$	5.25 <sup>b</sup> $\pm 0.62$	5.51 $\pm 0.10$
A2H	3.17 <sup>b</sup> $\pm 0.35$	3.13 $\pm 0.59$	4.31 <sup>b</sup> $\pm 0.09$	2.66 <sup>a</sup> $\pm 0.09$	4.68 $\pm 0.80$	5.31 <sup>b</sup> $\pm 0.30$	5.10 $\pm 0.37$
Control -m	2.07 <sup>a</sup> $\pm 0.15$	2.49 <sup>a</sup> $\pm 0.29$	2.38 <sup>a</sup> $\pm 0.35$	2.84 $\pm 0.11$	4.30 $\pm 0.39$	4.49 <sup>a</sup> $\pm 0.47$	3.97 <sup>a</sup> $\pm 0.53$
A1L-m	2.76 <sup>b</sup> $\pm 0.27$	3.42 <sup>b</sup> $\pm 0.29$	4.19 <sup>b</sup> $\pm 0.59$	4.06 $\pm 0.56$	5.33 $\pm 0.96$	5.15 <sup>b</sup> $\pm 0.45$	5.61 <sup>b</sup> $\pm 0.39$
A1H-m	3.33 <sup>b</sup> $\pm 0.31$	3.52 <sup>b</sup> $\pm 0.27$	4.10 <sup>b</sup> $\pm 0.81$	3.86 $\pm 0.61$	4.57 $\pm 0.92$	5.36 <sup>b</sup> $\pm 0.05$	5.26 <sup>b</sup> $\pm 0.40$
A2L-m	2.94 <sup>b</sup> $\pm 0.49$	3.29 <sup>b</sup> $\pm 0.33$	4.23 <sup>b</sup> $\pm 0.54$	3.70 $\pm 0.62$	5.47 $\pm 1.15$	5.34 <sup>b</sup> $\pm 0.12$	5.22 <sup>b</sup> $\pm 0.67$
A2H-m	3.05 <sup>b</sup> $\pm 0.40$	3.42 <sup>b</sup> $\pm 0.14$	5.18 <sup>b</sup> $\pm 0.82$	3.73 $\pm 0.13$	5.13 $\pm 0.38$	5.47 <sup>b</sup> $\pm 0.17$	4.95 <sup>b</sup> $\pm 0.47$

Table 3.19. Mean value ( $\pm$  SD) of C/N ratio in F2 soil.

Treatment	Sampling I	Sampling II	Sampling III	Sampling IV	Sampling V	Sampling VI	Sampling VII
Control	4.08 $\pm 0.15$	4.08 $\pm 0.50$	3.53 $\pm 0.26$	3.94 <sup>a</sup> $\pm 0.18$	6.05 $\pm 1.27$	5.94 $\pm 0.74$	5.78 $\pm 0.59$
A1L	4.27 $\pm 0.09$	4.78 $\pm 0.20$	4.77 $\pm 0.70$	4.18 <sup>ab</sup> $\pm 0.06$	7.39 $\pm 0.57$	7.53 $\pm 0.19$	6.34 $\pm 0.77$
A1H	4.02 $\pm 0.12$	4.28 $\pm 0.58$	4.73 $\pm 0.45$	4.36 <sup>ab</sup> $\pm 0.63$	8.14 $\pm 0.86$	7.34 $\pm 1.59$	7.12 $\pm 0.30$
A2L	3.99 $\pm 0.20$	3.99 $\pm 0.23$	5.29 $\pm 0.31$	4.87 <sup>b</sup> $\pm 0.05$	7.93 $\pm 0.86$	7.15 $\pm 0.79$	7.19 $\pm 0.38$
A2H	4.33 $\pm 0.49$	4.66 $\pm 0.55$	4.88 $\pm 2.09$	4.18 <sup>ab</sup> $\pm 0.27$	7.35 $\pm 1.55$	6.87 $\pm 0.12$	6.29 $\pm 0.46$
Control -m	4.02 <sup>a</sup> $\pm 0.14$	3.77 $\pm 0.47$	4.14 <sup>a</sup> $\pm 0.16$	3.91 $\pm 0.28$	5.81 $\pm 0.61$	6.07 $\pm 0.37$	5.84 $\pm 1.17$
A1L-m	4.51 <sup>b</sup> $\pm 0.18$	4.71 $\pm 0.83$	6.39 <sup>b</sup> $\pm 0.44$	4.18 $\pm 0.37$	7.46 $\pm 0.84$	7.45 $\pm 0.39$	7.32 $\pm 1.93$
A1H-m	3.95 <sup>a</sup> $\pm 0.09$	4.48 $\pm 0.19$	5.35 <sup>c</sup> $\pm 0.50$	4.54 $\pm 0.31$	8.02 $\pm 0.89$	6.76 $\pm 1.18$	6.16 $\pm 0.20$
A2L-m	4.55 <sup>b</sup> $\pm 0.33$	4.45 $\pm 0.26$	4.92 <sup>c</sup> $\pm 0.16$	4.57 $\pm 0.17$	7.96 $\pm 0.66$	6.81 $\pm 0.36$	6.31 $\pm 0.47$
A2H-m	4.19 <sup>ab</sup> $\pm 0.22$	5.04 $\pm 0.22$	5.40 <sup>c</sup> $\pm 0.34$	4.39 $\pm 0.09$	6.91 $\pm 1.85$	7.03 $\pm 0.58$	6.09 $\pm 0.67$



### *3.3.2. Effect of organic amendment on soil biological properties and microbial indices*

The content of microbial biomass C ( $C_{mic}$ ) is an appropriate indicator of soil quality because many scientific studies have highlighted the prompt and effective response in soil of this parameter to certain stress or disturbance conditions induced by human activities for different environments.

In this study, microbial biomass turned out also to be a quick and suitable indicator of soils quality.

After amending treatments, an increase in  $C_{mic}$  content was found, compare to control, in all treated plots, ranging from 0.07 mg g<sup>-1</sup> till to 0.68 mg g<sup>-1</sup> in F1 soil and from 0.06 mg g<sup>-1</sup> till to 0.67 mg g<sup>-1</sup> in F2 soil (figs 3.11 and 3.13) and the percentage variation, compared to control, ranged from -37.39% till to ~500% and from -39.95% till to 491.95% in F1 and F2 respectively (figs 3.12 and 3.14). However, only few results were statistically significant, due to the high variability of data. On the other hand, no considerable difference among treated plots, due to different organic amendments or mineral fertilizer, was clearly observed. The increasing trend in microbial biomass was more pronounced in F2 than F1 soil, moreover, significant differences ( $p < 0.001$ ) were also found in sampling times by two way ANOVA.

The microbial quotient represents the fraction of organic carbon consists of microbial carbon ( $C_{mic}/C_{org} = \text{mg } C_{mic} \text{ g}^{-1} C_{org}$ ). According with total microbial biomass data, microbial quotient was generally higher in treated plots compared to control (Figs 3.15 and 3.18) but this increase was not always significant, ranging from 5.83 till to 45.95 mg  $C_{mic} \text{ g}^{-1} C_{org}$  and from 4.02 till to 39.68 mg  $C_{mic} \text{ g}^{-1} C_{org}$  in F1 and F2 soils, respectively. However, no remarkable variation was found among the different amendments or among plots treated with or without mineral fertilizer. Two-ways ANOVA showed also a highly significant ( $p < 0.001$ ) variation in sampling time (Tables 3.6 and 3.7).

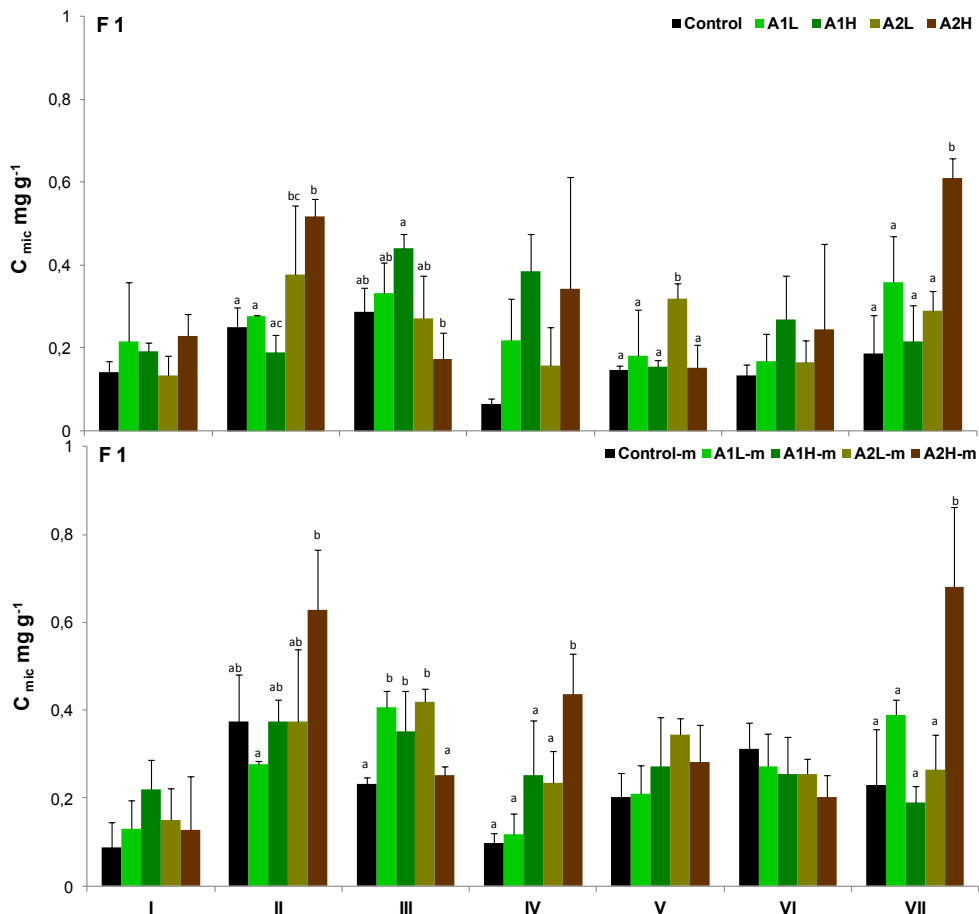


Figure 3.11 Effect of organic amendment on microbial biomass of F1 soil.

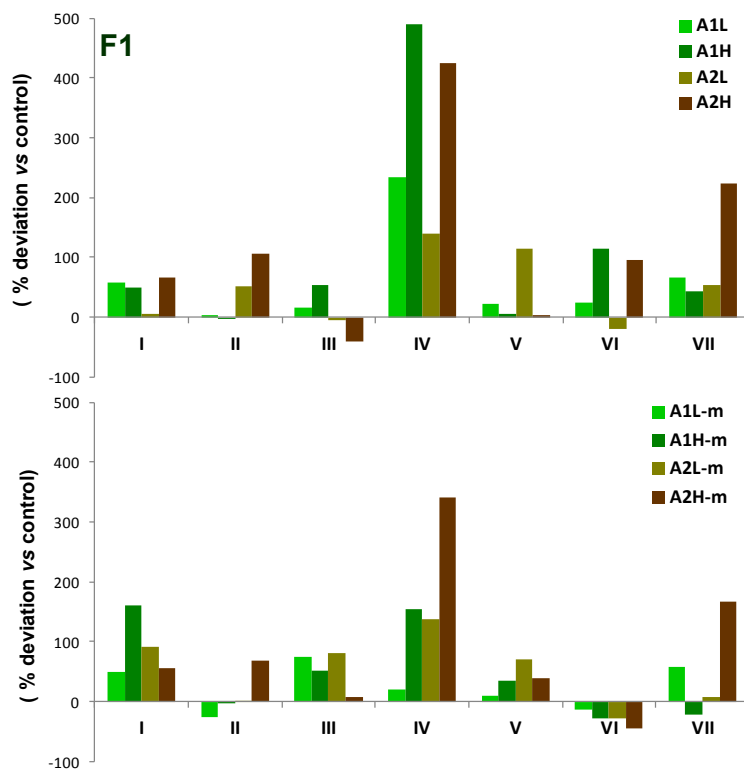


Figure 3.12 Percentage variations vs. control of  $C_{mic}$  in F1 soil.

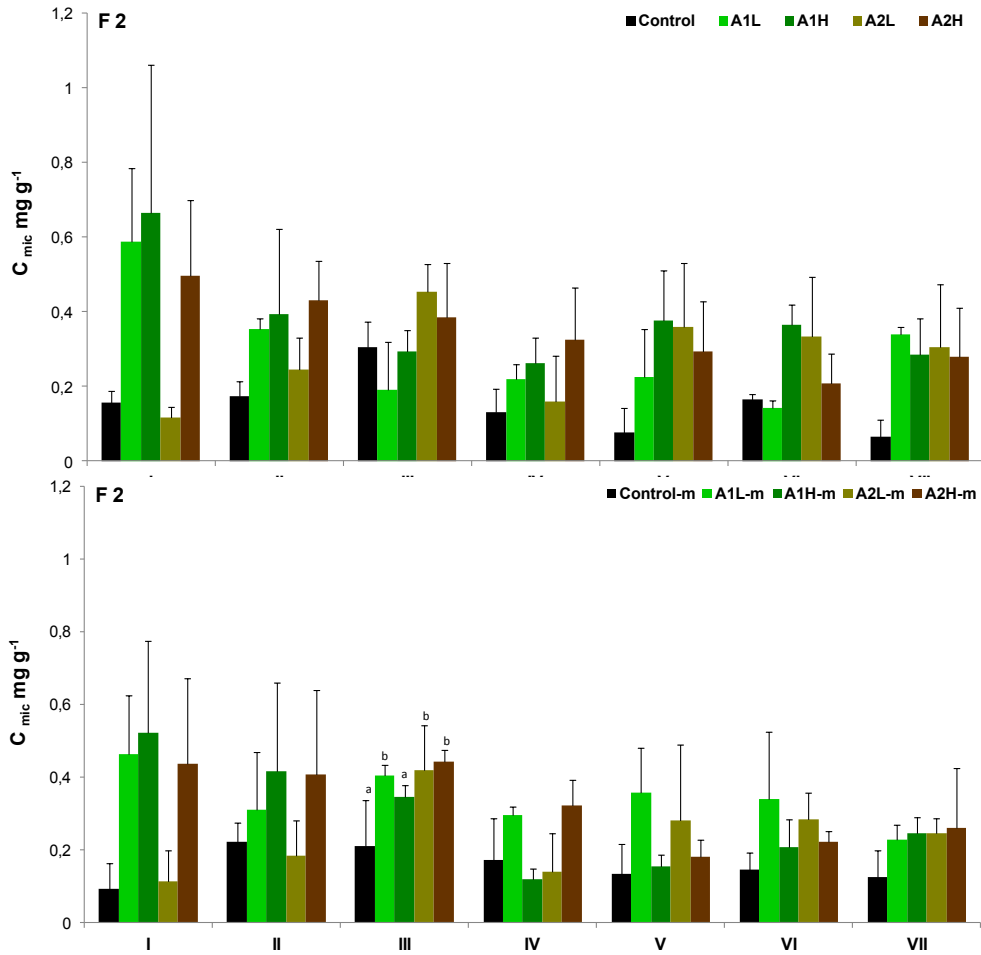


Figure 3.13 Effect of organic amendment on microbial biomass of F2 soil.

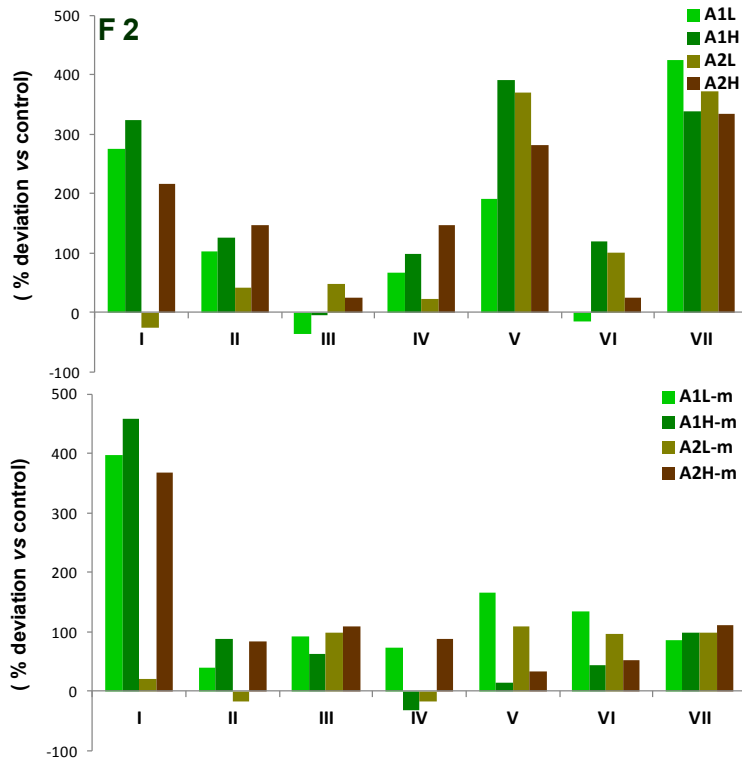


Figure 3.14 Percentage variations vs. control of  $C_{mic}$  in F2 soil.

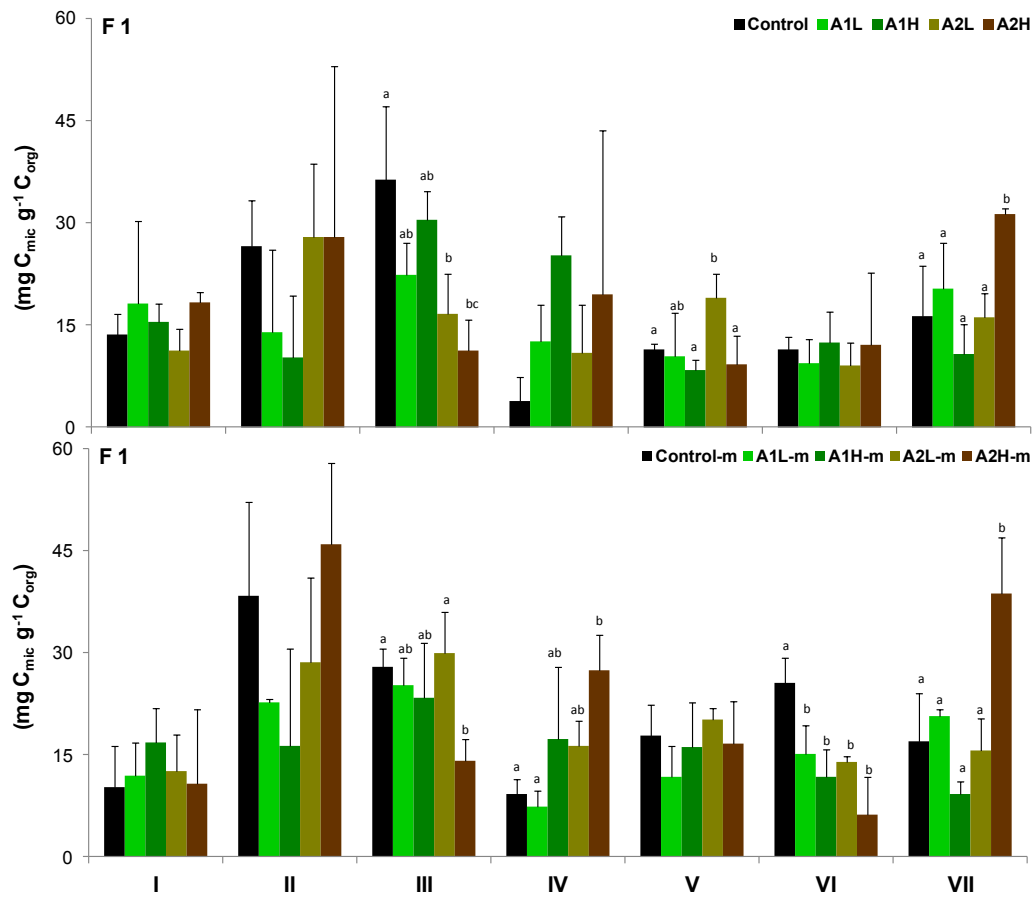


Figure 3.15 Effect of organic amendment on soil microbial quotient in F1 soil

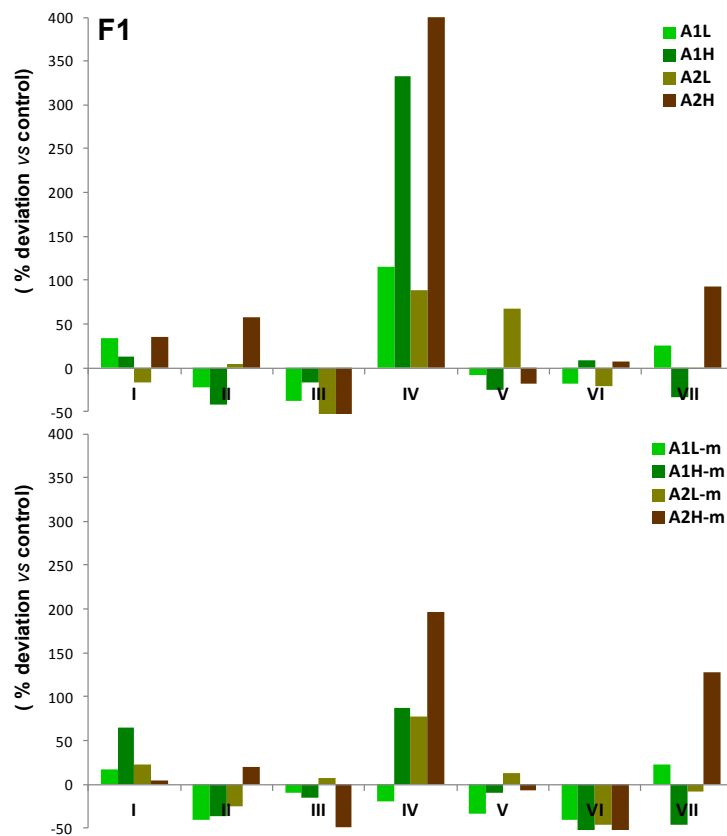


Figure 3.16 Percentage variations vs. control of microbial quotient in F1 soil

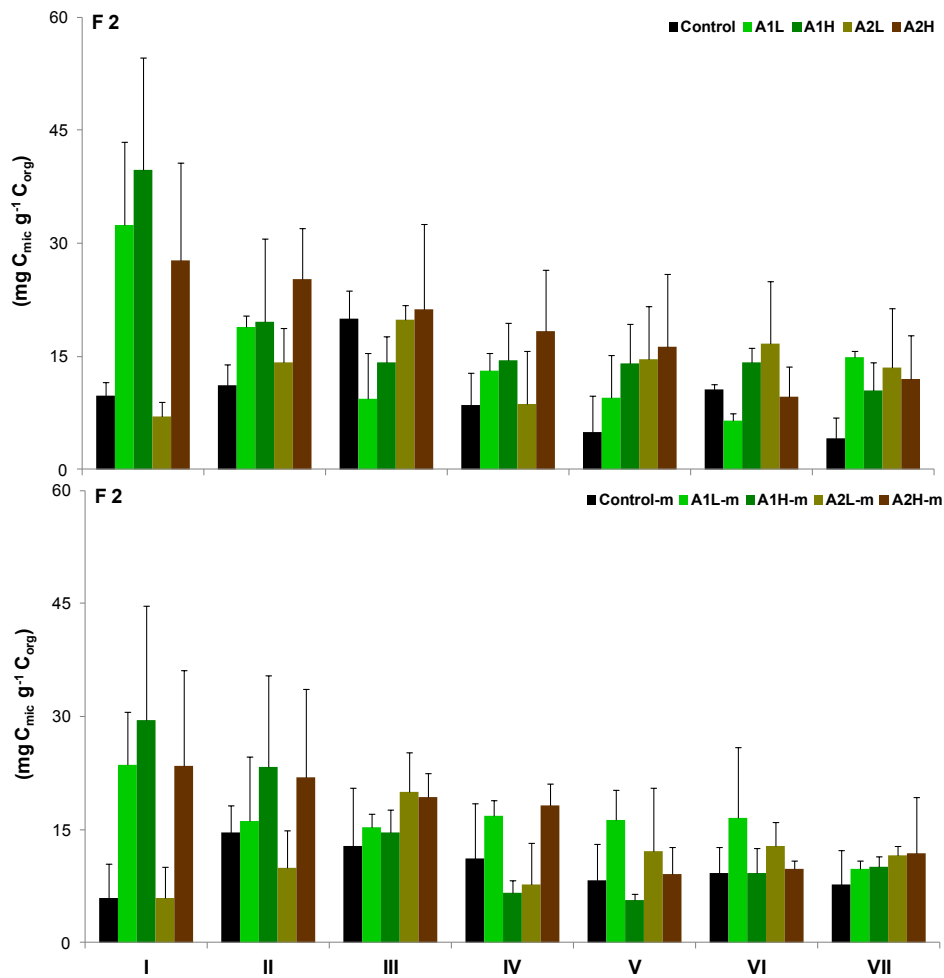


Figure 3.17 Effect of organic amendment on soil microbial quotient in F2 soil.

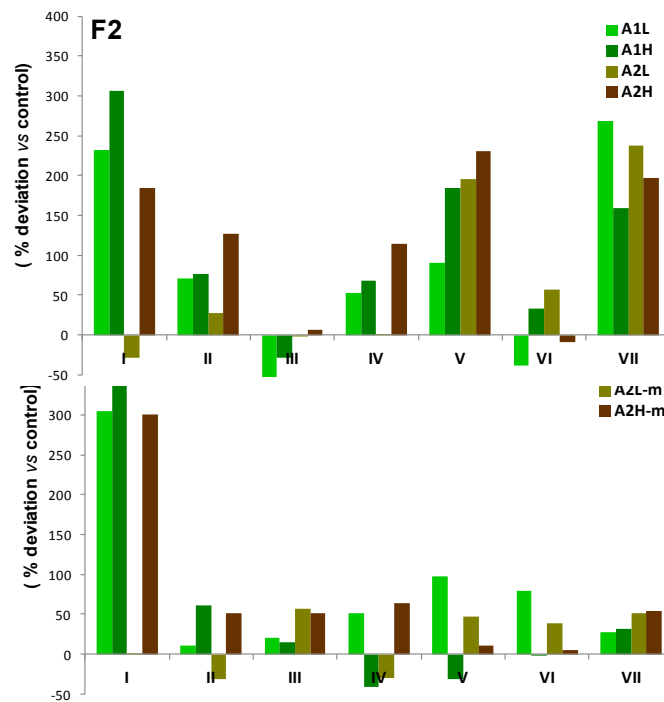


Figure 3.18 Percentage variations vs. control of microbial quotient in F2 soil.

Total microbial activity of soils was measured as soil potential respiration, and was reported in figs 3.19 and 3.21 (F1 and F2 soil, respectively). Moreover percentage variations *vs.* control were also shown in figs 3.20 and 3.22.

In this study, addition of slow-degradable organic fertilizers caused generally a prompt increase in soil potential respiration in treated plots of both farms, but the most significant and marked effects were found F1 soil and, in particular, in plots treated also with mineral fertilizer, where the highest number of sampling dates and treated plots showed a significant increase in soil potential respiration. However, in F2 a higher effect on soil respiration was found after the second amendment (i.e. starting from 4<sup>th</sup> sampling). The percentage increase in F1 soil ranged from 14.25% till to 307.87%, whereas in F2 soil ranged from 29.44% till to 139.75%. Moreover, mean value of this parameter in amended plots of the F1 soil ( $36.41 \mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) was higher than mean value measured in amended plots of the F2 soil ( $24.75 \mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ ).

No clear difference could be ascribed to amendment doses, whereas the additional mineral fertilizing treatment increased soil response in F1.

Finally, two ways ANOVA showed significant differences due to sampling time, amendment dose and interaction between these variables in both farms.

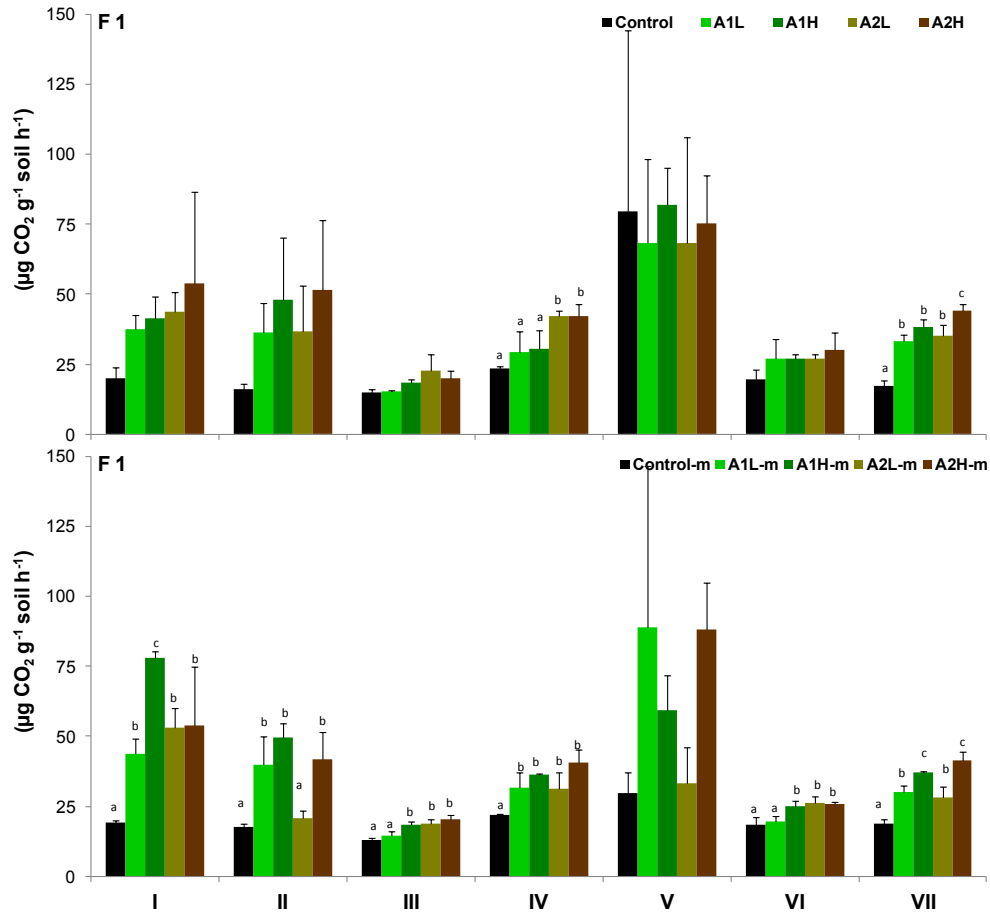


Figure 3.19 Effect of organic amendment on soil potential respiration in F1 soil.

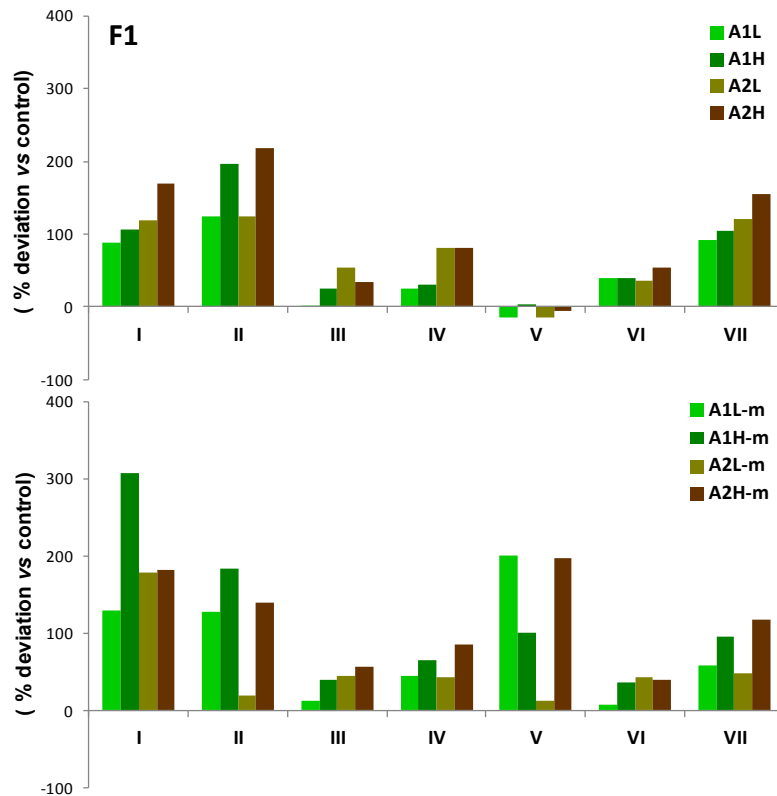


Figure 3.20 Percentage variations vs. control of potential respiration in F1 soil.

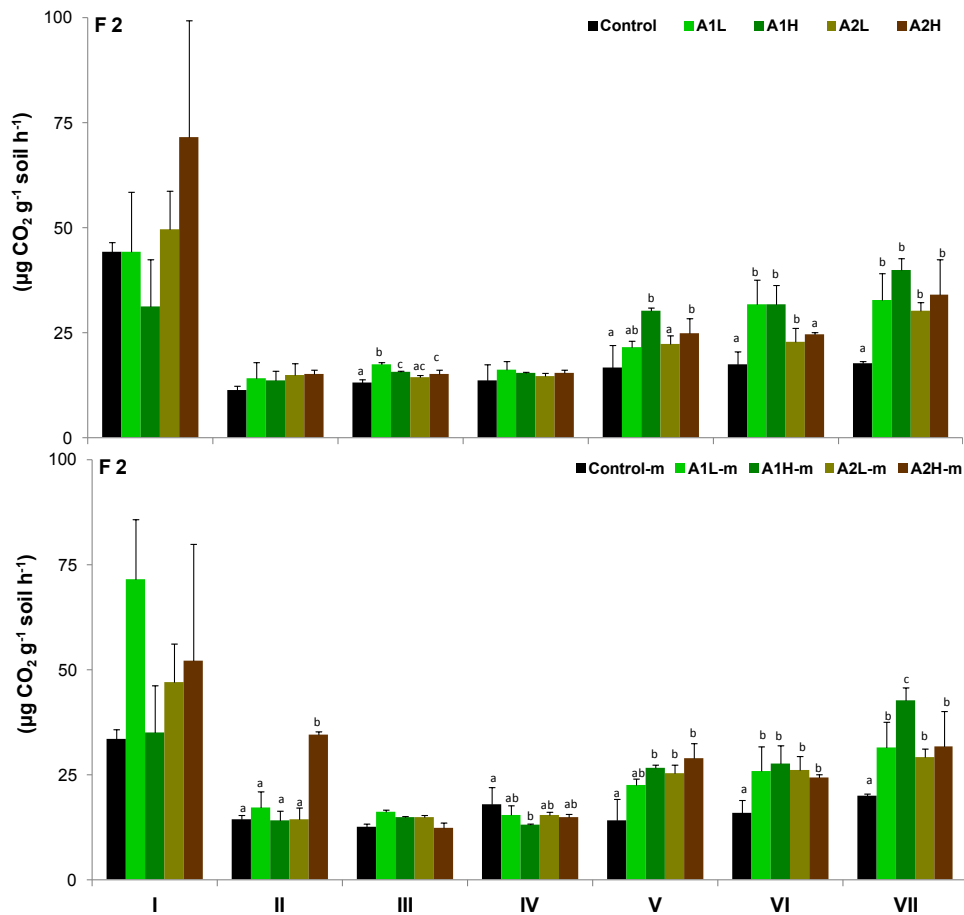


Figure 3.21 Effect of organic amendment on soil potential respiration in F2 soil.

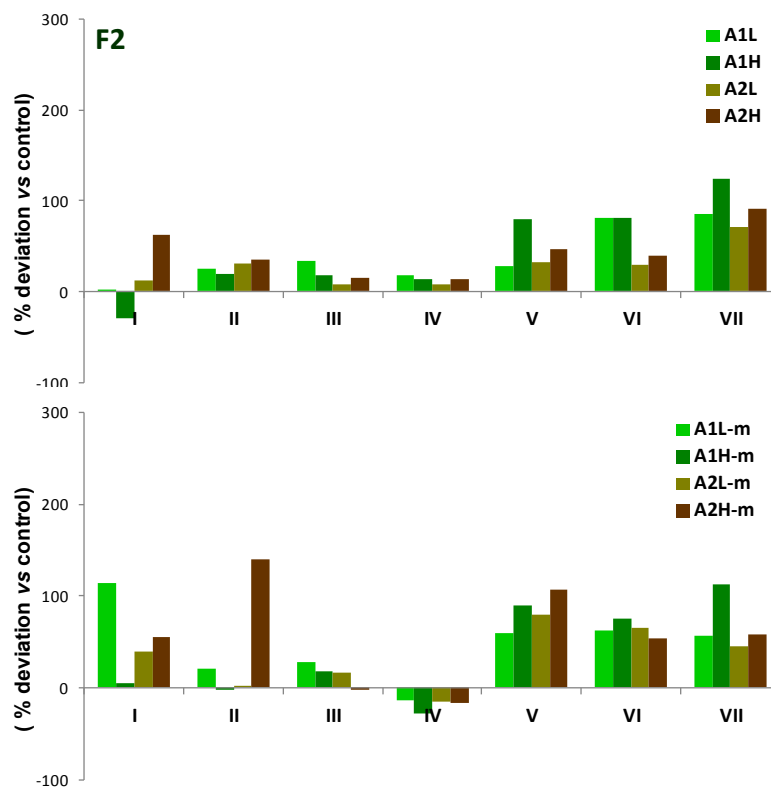


Figure 3.22 Percentage variations vs. control of potential respiration in F2 soil.



It has shown that metabolic quotient ( $qCO_2$ ), i.e. a specific respiration rate expressed in this study as  $\mu g C_{CO_2} g^{-1} C_{mic} h^{-1}$ , is a useful quantitative tool to assess the effects of anthropogenic stress or disturbance on soil microbial community, allowing understanding the efficiency of microbial community to utilize carbon sources. It is also a good indicator of maturity of the soil microbial community.

Metabolic quotient values, calculated for F1 and F2 soils, are reported in figures 3.23 and 3.25, whereas percentage variations compared to controls are shown in figures 3.24 and 3.26 (F1 and F2 respectively).

In this study, the  $qCO_2$  showed an increasing trend in F1, but increased values were only significant in some plots (A1L-m, A1H-m plots in 2<sup>nd</sup> sampling, A2H, A2H-m plots in 3<sup>rd</sup> sampling, A1L-m plot in 5<sup>th</sup> sampling and A1H-m plot in 7<sup>th</sup> sampling). By contrast, F2 soil did not show increases in  $qCO_2$ , except for A2H plot in the 2<sup>nd</sup> sampling.

However, percentage variation of this index ranged from -77.58% till to +328.79% in F1 soil and from -86.18% till to +196.71% in F2 soil. Moreover,  $qCO_2$  mean value obtained in F1 soil ( $51.71 \mu g C_{CO_2} g^{-1} C_{mic} h^{-1}$ ) was 45% higher than mean value obtained in F2 soil ( $35.42 \mu g C_{CO_2} g^{-1} C_{mic} h^{-1}$ ). No clear difference was found among different treatments in both farms. However, the two way ANOVA showed a sampling time effect ( $p < 0.001$ ) in both farms.

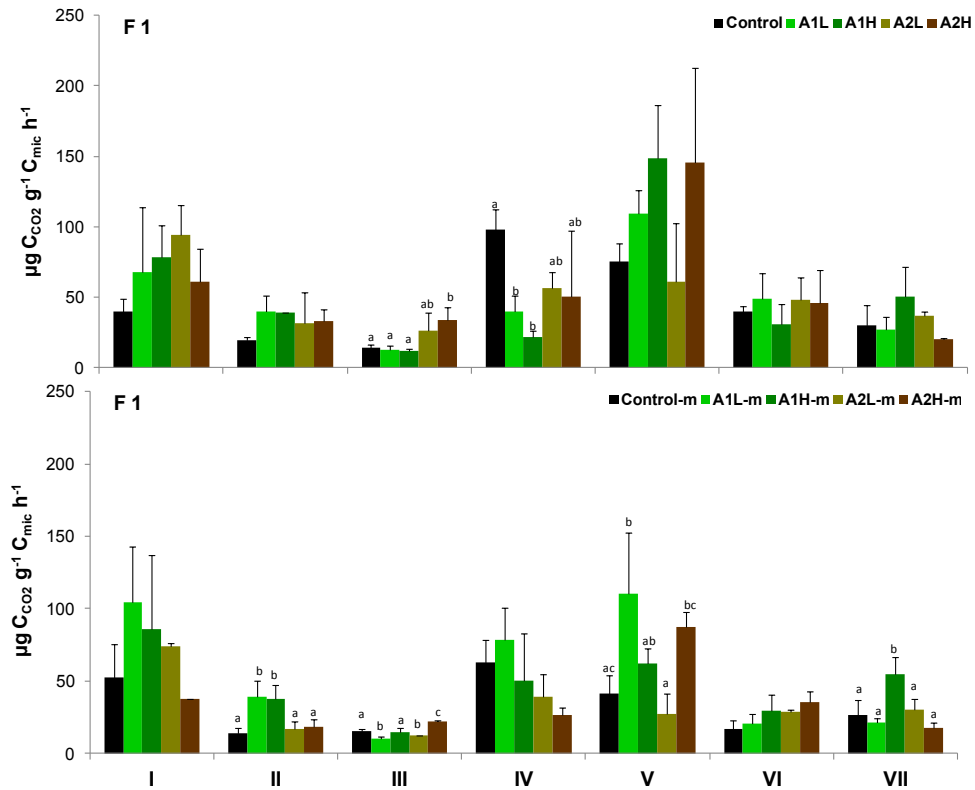


Figure 3.23 Effect of organic amendment on  $qCO_2$  of F1 soil

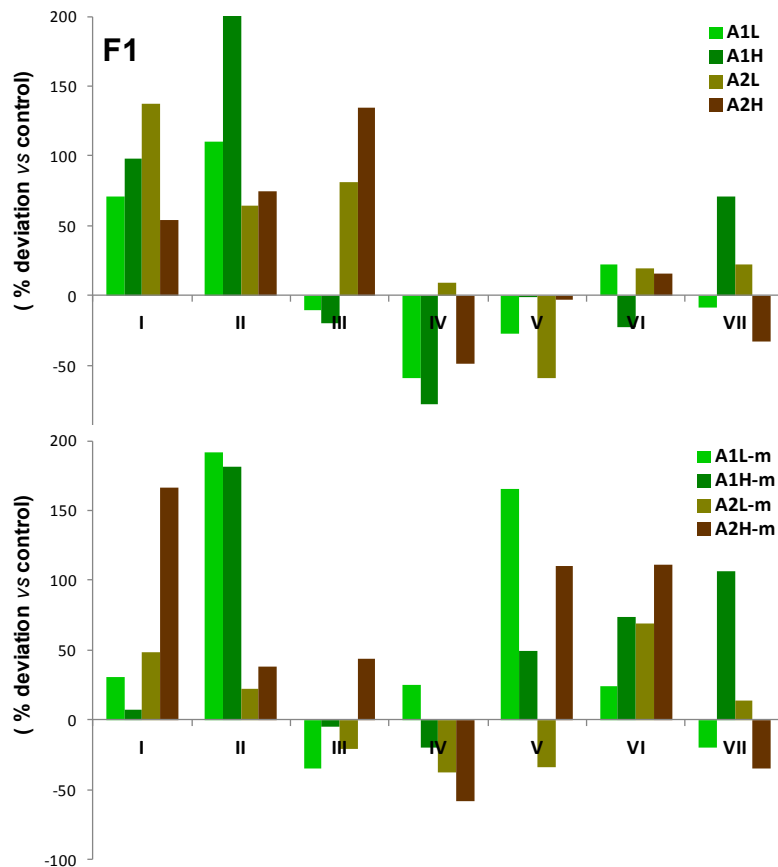


Figure 3.24 Percentage variations vs. control of  $qCO_2$  in F1 soil.

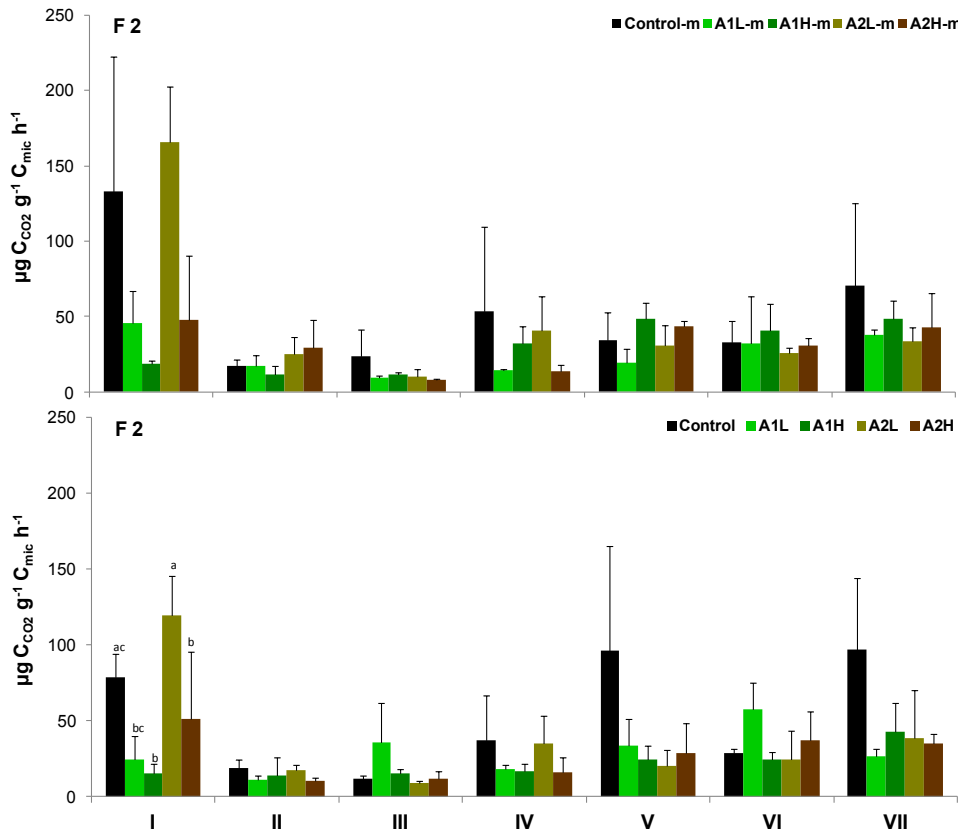


Figure 3.25 Effect of organic amendment on soil  $qCO_2$  value of F2 soil

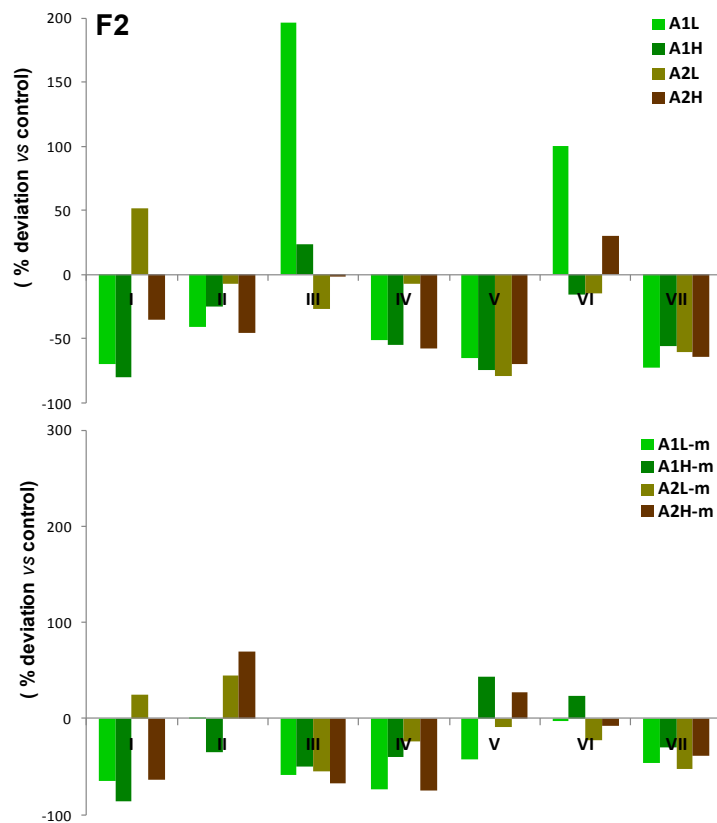


Figure 3.26 Percentage variations vs. control of  $qCO_2$  in F2 soil

Coefficient of endogenous mineralization (CEM) allows us to understand better changes in soil activity and organic carbon dynamics. It is a specific respiration rate (expressed in this study as  $\text{mg C}_{\text{CO}_2} \text{g}^{-1} \text{C}_{\text{org}} \text{h}^{-1}$ ) that take also into account the quality of organic carbon, because when potential respiration data are normalized per unit of organic carbon, the differences showed by the index can be related with the capacity of the microbial community to mineralize that specific available source of organic carbon in soil. Higher values of CEM indicate a faster mineralization process, and, as a consequence, a faster loss of organic matter from soil and a higher input of  $\text{CO}_2$  in atmosphere, but also a faster nutrient cycle and thus a higher input of available nutrients to soil.

CEM values, in studied soils, were represented in figures 3.27 and 3.29 and percentage variations vs. each control were shown in figure 3.28 and 3.30 (F1 and F2 soil, respectively).

CEM generally increased in F1 soil in response to addition of organic and mineral fertilizers, in particular after the first treatment, by contrast, in F2 soil the only increase of this index was found in A2H-m plot of the 2<sup>nd</sup> sampling. Moreover, percentage increase of CEM in F1 soil ranged from 10% up to 139.75%, whereas in F2 soil percentage increase ranged from 5% up to +98.26%. Moreover, CEM mean value in F1 soil was higher than in F2 soil ( $0.68$  vs.  $0.34 \text{ mg C}_{\text{CO}_2} \text{g}^{-1} \text{C}_{\text{org}} \text{h}^{-1}$ , respectively). No obvious differences were found among different treatments (i.e. dose or quality of amendments). However, two way ANOVA showed a sampling time effect ( $p < 0.001$ ) for both farms.

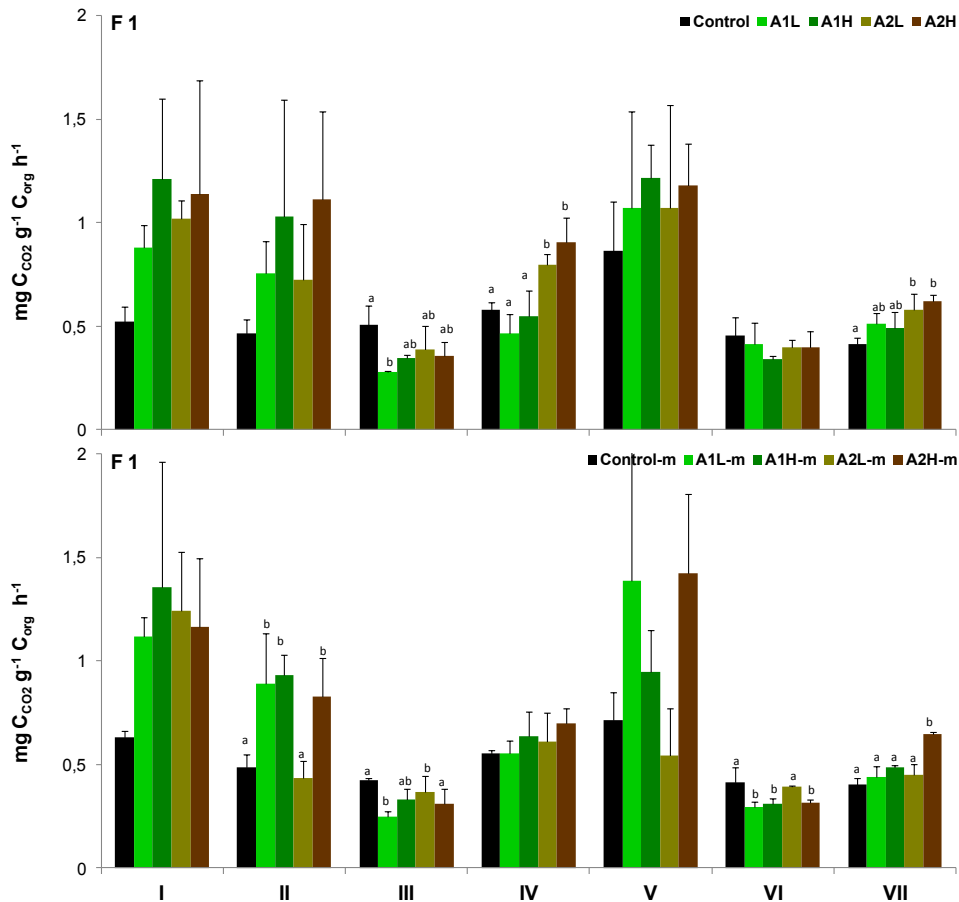


Figure 3.27 Effect of organic amendment on CEM values in F1 soil

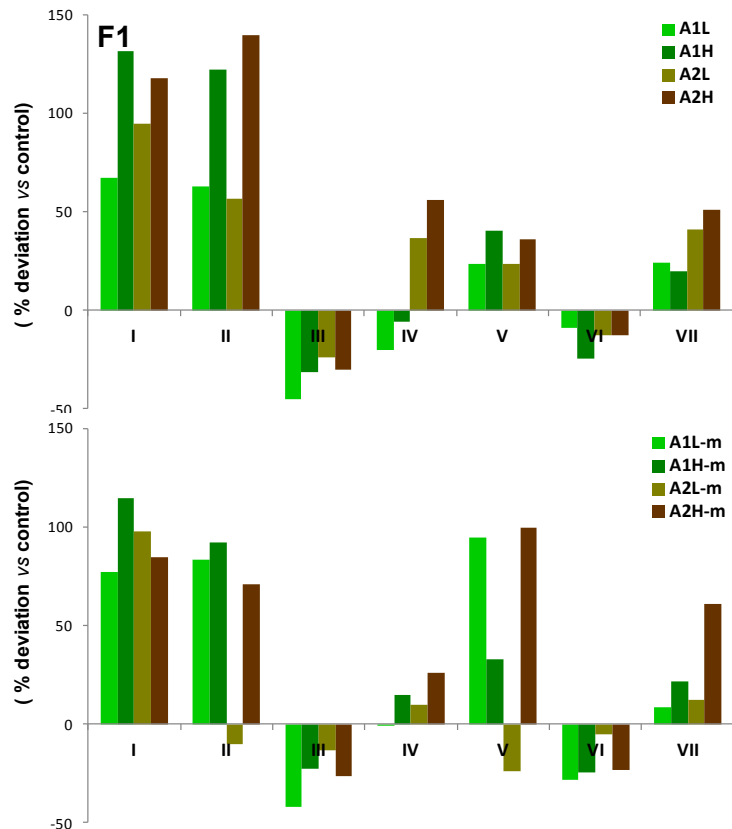


Figure 3.28 Percentage variations vs. control of CEM in F1 soil.

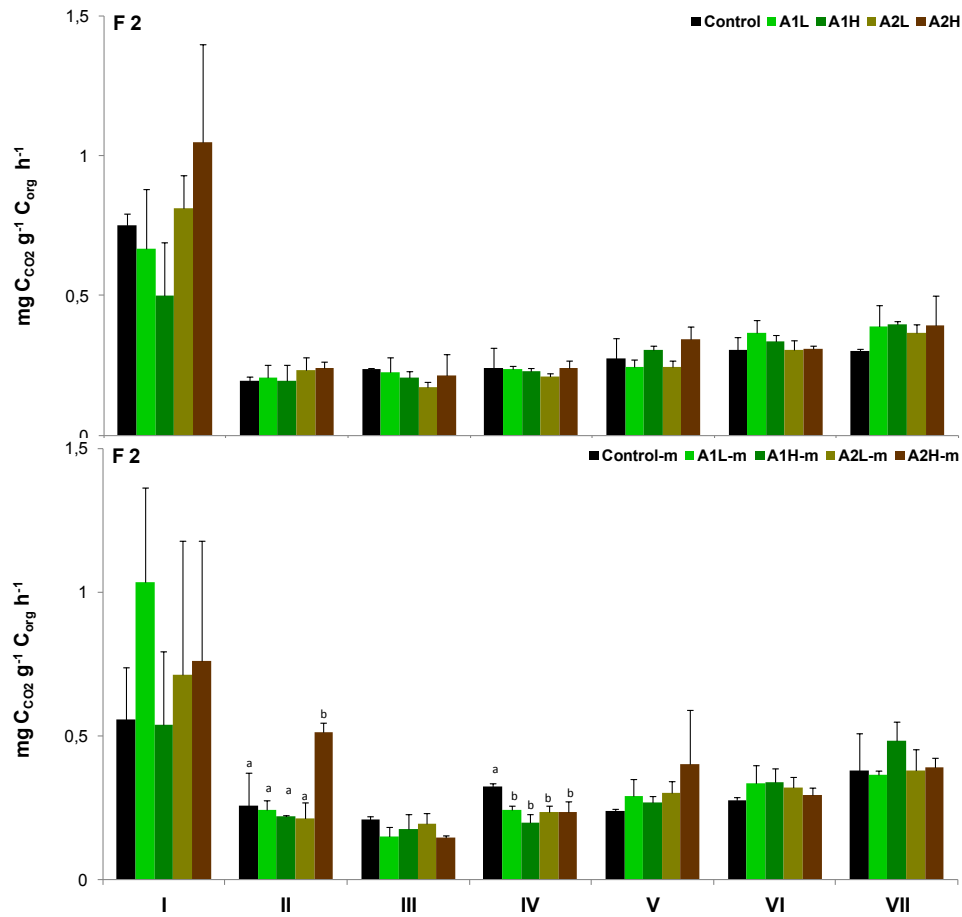


Figure 3.29 Effect of organic amendment on CEM values in F2 soil.

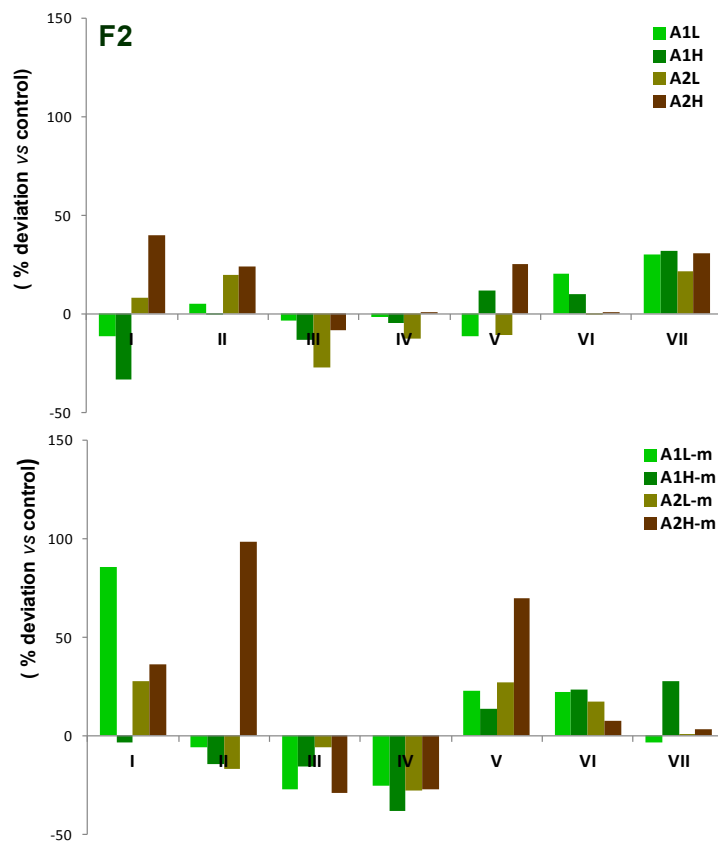


Figure 3.30 Percentage variations vs. control of CEM in F2 soil.

### *3.3.3 Effect of organic amendment on functional diversity of the soil microbial community*

Catabolic fingerprint of the soil microbial community, assessed as respiration responses induced by addition of simple organic compounds, was not greatly affected by the organic amendments used in this study, at tested doses, ever after long term addition. However, after treatments a general increase in the respiration responses was found and a certain number of respiration responses significantly differed from controls.

In particular, in F1 soil, plots treated with organic amendments showed significant changes, compared to control, in the 3<sup>rd</sup> and 5<sup>th</sup> samplings, with a percentage of changed responses of 36% and 20%, respectively (Fig. 3.31A and B), whereas, in the same soil, plots treated with organic amendments and mineral fertilizer showed significant changes, compared to control, in all sampling dates, with a percentage of changed responses ranging from 8% up to 48% (1<sup>st</sup> and 5<sup>th</sup> samplings, respectively, Fig. 3.32 A and B). It has to be emphasized that in F1 plots the addition of the mineral fertilizer caused an higher percentage of changed responses to organic substrates compared to F2 plots, although F1 soil was characterized by lower values of C/N ratio and higher values of total N, compared to F2 soil (see table 3.1). Moreover, in F1 soil, the mean values of respiration response in plots (data not showed, calculated for each plot by summarize all respiration responses to substrates and dividing by the total number of substrates) were generally higher than in control plot, with more marked effect in plots amended with mineral fertilizer.

On the other hand, in F2 soil, plots showed significant changes, compared to control, in all sampling dates, with a percentage of changed responses ranging from 8% up to 56% (1<sup>st</sup> and 3<sup>rd</sup> samplings, respectively, in plot treated with mineral fertilizer), but the effect was comparable in plot with or without mineral fertilizer (Fig. 3.33A and B, Fig. 3.34A and B).

Moreover, in F2 soil, the mean values of respiration response in plots (data not showed) were generally higher than in control plot, but the more marked effect was found in plots without mineral fertilizer.

Catabolic fingerprints of soil microbial community were also affected by sampling times. In fact, when the percentage of changed responses was calculated, for each plot, compared to 1<sup>st</sup> sampling, in both soils and in each sampling date differences were found (with a percentage variation ranging from 4% still to 60%), except for F1 plots treated with mineral fertilizer in 2<sup>nd</sup> sampling.

However, no remarkable difference was found due to different doses or types of amendment or to the mineral fertilizing treatment.

Furthermore, catabolic evenness was calculated from catabolic response profiles and the mean values of this index were represented in figure 3.35 and figure 3.36 (F1 and F2 soils, respectively).

In F1 soil, the only significant change in catabolic evenness was found in the 3<sup>rd</sup> sampling in plots treated with organic and mineral fertilizers. In particular, higher catabolic evenness was found in plots A2L-m and A2H-m, characterized by 25 C/N ratio.

In F2 soil, significant changes in catabolic evenness were found in 2<sup>nd</sup> sampling, in plots treated with and without mineral fertilizer. In particular, higher catabolic evenness was found in plots A1L, A2L, A1L-m and A1H-m.



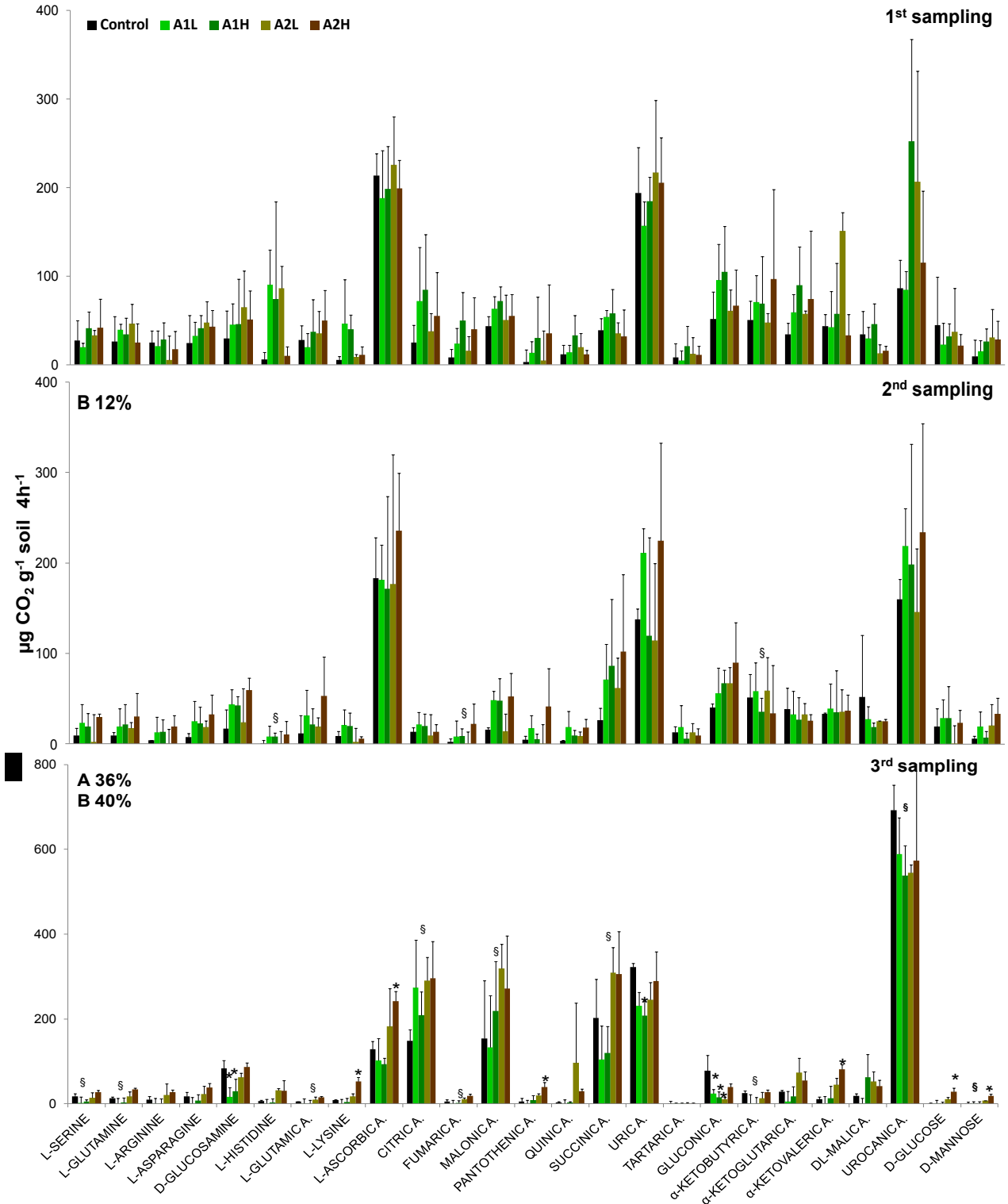


Figure 3.31 (A) Mean value and standard deviation of functional diversity data, as assessed by catabolic response profiles, in F1 plot untreated with mineral fertilizer (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> samplings). In the top on the left of each graph, the percentage of changed responses in amended plots compared to control (A) and the percentage of changed responses in all plots compared to each corresponding plot in 1<sup>st</sup> sampling (B) are shown. Significant differences compared to 1<sup>st</sup> sampling (§) and compared to control (\*) are indicated on each bar.

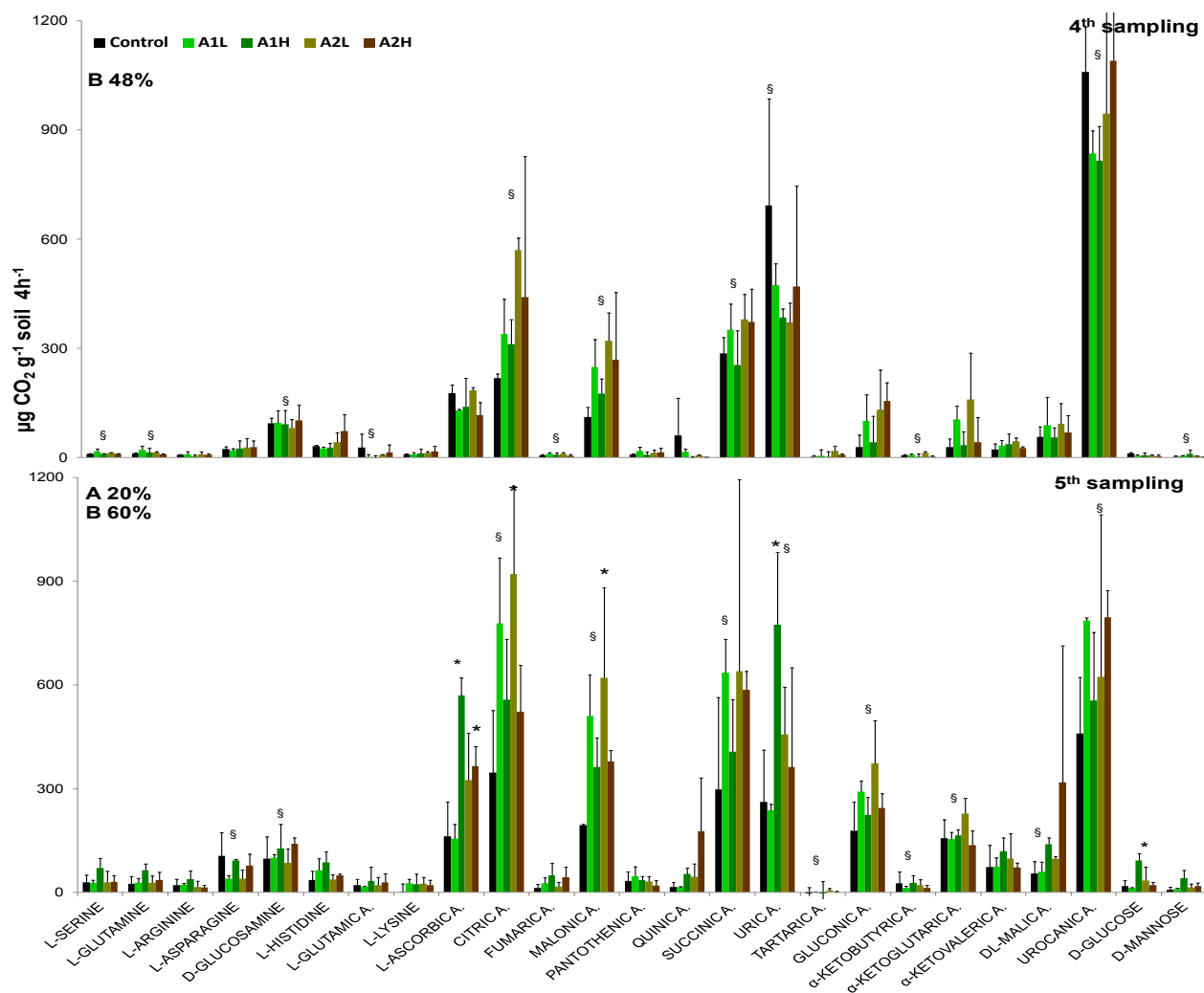


Figure 3.31 (B) Mean value and standard deviation of functional diversity data, as assessed by catabolic response profiles, in F1 plot untreated with mineral fertilizer (4<sup>th</sup> and 5<sup>th</sup> samplings). In the top on the left of each graph, the percentage of changed responses in amended plots compared to control (A) and the percentage of changed responses in all plots compared to each corresponding plot in 1<sup>st</sup> sampling (B) are shown. Significant differences compared to 1<sup>st</sup> sampling (§) and compared to control (\*) are indicated on each bar.

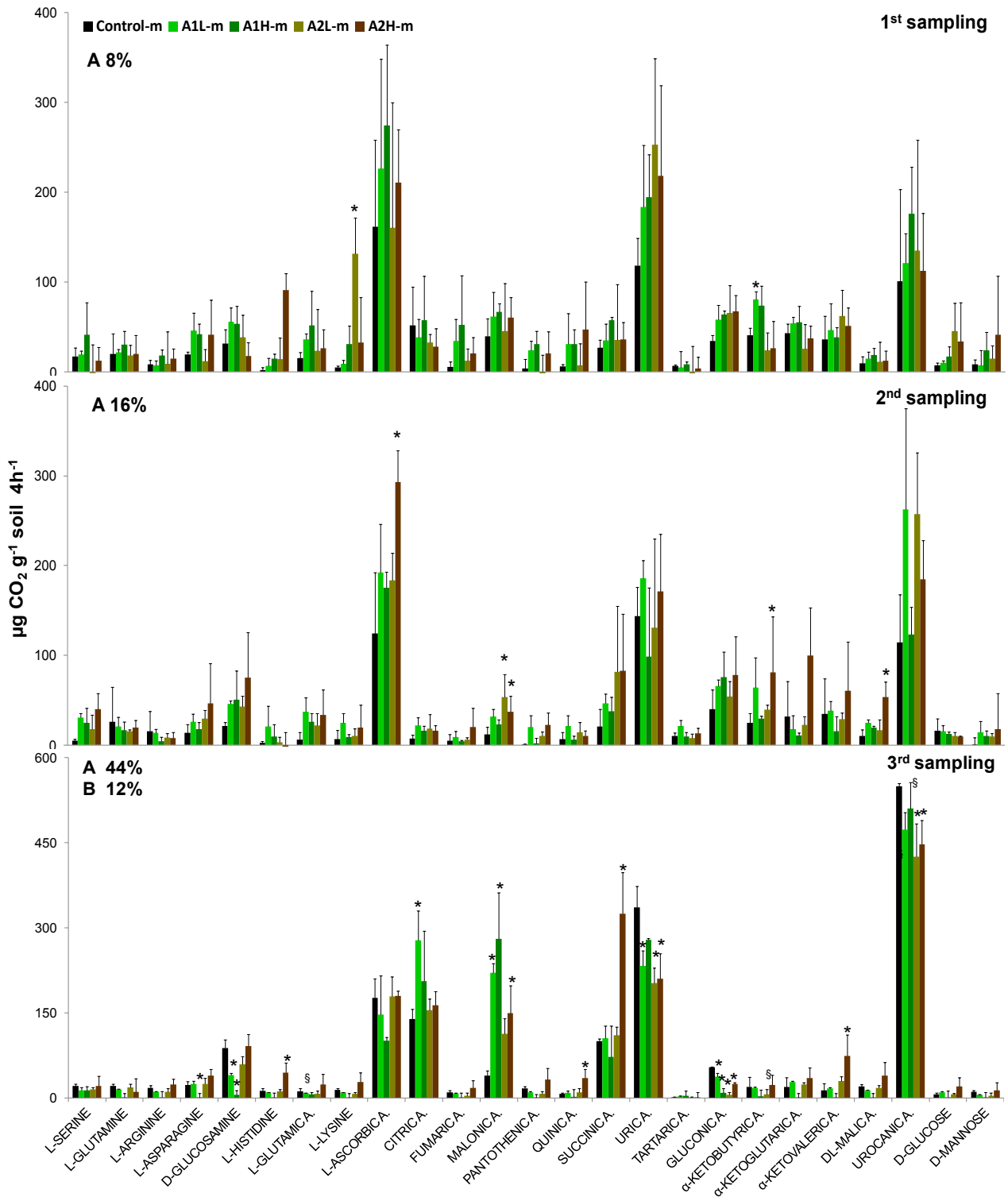


Figure 3.32 (A) Mean value and standard deviation of functional diversity data, as assessed by catabolic response profiles, in F1 plot treated with mineral fertilizer (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> samplings). In the top on the left of each graph, the percentage of changed responses in amended plots compared to control (A) and the percentage of changed responses in all plots compared to each corresponding plot in 1<sup>st</sup> sampling (B) are shown. Significant differences compared to 1<sup>st</sup> sampling (§) and compared to control (\*) are indicated on each bar.

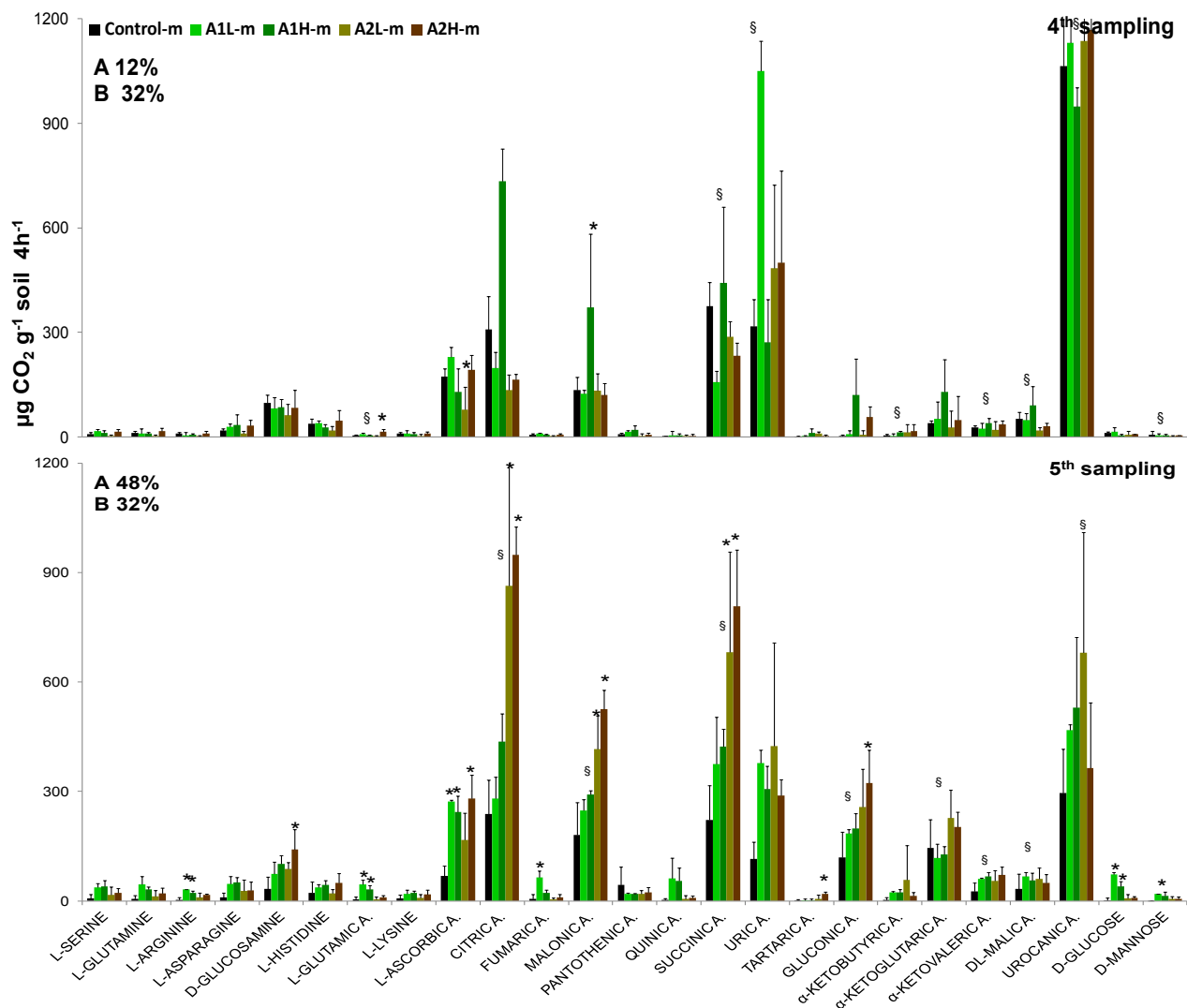


Figure 3.32 (B) Mean value and standard deviation of functional diversity data, as assessed by catabolic response profiles, in F1 plot treated with mineral fertilizer (4<sup>th</sup> and 5<sup>th</sup> samplings). In the top on the left of each graph, the percentage of changed responses in amended plots compared to control (A) and the percentage of changed responses in all plots compared to each corresponding plot in 1<sup>st</sup> sampling (B) are shown. Significant differences compared to 1<sup>st</sup> sampling (§) and compared to control (\*) are indicated on each bar.

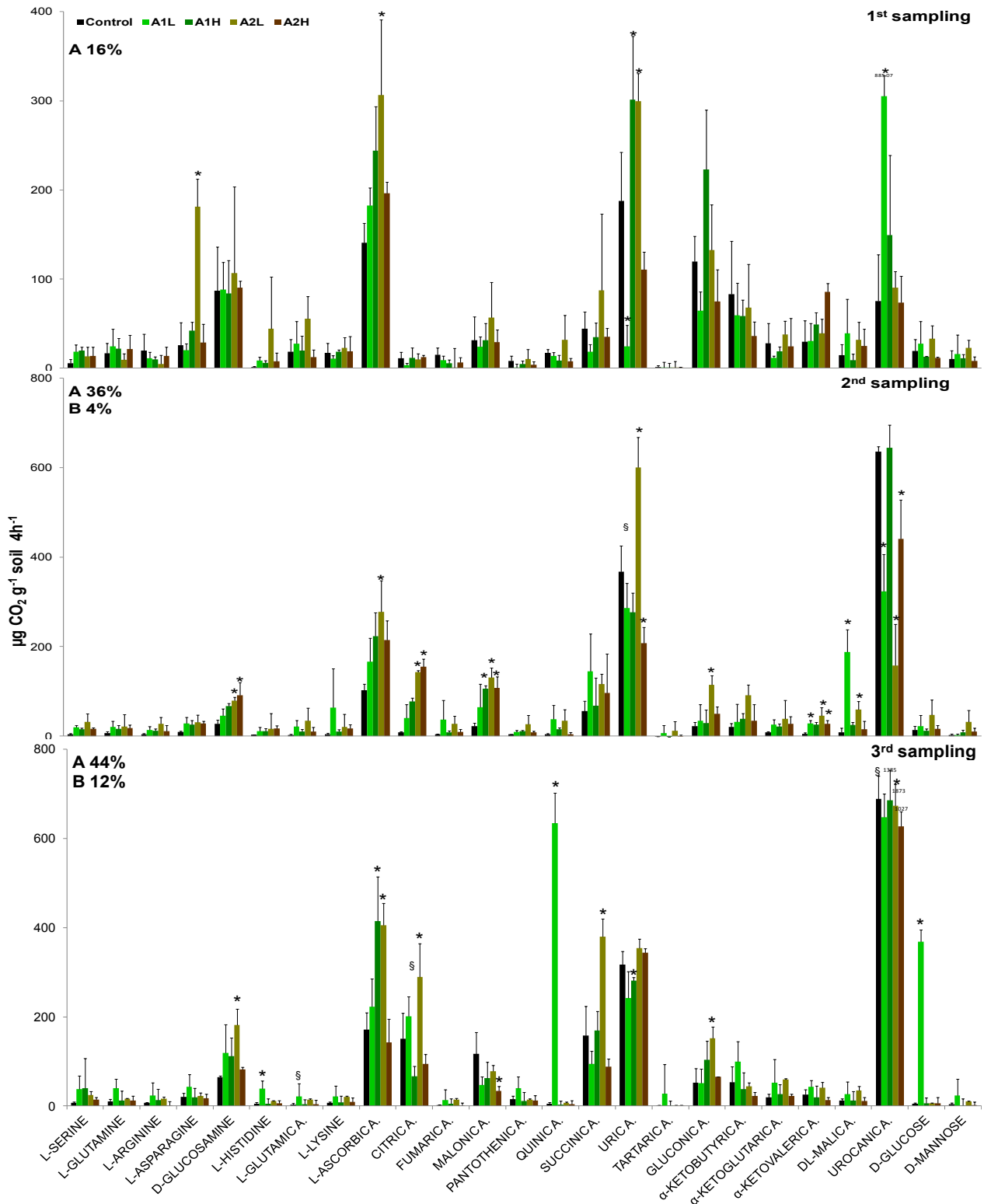


Figure 3.33 (A) Mean value and standard deviation of functional diversity data, as assessed by catabolic response profiles, in F2 plot untreated with mineral fertilizer (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> samplings). In the top on the left of each graph, the percentage of changed responses in amended plots compared to control (A) and the percentage of changed responses in all plots compared to each corresponding plot in 1<sup>st</sup> sampling (B) are shown. Significant differences compared to 1<sup>st</sup> sampling (§) and compared to control (\*) are indicated on each bar.

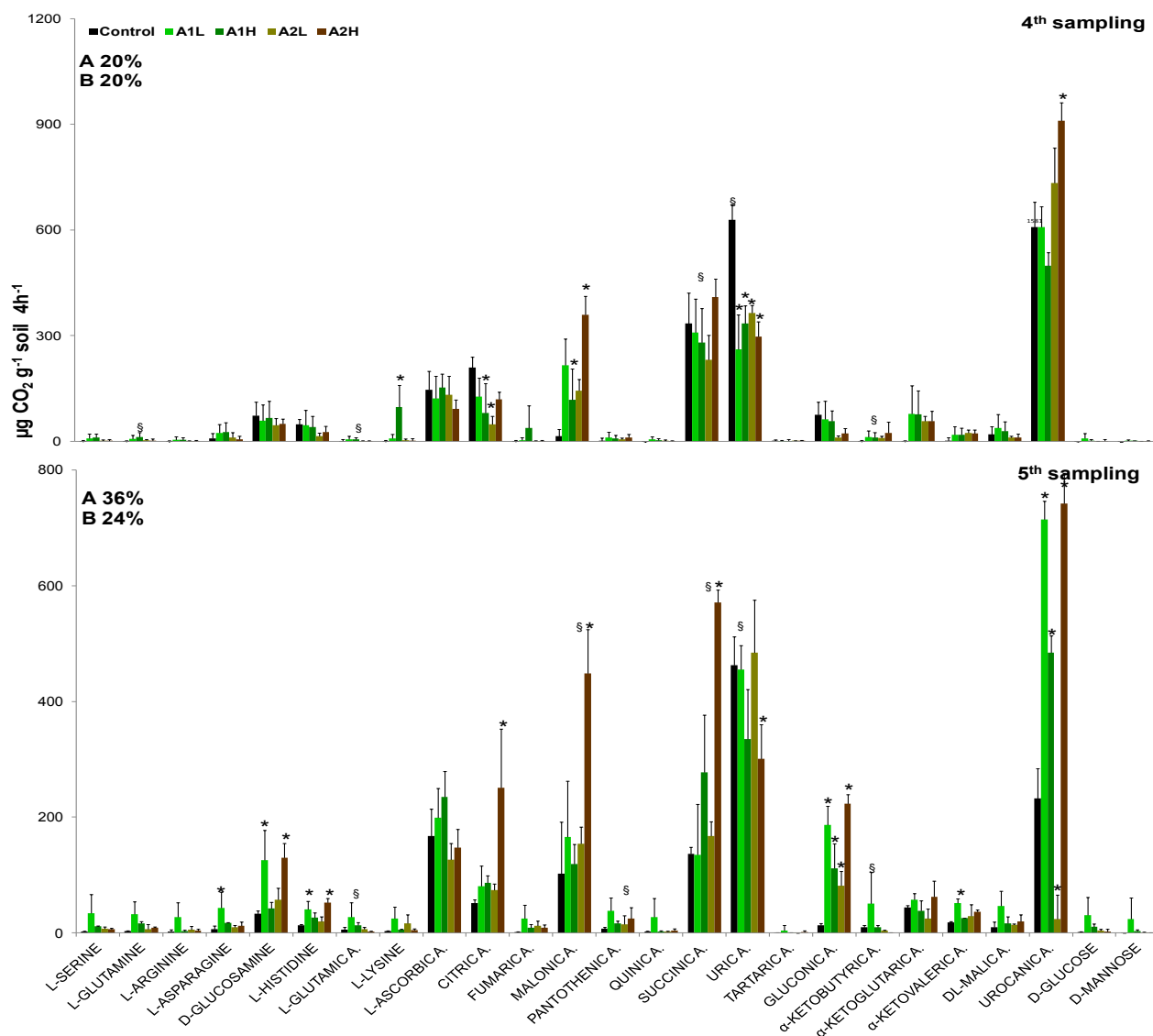


Figure 3.33 (B) Mean value and standard deviation of functional diversity data, as assessed by catabolic response profiles, in F2 plot untreated with mineral fertilizer (4<sup>th</sup> and 5<sup>th</sup> samplings). In the top on the left of each graph, the percentage of changed responses in amended plots compared to control (A) and the percentage of changed responses in all plots compared to each corresponding plot in 1<sup>st</sup> sampling (B) are shown. Significant differences compared to 1<sup>st</sup> sampling (§) and compared to control (\*) are indicated on each bar.

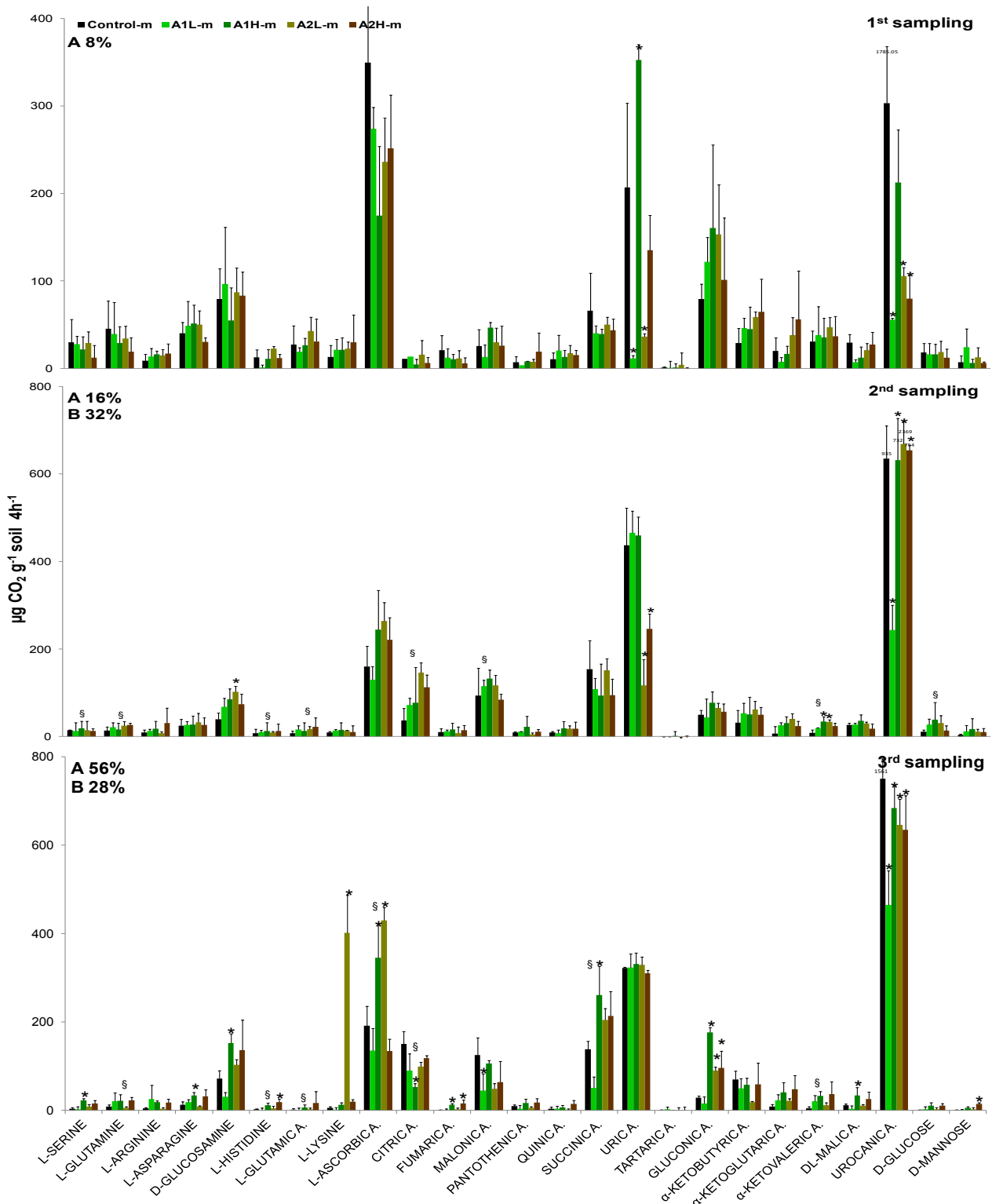


Figure 3.34 (A) Mean value (standard deviation of functional diversity data, as assessed by catabolic response profiles, in F2 plot treated with mineral fertilizer (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> samplings). In the top on the left of each graph, the percentage of changed responses in amended plots compared to control (A) and the percentage of changed responses in all plots compared to each corresponding plot in 1<sup>st</sup> sampling (B) are shown. Significant differences compared to 1<sup>st</sup> sampling (§) and compared to control (\*) are indicated on each bar.

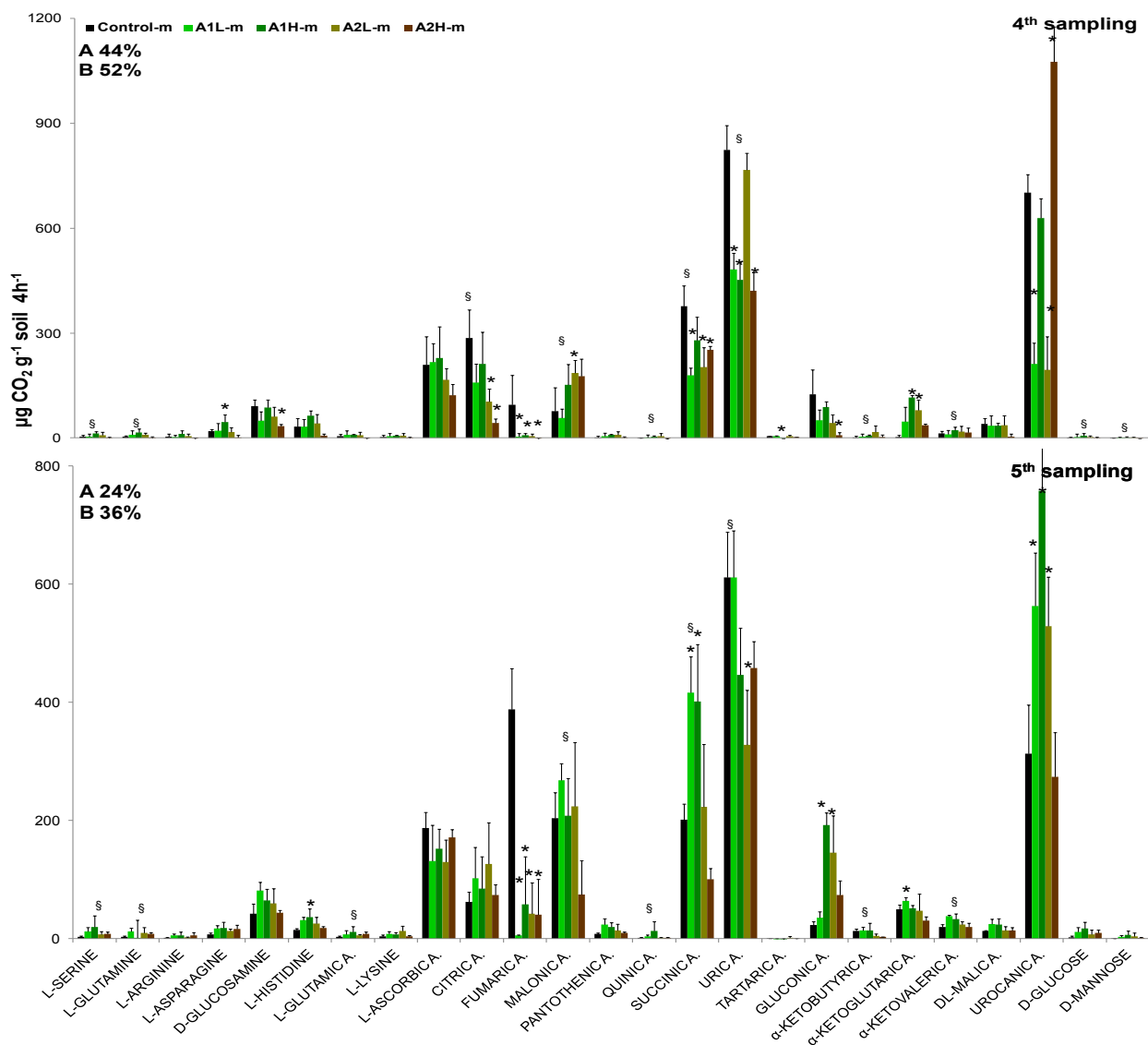


Figure 3.34 (B) Mean value and standard deviation of functional diversity data, as assessed by catabolic response profiles, in F2 plot treated with mineral fertilizer (4<sup>th</sup> and 5<sup>th</sup> samplings). In the top on the left of each graph, the percentage of changed responses in amended plots compared to control (A) and the percentage of changed responses in all plots compared to each corresponding plot in 1<sup>st</sup> sampling (B) are shown. Significant differences compared to 1<sup>st</sup> sampling (§) and compared to control (\*) are indicated on each bar.



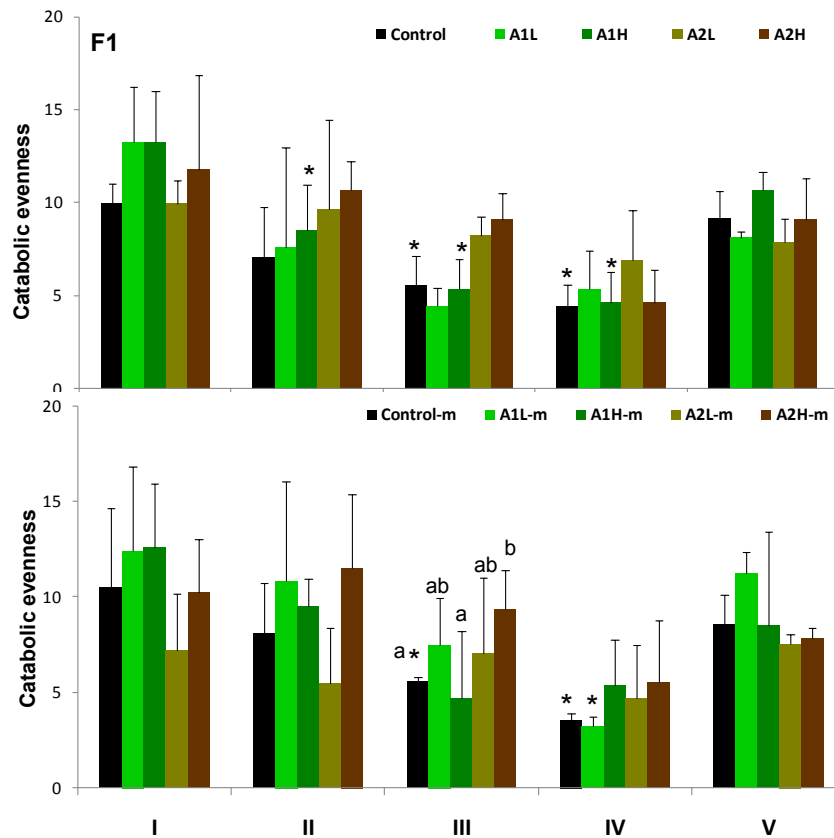


Figure 3.35 Effect of organic amendment on catabolic evenness in F1 soil. Significant differences compared to 1<sup>st</sup> sampling (\*) and compared to control (small letters) are indicated on bars.

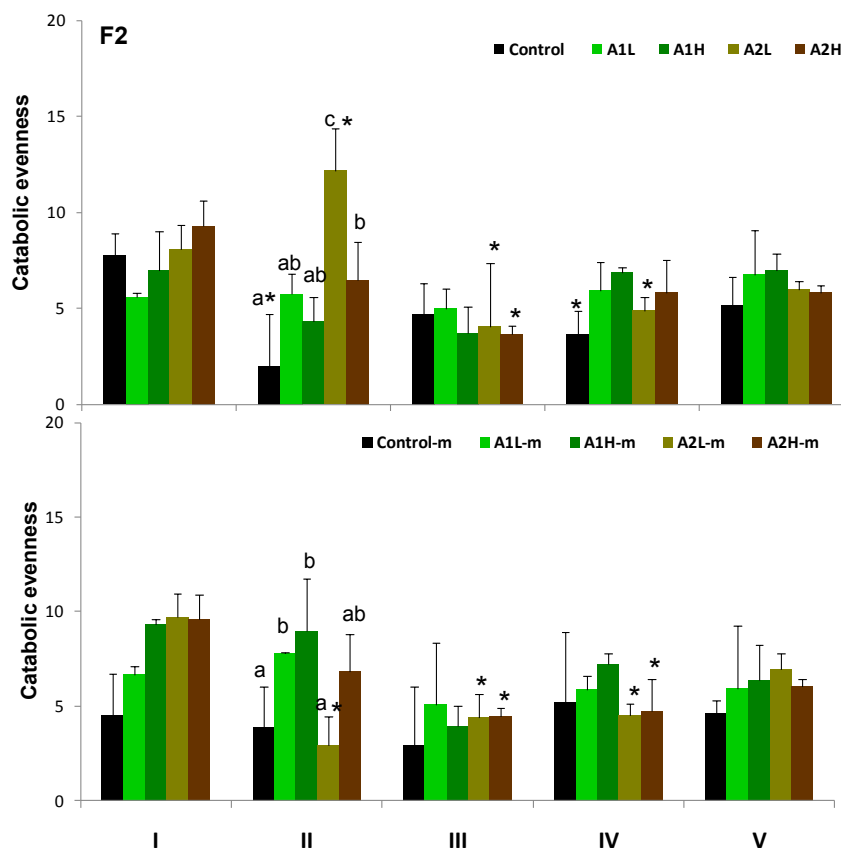


Figure 3.36 Effect of organic amendment on catabolic evenness in F2 soil.

### 3.5 Discussion

Proper assessment of soil quality requires the determination of a large number of indicators, as soil physical, chemical and biochemical/biological properties. In this study, the large amount of data, due to the number of parameters analyzed, treatments and soils tested and samplings carried out, made difficult to clearly interpret results. For this purpose, suitable statistical analysis was applied to the whole data set (except for soil water content that was not included considering its dependence from the local artificial irrigation) in order to better understand relationships among measured parameters and to clearly test differences among treatments or sampling times. In particular, Pearson's product-moment correlation coefficients and principal component analysis were elaborated, and, considering different geopedological properties of the studied soils, the statistical analyses were performed separately for the data set of the farm 1 and farm 2.

Regarding the effect of organic amendment on physical and chemical properties of the studied soils, it is important to underline that the effect of amendment on soil water content and water holding capacity can be very important in agricultural areas, as soil fertility status is strongly related to water availability. In general, addition of organic matter to the soil increases the water holding capacity, because water is held by adhesive and cohesive forces within the soil and an increase in the pore space will lead to an increase in water holding capacity of the soil (Reicosky et al., 2003). Hudson (1994) showed that for each 1% increase in organic matter, soil water holding capacity increased by 3.7%; as a consequence, less irrigation water is needed to irrigate the same crop (Verheijen et al., 2010; FAO report, 2005). Moreover, organic amendment has been shown to improve the water retention in sandy soils, when organic amendment applied at relatively high rates (Brockhoff et al., 2010), but also to decrease moisture content in clay soils (Verheijen et al., 2010). Concerning water retention, soils with coarse texture are substantially more sensitive to the amount of organic carbon compared with fine-

textured soils, thus the effect of changes in organic carbon content on soil water retention depends on the proportion of textural components, but also on the amount of organic carbon in soil (Rawl et al., 2003). In fact, at low carbon content in soil, an increase in organic carbon leads to an increase in water retention in coarse-texture soils and to a decrease in fine-texture soils, whereas, at high carbon content in soil, an increase in organic carbon results in an increase in water retention for all texture types (Rawls et al., 2004). In this study, after addition of organic amendments, soil water content and water holding capacity did not showed significant increases, except for F2 soil (a sandy loam soil with a good content of organic carbon), that showed, at the last sampling, an increase in these parameters in amended plots. It has to be emphasized that increases in soil water content and water holding capacity are particularly relevant for a sandy soil, because its fertility is markedly limited by small amounts of plant available water, especially in dry periods. According with this result, Pandey & Shukla (2006) showed that compost addition to a sandy soil resulted in higher retention of rainfall, if application levels are sufficiently high. Aggelides & Londra, (2000) noticed that, after addition, physical properties of the amended soils (including saturated and unsaturated hydraulic conductivity and water retention capacity) were improved, and, in most of the cases, the improvements were proportional to the application rates of the compost and they were greater in the loamy soil than in the clay soil. By contrast, Weber et al. (2007) demonstrated that MSW compost application increased soil water holding capacity and the amount of plant available water, but only in the short time. Moreover, many researches indicated that fertilization significantly influenced soil water content, because fertilization stimulates plant growth and thus use from plants of soil water (Ouattara et al., 2006; Ritchie and Johnson 1990; Song et al., 2010). In this study, no considerable differences were found among plots treated with or without mineral fertilizing, as well as no correlation was found between water content or water holding capacity and organic carbon content (tables 3.20 and 3.21).

Table 3.20 Matrix of Pearson correlation coefficients among soil physical (water content, water holding capacity), chemical (pH, available phosphorus, total and mineral nitrogen, organic carbon) and biological (microbial biomass carbon, microbial quotient, potential respiration, coefficient of endogenous mineralization, metabolic quotient, catabolic evenness) parameters measured in F1 plots.

WHC	0.129																						
pH	0.590***	0.008																					
P	0.089	0.281***	0.077																				
N	0.148*	0.057	-0.161*	-0.335***																			
NH <sub>4</sub> <sup>+</sup> -N	-0.433***	0.118	-0.394***	0.442***	-0.165*																		
NO <sub>3</sub> <sup>-</sup> -N	0.020	-0.394***	0.084	0.273***	-0.180*	0.139*																	
C <sub>org</sub>	0.134	-0.075	0.170*	0.478***	0.042	0.139*	0.573***																
C/N ratio	0.033	-0.104	0.212**	0.598***	-0.454***	0.220**	0.599***	0.865***															
C <sub>mic</sub>	0.091	0.018	0.066	-0.130	0.083	0.000	0.318***	0.165*	0.111														
MQ	0.020	0.083	-0.034	-0.312***	0.069	-0.048	0.038	-0.253***	-0.259***	0.877***													
RES	-0.416***	0.000	-0.333***	0.298***	0.043	0.621***	0.105	0.160*	0.136*	-0.029	-0.119												
CEM	-0.431***	-0.032	-0.385***	0.064	0.054	0.494***	-0.090	-0.186**	-0.177*	-0.109	-0.056	0.901***											
qCO <sub>2</sub>	-0.284***	0.027	-0.219**	0.143*	0.076	0.318***	-0.121	-0.045	-0.067	-0.539***	-0.528***	0.634***	0.679***										
CE	-0.283***	-0.457***	-0.248**	-0.145	-0.099	0.270***	0.238**	0.003	0.056	-0.122	-0.122	0.335***	0.374***	0.268**									
WC		WHC	pH	P	N	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	C <sub>org</sub>	C/N ratio	C <sub>mic</sub>	MQ	RES	CEM	qCO <sub>2</sub>									

\*\*\* =  $P < 0.001$ ; \*\* =  $0.001 < P < 0.01$ ; \* =  $0.01 < P < 0.05$ ; n = 210

Table 3.21 Matrix of Pearson correlation coefficients among soil physical (water content, water holding capacity), chemical (pH, available phosphorus, total and mineral nitrogen, organic carbon) and biological (microbial biomass carbon, microbial quotient, potential respiration, coefficient of endogenous mineralization, metabolic quotient, catabolic evenness) parameters measured in F2 plots.

<b>WHC</b>	-0.141*																										
<b>pH</b>	0.118	-0.226***																									
<b>P</b>	0.023	-0.253***	0.290***																								
<b>N</b>	0.179*	0.251***	-0.368***	-0.179*																							
<b>NH<sub>4</sub><sup>+</sup>-N</b>	0.577***	0.120	-0.167*	-0.133	0.373***																						
<b>NO<sub>3</sub><sup>-</sup>-N</b>	0.048	-0.143*	0.482***	0.334***	-0.485***	0.033																					
<b>C<sub>org</sub></b>	-0.050	0.021	0.333***	0.158*	-0.083	-0.171*	0.461***																				
<b>C/N ratio</b>	-0.142*	-0.150*	0.454***	0.213**	-0.717***	-0.350***	0.621***	0.730***																			
<b>C<sub>mic</sub></b>	0.079	0.191*	-0.227***	-0.122	0.250***	0.159*	-0.140*	0.137*	-0.092																		
<b>MQ</b>	0.107	0.205**	-0.312***	-0.178*	0.275***	0.209**	-0.257***	-0.135	-0.288***	0.953***																	
<b>RES</b>	0.536**	0.014	0.034	-0.161*	0.086	0.575***	0.201**	0.0875	0.055	0.140*	0.118																
<b>CEM</b>	0.548***	0.021	-0.085	-0.220**	0.130	0.626***	0.036	-0.146*	-0.153*	0.110	0.161*	0.955***															
<b>qCO<sub>2</sub></b>	0.340***	-0.095	0.079	-0.044	-0.067	0.339***	0.101	-0.122	0.008	-0.528***	-0.496***	0.470***	0.513***														
<b>CE</b>	0.231**	-0.002	-0.225*	-0.105	0.044	0.311***	0.111	0.008	0.006	0.054	0.064	0.360***	0.343***	0.171*													
<b>WC</b>		<b>WHC</b>	<b>pH</b>	<b>P</b>	<b>N</b>	<b>NH<sub>4</sub><sup>+</sup>-N</b>	<b>NO<sub>3</sub><sup>-</sup>-N</b>	<b>C<sub>org</sub></b>	<b>C/N ratio</b>	<b>C<sub>mic</sub></b>	<b>MQ</b>	<b>RES</b>	<b>CEM</b>	<b>qCO<sub>2</sub></b>													

\*\*\* = P<0.001; \*\* = 0.001<P<0.01; \* = 0.01<P<0.05; n = 210

Soil pH affects organic C solubility (Andersson et al., 2000) and increases the availability of biologically toxic heavy metals, affecting in turn microbial community structure (Anderson, 1998; Zelles, 1999; Marstorp et al., 2000) and microbial activity (Bååth and Anderson, 2003). Wardle (1992) concluded that soil pH is probably at least as important as soil C and N concentrations in influencing the size of the microbial biomass. However, in this study the addition of organic and mineral fertilizers caused only a slight increase in pH of F1 and F2 soils, and soil pH was positively correlated with organic carbon content in both farms (tables 3.20 and 3.21).

Inorganic and organic P fractions can act as source or sink of soluble P for the soil solution, depending on soil mineralogy, environmental conditions, fertilizer use and management system (Novais and Smyth, 1999). In natural ecosystems, where there is no P addition, availability is closely related with the cycle of organic P forms. Changes can be induced in the system through introduction of new plant species or increases in biomass yield and fertilization, which results in increased microorganism activity and mineralization rate (Condrón et al., 1985). However, when fertilizer is applied, all inorganic P fractions are increased and this effect is more important for labile and moderately labile forms, which are usually responsible for P buffering in soil solution (Gatiboni et al., 2007). In studied soils, available phosphorus content did not show remarkable increases in plots treated with different types and doses of organic amendment, but a slight decreasing trend in available P was found in the 1<sup>st</sup> sampling for both farms, probably linked to a “dilution effect” due to the compost-wood addition to the soil upper layer. This effect was probably counterbalanced in time by microorganism activity, and then in the final sampling values measured in amended plot did not differ from that measured in control plot, but F1 soil showed an increase in available P in amended plots treated also with mineral fertilizing. However, in all other samplings, no remarkable effect was found due to mineral fertilizing. Available P content, in both soils, was positively correlated with organic carbon content (tables 3.20 and 3.21).

Knowledge of the short and long term availability of N following organic amendment and mineral fertilizer application is essential in order to meet crop requirements and ensuring environmental protection from excessive nitrate leaching. Increasing the N use efficiency of organic amendments and understanding the N dynamics in compost amended soils remain important issues for research (Amlinger et al., 2003; Gutser et al., 2005). Long-term field experiments provide direct observations of changes in soil organic carbon storage and N balance over the decades that are critical for predictions of future soil productivity and soil-environment interactions (Richter et al., 2007). In this study, after 2 successive additions of organic amendments, with different types (i.e. C/N ratio) and amounts, and mineral fertilizer, an increasing trend of total N content was found in both farms. It has to be underlined that average values of total N in both farms (4.13 and 3.90 g kg<sup>-1</sup>, F1 and F2 soils respectively) were higher than the normal content of an agricultural soil (0.7 to 2 g kg<sup>-1</sup>) (Sequi, 2005), whereas in treated plots these values were, on average, 52% (F1) and 38% (F2) higher than respective control plots. Differently from chemical fertilization that determine a rapid N release, organic amendments determine a slow N release extended over time (Claassen and Carey, 2006), due to mineralization process. In fact, the organic portion of total N (not readily available for plants) can be mineralized, and then potentially taken up by the plants, immobilized, denitrified, volatilized, fixed within the clay minerals and/or leached (Kokkora, 2008). Notwithstanding, N dynamics in compost amended soils is influenced by various factors related to compost parameters, climatic conditions, crop types, soil properties and soil management practices (Amlinger et al., 2003). In studied soils, organic amendments caused an increase of total N and a slow release of mineral N from organic matter, sufficient to increase in time nitric N content in soils, in spite of the six crop cycles and leaching processes occurred during the experimental time. Stable linear trend in total N is particularly important for agricultural ecosystems, because N mineralization potentially increases N losses through leaching and

gaseous emissions (Malhi and Lemke, 2007). This study showed that a more marked and lasting effect of organic amendments on total nitrogen content of soils (quite apart from addition of mineral fertilizers) can be obtained by repeated additions of organic amendments, although N content in control plots decreased in time, probably due to absence of an additional mineral fertilization in the studied soils of farms. However, the increase in total nitrogen content had slight or no effect on ammoniacal N content in soils, that increased only in some plots and sampling times, with no clear trend, but caused the increase of nitric nitrogen in the final sampling. Negative correlations were found between nitric and total N contents (tables 3.20 and 3.21), showing that a decrease in total N corresponded to an increase in nitric N. It has also to highlight that in 3<sup>rd</sup> and 4<sup>th</sup> samplings, both farms showed remarkably lower values of NO<sub>3</sub><sup>-</sup>-N content compared to other sampling times, which may be the result of a seasonal fluctuation of this parameter, considering that 3<sup>rd</sup> and 4<sup>th</sup> samplings were carried out in autumn and winter. On the contrary, Carfora, (2008) found that in a mediterranean soil of southern Italy net nitrogen mineralization rate was significantly influenced by the sampling date, showing a strong seasonal trend with higher rates in autumn and winter, intermediate values in spring and lower rates in summer and spring. However, because mineralization and nitrification processes are strongly influenced by supplying organic substances, pH, temperature and moisture (Nobela, 2011), data suggest that in studied soils nitrification process was minimized in autumn and winter, probably due to the low temperatures.

Organic matter content is universally recognized as usefull indicator of soil quality under agricultural management practices (Goyal et al., 1999; Simek et al., 1999; Juan et al., 2008). In particular, total organic C content is considered a stable parameter compared to labile C, as light C fractions or microbial biomass (Haynes, 2000). In fact, several years of different land use are required to detect significant changes in the total pool of soil organic carbon (Gregorich et al., 1994). Our results showed a prompt and lasting increase in soil organic carbon after amendment, with



more marked effects due to the second addition. However, the increase of this parameter immediately after first organic amendment addition was not always significant, probably due to no heterogeneous distribution of amendments on soils that corresponded to a high variability of data measurements. Similarly, the significant increase, in both farms, of this parameter measured immediately after second organic amendment addition was lower than increases measured in following sampling times. It has to be emphasized that no considerable effect was due to doses or types of amendments, because the lowest amount of A1 mixture (with the lowest C/N ratio) already led to significant advantages in terms of organic matter recovery, but more marked and positive effects were found in F1 soil, showing the lowest starting values of organic carbon and C/N ratio. Therefore, our results allow to hypothesize that, in the studied area of southern Italy affected by high-intensity agricultural management for a long time, the sole annual addition of organic amendments, similar to A1 mixture and supplied with 30 t ha<sup>-1</sup> amount, could be sufficient to recovery and preserve soil quality. However, the clay nature of soil from farm 1 had probably an important role in this recovery of organic carbon, because clay particles are involved in biophysical and chemical processes of C stabilization (Christensen, 1996) by forming organo-mineral complexes that protect soil organic matter, delaying its mineralization. Moreover, in soils with clay texture, the low porosity within soil aggregates makes less aired and not accessible environment for microorganisms, slowing down microbial activity and organic matter decomposition (Bossio et al., 1998; Cookson et al., 2005; Jarvis et al., 1996; Lunquist et al., 1999; Schulten and Leinweber, 2000). On the other hand, the sandy loam nature of soil from farm 2 favoured oxygenation of edaphic environment, stimulating microbial activity and growth, contributing to enhance humification and mineralization processes (Christensen, 1996; Feller and Beare, 1997; Hassink, 1995) and favouring release of nutrients.

In the studied soils, the clay-loam soil of farm 1 showed lower value of C/N ratio compared to the sandy-loam soil of farm 2 (2.5 and 4.1, respectively). It has to be

underlined that the values of total nitrogen measured in soils of both farms were amazing high for agricultural soils and, consequently, C/N ratio values were extremely low. This last factor can affect negatively organic carbon stock in soil by favoring microbial growth and activity and, consequently, a quick mineralization of soil organic matter. Therefore, in agricultural areas, additional input of organic matter with high C/N ratio, like wood, could counterbalance input of mineral nitrogen due to chemical fertilization. When organic matter is incorporated into soil, microorganisms start to decompose it, releasing nutrients that can be used by bacteria and fungi, and, if they are in excess, also by other soil organisms, as plants (Borken et al., 2002). In this study, experimental design provided for addition of compost-wood mixtures in order to have organic amendments with a high C/N ratio, more resistant to decomposition. Notwithstanding, the resultant values of C/N ratio in soils of both farms were still too low to cause a nitrogen deficiency risk for plants or microbes. However, in both farms, C/N ratio values showed an increasing trend in amended plots, with more marked effects in F1 soil, but no remarkable effect was due to mineral fertilizing treatment and to different doses or types of amendments. The C/N values in both farms were strongly positively correlated with organic carbon content in soil (tables 3.20 and 3.21).

The organic matter supply in soil causes changes in soil physical and chemical characteristics, affecting in turn soil biochemical and biological properties. Among these, soil microbial growth and activity are considered quick and sensitive indicators of soil quality (Insam & Domsch, 1988; Powlson and Jenkinson 1976; Sparling, 1992). After Powlson and Jenkinson (1976) the microbial biomass is a very sensitive indicator of variations in soil status. In this study, microbial biomass turned out generally to be a suitable indicator of soil quality, affected positively by organic amendment addition. Mean values of microbial biomass carbon in amended soils (0.28 and 0.29 mg g<sup>-1</sup> in F1 and F2 soil, respectively) were clearly higher than mean values measured in other agricultural soils of the Sele River Plane (0,15 mg g<sup>-1</sup>; Bonanomi et al., 2011), under intensive cultivation, and in

other agricultural soils of the Campania Region (Maddaloni, Caserta), under non-intensive cultivation (Marzaioli et al., 2010). Moreover, microbial biomass was generally higher in amended than in control plots, but few results were statistically significant due to the high variability of data. The increase in microbial biomass was more pronounced and lasting in time in amended soil of the farm 2 than farm 1, although values measured in control plots of both farms were comparable. This effect probably depended on the higher starting content of organic carbon in soil of the farm 2 compared to the farm 1, determining conditions more favourable for microbial growth and, generally, for soil biological properties. However, some changes were also found among sampling times that could be explained by seasonal effect. In fact samplings were carried out in different seasons, and in particular 3<sup>rd</sup> and 6<sup>th</sup> in autumn, 4<sup>th</sup> and 7<sup>th</sup> in winter, when soil biological growth and activity is reduced (McGill et al., 1986; Ros et al., 2003) compared to the spring and summer seasons (1<sup>st</sup>, 2<sup>nd</sup> and 5<sup>th</sup> samplings).

Some authors (Insam & Domsch, 1988; Sparling, 1992) have suggested that microbial quotient ( $C_{mic}/C_{org}$  ratio) suits better to point out changes in soil processes than organic carbon or microbial biomass separately. Consequently, use of the quotient avoids the problems of comparing trends in soils with different organic matter or microbial biomass content (Sparling, 1997) and appears to provide more sensitive indications of soil changes than biomass measurements alone (Anderson, 2003; Brookes, 1995; Dilly and Munch, 1998). The ( $C_{mic}/C_{org}$ ) index could be used as a stability indicator for quick recognition of soil ecological changes, for instance, to predict long term trends in soil organic matter and monitor land degradation or restoration (Anderson, 2003; Anderson and Domsch 1989; Hart et al. 1989; Ross et al. 1982; Sparling, 1992). However, the microbial quotient is affected by clay content, mineralogy, organic matter, vegetation and management history (Anderson, 2003). In studied amended plots an increasing trend in microbial quotient was found, but more marked increases were found in F2 soil. This trend indicates larger substrate availability for the soil

microorganisms (Anderson, 2003) and a positive trend for organic C accumulation in the intensive farming systems due to the easily available carbon fraction (Marinari et al., 2006), supporting the hypothesis of Anderson and Domsch (1986) that a larger ratio imply an increased availability of fresh substrates.

Respiration activity is an indicator of the soil metabolism (Šantrůčková, 1993; Tesařová and Gloser, 1976). Soil microbial community is able to respond quickly to changes in environmental conditions and its response can generally be measured as an increase or decrease in total microbial activity, i.e. soil respiration. In fact, changes in microbial biomass can affect directly soil respiration rate, but soil activity can also change in response to variations of a large number of environmental factors, as soil organic matter and nutrient content, temperature, water content (Alvarez et al., 1995; Brookes, 1995; Orchard & Cook, 1983), pH, soil type, plant type and anthropogenic disturbance (Balogh et al., 2011; Boone et al., 1998; Conant et al., 2004; Hanson et al. 2000; Knorr et al. 2005; Li et al., 2008; Luo & Zhou 2006; Ma et al., 2005; Olsson et al. 2005; Wu et al., 2010) and presence of some contaminants (Crecchio et al. 2004; García-Gil et al., 2000; Marcote et al., 2001; Ros et al., 2003). In this study, addition of slow-degradable organic fertilizers caused generally a prompt increase in soil potential respiration, but more marked effects are found in plots of F1 soil treated also with mineral fertilizer, whereas a higher effect on F2 amended plots was found after the second addition. Mean value of potential respiration in amended plots of the F1 soil ( $36.41 \mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) was clearly higher than value measured in amended plots of the F2 soil ( $24.75 \mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ ), in soils under intensive cultivation of the Sele River Plane ( $26.2 \mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ , Bonanomi et al, 2011) and in other agricultural soils of the Campania Region (Maddaloni, Caserta) under non-intensive cultivation (Marzaioli et al., 2010). Increases in microbial activity were recognised by Fresquez and Lindemann (1982) in organic-amended soils of the USA. Moreover, increases in soil respiration and enzyme activities after organic amendment, in Mediterranean soils, have been widely reported (Bastida et al., 2008; Crecchio et

al., 2004; García-Gil et al., 2000; Pascual et al., 1999; Perucci, 1992; Ros et al., 2003). It has to be emphasized that increases in soil respiration can be negatively related to soil quality and indicate stress or disturbance conditions in ecosystem (Islan and Weil 2000; Růžek et al., 2006). In fact, a higher soil respiration rate can be related to a growth of microbial community, but also indicates a high biological activity that causes a more rapid decomposition process. Therefore an increase in respiration rate non counterbalanced, at the same time, by an adequate microbial growth, corresponds to a predominance of catabolic processes over anabolic ones and can mean an increase in carbon mineralization process with a loss of organic matter from soil. Our results showed that soil potential respiration in F1 soil was also positively correlated to organic carbon, but was not correlated with microbial biomass carbon; on the contrary respiration in F2 soil was positively correlated with microbial biomass, but not correlated with organic carbon (tables 3.20 and 3.21). To better understand the trend of biological activity in studied soils, two indices were also calculated: metabolic quotient ( $qCO_2$ ) and coefficient of endogenous mineralization (CEM).

The metabolic quotient ( $qCO_2$ ) represents the respiration rate per unit of microbial biomass ( $qCO_2 = mg\ C-CO_2\ g^{-1}\ C_{mic}\ h^{-1}$ ; Anderson & Domsch, 1993) and reflects the maintenance energy requirement for soil microbes (Anderson, 2003) and can be a relative measure of how efficient soil microbial biomass is to utilize C resources, as well as the degree of substrate limitation for soil microbes (Wardle and Ghani, 1995; Dilly and Munch, 1998). Additionally, as reported by Odum (1969) in the “*The Strategy of Ecosystem Development*”, in the course of an ecological succession, we find, within a younger ecosystem, less competition for resources to assimilate, and this determines the presence of organisms with lower energy efficiency, whereas, when in more mature stages of an ecosystem there is a greater competition for resources and this leads to a greater selective pressure that favors individuals with higher energy efficiency (Insam & Haselwandter, 1989; Odum, 1971). So, in the case of edaphic communities, during a succession, respiration rate

per unit of biomass tends to decrease and then, in a mature ecosystem, we will find a greater amount of microbial carbon and a lower rate of respiration. In fact, Insam and Haselwandter (1989) found a reductional trend with time of this ratio tested by studying two primary successions on recessional moraines. Killham (1985) and Killham and Firestone(1984) showed that soil microorganisms divert more energy from growth into maintenance as stress increases and thus the metabolic quotient can be a much more sensitive indicator of stress than respiration alone. The  $qCO_2$  has been widely applied in the assessment of the effect on soil due to cultivation regime (Anderson and Domsch, 1990), pollution gradients (Ohtonen,1994), temperature (Anderson and Domsch, 2010; Anderson and Gray, 1991), forest ecosystems (Anderson and Domsch, 1993) and acidification (Wolters, 1991). However, Wardle and Ghani (1995) have questioned the use of  $qCO_2$  as a bioindicator, because it failed to distinguish between effects of disturbance and stress but several authors have observed increases in the  $qCO_2$  after disturbance (e.g. Fritze et al., 1994; Sawada et al., 2009; Xu et al., 2007). In studied soils, metabolic quotient showed an increasing trend in some amended plots of F1 soil only in which the mean value of this index ( $51.71 \mu g C_{CO_2} g^{-1} C_{mic} h^{-1}$ ) was 45% higher than the mean value calculated for F2 soil ( $35.42 \mu g C_{CO_2} g^{-1} C_{mic} h^{-1}$ ). Moreover, mean value calculated for F1 soil was lower than that measured in soils from other farms of the Sele River Plain under intensive cultivation ( $78.8 \mu g C_{CO_2} g^{-1} C_{mic} h^{-1}$ , Bonanomi et al., 2011). Gupta, et al., 1994, reported changes in  $qCO_2$  after enrichment of the soil with crop residues. Fließbach and Mäder, (1997), comparing a soil with organic farming with a soil under traditional agricultural system, found lower values of  $qCO_2$  in soils with organic farming, indicating a higher efficiency of microbial populations and suggesting better environmental conditions. Moreover, according with our results, Hu et al., (2011) showed that long-term fertilization had significant effects on soil microbial functional diversity and metabolic quotient, whereas, organic amendments could affect microbial parameters in different ways from mineral fertilizers and could play a greater role

in decreasing soil metabolic quotient. After Anderson (2003) above  $2.0 \text{ mg C}_{\text{CO}_2} \text{ g}^{-1} \text{ C}_{\text{mic}} \text{ h}^{-1}$  there is a critical threshold for the “baseline performance” of microbial community, but in this study values are very lower than the threshold value.

The coefficient of endogenous mineralization (CEM) is the respiration rate per unit of microbial biomass (expressed as  $\text{mg C}_{\text{CO}_2} \text{ g}^{-1} \text{ C}_{\text{org}} \text{ h}^{-1}$ ). It represents the fraction of organic carbon mineralized in the unit of time and can provide useful information on the potential ability of soil carbon to be easily decomposed or accumulated in soil (in fact, a greater presence of labile fraction results in a greater increase in the rate of mineralization).

In this study, coefficient of mineralization quickly increased after addition of organic amendments in F1 soil, by contrast in F2 soil this index increased only in one plot of the 2<sup>nd</sup> sampling. Moreover, mean value of coefficient of mineralization in F1 soil ( $0.68 \text{ mg C}_{\text{CO}_2} \text{ g}^{-1} \text{ C}_{\text{org}} \text{ h}^{-1}$ ) was double than that in F2 soil ( $0.34 \text{ mg C}_{\text{CO}_2} \text{ g}^{-1} \text{ C}_{\text{org}} \text{ h}^{-1}$ ) and higher than mean values measured in soils under intensive cultivation of the Sele River Planes ( $0.60 \text{ mg C}_{\text{CO}_2} \text{ g}^{-1} \text{ C}_{\text{org}} \text{ h}^{-1}$ , Bonanomi et al, 2011) and in other agricultural soils of the Campania Region (Maddaloni, Caserta) under non-intensive cultivation (Marzaioli et al., 2010). However, CEM values in soils of both farms were positively correlated with metabolic quotient, showing that in plots where there is a microbial community with lower energy efficiency there is also a higher rate of mineralization. Pascual et al. (1998) found that, after the addition of sewage, solid waste and compost and incubation of soils, the CEM was significantly higher in the treated soils compared to the untreated control soil, although the extent of its variation was dependent on the nature of the amendment, in fact the treated soil with fresh solid waste showed higher values of CEM compared to the treated soil with compost. Moreover, increases in CEM values were found after stress or disturbance in soils, such as fire and crop rotation (Gijssman et al., 1997; Rutigliano et al., 2002; Rutigliano et al., 2004; De Marco et al., 2005).

In conclusion, the quickly and lasting increase in respiration,  $q\text{CO}_2$  and CEM in amended plots of the F1 soil indicated an acceleration of mineralisation process, with a more marked tendency to lose organic carbon from soil, and a microbial community characterized by a less-fully development. All these effects were probably related to the lower starting content of organic carbon and, especially, to the lower value of C/N ratio in soil of the farm 1 compared with soil of farm 2, even if influence of further environmental factors, not analyzed in this study, cannot be excluded.

It has been hypothesized that soil microbial diversity is key tool for the maintenance of soil processes (Giller et al., 1997) and is a good indicator of soil resilience to stress or disturbance (Degens et al 2001). In this study, we hypothesized that functional diversity of the soil microbial community could be positively affected by organic matter addition. Really, catabolic respons profiles of the soil microbial community were not greatly affected by the used compost-wood mixtures, at the tested dosed, even after long term addition. In this respect, we can suppose that application of organic amendments, providing additional resources for microbes, did not greatly stimulate competition among microbial populations and thus clear changes in soil functional diversity. However, after treatments a general increase in the respiration responses was found and a certain number of respiration responses significantly differed from controls, with a percentage of changed responses, in amended plots compared to control, ranging from 8% to 56%, and more marked effects, for F1 soil, in plots treated with mineral fertilizer. Moreover, catabolic respons profiles of studied soils were also affected by sampling times, with a percentage variation of changed responses, in all plots compared to each corresponding plot in 1<sup>st</sup> sampling, ranging from 4% to 60%. This effect was probably due to changes in environmental conditions occurred in time and to the different crop types, all factors having a great impact on microbial functional diversity (Kennedy and Stubbs, 2006). In fact, soil microbial community can be strongly influenced by plant species and degree of cropping intensity, because



plants can be a selective force for microbial populations of the rhizosphere through their influences on exudation patterns (Meharg and Killham, 1995) and soil nutrients (Jensen and Nybroe, 1999; Pennanen et al., 1999), and the composition of the plant community may drive the composition of the soil microbial community (Achouak et al., 2000; Minarnisawa et al., 1999; Westover et al., 1997).

It has been established that microbial catabolic diversity in soils was highly linked with organic C pools and decreases in catabolic evenness values have also been related to decreases in organic carbon availability (Degens et al., 2000; Schipper et al., 2001). Catabolic diversity depended on both richness and evenness of the use of substrates (Zak et al., 1994; Degens et al., 2000). In our case richness was not affected by the addition of organic amendments because all used substrates were metabolized, whereas no relevant effect of organic amendment was found on catabolic evenness index that was not correlated with soil content of organic carbon. However, others authors did not find any correlation between organic carbon and catabolic evenness (D'Ascoli et al., 2005; Lalor, et al., 2007; Shillam, 2008).

Finally, the principal component analysis applied to the whole set of data (Figs. 3.37 and 3.38, respectively), summarizing our results, showed in F1 soil a clear distance among treated plots and control plots, in fact a lot of control plots is grouped in bottom on the left of the axes, but a clear distance was also found among sampling times, with 1<sup>st</sup> and 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> samplings grouped together, respectively. Moreover, axis 1 (i.e. factor 1) was strongly positively correlated with ammoniacal N, soil respiration, CEM, qCO<sub>2</sub>, and negatively correlated to soil pH, whereas axis 2 (i.e. factor 2) was strongly positively correlated with available P, nitric N, organic carbon, C/N ratio and microbial biomass carbon too.

In F2 soil a clear distance among treated plots and control plots was found in all samplings, except for the 1<sup>st</sup> sampling, and a great clear distance was found among sampling times, with 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> samplings, and 5<sup>th</sup>, 6<sup>th</sup> samplings grouped

together, respectively; moreover 1<sup>st</sup> and 7<sup>th</sup> samplings was clearly separated and definitely cutted off from the other samplings. Axis 1 (i.e. factor 1) was strongly positively correlated with soil pH, available P, nitric N, organic carbon, C/N ratio, but negatively correlated to total N, whereas axis 2 (i.e. factor 2) was strongly positively correlated with ammoniacal N, soil respiration, CEM and qCO<sub>2</sub>.

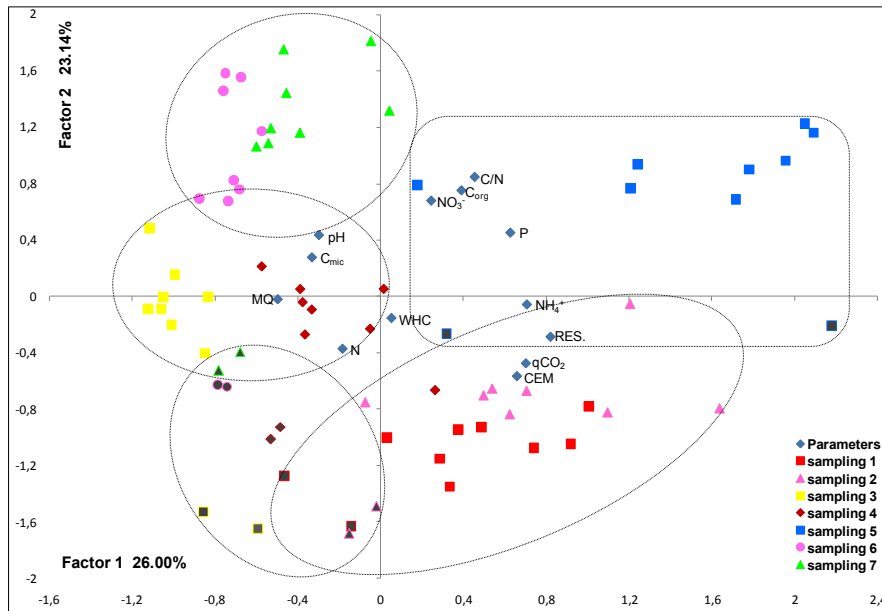


Fig. 3.37 Biplot of analysed soil properties in F1 soil. Control plots have grey colour (a lot of them is enclosed in the circle in bottom on the left).

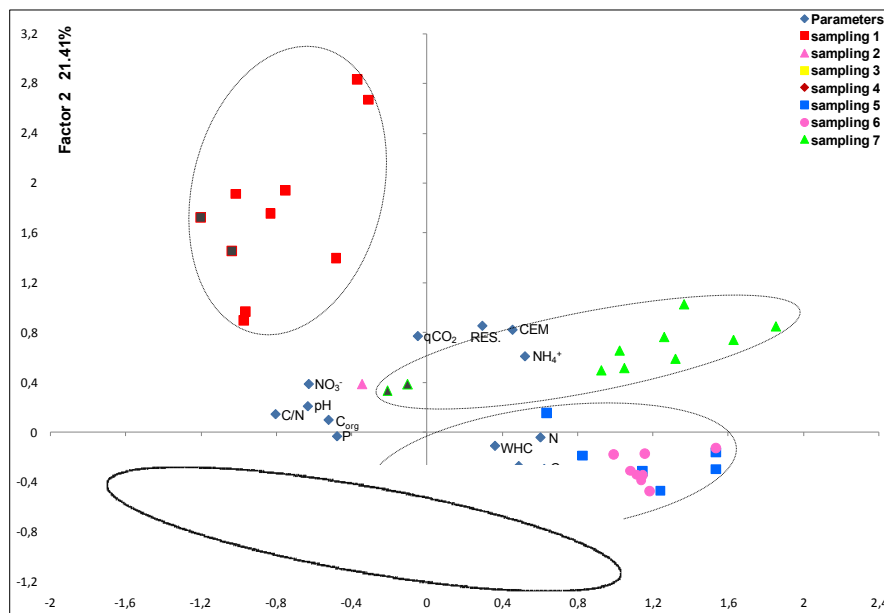


Fig. 3.38 Biplot of analysed soil properties in F2 soil. Control plots have grey colour.

### **3.5. Conclusions**

This study showed that successive applications of slow-degradable organic amendments (compost and wood mixtures) had positive effects, on the long term, on chemical, biochemical and biological properties of the studied soils, affected for long time by intensive agricultural management under permanent plastic cover.

In particular, a significant increase in organic C, total and nitric N, C/N ratio and in microbial activity and growth was generally found in amended plots compared to control, with more marked and lasting effects after treatment repetition.

Use of organic amendments also increased soil respiration response to addition of simple organic compounds, although did not greatly affect soil functional diversity, as assessed by catabolic response profile and catabolic evenness. In this respect, it can be supposed that application of organic amendments, providing additional resources for microbial populations, did not greatly stimulate competition among these populations and thus clear changes in soil functional diversity.

No considerable effect was generally observed, on studied soils, due to the tested doses or types of organic amendment mixtures. In fact, use of the lowest amount of A1 mixture (with the lowest C/N ratio) already led to significant advantages, in particular in terms of organic matter recovery and improvement in soil biological properties. Moreover, additional use of a mineral fertilizer did not affect greatly soil properties, except for microbial activity. These results allow us to hypothesize that, in the studied area of southern Italy affected by high-intensity agricultural management for a long time, the sole annual addition of organic amendments, similar to A1 mixture and supplied with 30 t ha<sup>-1</sup> amount, could be sufficient to recovery and preserve soil quality.

Although, at tested doses, it was not possible to discriminate among the used amendments, it has also to be emphasized that tested amendments caused more marked positive effects on biological properties of the soil with the highest starting values of organic carbon and C/N ratio (i.e. soil of farm 2), indicating a key role of

organic matter content also in promoting soil recovery by sustainable agricultural practices, by a positive feedback mechanism.

In conclusion, all together data substantiated the hypothesis that soil management practices including use of organic amendments, quite apart from addition of mineral fertilizers, are a key tool to maintaining, in time, soil quality and sustainability in intensive agricultural systems.

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## **Chapter 4**

# **Effect of sludge addition on bacterial community structure and chemical/biochemical properties of a Chilean volcanic soil**

### **4.1 Introduction**

Over 370 million tonnes of paper have produced worldwide in 2009/2010 years from the pulp and paper industry, whose demand is continuing to increase (i.e., in Europe in 2009 production rate increased 8.4% and consumption rate increased 9.3%; CEPI, 2011). During the various stages of cellulose processing, in the pulp and paper industry, a large amount of sludge residues (approximate, 60 m<sup>3</sup> per ton of paper) are produced as a by-product (Thompson et al., 2001). The production of solid waste generates around 45% wastewater sludge (0.2-1.2 kg dry matter per kg of biological oxygen demand removed), 25% ash, 15% wood cuttings and waste, and 15% other solid waste (Zambrano et al., 2003; Gallardo et al., 2010a). For this reason, paper pulp sludge can be considered as one of the serious environmental threats (Oral et al., 2005) and as a significant taxpayer of discharge of pollutants to the environment that needs to be solved (Amat et al., 2005; Savant et al., 2006; Natajarat et al., 2007; Wang et al., 2007; Ramos et al., 2009). Environmental problems, connected with this waste, have not been solved satisfactorily in the past, leading to an unsatisfactory situation in many countries (Oral et al., 2005). For example, in Chile, a country with emerging pulp and paper mill production, where cellulose exports grew by 50% in the last decade and the current production reaches 3.0 million tonnes per year (Espinosa, 2002), the high production of pulp and paper as well as broader application of activated sludge have amplified sludge

management problems and increased stringent environmental regulations of pulp sludge production. Environmental regulations, as in Europe and other American countries, prohibiting the landfilling of organic waste have led to significant reductions in this practice since 1990 (Blanco et al., 2004; Fraser et al., 2009). It has to be emphasize that pulp mill sludge has a high potential as source of organic carbon (including cellulose and lignin), microorganisms and inorganic substances (N, P, K, S, B, Mn), as widely reported in literature (Nkana et al., 1999; Gagnon et al., 2000; Foley and Cooperband, 2002; Jordan and Rodriguez, 2004; Zhang et al., 2004), but also contains low concentrations of trace metals (Cd, Cr, Cu, Ni, Pb, Zn and Al) and organic pollutants (Gagnon et al., 2000; Gallardo et al., 2010a). Therefore, application of this type of sludge, at recommended rates and properly managed in agricultural land as a partial substitute of chemical fertilizers, is considered an environmentally friendly disposal (Snyman et al., 1998; Gallardo et al., 2007). In fact, the controlled disposal of sludge in soils partially depletes soil acidification (Zambrano et al., 2003; Aravena et al. 2007; Gallardo et al., 2007), enhances nutrient transportation, water-holding capacity and cation exchange capacity (Andrade et al., 2000), as well as structure and texture (Barral et al., 2009), reduces erosion, improving soil quality consequently.

Soil microorganisms have long been documented as sensible indicators in soil quality assessment, because of their abundant distribution and metabolic activities which are determined by nutritional and other soil physical-chemical conditions (Bossio et al., 1998; Buyer et al., 1999; Girvan et al., 2003; Fierer and Jackson, 2006; Singh et al., 2007). Moreover, they show a prompt response to anthropogenic changes (Sparling et al., 2004; Araujo and Monteiro, 2007; Truu et al., 2008) and are involved in redox and immobilization processes of mineral elements as well as in mineralization of organic matter in soil (Chander and Brookes, 1993). Understanding the changes in soil enzyme activities as well as in microbial community structure and composition, following application of organic amendments, can lead to expansion of healthier soil management practices

(Dolfing et al., 2004). In fact, changes in soil bacterial abundance and community structure have consequences for nutrient cycling, C-sequestration and long-term sustainability. There is a overwhelming evidence that culture-independent molecular approaches using the 16S rDNA has provided major insights into species and functional diversity of bacterial populations in soils under different management practices (Nannipieri et al., 2003; Prosser, 2002). Additionally this technique has been used to distinguish microbial community composition (Muyzer et al., 1993), to screen population shifts (Ferris and Ward, 1997), and to follow the succession of bacterial populations over time (Simpson et al., 2000).

Several studies have been carried out on the short term effects due to the pulp mill sludge addition on soil physical, chemical and biochemical properties and plant production (Cabral and Vasconcelos, 1993; Thompson et al., 2001; Gilbridea et al., 2006; Nunes et al., 2007; Gallardo et al., 2010a; Gallardo et al., 2010b; Torkashvand, 2010), but there is lacking in information on the long term effects due to addition of this type of sludge on soil biochemical/biological properties. To assist it as effective and safe means of soil amendment, it is necessary to evaluate the potential impact or benefit of pulp mill sludge on both soil chemical and biochemical/biological properties on the long term. In particular, the complete description of the dynamic succession of the bacterial populations in response to sludge amendment has yet to be evaluated. This work extends the short term studies previously carried out by Gallardo et al. (2010a) and presents novel findings, by investigating the changes in soil chemical and biochemical/biological properties after pulp mill sludge amendment, in order to bridge the gap in understanding the long term effects of this type of organic amendment on soil.

In particular, this study aimed to investigate, on an annual period, the effects of pulp mill sludge application on bacterial community structure (assessed by PCR-DGGE, 16rDNA gene) in a volcanic soil of southern Chile. Moreover, to test a more completed data set, some chemical properties of soil and used pulp mill sludge (i.e., organic matter, total N, available P, pH, exchangeable K, Na, Ca, Mg

and available Zn and Mn) and some enzymatic activity (i.e., FDA-hydrolase and acid phosphatase) were also reported (unpublished data from Gallardo et al.).

This study was performed, during the period of Ph.D. doctorate foreign, at the Scientific and Technological Bioresource Nucleus, Universidad de La Frontera, Temuco, Chile, under the supervision of Prof. Milko A. Jorquera, and was funded by FONDECYT no.1080427, 1100625 and 11080159.

## **4.2 Materials and methods**

### *4.2.1 Study site and experimental design*

The field study was carried out in an experimental farm of the Universidad de La Frontera, Temuco, an area of southern Chile (38° 42' S, 73° 35' W) during August 2009–November 2010. Climatic condition of this location is predominantly temperate, with a mean monthly temperature ranging between 16 °C in January and 7 °C in August and a mean annual rainfall ranging from 185 mm, mainly distributed in winter, to <50 mm, in spring and summer (average 8 years of observation from local metrological station). The soil was a volcanic soil (Andisol) and in the experimental field *Lolium perenne* grass was cultivated, as common in this area, and ryegrass pasture was established as described by Mora et al. (2002). The experimental field was divided into 12 subplots (6×3 m) in a factorial array, in order to have 3 field replicates for each treatment and 3 untreated subplot used as control, in a randomized complete block design. In particular, pulp mill sludge was added at the rate of 10, 20 and 30 t ha<sup>-1</sup>, manually incorporated into the soil and mixed throughout the upper 10 cm. During the whole experimental period (15 months), five successive applications of sludge have been carried out, with an interval of three months.

### *4.2.2 Pulp sludge collection and preparation*

The sludge was collected from a biological wastewater treatment plant of a pulp and paper industry. During cellulose process, sludge is produced as primary and secondary by-products. In this study, secondary sludge from the bleached pulp mill wastewater treatment plant was used. It was collected from a landfill after one year disposal, because in this condition the sludge becomes naturally stable and fulfills the requirements of the Chilean Normative (Gallardo et al., 2010a). The chemical

characteristics of sludge after stabilization and the chemical properties of the Temuco soil, before treatments, are shown in Table 4.1.

Table 4.1 Chemical characterization of Temuco volcanic soil and pulp mill sludge at the beginning of the experiment. Results are the means of three replicates (Sludge characteristics from Gallardo et al., 2010a).

Parameters	Unit	Soil	Sludge
Nitrogen (N-NH <sub>4</sub> <sup>+</sup> + N-NO <sub>3</sub> <sup>-</sup> ) <sup>a</sup>	mg kg <sup>-1</sup>	19.12	586.00
Posphorous <sup>a</sup>	mg kg <sup>-1</sup>	17.50	313.00
pH		5.74	6.97
Organic Carbon	%	6.09	41.12
Organic Matter	%	10.50	76.07
Sodium <sup>a</sup>	cmol <sup>+</sup> kg <sup>-1</sup>	0.08	41.55
Calcium <sup>a</sup>	cmol <sup>+</sup> kg <sup>-1</sup>	3.50	27.95
Magnesium <sup>a</sup>	cmol <sup>+</sup> kg <sup>-1</sup>	0.92	13.68
Potassium <sup>a</sup>	cmol <sup>+</sup> kg <sup>-1</sup>	0.89	3.62
Aluminum <sup>a</sup>	cmol <sup>+</sup> kg <sup>-1</sup>	0.07	0.03
CEC	cmol <sup>+</sup> kg <sup>-1</sup>	8.82	86.83
Aluminium saturation	%	0.78	0.04
Zinc <sup>a</sup>	mg kg <sup>-1</sup>	0.83	376.30
Manganese <sup>a</sup>	mg kg <sup>-1</sup>	11.62	111.05
Copper <sup>a</sup>	mg kg <sup>-1</sup>	3.29	5.04
Iron <sup>a</sup>	mg kg <sup>-1</sup>	29.72	18.47
Cadmium <sup>b</sup>	mg kg <sup>-1</sup>	--	1.77
Chromium <sup>b</sup>	mg kg <sup>-1</sup>	--	22.25
Nickel <sup>b</sup>	mg kg <sup>-1</sup>	--	26.30

Organic matter = Organic carbon × 1.724 factor.

CEC =Cation exchange capacity (Σ Ca, Mg, K, Na).

Aluminium saturation (%) = [Al / (Σ Ca, Mg, K, Na and Al) × 100].

<sup>a</sup> Available elements.

<sup>b</sup> Total elements.

#### 4.2.3 Soil sampling and storage

Soil samples were collected in all subplots, five times over all the study period. The first sampling was carried out after one month from the sludge application whereas the following four samplings were carried out at once before each sludge application (see Table 4.2). Fresh soil samples were collected from the soil surface



layer to 20 cm depth, sieved at 2 mm mesh, air dried at room temperature for chemical analyses, or stored in plastic bags at 5 °C and -20 °C for biochemical assays or DGGE analysis, respectively.

**Table 4.2** Time schedule of the study project

<b>Scheme</b>	<b>Time duration</b>
1 <sup>st</sup> amendment addition	06 August 2009
1 <sup>st</sup> sampling	08 September 2009
2 <sup>nd</sup> amendment addition	05 November 2009
2 <sup>nd</sup> sampling	05 February 2010
3 <sup>rd</sup> amendment addition	05 February 2010
3 <sup>rd</sup> sampling	11 May 2010
4 <sup>th</sup> amendment addition	11 May 2010
4 <sup>th</sup> sampling	11 August 2010
5 <sup>th</sup> amendment addition	11 August 2010
5 <sup>th</sup> sampling	11 November 2010

#### *4.2.4 Soil chemical analyses*

The chemical characteristics of the soil were determined according to the methodology described by Sadzawka et al. (2004). The organic carbon content was determined by the dichromate oxidation method and colorimetric determination of the reduced chromate; pH was measured by potentiometric method in 1:2.5 soil/water extracts; available P was extracted with sodium bicarbonate (0.5 M, pH 8.5), by the Olsen method (Sparks, 1996), and determined colorimetrically by the molybdate-ascorbic acid method. Total N was measured by the Kjeldahl method (Bremner and Mulvaney, 1982). Cation contents were

determined by atomic absorption spectrophotometry (Shimadzu GBC SensAA), after extraction of Ca, Mg, K and Na with ammonium acetate 1 M at 7.0 pH and extraction of Mn and Zn by DTPA (diethylenetriaminepentacetic acid), calcium chloride and TEA (triethanolamine) solution, buffered at 7.3 pH.

#### 4.2.5 Enzymatic analyses

Total microbial activity was determined by FDA-hydrolysis according to Adam and Duncan (2001). Fresh soil samples (1.5 g) were mixed with 9.9 ml of sodium phosphate buffer (pH 7.6) and then incubated in a 25 mL flask, for 1 hour at 25 °C in an incubation bath. At the end of incubation, the reaction was stopped by adding 10 ml of acetone. FDA stock solution was added to a triplicate set of samples whereas blank controls were prepared with buffer only. After filtering the solutions (Whatman No. 42), absorbance was measured at 490 nm by UV Visible Spectrophotometer (Cary 50, Varian Australia Pty Ltd.). The concentration of the released fluorescein was calculated by a calibration curve with standard quantities of fluorescein, and the results were expressed as  $\mu\text{g FDA g}^{-1} \text{ h}^{-1}$ . Acid phosphatase activity in soil was measured using the method of Tabatabai and Bremner (1969), using p-nitrophenyl- $\beta$ -glucopyranoside as substrate. In plastic tubes fresh soil samples (1 g) were placed and the reaction started with the application of p-nitrophenyl- $\beta$ -glucopyranoside. After incubation for 1 hour at 37 °C the release of p-nitrophenol (pNP) was measured by determining spectrophotometrically absorbance at 400 nm. Four replicates, including one blank, were used for each soil sample. The acid phosphatase activity was expressed as  $\mu\text{mol PNF g}^{-1} \text{ h}^{-1}$ .

#### *4.2.6 Bacterial community structure analysis*

The genetic structure of bacterial community in soil was determined by extraction of gene fragments encoding for 16sr DNA, amplification by polymerase chain reaction and separation by denaturing gradient gel electrophoresis (DGGE) (Muyzer et al., 1993). Briefly, total DNA was extracted from 0.25 g of each soil sample using an Ultra Clean Soil DNA extraction kit (Mo Bio, Carlsbad, CA, USA), following the instructions of manufacturer. The eubacterial primer set EUBf933-GC/EUBr1387 was used to amplify fragments of 16S rDNA gene (Heuer et al., 1997) by touchdown polymerase chain reaction (PCR). All PCR amplifications were carried out with reagents supplied with GoTaq® DNA Polymerase (Promega, Co. Madison, WI, USA). Successful extraction and amplification of DNA fragments was verified by horizontal electrophoresis on 1xTAE (tris acetate EDTA ) buffer in 1% (w/v) agarose gel with ethidium bromide staining (Fig. 4.1).

DGGE was performed using the Bio Rad Dcode Universal Mutation Detection System™. Twenty two micro liters of PCR product for each sample were applied to a lane of the gel. The separation was performed on an 8% (w/v) acrylamide gel in 1xTAE (40 mM Tris acetate pH 8.0, 1 mM Na<sub>2</sub>EDTA) containing a linear denaturant gradient ranging from 40% to 70%: 100% denaturant consisted of 7 M urea and 40% formamide as in Muyzer et al. (1993). The gel was run at constant temperature (60 °C for 720 minutes) and at constant voltage (100 V) in 1xTAE buffer. After electrophoresis, the gel was stained for 30 minutes, with mild agitation in dark, in 10µl SYBR Gold (Molecular Probes, Invitrogen Co.) into 100 ml distilled water and photographed on an UV transilluminator.

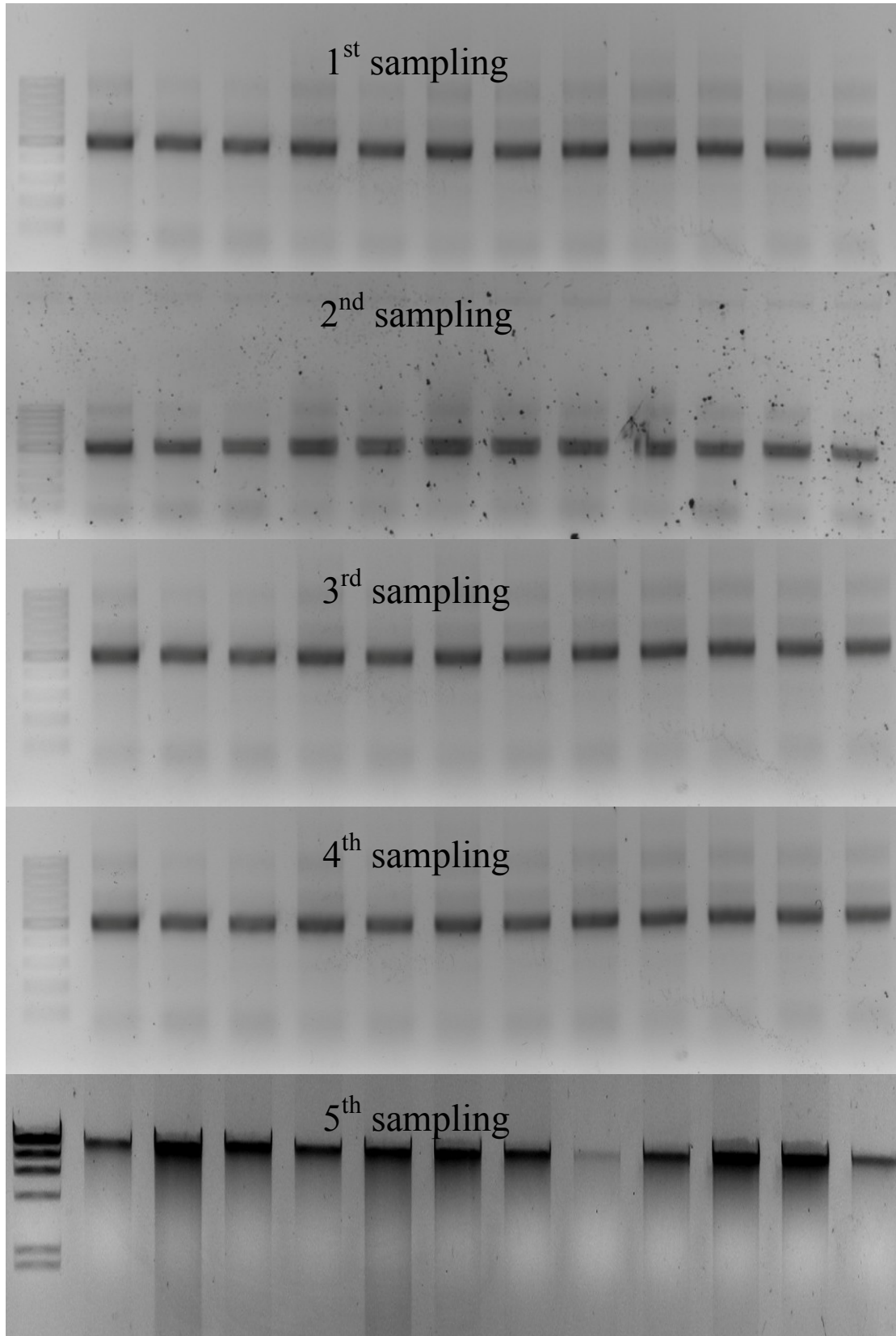


Figure 4.1. Total extracted DNA was visualized by agarose electrophoresis in 1% corresponding to lane 2 to 12.

#### *4.2.7. Cluster analysis*

The hierarchical cluster analysis was carried out to determine the relationship among the profiles of DGGE banding. The cluster analysis was performed using unweighted pair group method with arithmetic mean (UPGMA) with the software package MEGA ([www.megasoftware.net](http://www.megasoftware.net)) and visualized as a dendrogram. The dendrograms were constructed based on presence-absence bands in DGGE gels with a bootstrap confidence value of 1,000.

#### *4.2.8 Statistical analysis*

Means and standard errors of three field replicates were reported in tables and graphs. Significant differences among treatments were tested by one-way ANOVA, followed by the Student-Newman-Keuls test ( $P < 0.05$ ; SigmaStat 3.1). Two-way ANOVA, followed by the Holm-Sidak test, was used to test the effects of sampling times and pulp sludge doses on chemical, biochemical and biological parameters ( $P < 0.05$ ; SigmaStat 3.1). Pearson correlation coefficients were calculated to determine relationships among chemical, biochemical and biological data ( $P < 0.05$ ;  $n = 60$ ; SigmaStat 3.1).

### 4.3 Results

In this study OM, available P, exchangeable Na, Ca and Mg showed an increasing trend in amended soils, in most of the sampling dates (Table 4.3). On the other hand, pH and exchangeable K generally decreased in treated soils, except for an increase in exchangeable K in amended soils of the first sampling. In particular, the repeated application of secondary pulp sludge (on annual period) rich in OM (76.07%, see Table 4.1) generally increased the content of OM in the volcanic soil from 11.83% (the lowest value in the control soils) up to 14.19% (the highest value in soil amended with 30 t ha<sup>-1</sup>, see Table 4.3), although a significant increase was only found at the 3<sup>rd</sup> sampling for soil amended with the highest dose, and a surprising and significant decrease in OM in amended soils (20 and 30 t ha<sup>-1</sup>) at the 1<sup>st</sup> sampling was found. In spite of the high content of mineral N in sludge (586.00 mg kg<sup>-1</sup>, Table 4.1), the increase in total N in amended soils was very slight and not statistically significant. Moreover, at the end of the study, a significant increase was detected in C/N ratio, that has a fundamental rule in regulating microbial activity and growth, and thus in the dynamic of decomposition process and nutrient cycles. As the high content of available P in pulp mill sludge (313.00 mg kg<sup>-1</sup>; Table 4.1), all amended soils showed a prompt and significant increase in this nutrient at the 1<sup>st</sup> sampling (ranging from 23 to 35% higher than control), but no significant increase was found at other sampling dates. Similarly, considering the high values of Na and Ca in pulp mill sludge (41.55 and 27.95 cmol<sup>+</sup>kg<sup>-1</sup>, respectively; Table 4.1), the prompt and significant increase in these exchangeable cations, in amended soils of the first sampling, did not amaze, whereas Mg content increased significantly at the 5<sup>th</sup> sampling and at 10 and 20 mg kg<sup>-1</sup> sludge doses. Moreover, the available Zn content showed generally an increasing trend in amended soils, with more marked effects at the end of study period and in soils treated with 20 and 30 t ha<sup>-1</sup> sludge doses (ranging from 3 to 9 times higher than in control; Table 4.3). An opposite trend was found for exchangeable K, that generally decreased with increasing sludge doses, except for 1<sup>st</sup> sampling.

**Table 4.3** Means  $\pm$  standard deviations of chemical properties are shown for control (0) and soils amended with different sludge doses (10, 20, 30 t ha<sup>-1</sup>), at the different sampling times. Significant differences among treatments, for each sampling time, were tested by one-way ANOVA followed by the Student-Newman-Keuls test ( $P < 0.05$ ,  $n=3$ ) and indicated by different superscript letters.

Dose (t ha <sup>-1</sup> )	pH	OM (%)	Total N (%)	C:N	P <sup>a</sup> (mg kg <sup>-1</sup> )	K <sup>b</sup> (cmol <sup>+</sup> kg <sup>-1</sup> )	Na <sup>b</sup> (cmol <sup>+</sup> kg <sup>-1</sup> )	Ca <sup>b</sup> (cmol <sup>+</sup> kg <sup>-1</sup> )	Mg <sup>b</sup> (cmol <sup>+</sup> kg <sup>-1</sup> )	Zn <sup>a</sup> (mg kg <sup>-1</sup> )	Mn <sup>a</sup> (mg kg <sup>-1</sup> )	
1 <sup>st</sup>	0	5.52 $\pm$ 0.04	13.30 $\pm$ 0.10 <sup>a</sup>	0.69 $\pm$ 0.01	11.23 $\pm$ 0.12 <sup>a</sup>	23.87 $\pm$ 0.43 <sup>a</sup>	0.80 $\pm$ 0.04 <sup>a</sup>	0.15 $\pm$ 0.01 <sup>a</sup>	5.25 $\pm$ 0.10 <sup>a</sup>	1.93 $\pm$ 0.20	0.66 $\pm$ 0.08	6.41 $\pm$ 1.15
	10	5.44 $\pm$ 0.06	13.30 $\pm$ 0.47 <sup>a</sup>	0.69 $\pm$ 0.02	11.23 $\pm$ 0.65 <sup>a</sup>	29.43 $\pm$ 3.60 <sup>b</sup>	0.89 $\pm$ 0.05 <sup>b</sup>	0.25 $\pm$ 0.02 <sup>b</sup>	5.21 $\pm$ 0.08 <sup>a</sup>	1.99 $\pm$ 0.03	1.35 $\pm$ 0.35	5.85 $\pm$ 0.81
	20	5.44 $\pm$ 0.15	12.48 $\pm$ 0.04 <sup>b</sup>	0.69 $\pm$ 0.01	10.53 $\pm$ 0.18 <sup>a</sup>	32.33 $\pm$ 3.09 <sup>b</sup>	0.93 $\pm$ 0.03 <sup>b</sup>	0.32 $\pm$ 0.01 <sup>c</sup>	5.30 $\pm$ 0.08 <sup>a</sup>	2.08 $\pm$ 0.07	1.85 $\pm$ 0.93	6.00 $\pm$ 0.18
2 <sup>nd</sup>	0	5.46 $\pm$ 0.10	10.97 $\pm$ 0.41 <sup>c</sup>	0.68 $\pm$ 0.01	9.33 $\pm$ 0.36 <sup>b</sup>	30.41 $\pm$ 1.14 <sup>b</sup>	0.98 $\pm$ 0.02 <sup>b</sup>	0.34 $\pm$ 0.03 <sup>c</sup>	5.46 $\pm$ 0.08 <sup>b</sup>	2.14 $\pm$ 0.02	2.45 $\pm$ 1.30	6.78 $\pm$ 1.22
	10	5.45 $\pm$ 0.03 <sup>a</sup>	11.83 $\pm$ 0.29	0.68 $\pm$ 0.04	10.04 $\pm$ 0.43	18.49 $\pm$ 1.44	2.38 $\pm$ 0.64 <sup>a</sup>	0.06 $\pm$ 0.03	6.87 $\pm$ 0.74	1.62 $\pm$ 0.14	0.30 $\pm$ 0.26 <sup>a</sup>	4.67 $\pm$ 2.94
	20	5.51 $\pm$ 0.02 <sup>b</sup>	12.07 $\pm$ 0.35	0.72 $\pm$ 0.03	9.71 $\pm$ 0.43	21.34 $\pm$ 2.23	2.02 $\pm$ 0.13 <sup>a</sup>	0.10 $\pm$ 0.02	7.01 $\pm$ 0.55	1.62 $\pm$ 0.08	0.20 $\pm$ 0.07 <sup>a</sup>	6.53 $\pm$ 2.64
3 <sup>rd</sup>	0	5.46 $\pm$ 0.01 <sup>a</sup>	12.76 $\pm$ 0.61	0.74 $\pm$ 0.02	10.07 $\pm$ 0.73	21.58 $\pm$ 1.26	1.65 $\pm$ 0.26 <sup>a</sup>	0.10 $\pm$ 0.04	6.39 $\pm$ 0.34	1.53 $\pm$ 0.05	2.01 $\pm$ 0.16 <sup>b</sup>	4.65 $\pm$ 3.57
	10	5.39 $\pm$ 0.02 <sup>c</sup>	12.21 $\pm$ 0.88	0.71 $\pm$ 0.01	9.92 $\pm$ 0.85	19.10 $\pm$ 1.71	1.38 $\pm$ 0.19 <sup>b</sup>	0.15 $\pm$ 0.04	6.63 $\pm$ 0.33	1.50 $\pm$ 0.08	1.99 $\pm$ 0.48 <sup>b</sup>	2.88 $\pm$ 0.27
	20	5.59 $\pm$ 0.18	11.83 $\pm$ 0.03 <sup>a</sup>	0.72 $\pm$ 0.03	9.54 $\pm$ 0.32	18.59 $\pm$ 0.92	1.76 $\pm$ 0.59 <sup>a</sup>	0.16 $\pm$ 0.02	5.72 $\pm$ 0.31	1.15 $\pm$ 0.24	0.53 $\pm$ 0.20	2.72 $\pm$ 0.99
4 <sup>th</sup>	0	5.46 $\pm$ 0.17	11.84 $\pm$ 0.07 <sup>a</sup>	0.73 $\pm$ 0.07	9.50 $\pm$ 0.88	18.87 $\pm$ 2.89	1.21 $\pm$ 0.14 <sup>a</sup>	0.23 $\pm$ 0.08	6.06 $\pm$ 0.83	1.31 $\pm$ 0.14	1.41 $\pm$ 0.41	3.83 $\pm$ 1.95
	10	5.56 $\pm$ 0.25	12.02 $\pm$ 0.47 <sup>a</sup>	0.72 $\pm$ 0.06	9.68 $\pm$ 0.93	18.66 $\pm$ 1.27	1.05 $\pm$ 0.36 <sup>a</sup>	0.25 $\pm$ 0.06	6.25 $\pm$ 0.72	1.27 $\pm$ 0.18	2.74 $\pm$ 3.17	7.30 $\pm$ 0.81
	20	5.54 $\pm$ 0.31	12.95 $\pm$ 0.04 <sup>b</sup>	0.75 $\pm$ 0.06	10.11 $\pm$ 0.86	20.28 $\pm$ 3.03	0.73 $\pm$ 0.12 <sup>b</sup>	0.91 $\pm$ 0.95	8.08 $\pm$ 2.29	1.53 $\pm$ 0.15	2.79 $\pm$ 0.60	2.47 $\pm$ 3.30
5 <sup>th</sup>	0	5.55 $\pm$ 0.26 <sup>a</sup>	12.48 $\pm$ 1.34	0.72 $\pm$ 0.06	10.04 $\pm$ 1.26	19.03 $\pm$ 6.06	1.49 $\pm$ 0.17	0.06 $\pm$ 0.03	6.09 $\pm$ 0.66	1.32 $\pm$ 0.12	0.29 $\pm$ 0.15	3.93 $\pm$ 1.67
	10	5.40 $\pm$ 0.03 <sup>a</sup>	11.73 $\pm$ 0.08	0.70 $\pm$ 0.02	9.67 $\pm$ 0.26	18.30 $\pm$ 1.21	1.06 $\pm$ 0.13	0.10 $\pm$ 0.02	6.10 $\pm$ 1.21	1.43 $\pm$ 0.29	0.82 $\pm$ 0.58	2.13 $\pm$ 2.35
	20	4.91 $\pm$ 0.08 <sup>b</sup>	12.55 $\pm$ 0.56	0.74 $\pm$ 0.05	9.91 $\pm$ 0.78	25.73 $\pm$ 2.98	0.63 $\pm$ 0.07	0.10 $\pm$ 0.04	7.40 $\pm$ 1.13	1.54 $\pm$ 0.10	4.64 $\pm$ 3.13	7.40 $\pm$ 3.00
6 <sup>th</sup>	0	4.73 $\pm$ 0.05 <sup>b</sup>	13.00 $\pm$ 0.52	0.78 $\pm$ 0.01	9.63 $\pm$ 0.41	26.33 $\pm$ 3.23	0.73 $\pm$ 0.73	0.15 $\pm$ 0.04	6.62 $\pm$ 0.75	1.35 $\pm$ 0.13	8.07 $\pm$ 6.17	6.45 $\pm$ 5.82
	10	5.17 $\pm$ 0.46	12.38 $\pm$ 0.43	0.76 $\pm$ 0.04	9.46 $\pm$ 0.28 <sup>a</sup>	26.53 $\pm$ 4.83	1.01 $\pm$ 0.32	0.47 $\pm$ 0.15	5.91 $\pm$ 0.40	1.58 $\pm$ 0.05 <sup>a</sup>	0.62 $\pm$ 0.16 <sup>a</sup>	7.67 $\pm$ 2.99
	20	5.26 $\pm$ 0.22	13.39 $\pm$ 0.63	0.78 $\pm$ 0.06	10.93 $\pm$ 0.31 <sup>a</sup>	28.58 $\pm$ 1.48	0.93 $\pm$ 0.13	0.40 $\pm$ 0.13	6.63 $\pm$ 1.13	1.73 $\pm$ 0.11 <sup>b</sup>	3.05 $\pm$ 1.04 <sup>ab</sup>	6.61 $\pm$ 2.08
7 <sup>th</sup>	0	4.93 $\pm$ 0.16	13.35 $\pm$ 0.14	0.77 $\pm$ 0.04	10.08 $\pm$ 0.66 <sup>a</sup>	29.52 $\pm$ 4.10	0.76 $\pm$ 0.19	0.44 $\pm$ 0.19	7.08 $\pm$ 0.19	1.71 $\pm$ 0.06 <sup>b</sup>	5.15 $\pm$ 1.39 <sup>b</sup>	7.14 $\pm$ 0.53
	10	4.81 $\pm$ 0.39	14.19 $\pm$ 1.06	0.73 $\pm$ 0.05	11.34 $\pm$ 0.84 <sup>b</sup>	30.36 $\pm$ 7.09	0.49 $\pm$ 0.20	0.29 $\pm$ 0.07	6.51 $\pm$ 0.35	1.53 $\pm$ 0.06 <sup>a</sup>	6.08 $\pm$ 3.19 <sup>b</sup>	8.16 $\pm$ 2.85
	20	4.81 $\pm$ 0.39	14.19 $\pm$ 1.06	0.73 $\pm$ 0.05	11.34 $\pm$ 0.84 <sup>b</sup>	30.36 $\pm$ 7.09	0.49 $\pm$ 0.20	0.29 $\pm$ 0.07	6.51 $\pm$ 0.35	1.53 $\pm$ 0.06 <sup>a</sup>	6.08 $\pm$ 3.19 <sup>b</sup>	8.16 $\pm$ 2.85

<sup>a</sup>Available elements  
<sup>b</sup>Exchangeable cations  
 OM = organic matter

Finally, a slight decrease in pH was found in studied soils at the 4<sup>th</sup> sampling (Table 4.3), ranging from moderately acid (5.55, in control soil) to strongly acid (4.91 and 4.73 in soils amended with 20 and 30 t ha<sup>-1</sup>, respectively) (USDA, 1951). However, the beneficial effect of pulp mill sludge addition on soil chemical properties, at the tested doses, was generally prompt but not marked or lasting. Considering biochemical soil properties, total microbial activity, as assessed by FDA-hydrolase enzyme activity, showed generally an increase in amended soils respect to the control, except for the 3<sup>rd</sup> sampling (Fig. 4.1 A), but the increase was not always related to higher values of pulp sludge addition (20-30 t ha<sup>-1</sup>).

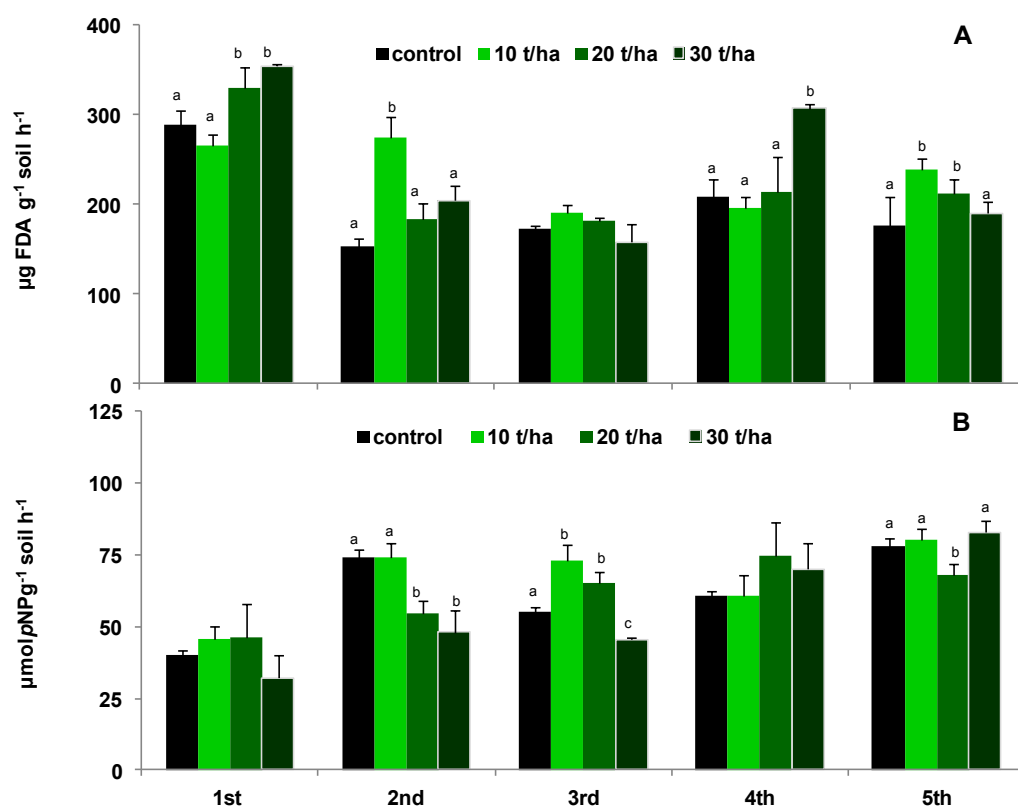


Fig. 4.1 Mean values (+ standard deviations) of FDA-hydrolase (A) and acid phosphatase (B) activities are shown in control and amended soils (0, 10, 20, 30 t ha<sup>-1</sup>), at the different sampling times (unpublished data from Gallardo et al.). Different superscript letters indicated significant differences among treatments (0, 10, 20, 30 t ha<sup>-1</sup>) for each sampling time, tested by one-way ANOVA followed by the Student-Newman-Keuls test ( $P < 0.05$ ;  $n=3$ ).



The increasing trend of total microbial activity was sustained until the end of the trial period, but a more clear and marked effect was found at the 1<sup>st</sup> sampling (one month after the 1<sup>st</sup> sludge addition) on soils treated with the highest amounts of sludge (20 and 30 t ha<sup>-1</sup>). On the contrary, a significant increase in acid phosphatase activity was only found at the 3<sup>rd</sup> sampling (Fig.4.1 B), in soils amended with pulp mill sludge at 10 and 20 t ha<sup>-1</sup> doses.

The fingerprint of the 16S rDNA gene fragments by DGGE revealed that bacterial community structure was affected by addition of pulp mill sludge, but marked effects were also due to the sampling time. A change, compared to control, was evident at the 1<sup>st</sup> sampling (that was carried out one month after 1<sup>st</sup> sludge addition) when a higher number of dominant bands was found in soil treated with 20 and 30 t ha<sup>-1</sup>, highlighting a variation in bacterial community structure (Figs 4.2 and 4.3). The hierarchical cluster analysis also confirmed this result, showing a clear distance between soils treated with 0 and 10 t ha<sup>-1</sup> and soils treated with 20 and 30 t ha<sup>-1</sup>.

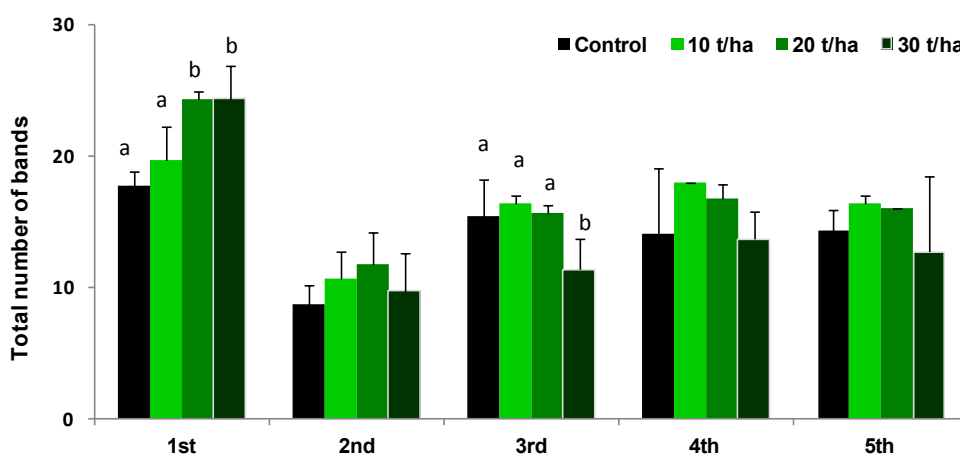


Fig. 4.2 Mean values (+ standard deviations) of number of bands, from DGGE analysis, are shown in soils amended with pulp mill sludge. Different superscript letters indicated significant differences among treatments (0, 10, 20, 30 t ha<sup>-1</sup>) for each sampling time, tested by one-way ANOVA followed by the Student-Newman-Keuls test ( $P < 0.05$ ;  $n=3$ ).

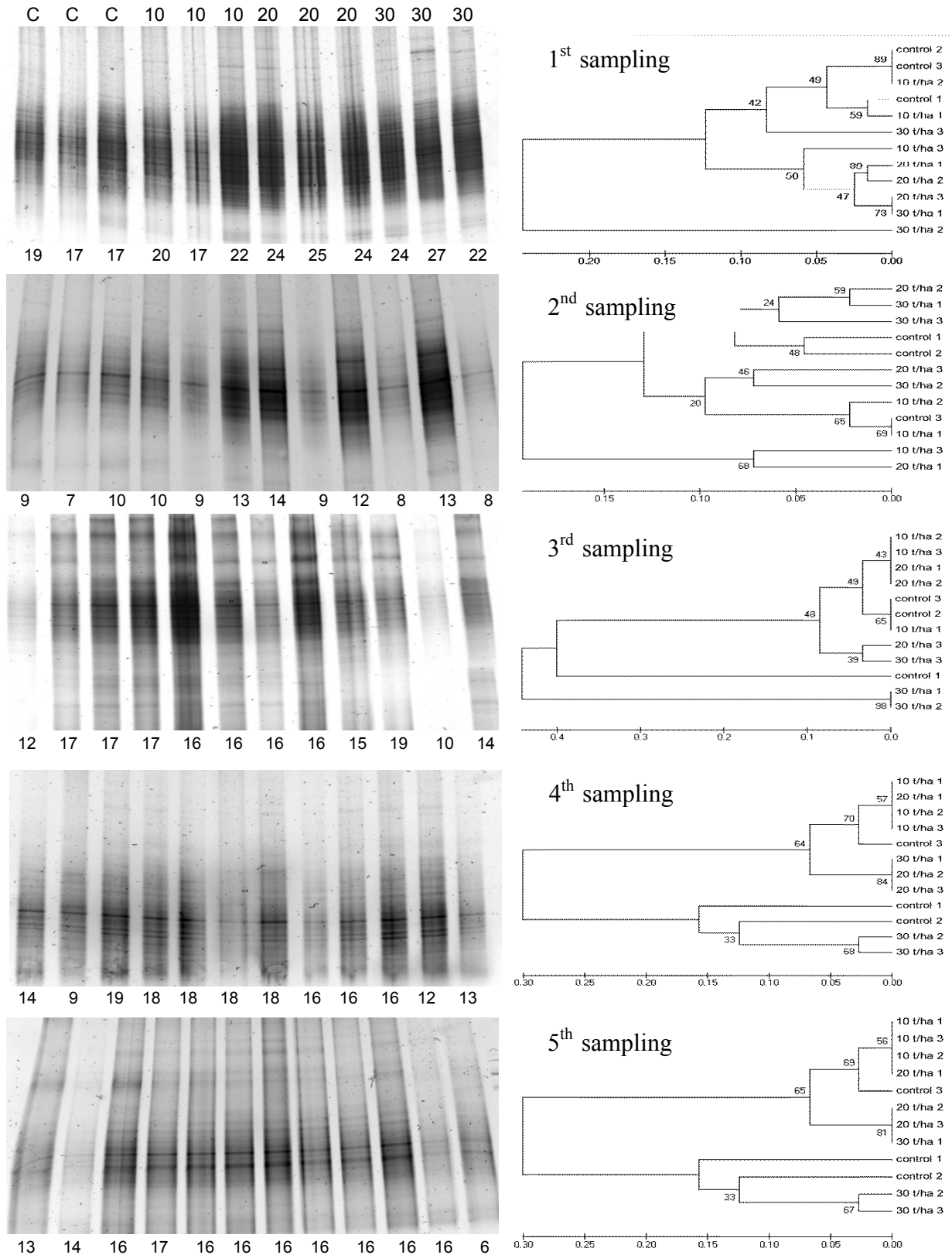


Fig. 4.3 DGGE profile of the bacterial 16S rDNA (eubacterial primer set EUBf933-GC/EUBr1387) and hierarchical cluster analysis (UPGMA, by MEGA 5.05) are shown for each treatment and sampling time. At the bottom of each fingerprint, the number of bands for each sample is reported.

## **4.5 Discussion**

Application of pulp mill sludge to soil generally represents a valuable resource practiced technique used to increase soil organic matter content (a key soil characteristic, affecting terrestrial ecosystem development and functioning), to improve nutrient availability and to get better yield (Dolar et al., 1972; Zibilske, 1987; Phillips et al., 1997; Nkana et al., 1999; Vance, 2000; Foley and Cooperband, 2002; Jordan and Rodriguez, 2004; Zhang et al., 2004; Battaglia et al., 2007; Gallardo et al., 2007; Gallardo et al., 2010a; Ribeiro et al., 2010). In this study, pulp mill sludge showed high contents of organic matter (OM), macronutrients (N, P, K, Ca and Mg), and micronutrients (Mn, Cu and Zn), but low content of Fe and Al. It has to be emphasize that the low Al content in this sludge is a particularly relevant fact because the concentrations of Al are often high in pulp mill sludge. In fact, the clays used in the paper making process and aluminum salt used in the clarification process lead to have high concentrations of aluminum (Camberato et al., 1997; Vance, 2000). As reported by other authors (Feldkinchner et al., 2003; Rotenberg et al., 2005; Gallardo et al., 2010a) a clear increase was generally found in OM, total N, available P, exchangeable Na and Ca and C/N ratio after addition of pulp mill sludge to soil. In this study, the high contents of OM, macronutrients and micronutrients in pulp sludge affected positively the trend of some chemical parameters, contributing to improve soil characteristics, although these effects were not lasting in time. Moreover, positive correlations were found between OM and N, P and C/N ratio ( $r=0.298$ ,  $n=60$  and  $P<0.05$ ,  $r=0.418$ ,  $n=60$  and  $P<0.01$ ,  $r=0.658$ ,  $n=60$  and  $P<0.01$ , respectively, see Table 4.4). The increase in extractable P, in amended soils, could be depend on the mineralization of organic P from the decomposition of pulp mill sludge. In fact, it has been showed that the incorporation of organic residues into the soil can increase the availability of P, depending on the capacity that the residue possesses to reduce the adsorption of P in the soil, on the contribution of different species of



P (Haynes and Mokolobate, 2001; Mokolobate and Haynes, 2002; Pypers et al., 2005) and on the microbiological capacity to degrade compounds of P with the subsequent release of phosphate. Similarly, Simard et al. (1998) reported that de-inking paper sludge increased extractable P in soils from Canada. On the contrary, other studies on Mediterranean soils (Cabral and Vasconcelos, 1993) have indicated that increasing amounts of combined primary/secondary pulp mill sludge did not cause any significant effect on available P due to the high C:P ratio of the sludge used.

In this study the high content of Zn and Mn found in sludge could be worrying, because high values of these elements are considered to be toxic for seeds germination and soil microorganisms (Gallardo et al, 2010b). Zn content was still 9 times higher than in control and a significant positive correlation was found between OM and Zn contents ( $r=0.332$ ,  $n=60$  and  $P<0.01$ ; Table 4.4). However, in spite of the increase in concentration of this heavy metal, the detected level in amended soils did not exceed that allowed by the Chilean regulation for this type of soil (CONAMA, 2001). Afterwards, the pH slightly decreased in amended soil of the last samplings still to the strongly acid values 4.91 and 4.73 (20 and 30 t ha<sup>-1</sup>, respectively, in the 4<sup>th</sup> sampling), and this decrease could be attributed to the weak organic acid released deriving from degradation of organic matters in the pulp sludge (Sims, 1990; Habteselassie et al., 2006).

Differences in organic matter composition can affect the decomposition process, modifying the availability of substrates, and, consequently, microbial succession and community structure (Marschner et al., 2003). After Kandeler (2007), the composition of the soil microbial community determines its potential to synthesize enzymes, and therefore, any change in the microbial community due to environmental factors should be reflected on the levels of enzymatic activity. In fact, changes in bacterial community structure, together with environmental effects and ecological interactions, have direct effects on the metabolic diversity and biological activity of soils (Zak et al., 1994). In this study the bacterial community

structure was affected by addition of pulp mill sludge, as shown by the increase in number and intensity of bands in soil treated with 20 and 30 t ha<sup>-1</sup>, and the number of bands was positively correlated with FDA-hydrolase activity ( $r=0.571$ ,  $n=60$  and  $P<0.01$ ; Table 4.4). However, marked effects were only found at the 1<sup>st</sup> sampling, as confirmed by the hierarchical cluster analysis, although a higher influence of sampling times compared to sludge doses was found too. In fact, PCR-DGGE analysis showed different profiles at different sampling times, probably due to a restructuring of bacterial communities in the different time. Crecchio et al. (2001) found variations in some enzyme activities (as dehydrogenase), after amendment of soil with compost from municipal solid waste, but did not find changes among genetic fingerprints, and explained these results hypothesizing that in the studied soils bacterial responded mainly by altering their metabolic activity (i.e. extracellular enzymes). Similarly, our data suggest, according to results of Gallardo et al. (2010a), that sludge application did not stimulate greatly changes in microorganism populations when soil shows a high starting content of OM and nutrients, probably due to a low competition among microorganism populations for resources. The results from two-way ANOVA test (Table 4.5) confirmed a high influence of sampling times on many parameters, including the number of bands, but a comparable dependence of FDA-hydrolase and acid phosphatase activities from both independent variables.

**Table 4.5.** Summarize results of two-way ANOVAs for chemical, biochemical and biological parameters. Sampling times and pulp sludge doses (0, 10, 20 and 30 t ha<sup>-1</sup>) were independent variables.

Parameter	Source	F	P-value
pH	Sampling time	15.054	<0.001
	Sludge dose	6.327	0.001
	Interaction	2.375	0.020
OM	Sampling time	8.662	<0.001
	Sludge dose	0.985	0.410 <sup>NS</sup>
	Interaction	5.855	<0.001
Total N	Sampling time	5.841	<0.001
	Sludge dose	0.529	0.665 <sup>NS</sup>
	Interaction	1.013	0.456 <sup>NS</sup>
C/N	Sampling time	3.496	0.015
	Sludge dose	0.025	0.994 <sup>NS</sup>
	Interaction	2.820	0.007
Posphorous	Sampling time	26.079	0.001
	Sludge dose	5.750	0.002
	Interaction	1.281	0.267 <sup>NS</sup>
Potassium	Sampling time	23.360	0.001
	Sludge dose	12.169	0.001
	Interaction	1.726	0.097
Sodium	Sampling time	8.800	<0.001
	Sludge dose	3.310	0.030
	Interaction	2.207	0.031
Calcium	Sampling time	6.007	<0.001
	Sludge dose	2.109	0.115 <sup>NS</sup>
	Interaction	1.504	0.164 <sup>NS</sup>
Magnesium	Sampling time	50.681	<0.001
	Sludge dose	2.057	0.121 <sup>NS</sup>
	Interaction	1.768	0.088 <sup>NS</sup>
Zinc	Sampling time	4.352	0.005
	Sludge dose	12.047	<0.001
	Interaction	1.368	0.221 <sup>NS</sup>
Manganese	Sampling time	3.351	0.019
	Sludge dose	1.164	0.336 <sup>NS</sup>
	Interaction	1.336	0.238 <sup>NS</sup>
FDA-hydrolase	Sampling time	98.160	<0.001
	Sludge dose	15.647	<0.001
	Interaction	14.114	<0.001
Ac. phosphatase	Sampling time	53.861	<0.001
	Sludge dose	7.981	<0.001
	Interaction	7.160	<0.001
N. of bands	Sampling time	33.674	<0.001
	Sludge dose	5.039	0.005
	Interaction	2.006	0.050 <sup>NS</sup>

NS = not significant, i.e. P ≥ 0.05

## 4.6 Conclusions

Our results showed that pulp mill sludge application can generally affect positively chemical and biochemical characteristics of soil, confirming that the beneficial effect is principally due to the increase in micro- and macronutrient contents and microbial activity. However, at the tested doses, no marked or lasting effects were found even after successive applications of sludge.

The analysis of fingerprints from 16S rDNA fragments by DGGE revealed that the application of sludge did not greatly modify the bacterial community structure, even when high doses of sludge were applied. The whole data set indicated that application of pulp mill sludge can improve soil properties, but further studies need to establish the appropriate rate of sludge application and to test the magnitude and stability of beneficial changes deriving from sludge use as well as what soil activities and microbial groups are stimulated by sludge application.



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## Chapter 5

### General conclusions

Understanding the changes in soil quality following application of organic amendments can lead to expansion of healthier soil managements. In this thesis, two field studies were carried out in order to test the effects of different organic amendments on chemical and biochemical/biological properties, and biodiversity of the soil.

Results of the first study, carried out in two farms of the Sele River Plane (southern Italy) under intensive management and with different geopedologic properties, showed that the continual application of slow-degradable organic fertilizers (compost from municipal wastes mixed with scraps from poplar pruning) can affect positively chemical and biochemical/biological properties of the studied soils, but no remarkable effect was found on the functional diversity of the microbial community. In particular, data showed a prompt and lasting increase in organic carbon content after amendment, but the ameliorant effects on the other properties of the soil were particularly evident after the second addition. Moreover, the presence of wood scraps in the amendment mixtures favoured a slight increase of C/N ratio, contributing to limit mineralization processes and organic carbon loss from soil in long-term. Although, at tested doses, it was not possible to discriminate among the used amendments, it has to be underlined that the soil with the highest starting values of organic carbon and C/N ratio (i.e. Farm 2) showed more marked beneficial effects deriving from the amendment, indicating a key role of organic matter content also in promoting soil recovery by sustainable agricultural practices. In conclusion, this study provided useful information for conservation and environmental sustainable management of agricultural soils highlighting that the continual application of organic matter to soil, even in absence of mineral fertilizing, can improve soil chemical and biological properties

and, thus, affect positively soil quality of areas managed for long time by intensive farming.

Results of the second study, carried out in an experimental farm of the Universidad de La Frontera (southern Chile) under *Lolium perenne* cultivation, showed that pulp mill sludge application had generally a positive effect on chemical and biochemical characteristics of soil, although, at the tested doses, no marked or lasting effects were found due to the successive applications. On the contrary, sludge addition did not greatly modify the bacterial community structure, even when high doses of pulp mill sludge were applied. According with previous studies, data showed that pulp mill sludge addition did not affect negatively soil, considering the heavy metal content in soil, and the beneficial effects deriving from its use were principally due to the increase in micro- and macronutrient contents and microbial activity.

Finally, both studies confirmed that the tested biochemical/biological parameters (as enzyme activities, soil potential respiration, microbial biomass carbon, and related indices) are prompt and sensitive indicators of the changes in the soil quality due to application of organic amendments. On the other hand, functional or genetic diversity of soil microorganisms was not greatly affected by amendment, probably because of the reduction of competition among microbial population due to the increase in resources, as organic matter and nutrient contents, in amended soils.

However, considering the complexity of the problems involved in preventing and mitigating the consequence of the wrong use of the soil, further multidisciplinary studies need to establish the appropriate rate of amendment applications and test the magnitude and stability of beneficial effects deriving from these agricultural practices.

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