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Effects of sustainable soil management on fertility of agricultural soils

Ph.D. Dissertation
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A Vincenzo, nonostante la sua età, ha nelle sue mani una piccola saggezza…

To Vincenzo, despite his age, has in his hands a little wisdom…

A Vincenzo, que a pesar de su edad, lleva una pequeña sabiduría en sus manos…

1 Introduction

1.1 Intensive agriculture

Intensive agriculture represents the change from traditional farming systems, based largely on the management of natural resources and ecosystem services, to the specialized systems availing themselves of biochemistry and engineering to crop production. Adoption of mechanization, standardization, labour-saving technologies and the use of chemicals to feed and protect crops had revolutionized traditional agriculture in the 20th century. Productivity increased largely through the use of heavy farm equipment and machinery powered by fossil fuel, intensive tillage, high-yielding crop varieties, irrigation, manufactured inputs, and ever increasing capital intensity (Kassam and Hodgkin, 2009).

From 1961 to 2007 there have been a great intensification of agriculture in the world (Figure 1.1) and, as a consequence, an increasing application of fertilizers and pesticides and the use of irrigation equipment, which induces great crop yields per unit of land (Figure 1.2).

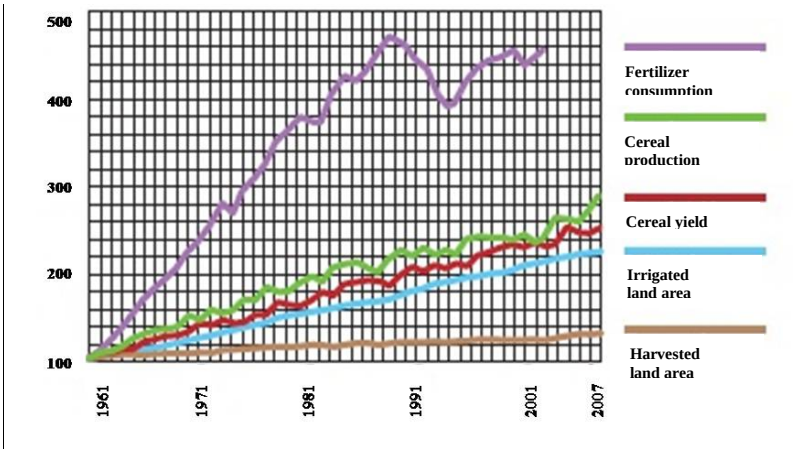

Figure 1.1 Indicators of global crop production intensification, 1961-2007. Index (1961=100) (http://www.fao.org/ag/save-and-grow/en/1/index.html).

As indicated by the June 2012 forecast of the IFA Agriculture Committee, global fertilizer consumption on a calendar year basis is projected to grow at an annual rate of 1.7%, to reach 192.3 million metric tonnes (Mt) nutrients in 2016 (Table 1.1). Increases in demand are projected for all three major nutrients, showing average annual growth rates of 1.3% for N, 2.1% for P, and 2.8% for K.

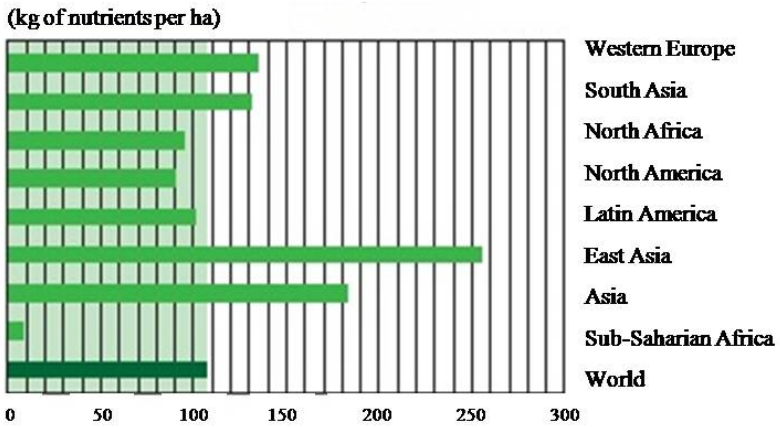

Figure 1.2. Use of mineral fertilizers during the two-year period 2008-2009 (http://www.fao.org/ag/save-and-grow/en/1/index.html).

Table 1.1 World fertilizer consumption.

Calendar Year Basis			
Mt nutrient *	**2011**	**2012**	**2016**
N	107.5	109.5	114.4
P_2O_5	40.9	41.9	45.3
K_2O	28.5	28.5	32.6
Total	*176.9*	*179.9*	*192.3*

*Million metric tones. (From Heffer and Prud'homme, 2012)

Total nutrient sales in the fertilizer and industrial sectors in 2016 are forecast at 245 Mt nutrients, representing a 9% increase compared with 2011 and an average annual growth rate of 1.8%.

The harmful environmental impacts of agriculture basically derives from the transformation of natural habitats to agricultural areas. Agricultural practices can change whole ecosystems through conversion of the landscape and the usage of fertilizers and pesticides or technologically (including use of genetically modified organisms) to obtain the maximum yield of a single provisioning ecosystem service (Bennett and Balvanera, 2007).

As reported in FAO database (2010), thanks to the increase in the use of agrochemicals cereal production has doubled in the past 40-50 years in order to satisfy increasing demand for food. On the positive side, the use of agrochemicals has saved natural habitats from conversion to agricultural land. However, fertilizers and pesticides (fungicides, herbicides, insecticides, etc.) are mostly nitrogen-(NOx, ammonium), phosphorus- or potassium-based and their use and over-use causes leaching into the soil and resultant soil degradation and groundwater pollution. Crops can take up only 30–50% of nitrogen informs of nitrate (NO_3^-) and ammonium (NH_4^+) and approximately 45%

of phosphorus fertilizers, thus a great amount of the applied components are lost in the soil where they pollute groundwater (Mózner et al., 2012).

Neverthless, serious environmental problems may derive from intensive agricultural practices. Alterations of environmental conditions, the growth and activity of soil microorganism with serious consequences on nutrient availability could occur thus determining further costs for increased fertilization, irrigation, and energy to maintain productivity of the degraded soils (Cassman, 1999). Reducing microbial biodiversity, intensive agricultural practices may also diminuish microbial efficience, because the ability of ecosystems to provide some services depends on both number and type of species in an ecosystem (Hector et al., 1999; Loreau et al., 2001).

Intensive agriculture also consists in a strong use of pesticides, in spite of the fact that pesticides themselves do not directly contribute to better crop yields but simply help to control the potential losses caused by animal pests (such as insects, mites, nematodes and rodents), plant pathogens (such as fungi, viruses and bacteria), and weeds (Oerke, 2006).

Fertilizers and especially bioaccumulating or persistent agricultural organic pollutants can pollute terrestrial and aquatic habitats, and then they can enter other ecosystems through leaching, increasing health and water purification costs (Tilman et al., 2002). Therefore this system can also contribute to eutrophication of aquatic habitats.

A very important problem related to intensive agriculture is frequently tillage, causing soil erosion and loss of soil quality (ATTRA, 2004). In the last decades a significant decrease in primary productivity has been observed worldwide as a consequence of several factors caused for human activity such as soil erosion, overgrazing, salinity and/or sodicity induced by irrigation, pollution by heavy metals and xenobiotics carry on to a reduction of soil organic carbon and loss of soil biodiversity due to the continuous application of pesticides.

Intensifying agriculture involves the use of improved crop varieties and the more intensive or more efficient use of water and plant nutrients.

The declining quality of the land and water resources available for crop production has major implications for the future. The United Nations Environment Programme (UNEP) has estimated that unsustainable land use practices result in global net losses of cropland productivity averaging 0.2 % a year (Nellemann et al., 2009).

1.2 Sustainable agriculture

Sustainable agriculture is of vital importance for world population as it offers the opportunity to meet human agricultural needs in agreement with the environment, something that conventional agriculture does not do.

Sustainability can be described as the ability to provide for core societal needs in a manner that can be readily mantained into the future without unwanted negative effects. Most definitions of sustainability are framed in terms of three broad social goals: environmental, economic, and social health or well-being. For example, a sustainable farming system might be considered a farm that provides food, feed, fiber, biofuel, etc. for society. In addition it allows producers and laborers to get economic returns, farm animals to be raised in according to cruelty-free practices, and consumers to have available safe, healthy, and affordable food, while at the same time maintains or enhances the natural resource base upon which agriculture depends (USDA-NAL, 2007). Thus, the environment friendly techniques ensure safe and healthy agricultural products and their permanence in time, capable of maintaining their productivity and usefulness to society indefinitely.

Critics of sustainable agriculture mainly claim that this method results in lower crop yields and higher land use. There is recent evidence, though, suggesting that over time, sustainably farmed lands can be as productive as conventional industrial farms (Dicks and Buckley, 1989). There is a challenge in the sustainable agriculture approach, as it involves a conflict between the need for providing food for a growing population and the ecological limits of increasing crop yields. Some areas in the world such as China, South Asia and Africa demand significant increases in yield to satisfy the increasing needs of growing population, but the environmental constraints will limit this outcome. According to Harris (1996), there is a conflict between the pressure to increase yields on the demand side and the requisites of long-term sustainability. There is an ecological cost to provide food for the global population and meeting conditions for sustainability.

Neo-classical economical approaches focus on yield increases as a result of technological advances and increasing inputs. In this way biophysical limits and carrying capacity are not taken into account. Neo-classical economists reject the necessity to take into account the focus on limits, arguing that technological advances and trading activities will solve the problem of the excessive use of agricultural land. In contrast, the ecological economic perspective is based on the environmental limits of the economic growth (Harris, 1996).

The concept of sustainability in the context of agricultural and food production is central to any future challenges (Pretty, 2008). It incorporates four key principles:

1. *persistence*: the capacity to continue to deliver desired outputs over long periods of time (human generations), thus conferring predictability;
2. *resilience*: the capacity to absorb, utilise or even benefit from perturbations (shocks and stresses), and so persist without qualitative changes in structure;
3. *autarchy*: the capacity to deliver desired outputs from inputs and resources (factors of production) acquired from key system boundaries;

4. *benevolence*: the capacity to produce desired outputs (food, fibre, fuel, oil) while sustaining the functioning of ecosystem services and not causing depletion of natural capitals (i.e.minerals, biodiversity, soil, clean water).

As Royal Society (2009) showed, sustainable agricultural systems are less vulnerable to shocks and stresses and also contribute to the delivery and maintenance of a range of valued public goods, such as clean water, carbon sequestration, flood protection, groundwater recharge and landscape amenity value. A sustainable production system exhibits most of the following attributes: 1) utilization of crop varieties and livestock breeds with high productivity per externally derived input; 2) abolition of the unnecessary use of external inputs; 3) use of agroecological processes such as nutrientcycling, biological nitrogen fixation, allelopathy, predation and parasitism; 4) minimum application of technologies or practices with adverse impacts on the environment and human health; 5) productive use of human capital in the form of knowledge and capacity to adapt and innovate, and social capital to resolve common landscape-scaleproblems; 6) quantification and minimization of management system impacts on externalities such as green house gas emissions, clean water availability, carbon sequestration, conservation of biodiversity, and minimum diffusion of pests, pathogens and weeds.

1.3 Farming systems

Crops are grown under a wide range of production systems. There is an intensive approach, in which most aspects of production are controlled by technological interventions such as soil tillage, protective or curative pest and weed control with agrochemicals, and the application of mineral fertilizers for plant nutrition. There are also production systems that consider a predominantly ecological approach and are both productive and more sustainable. These agro-ecological systems are generally characterized by minimal disturbance of the natural environment, plant nutrition from organic and non-organic sources, and the use of both natural and managed biodiversity to produce food, raw materials and other ecosystem services. Furthermore these systems sustain the health of farmland already in use, and can regenerate land left in poor condition by past misuse (Doran and Zeiss, 2000).

Farming systems for sustainable crop production intensification will offer a range of productivity, socio-economical and environmental benefits to producers and to society at large, including high and stable production and profitability, adaptation and reduced vulnerability to climate change, enhanced ecosystem functioning and services, and reductions in agriculture greenhouse gas emissions and “carbon footprint”.

1.3.1 Conventional farming

Conventional crop production is based on the use of synthetic pesticides and herbicides, and the supply of synthetic fertilizer, in addition or in replacement of those generated on the farm (manure), to maintain soil fertility.

Fields are more frequently planted in few rotations of marketable crops than left fallow or planted with cover crops. Conventional corn, soybean, and cotton farms are increasingly planted with seeds that are genetically engineered to facilitate weed control or to reduce pest losses (and pesticide use) (Committee on the Impact of Biotechnology on Farm - Level Economics and Sustainability and National Research Council, 2010).

There are several types of resource-conserving technologies and practices that can be used to improve the stocks and use of natural capital in and around agroecosystems and environmental performance of conventional agriculture. These are:

a) *Crop rotation*: diversified crop rotations are used to riduce possible weed, disease, and pest problems, to utilize the beneficial effects of some crops on soil conditions and on the productivity of subsequent crops, and to utilize breaking crop.
b) *Retention of adequate levels of crop residues and soil surface cover*: the retention of sufficient crop residues helps to protect soil from water and wind erosion, to reduce water runoff and evaporation, to improve water productivity and to enhance soil physical, chemical, and biological properties associated with long-term sustainable productivity.
c) *Integrated pest management (IPM)*: use of pesticides to pest, disease and weed control when other options are ineffective.
d) *Integrated nutrient management*: it has the aim to both balance the need to fix nitrogen within farm systems with the need to import inorganic and organic sources of nutrients and reduce nutrient losses through erosion control (Crews and Peoples, 2004; Leach et al., 2004; Goulding et al., 2008; Moss, 2008).
e) *Conservation tillage*: tillage practices have significantly improved soil conditions, reduced degradation and enhanced productivity in many parts of the world.
f) *Precision farming practices*: combination of specific information about soil conditions and indicators of crop performance to target fertilization and other crop management practices where they are most needed.
g) *Best management practices (BMPs)*: BMPs include the use of buffer or filter strips, manure handling and management, nutrient management planning, wildlife habitat enhancement within agricultural landscapes, composting to process agricultural wastes, and practices designed to increase irrigation water use efficiency (USDA-NRCS, 2009).

h) *Development of crops and animals that have enhanced genetic resistance to climatic extremes,pests, and other threats, often with the use of new genetic engineering tools*: millions of lives depend upon the extent to which crop genetic improvement can keep place with the growing global population, changing climate, and shrinking environmental resources. Genetically improved seed is only part of the solution. Such seed must be integrated into ecologically based farming systems and evaluated in light of their environmental, economic, and social impacts - the three pillars of sustainable agriculture (Ronald, 2011). Thus, an important goal for genetic improvement of agricultural crops is to adapt our existing food crops to increasing temperatures, decreased water availability in some places and flooding in others, rising salinity, and changing pathogen and insect threats (World Bank, 2007; Gregory et al., 2009; Royal Society, 2009). Such improvements will require diverse approaches that will enhance the sustainability of our farms. These strategies must be evaluated in light of their environmental, economic, and social impacts - the three pillars of sustainable agriculture (Committee on the Impact of Biotechnology on Farm - Level Economics and Sustainability and National Research Council, 2010).

i) *Agroforestry*: it incorporates multifunctional trees into agricultural systems and collective management of nearby forest resources (Leakey et al., 2005).Agroforestry systems, involving the cultivation of woody perennials and annual crops, are increasingly practiced on degraded land, usually with perennial legumes. Conservation agriculture works well with agroforestry and several tree crop systems, and farmers in both developing and developed regions practise it in some form. These systems could be further enhanced by improved crop associations, including legumes, and integration with livestock. Alley cropping is one innovation in this area that offers productivity, economic and environmental benefits to producers (Weber, 1996). Another example is the use of varying densities of “fertilizer trees” that enhance biological nitrogen fixation, conserve moisture and increase production of biomass for use as surface residues.

j) *Aquaculture*: it incorporates fish, shrimps and other aquatic resources into farm systems, such as into irrigated rice fields and fish ponds, and so leads to increases in protein production (Bunting, 2007).

k) *Water harvesting in dryland areas*: it means formerly abandoned and degraded lands can be cultivated, and additional crops can be grown on small patches of irrigated land owing to better rain water retention (Pretty, 1995), and improving water productivity of crops (Morison et al., 2008).

l) *Livestock integration into farming systems*: use of dairy cattle, pigs and poultry, including zero-grazing cut and carry systems (Altieri, 1995; Wilkins, 2008).

Nevertheless conventional farming can produce changes in soil quality and productivity providing critical signs of environmental degradation and transformation, since soil acts as a source of nutrients and water and a sink for pollutant chemicals. For example, changes in soil aggregation and structure through conventional agricultural management practices aggravate surface runoff and losses of nutrients and soil to water bodies. On the other hand conservation tillage practices help to maintain the soil carbon and nutrient pool, which also promote higher productivity (Bhardwajet al., 2011).

As regards soil organic carbon sequestration Piccolo (2012) point out that there are no solid scientific bases to justify the belief that reduced or removed tillage methods in cropland soils increases SOC sequestration. Long term of these practices is required for keep a significant improve SOC content in crop soil. Also, the reduced tillage practices do not guarantee a persistent organic carbon sequestration, as tillage is resumed (possibly by lack of sufficient incentives to farmers), the fixed carbon is rapidly lost again from soil, carriying to reduced crop productivity; not significant and unstable carbon fixation and temporary sequestration until traditional tillage practice is resumed. Carbon sequestration in cropland by adopting reduced-tillage practices has been estimated (Figure 1.3) to be rather small (<0.5 ton C ha^{-1} $year^{-1}$) and extremely variable (>50% error), thereby showing their little use in off-setting GHG emissions in Europe (Freibauer et al., 2004; Smith et al. 2007). Therefore, it would be desirable to find better alternatives to these soil management practices for organic carbon sequestrationin agriculture, as providing to soil SOM of high quality.

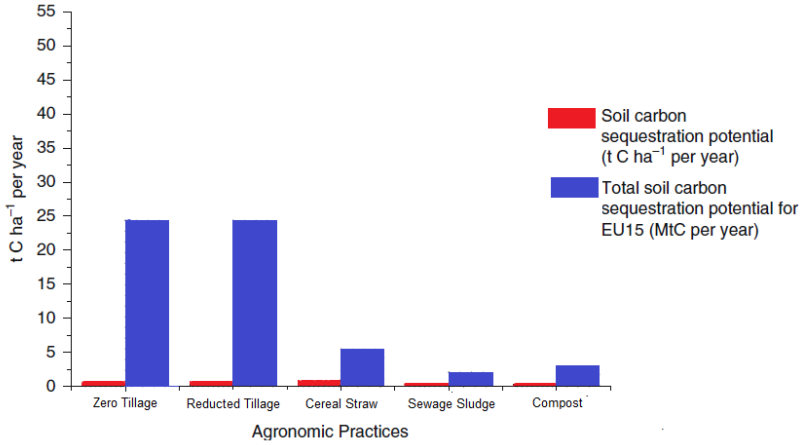

Figure 1.3 Carbon sequestration potentials of agronomic practices (adapted from Piccolo, 2012)

1.3.2 Organic farming

The organic farming systems emphasize the use of renewable resources and the conservation of soil and water to enhance environmental quality for future generations. They typically rely on crop rotations, green manures, composts, naturally derived fertilizers and pesticides, biological pest

controls, mechanical cultivation, and modern technology. Organic meat, poultry, eggs, and dairy products come from animals that are not given any antibiotics or growth hormones. Organic food is produced without the use of most conventional pesticides, fertilizers made with synthetic ingredients or sewage sludge, bioengineering, or ionizing radiation.

Organic farming, when practiced in combination with conservation agriculture, can lead to improved soil health and productivity, increased efficiency in the use of organic matter and energy savings. Organic Conservation Agriculture (OCA) farming serves mainly niche markets and is practised in parts of Brazil, Germany and the United States of America, and by some subsistence farmers in Africa. Shifting cultivation entails the clearing for crop production of forest land that is subsequently abandoned, allowing natural reforestation and the recoveryof depleted plant nutrients (FAO, 2011). Although shifting cultivation is often viewed negatively, it can be adapted to follow sustainable crop production intensification principles. In place of slash-and-burn, shifting cultivators could adopt slash-and-mulch systems, in which diversified cropping (including legumes and perennials) reduces the need for land clearing. Other ecosystem-based approaches, such as the system of rice intensification, have also proven, in specific circumstances, to be successful as a basis for sustainable intensification (Chabi-Olaye et al., 2006).

Table 1.2. Organic agricultural land by country 2010 in Europe (from http://www.organic-world.net).

	Organic agriculture area (ha)	**Organic area/total arable land**
World	*37,041,005*	*0.8*
Australia	12,001,724	2.9
Argentina	4,177,653	3.0
USA	1,948,946	0.6
Brasil	1,765,793	0.7
Spain	1,456,672	5.9
China	1,390,000	0.3
Italy	1,113,742	8.7
Germany	990,702	5.9
Uruguay	930,965	6.3
France	845,442	3.1

The total area of the biological agriculture in the world is around 37 million hectares, 83% of which concentrated in Oceania, Europe and Latin America. Italy remains among the top ten countries in the world for acreage organically and, among these, the one with the highest percentage of the total arable lands (Table 1.2).

Ten million hectares of agricultural land are organic (including in conversion areas) and constitute 2.1 percent of the agricultural land in Europe (Figures 1.4 and 1.5). The organic agricultural land increased by 0.8 million hectares or nine percent in 2010. A most 280.000 producers were reported.

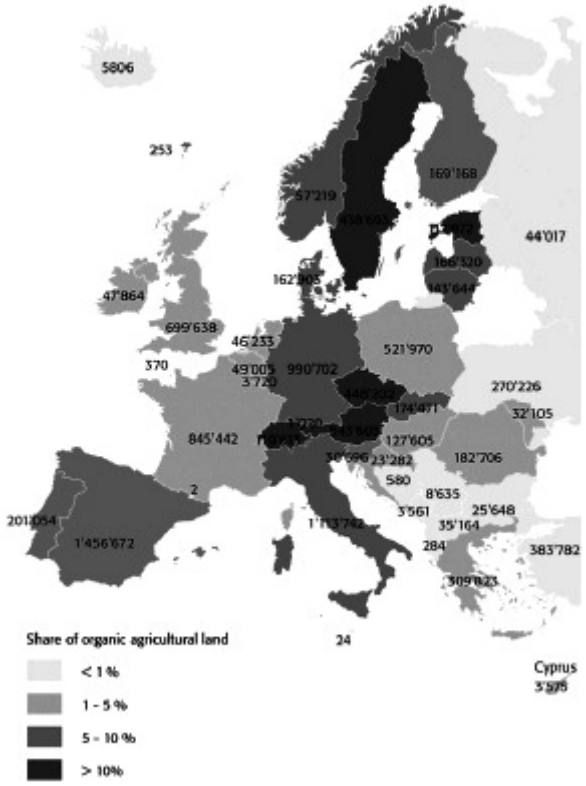

Figure 1.4. Organic agriculture areas in Europe (from http://www.organic-world.net)

Italy is among the first countries of the world that present lands crop with conservational methods, and among these Italy presents the highest percentage respect to the total Utilised Agricultural Area (UAA) (IFOAM, 2012).

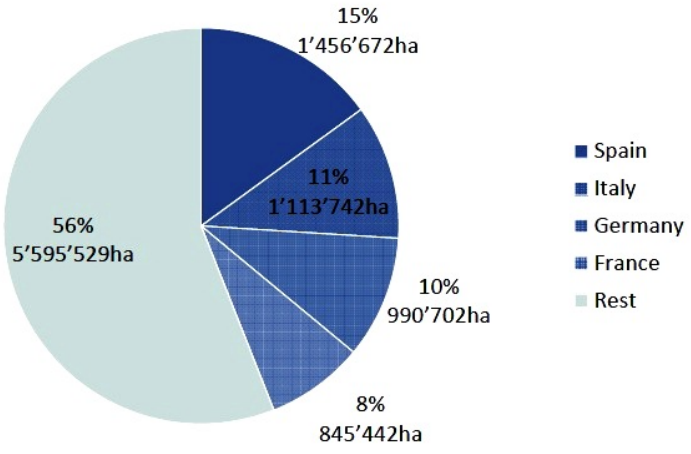

Figure 1.5. Distribution of organically managed agricultural lands (10 million hectares) in Europe

The market reached 19.6 billion euros, an increase of roughly eight percent compared with 2009. The largest market for organic products in 2009 was Germany with a turnover of 6.020 million euros, followed by France (3.385 million euros) and the UK (2.000 million euros). As a portion of the total market share, the highest levels have been reached in Denmark, Austria and Switzerland, with five percent or more for organic products. The highest per capita spending is also in these countries and in Luxembourg.

1.3.3 Biodynamic farming

Biodinamic farming system typically use the full range of organic production practices, but also use a series of eight soil, crop, and compost amendments, called preparations, made from cow manure, silica,and various plant substances. Biodynamic farming also places greater emphasis on i) the integration of animals to create a closed nutrient cycle, ii) the use of an astronomical calendar to determine auspicious planting, cultivating, and harvesting times, and iii) an awareness of spiritual forces in nature. Biodynamic farmers view the soil and the whole farm as an integrated, living organism and self-contained individuality. More than a production system, biodynamic agriculture is a practice of living and relating to nature in away that focuses on the health of the bioregion, landscape, soil, and animal, plant, and human life, and it promotes the inner development of each practitioner. The Demeter Association has certification programs for food and feed produced by strict biodynamic farming methods in different countries (Committee on Twenty-First Century Systems Agriculture, 2010).

1.3.4 Mixed farming

United Nations of Food and Agricultural Organization (FAO) defined conservation agriculture as the use of resource-conserving but high-output agricultural systems. Generally, conservation farming involves the integrated use of minimal tillage systems, cover crops, and crop rotations.

Reduced- or low-input farming is based on a reduction of materials imported from outside the farm, such as commercially purchased chemicals and fuels. Low-input farming is structured in such a way that tightens flow loops and provides ecosystem services internal to the farm and field, and therefore reduces input use. Biological pest controls, solar or wind energy, biologically fixed nitrogen, and other nutrients released from green manures, organic matter, or soil reserves represent some of the internal resources used in low-input farming system. In some cases, external resources are replaced by resources found on or near the farm. Many reduced-input or low-input farming systems are examples of integrated farming systems (Committee on Twenty-First Century Systems Agriculture, 2010).

Combination of conventional and organic production systems develop integrated farming in order to balance environmental quality and economic profit. For example the application of composts and green manure together with some synthetic fertilizers to the soils; the use of some synthetic or natural pesticides in addition to biological, cultural, and mechanical pest control practices. Alternative livestock production systems refer to farms that use lower-confinement housing and rely more on pastures than conventional and industrial livestock farms.

Another example of mixed farming ir represented by mixed crop-livestock farming: in some livestock farm, a significant fraction of the animal feed inputs are generated on cropland and pastures that are under the direct control of the livestock farmer. This system allow to efficiently recycle nutrients, promote crop rotations, and insulate livestock farmers from price fluctuations in feed and input markets. They reflect the resurgence of traditional mixed crop-livestock farming systems that characterized most production units in the first half of the 20th century (Committee on Twenty-First Century Systems Agriculture, 2010).

1.4 Soil biodiversity

Soil biota indicates the entire microbial community living in soil which expresses the vital functions of soil; it is also characterized by a significant spatial diversity with macroscopic differences between soil rhizospheric and non-rhizospheric, between macropores and micropores, between different horizons along the profile, etc. Biota interact with many factors such as soil spatial and temporal heterogeneity (the retention of water, the presence of nutrients, aggregation, granulometric composition, etc.). Several studies reported a higher microbial biomass and community structure in smaller size fractions and that smaller size fraction shost higher diversities of microbes than larger size particles (Lagomarsino et al., 2012; Bailey et al., 2013; Helgason et al., 2010). The diversity and richness of soil bacterial communities differed by ecosystem type, and these differences could largely be explained by soil pH (Fierer and Jackson, 2006). Environmental factors and the type of soil influence soil microbial diversity; it is often the type of agricultural practice used or the type of treatment applied that can determine significant alterations of biodiversity (Gomez et al., 2006) with consequences sometimes difficult or impossible to retrieve (Mocali et al., 2008).

All the life forms present in soil, in particular micro-organisms, which represent a great amount of "invisible life" of fundamental importance to all life on earth, represent the 95 to 98% of the Earth's biodiversity. In fact, the microflora is the most relevant part of soil biomass, that most affects its biological properties.

Some beneficial microorganisms are those that fix atmospheric N, decompose organic wastes and residues, detoxify pesticides, suppress plant diseases and soil-borne pathogens, enhance nutrient cycling and produce bioactive compounds such as vitamins, hormones and enzymes that stimulate

plant growth. The rhizospheric soils contain diverse type of efficient microbes with beneficial effects on crop productivity. The plant growth promoting rhizobacteria (PGPR) and cyanobacteria are rhizospheric microbes and produce bioactive substances to promote plant growth and/or protect them against pathogens (Glick, 1995; Harish et al., 2009).

1.5 Soil quality

Many definitions of soil quality have been proposed in the last decades (Arshad and Coen, 1992; Doran and Parkin, 1994; Karlen et al., 1997). The most recent, proposed by Karlen et al. (1997) is as follows: "the capacityof a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation". As decribed by Sombroek and Sims (FAO, 1995), soil has various functions, as follows: 1) production; 2) biotic environmental; 3) climate-regulative; 4) hydrologic; 5) storage; 6) waste and pollution control; 7) living space; 8) archive or heritage; 9) connective space.

Changes in soil quality and productivity can provide critical signs of environmental degradation and transformation, since soil acts as a source of nutrients and water and a sink for pollutant chemicals. For example, changes in soil aggregation and structure through conventional agricultural management practices increase surface runoff and losses of nutrients. On the other hand, conservation tillage practices help to maintain the soil carbon and nutrient pool, which also promote higher productivity (Bhardwaj, 2011).

The determination of the quality-related properties of soil which are sensitive to changes caused by management practices and environmental stress may help to monitor the changes in its sustainability and environmental quality. Besides soil physical and chemical properties, also soil microorganisms can respond to external disturbs, but as they can do it rapidly they are considered essential in monitoring soil status. However, it is still unclear if naturally occurring environmental factors can damage the genotypic ability of the soil microbiota to recover after averse conditions thus becoming healthy (Schloter et al., 2003).

1.6 Soil quality indicators

Soil quality, which is a complex functional concept (Stocking, 2003), cannot be measured directly but may be assessed from management-induced changes in soil attributes. Assessing soil quality is a challenge because there are no established standards; soils vary spatially and temporally and are readily affected by management (Karlen et al., 1994; Stocking, 2003). Changes in soil quality can be measured through indicators which include physical, chemical and biological processes and

characteristics. Soil quality indicators can be classified into four categories that include visual, physical, chemical, and biological indicators (USDA, 2006).

Physical indicators are related to the organization of the particles and pores, reflecting effects on root growth, speed of plant emergence and water infiltration; they include depth, bulk density, porosity, aggregate stability, textureand compaction (Martinez-Salgado et al., 2010).

Chemical indicators include pH, salinity, organic matter content, phosphorus availability, cation exchange capacity,nutrient cycling, and the presence of contaminants such as heavy metals, organic compounds, radioactive substances,etc. (Martinez-Salgado et al., 2010).

Biological indicators have been increasingly recognized as indicators of soil health, including measurements of micro- and macro-organisms, and their activities or functions (Kennedy and Papendick, 1995; Elliott et al., 1996; Ruzek et al., 2004). Concentration or population of earthworms, nematodes, termites, ants, as well as microbial biomass, fungi, actinomycetes, or lichens, can be used as indicators, because of their role in soil development and conservation, nutrient cycling and specific soil fertility (Anderson, 2003); biological indicators also include metabolic processes such as respiration, used to measure microbial activity related to decomposition of organic matter in soil, and the metabolic quotient (qCO_2), defined as the respiration to microbial biomass ratio, which is associated to mineralization of organic substrate per unit of microbial biomass (Bastida et al., 2008). Other biological indicators that have been widely studied are the chemical compounds or metabolic products of organisms, in particular enzymes related to specific functions of substrates degradation or mineralization of organic N, S or P.

Soil enzymatic activities act as potential indicators of ecosystem quality being operationally practical, sensitive, integrative; they are defined as "biological fingerprints" of past soil management, and relate to soil tillage and structure (Dick, 2000). Determination of rates of decomposition of plant debris in bags or measurements of the numbers of weed seeds, or the presence and quantification of the population of pathogenic organisms can also serve as biological indicators of soil quality (Janssens et al., 2006).

Soil quality indicators should also be linked to soil functions (Larson and Pierce, 1994; Acton and Gregorich, 1995; Karlen et al.,1996; Doran et al., 1996) which may individually or collectively serve as a medium for plant growth, as an environmental filter, as a buffer and transformer, and as a habitat for biota (Seybold et al., 1997; Brady and Weil, 2002).

Selection of soil quality indicators and their integration into a single index using a valid model could help to provide early indications of soil quality changes (Granatstein and Bezdicek, 1992; Mandal et al., 2008). Selection ofrepresentative soil characteristics that play critical roles in ecologicalfunctions is crucialto effective soil quality assessment (Govaerts et al., 2006;Gregorichet al., 1994; Lee et al., 2006; Mandal et al., 2008).

Measurements of changes in soil quality implicate lots of challenges, for example the site specificity: in some places a single property or subset of properties may be disproportionately important, whereas in other places different properties may matter more. Another challengeis that agricultural management affects all major components – physical, chemical and biological – of a soil system, and evaluation of soil quality thus should ideally involve all of them. The biggest drawback in this approach is that many soil properties are variable and every property does not have the same degree or evendirection of response (Bhardwaj et al., 2011).

1.6.1 Chemical indicators

As described above, chemical indicators include pH, salinity (expressed as electrical conductivity, EC), cation exchange capacity, phosphorus availability, organic matter content, nutrient cycling, the presence of contaminants such as heavy metals, organic compounds, radioactive substances (Martinez-Salgado et al., 2010; Heil and Sposito, 1997), soluble complexes, and plant available nutrients. These properties, or chemical attributes of soil quality, may be used to assess soil quality and to monitor changes caused by degradation (Larson and Pierce, 1991; Arshad and Coen, 1992; Karlen and Stott, 1994). These indicators determine the presence of soil-plant-related organisms, nutrient availability, water for plants and other organisms, and mobility of contaminant (Martinez-Salgado et al., 2010). The primary function of soil in relation to its chemical quality for crop production is to provide nutrients for crop growth. Because of the use of pesticides and fertilizers and the application of sewage sludges and other wastes to agricultural lands, the capacity of a soil to immobilize or detoxify pesticides and heavy metals must also be considered in determining the chemical aspects of soil quality (Heil and Sposito, 1997). Närhi et al. (2013) reported a study about the long-term effects of mechanical site preparation on soil chemical properties. They measured soil dielectric permittivity as dependent on water content, electrical conductivity, temperature, pH, as well as ammonium acetate extractable concentrations of mineral soil elements: the loss of soil nutrients was considerably high, particularly with exchangeable Ca^{+2} (40%) and Mg^{+2} (51%), Ca:Al ratio (57%), and soil electrical conductivity (53%). The disk trenching had no considerable long-term effect on soil water content, thus intensive mechanical site preparation is a risk for long-term soil fertility.

1.6.1.1 pH

Soil pH is defined as the negative logarithm (base 10) of the H^+ activity (moles per liter) in the soil solution. As the activity of H^+ in the soil solution increases, the soil pH value decreases.

Soil pH is a function of parent material, time of weathering, vegetation and climate; it is considered as one of the dominant chemical indicators of soil health, identifying trends in change for a range of

soil biological and chemical functions including acidification, salinisation, crop performance, nutrient availability and cycling and biological activity (Dalal and Moloney, 2000).

Integrative soil health tests value include pH to assess impacts of land use change and agricultural practices (Gil et al., 2009; Idowu et al., 2009; Pattison et al., 2008; Schindelbeck et al., 2008). Conversely Brinkman and Sombroek (1999) suggested that most soils would not be subjected to rapid pH changes resulting from drivers of climate change such as elevated temperatures, CO_2 fertilization, variable precipitation and atmospheric N deposition (DeVries and Breeuwsma, 1987; McCarthy et al., 2001), it is likely, however, that these drivers of climate change will affect organic matter status, C and nutrient cycling, plant available water and hence plant productivity, which in turn will affect soil pH (Reth et al., 2005).

Eckert and Sims (2011) reported the soil buffer capacity: soil pH is buffered by several components of the solid phase, including hydroxyaluminum monomers and polymers, the soil organic matter, and (in alkaline soils) the undissolved carbonate compounds. An equilibrium condition exists between these components and the soil solution in such a way that when acid or base is added to the solution, the buffering agents may maintain the initial equilibrium.

The addition of limestone and other basic materials is normally used to maintain soil pH in a desirable range. Although organic matter additions may not directly affect soil pH, soils that receive significant amounts of organic materials tend to maintain (buffer) soil pH values for longer periods of time (Evanylo and McGuinn, 2009).

1.6.1.2 Soluble salts in soils and electrical conductivity

Gartley (2011) defined soluble salts, for soils, technically, as those dissolved inorganic solutes that are more soluble than gypsum ($CaSO_4 \cdot 2H_2O$; solubility of 0.24 g 100 mL^{-1} at 0 °C). The most common soluble salts in soils are the cations calcium (Ca^{+2}), magnesium (Mg^{+2}), and sodium (Na^{+}) and the anions chloride (Cl^{-}), sulfate (SO_4^{-2}), and bicarbonate (HCO_3^{-}). Smaller quantities of potassium (K^{+}), ammonium (NH_4^{+}), nitrate (NO_3^{-}), and carbonate (CO_3^{-2}) are also found in most soils. The usual method to quantify the soluble salts concentration in soils is to measure the electrical conductivity (EC) of either the soil solution or a soil-water extract. Electrical conductivity refers to the ability of a material or solution to conduct an electrical current. As soluble salts increase in the soil, the soil solution becomes a better conductor of electricity and EC increases. The unit most commonly used for EC in soil solutions or in soil-water extracts is mmhos cm^{-1} but the official international unit for EC is siemens per meter (1 mmhos cm^{-1} is equal to 0.1 S m^{-1}).

Soil electrical conductivity (EC) is considered a reliable indicator of soil quality/health and easy to measure (Arnold et al., 2005). Information about trends in salinity, crop performance, nutrient cycling (particularly nitrate) and biological activity could be found out by EC values and in addition

a surrogate measure of soil structural decline especially in sodic soils could derive from this parameter, along with pH (Arnold et al., 2005; Dalal and Moloney, 2000).

Electrical conductivity has been used as a chemical indicator to express soil biological quality in response to crop management practices (Gil et al., 2009). Using elevation gradient as a surrogate for increasing temperatures and decreasing precipitation under climate change scenarios, Smith et al. (2002) found that EC decreased and pH increased in a semi-arid environment. Pariente (2001) examined the dynamics of soluble salts concentration in soils from four climatic regions (Mediterranean, semi-arid, mildly arid and arid) and found a non-linear relationship between the soluble salts content and rainfall, with sites that received <200 mm rainfall contained significantly high soluble contents and vice versa. Clearly, there is a need for comprehensive assessment of the influence of drivers of climate change on soil EC as an important soil health indicator in different ecosystems. Sources of soluble salts in soils include commercial fertilizers, animal manures, municipal sewage sludges, soil organic matter, runoff from areas where salt or ice-melt products have been used and irrigation water that is high in dissolved salts. At “normal” concentrations, soluble salts have little harmful effect on plant growth; however, if excessive soluble salts exist, plant injury, such as reduced germination rates and leaf burning, or death may occur (Gartley, 2011).

1.6.1.3 Cation Exchange Capacity

Measurements of the cation exchange capacity (CEC) show significant soil properties, in particular its ability to retain the cations because of their mobility in the soil (Saidi, 2012). Ross and Ketterings (2011) observed that the cation exchange capacity (CEC) of a soil is a measure of the quantity of negatively charged sites on soil surfaces that can retain positively charged ions (cations) such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^{+}), by electrostatic forces. Cations retained electrostatically are easily exchangeable with cations in the soil solution so a soil with a higher CEC has a greater capacity to maintain adequate quantities of Ca^{2+}, Mg^{2+} and K^{+} than a soil witha low CEC. Soils with high CEC hold more nutrients, and furthermore they are better able to buffer, or avoid rapid changes in soil solution levels of these nutrients by replacing them as the solution becomes depleted. Generally, the inherent fertility, and long-term productivity of a soil is greatly influenced by its CEC. Lower values of CEC (less than 5 meq $100g^{-1}$) in soils result in a lower clay and organic matter content, a lower water holding capacity, require more frequent lime and fertilizer additions, and is subjected to leaching of NO_3, B, NH_4, K and perhaps Mg. Such soils will have lower yield potential than soils with higher CEC under the same level of management, but high productivity can be maintained by intensive management. These soils will usually be easier to cultivate than soils with higher CEC since they drain more rapidly, and added nutrients are highly

available for plant uptake. Soil CEC is normally expressed in one of two numerically equivalent sets of units: meq100 g^{-1} (milliequivalents of charge per 100 g of dry soil) or cmolckg^{-1} (centimoles of charge per kilogram of dry soil) (Ross and Ketterings, 2011).CEC is a good indicator of the degradation of soil surface formations, as it is directly related to the SOC storage capacity in Mediterranean environments (Ruiz Sinoga et al., 2012).

1.6.1.4 Soil Organic Carbon

Soil organic carbon (SOC) content is a key factor of soil quality. It is the most often reported attribute from long-term studies and is chosen as the most important indicator of soil quality and agronomic sustainability because of its impact on other physical, chemical and biological indicators of soil quality. It strongly impacts on soil physical-mechanical quality by favorable changes in surface area, formation and stabilization of aggregates, total porosity and pore size distribution, aggregate strength, erodibility and susceptibility to crusting and compaction. In addition, SOC contributes positively to a range of biological, physical and chemical properties important to defining the potential productivity of a soil.

Principal impacts of SOC content on soil hydrologic properties include increase in plant available water capacity because of alteration in soil moisture characteristic curves (pF) which favor retention of water at low potential (-0.01 to 0.03 MPa range). Among other impacts of SOC on hydrological properties increase in water infiltration rate (infiltrability), and decrease in surface runoff (rate and amount) could be noted. Improvements in these soil hydrological properties are important to reduce susceptibility of agro-ecosystems to pedological/agronomic droughts (Lal et al., 2012). Key parameters of soil chemical quality improved by increase in SOC pool and its quality include charge properties affecting both anion exchange capacity and cation exchange capacity, thereby enhancing the nutrient retention by reducing losses through leaching and volatilization. Increase in charge characteristics also improves soils buffering capacity against sudden changes in reaction (pH), and elemental transformations. Attributes of soil biological quality enhanced by improvements in SOC concentration include activity and species diversity of soil organisms including earthworms, which accentuate bioturbation and enhance soil structures, microbial biomass which affects C turnover and rhizospheric processes including nitrification/denitrification. The overall improvement in soil quality also enhances ecological processes such as elemental cycling, oxidation/uptake of CH_4, and use efficiency of input (fertilizers, water, decline in sedimentation, non-point source pollution). There is an improvement in land value, and also enhancement in aesthetic/cultural attributes. Strategies of enhancement of SOC pool in agroecosystems include those that create a positive C budget. In this regard, the importance of retention of crop/animal residues by surface application of by-product (e.g., mulch, manure) cannot

be over emphasized. There are numerous advantages of crops residue retention, which impact SOC dynamics and enhance provisioning of important ecosystem services. The significance of growing perennial grain crops is also being considered (Glover et al., 2010, 2012).

There is a wide range of soil quality indices (Bastida et al. 2008; Erkossa et al., 2007; Lal, 1994; Schloter et al., 2003). Most indices, which involve SOC concentration/pool, are based on critical limits of SOC and other parameters (Arshad and Martin, 2002; Aune and Lal, 1998). With multiparametric indices, standardization of soil quality attributes and creation of minimum data-set are important considerations (Bastida et al., 2008; Nortcliff, 2002; Rezaei et al., 2006). Some indices involve the soil management assessment framework (Andrews et al., 2004), microbiological and biochemical parameters (Arias et al., 2005; Hofman and Dusek, 2003), and can be used at plot or preferably at a watershed scale (Cambardella et al., 2004).

1.6.1.5 Plant Available Nutrients

In their identification of basic soil properties to meet requirements of indicators for screening soil quality/health, Doran et al. (1999) point out the extractable nutrients nitrogen, phosphorus, potassium, since “they provide information on plant available nutrients and potential loss from soil providing indication of productivity and environmental quality”. Measurement of extractable nutrients may provide indication of a soil capacity to support plant growth; conversely, it may identify critical or threshold values for environmental hazard assessment (Dalal and Moloney, 2000). Nutrient cycling, especially N, is intimately linked with soil organic carbon cycling (Weil and Magdoff, 2004), and hence drivers of climate change such as elevated temperatures,variable precipitation and atmospheric N deposition are likely to impact on N cycling and possibly the cycling of other plant available nutrients such as phosphorus and sulphur, although direction and exact magnitude of change in plant available nutrients need to be investigated in detail.

1.6.2 Biochemical indicators

Soil biochemical parameters has been used as a trusted indicator of the quality of the soil because that estimate the changes in the dynamics and distribution of soil microbial processes in different land use systems. Among them, soil enzymes involved in the cycling of bio- elements (C, N, P and S) can be considered as good indicators of soil biological quality and fertility because of their essential role in soil biology, ease of measurement, and rapid response to changes in soil management such as use of fertilizers, amendments, vegetation cover and pesticides (Gianfreda and Bollag, 1996), the soil enzymes possessing major sensibility to the change of biotic and abiotic factors. The activities of extracellular enzymes are a measure of the potential of soil to carry out

biochemical processes responsible for the release of nutrients to plants and microorganisms through the transformation of organic matter.

1.6.2.1 Soil enzymes

Enzymes are a measure of the potential of soil to carry out biochemical processes responsible for the release of nutrients to plants and microorganisms through the transformation of organic matter. Enzymes activities have been suggested as suitable indicators of soil quality because: (a) they are a measure of the soil microbial activity and therefore they are strictly related to the nutrient cycles and transformations; (b) they rapidly may respond to the changes caused by both natural and anthropogenic factors (Gianfreda and Bollag, 1996; Drijber et al., 2000; Calderon et al., 2000; Colombo et al., 2002; Nannipieri et al., 2002). Soil enzymes are related to soil fertility, microbial activity, biochemical cycling of various elements (C, N, S). The main groups of enzymes involved in nutrient cycles include dehydrogenases, glucosidases, urease, amidases, phosphatases, arylsulphatase, cellulases, and phenol oxidases as shown in Figure 1.6.

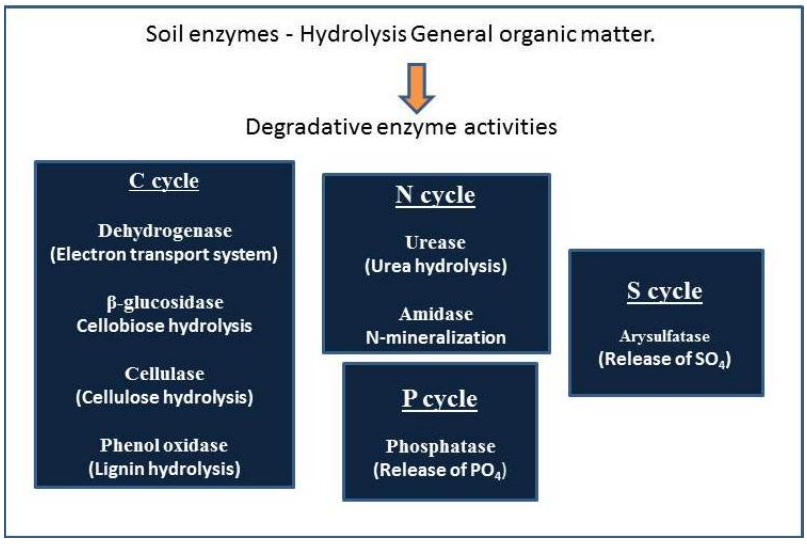

Figure 1.6 Soil enzymes as indicators of soil health

Nannipieri et al. (2012) claims that there is a great problem to use enzyme activities as indicators of soil functions because sometimes there is not an accurate reflection of soil quality in them, in particular (1) the enzyme assays determine potential and not real enzyme activities; (2) the meaning of measured enzyme activities is not known; (3) the assumption that a single enzyme activity is an indicator of nutrient dynamics in soil neglects that the many enzyme activities are involved in such dynamic processes; (4) spatio-temporal variations in natural environments are not always considered when measuring enzyme activities; and (5) many direct and indirect effects make difficult the interpretation of the response of the enzyme activity to perturbations, changes in the soil management in general, etc.

1.6.2.1.1 Dehidrogenases

Soil dehydrogenases (EC 1.1.1.) are the major representatives of the oxidoreductase enzymes class (Gu et al., 2009). They play a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors. Dehydrogenase (DHY) is one of the most important enzyme used as indicator of biological activity in soil; these enzymes occur in all living microbial cells (Moeskops et al., 2010; Zhao et al., 2010; Yuan and Yue, 2012) and because of their extracellular nature, they can reflect the metabolic status of microorganisms in soil. On the other hand, extracellular enzymes are quickly mineralized by other enzymes (i.e. proteases) in the degradative process or immobilized by humic molecules and clays.

Several environmental factors, including soil moisture, oxygen availability, oxidation reduction potential, pH, organic matter content, depth of the soil profile, temperature, season of the year, heavy metals contamination and soil fertilization or pesticide use, can affect significantly DHY in the soil environment. Wolińska and Stępniewska (2012) mentioned pH as an important parameter affecting soil DHY being their optimal activity range between 5.5-5.73. Brzezinska et al. (1998) suggested that soil water content and temperature influence dehydrogenase activity indirectly by affecting soil redox status.

The most common laboratory procedure used for DHY determination is the method developed by Casida et al. (1964) and Thalmann (1968). Soil activation is determined indirectly, using hydrolytic reaction leading to formazan evolving. The formazan concentration is directly proportional to the vitality level of community of soil non-photosynthetic microorganisms. The activation rate can be expressed as activity of soil dehydrogenases or relatively in percentage as comparison to control. The mothod is based on the evaluationof the triphenyltetrazolium chloride (TTC) reduction rate intriphenylformazan (TPF) after incubation for 24 h at 30 ºC as described in Figure 1.7.

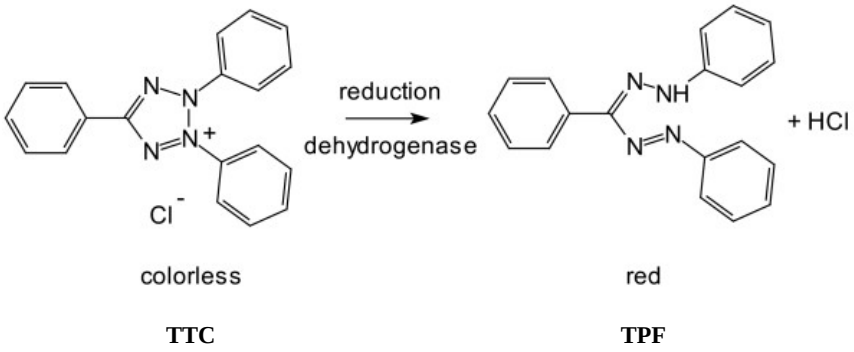

Figure 1.7 Hydrolysis of TTC to TPF

1.6.2.1.2 Phosphatases

Because of their participation in phosphorus cycle, phosphatases enzymes release inorganic phosphate which can be taken up by plants or microorganisms from organic moiety and complex

inorganic materials. Phosphorus is the maker of the energy currency and it plays important roles in enumerable metabolic pathways in living systems (Rasol and Reshi, 2010).

Phosphatases (PHO) catalyze the hydrolysis of phosphate both ester of phosphoric acid and are enzymes specific, capable of acting on member of different structurally related substrates (Figure 1.8). Soil phosphatase activity depends heavily on soil moisture content and environmental temperature (Huang et al., 2011). They are usually classified according to their pH optimum as: neutral (EC 3.1.3), alkaline (EC 3.1.3.1) and acid (EC 3.1.3.2) (Akanji and Adesokan, 2005; Raghava et al., 2011). They have received trivial names and sub classifications according to their substrates, such as phytases, nucleotidases, sugar phosphatases and glycerophosphatase belong to the group of phosphoric monoester hydrolases, phosphoric monoester hydrolases or phosphomonoesterases (EC 3.1.3); they also can be classified as phosphoric diester hydrolases or phosphodiesterases (EC 3.1.4), triphosphoric monoester hydrolases (EC 3.1.5) and enzymes acting onphosphoryl-containing anhydrides (EC 3.6.1) and on P-N bonds (EC 3.9). Phosphomonoesterases include acid and alkaline phosphomonoesterases (which hydrolyze monoester bonds including mononucleotides and sugar phosphates), phosphoprotein phosphatases (which hydrolyse phosphoester bonds of phosphoserines, phosphothreonines or phosphotyrosines), phytases (EC 3.1.3.26 for 4-phytase and EC 3.1.3.8 for 3-phytase, which hydrolyse all six phosphate groups from inositol hexaphosphate) and nucleotidases. Phosphodiesterases hydrolyse one or two ester bonds in phosphodiester compounds and include nucleases, which catalyse the hydrolysis of phosphodiester bonds of nucleic acids to produce nucleotide units or mononucleotides but not inorganic phosphates. (Nannipieri et al., 2011).

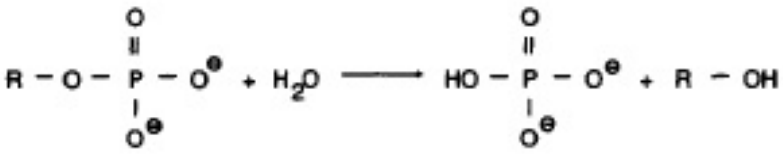

Figure 1.8 Phosphatase reaction

Apart from being good indicators of soil fertility, phosphatase enzymes plays a key role in the soil system (Eivazi and Tabatabai, 1977; Dick et al., 2000). For example, when there is a signal indicating P deficiency in the soil, acid phosphatase secretion from plant roots is increased to enhance the solubilization and remobilization of phosphate, thus influencing the ability of the plant to cope with P-stressed conditions (Karthikeyan et al., 2002; Mudge et al., 2002; Versaw and Harrison, 2002).

The most common laboratory procedure used for PHO determination is the method of Tabatabai and Bremner (1969), that is based on the incubation of soil samples mixed with a buffer solution in

the presence of *p*-nitrophenylphosphate (*p*-NPP) for 1 hr at 37ºC. The released *p*-nitrophenol is stained, so it can be measured spectrophotometrically at 400 nm,

1.6.2.1.3 β-glucosidase

β-glucosidase (EC 3.2.1.21) is the rate limiting enzyme in the microbial degradation of cellulose to glucose (Figure 1.9) involved in hydrolysys of maltose and cellobiose. The final product of the reaction is glucose, an important C energy source of life to microbes in the soil (Esen, 1993).

β-glucosidase (GLU) is considered a soil quality indicator because of its sensitivity to changes in pH thus representing a good biochemical indicator resulting from soil acidification and ecological changes. It is also one of the immobilized enzymes most often reported in literature and suggested as an indicator of management practices effects (Acosta-Martínez and Tabatabai, 2000; Madejón et al., 2001).

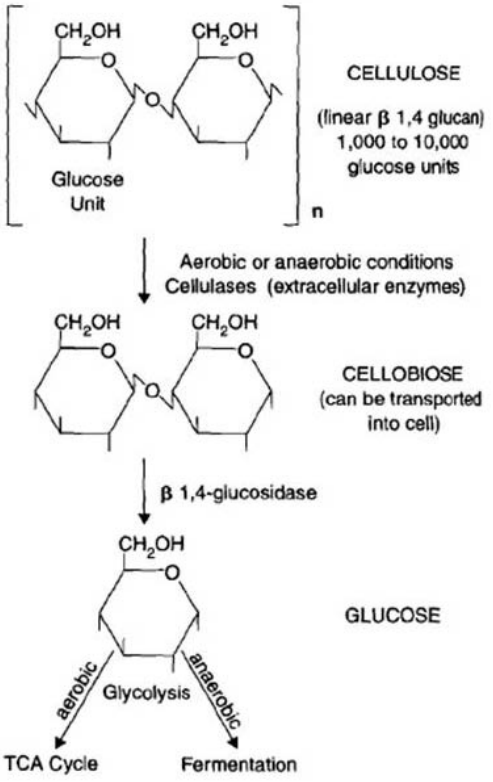

Figure 1.9 The decomposition of cellulose (from Sylvia et al., 2005)

GLU is strictly related to soil microbial biomass, therefore it is considered a sensitive indicator to monitor short-term variations of soil quality, and may give information about the past biological activity, the capacity of soil to stabilize soil organic matter, and it is affected by management of soil (Das and Varma, 2001). GLU activity is limited under high salinity conditions, and partially inhibited by inorganic N fertilization; it is proportional to N apply over time, probably because the added N stimulate the release of root exudates which in turn stimulate β-glucosidase activity (Eivazi and Tabatabai, 1990), whereas uptake by plant roots keep the amount of inorganic N in the soil

solution low, thus reducing the risk of enzyme inhibition. GLU is a sensitive index of changes in soil organic matter content, viable microbial count and vegetation cover, therefore, it is the main enzyme to be examined in soil and its activity is the best of the physic-chemical indicators of soil organic matter turnover.

The classical method used to measure this enzyme activity was proposed by Eivazi and Tabatabai in 1977 and improved in 1988 from the same authors. It is based on the conversion of the artificial substrate *p*-nitrophenyl-β-D-glucopyranoside to *p*-nitrophenol in 1h incubation of soil at 37 °C.

1.6.2.1.4 Invertase

The enzyme invertase (EC 3.2.1.26) catalyzes the hydrolysis of sucrose (Figure 1.10) and yields glucose and fructose (Figure 15), and is widely distributed in microorganisms, animals and plants. The optimum conditions of pH and temperature are 5.0-5.6 and 50 ºC, respectively.

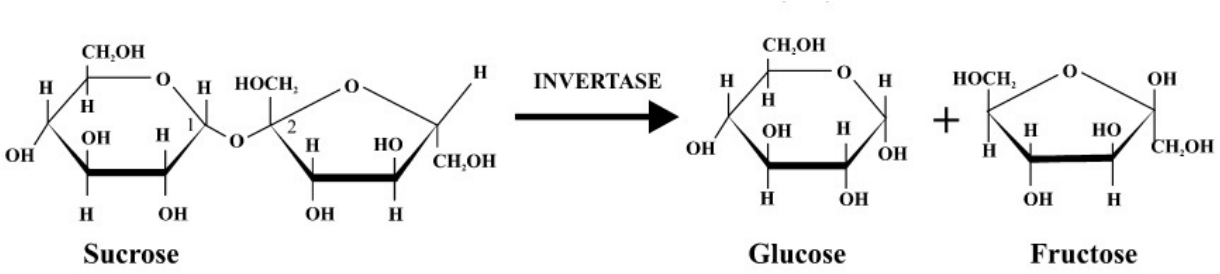

Figure 1.10 Schematic representation of sucrose hydrolysis. From Sagar et al. 2012.

Invertase activity is only partly associated with light organic matter fractions; in general, the enzyme is linked to the soil organic fraction of high density, clay minerals and microbial biomass (Ross, 1983; Stemmer et al., 1998). Soil organic matter (SOM) can influence invertase activities in higher plants (Malcolm and Vaughan, 1979) and, therefore, it could be expected that soil invertase is also influenced by the SOM in solution. This enzyme can be affect by pesticide application, in fact Glyphosate and paraquat increased invertase activity of several soils (Sannino and Gianfreda, 2001). This enzyme response greatly to the change of cover crop and deph of soil than to seasonal effect. The most common procedure used to measure invertase activity, is based on the method described by Kandeler (1999) in which the moist soil fraction is incubated with sucrose solution of for 3h at 50 ºC. Later, reducing sugars areevaluatedas described by Schinner and von Mersi (1990) and Schinner et al. (1996).

1.6.2.1.5 Fluorescein diacetace (FDA) hydrolysis

Fluorescein diacetate (FDA) is one of the parameters to measure microbial activity in soils. It is considered an indicator of soil quality because the size, diversity and activity of microbial

population is of great importance due to its rapid response to external factors such as climatic changes, environmental pollution, ecosystem diversification. The total microbial activity provides a general measure of organic matter turnover in natural habitats as about 90% of the energy in the soil environment flows through microbial decomposers (Schnurer and Rosswall, 1982).

FDA is hydrolyzed by a number of different enzymes, such as proteases, lipases, and esterases. The equation of the reaction is reported below (Figure 1.11).

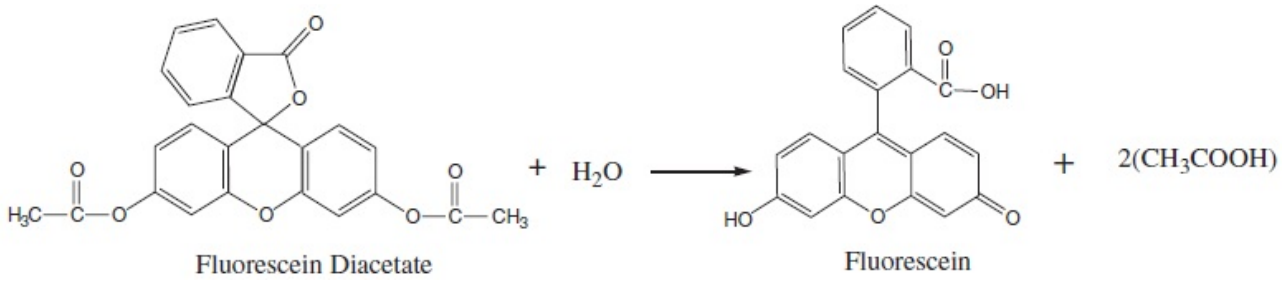

Figure 1.11 Fluorescein diacetate hydrolysis

The measurement of FDA activity is based on the method of Green et al. (2006). The product of this enzymatic conversion is fluorescein, which can be visualized within cells by fluorescence microscopy. Fluorescein can also be quantified by fluorometry or spectrophotometry.

1.6.2.1.6 Urease

Urease enzyme is involved in the hydrolysis of urea to carbondioxide (CO_2) and ammonia NH_3, which can be assimilated by microbes and plants (Figure 1.12). It acts on carbon-nitrogen (C–N) bonds other than peptide linkage, the hydrolysis of urea cause the soil acidification and this, in turn, results in a rapid N loss to the atmospherethrough NH_3 volatilization, therefore, the urea activity is considered a vital process in the regulation of N supply to plants after urea fertilization. Soil urease originates mainly from plants (Polacco, 1977) and microorganisms found as both intra- and extra-cellular enzymes (Burns, 1986; Mobley and Hausinger, 1989). Urease extracted from plants or microorganisms is rapidly degraded in soil by proteolytic enzymes (Pettit et al., 1976; Zantua and Bremner, 1977). This suggests that a significant fraction of the activity of this enyme in the soil is carried out by extracellular urease, which is stabilized by immobilization on organic and mineral soil colloids.

Urease activity in soils is influenced by many factors as management of soil, organic matter content, organic input, heavy metals, and environmental factors as temperature.

Since urease plays a vital role in the hydrolysis of urea fertilizer, it is important to uncover other unknown factors that may reduce the efficiency of this enzyme in the ecosystem.The urease increment under low and normal N application rates (but not high N application rate). Long-term N

fertilization significantly decreased urease activity, in fact urease enzyme has also been widely used in the evaluation of changes in soil quality due to soil management. Its activity increased due to organic fertilization (Pascual et al., 1999) and addition of cattle slurry (Kandeler and Eder, 1993) and decreased as a consequence of intensive tilling (Saviozzi et al., 2001). Soil urease showed close relation with urea hydrolyzation and increased the utilization rate of nitrogen fertilizer (Cookson and Lipiece, 1996; Klose and Tabatabai, 1999).

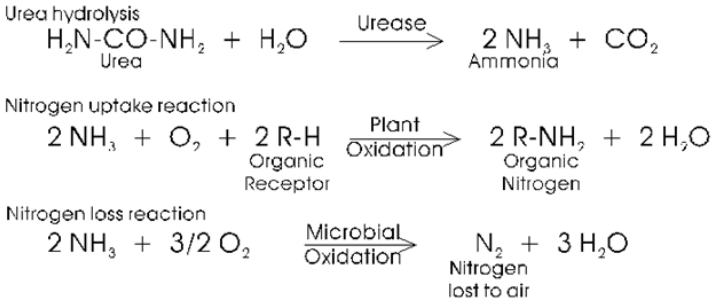

Figure 1.12 Urease hydrolysis

The method to evaluate urease activity in soil consist in the incubation of soil with an aqueous or buffered urea solutionfor 2h at 37 ºC (Kandeler and Gerber, 1988). The method is characterizated by high sensitivity and stability of the coloured complex formed.

1.6.2.1.7 Phytase

Organic phosphate represents a major reservoir of inorganic phosphorus (Pi), essential element for plants growth. One of the major forms of organic phosphorus is phytate (myo-inositol hexakisphosphate). Plants accumulate phytate in their seeds, roots, and other tissues during ripening. The phytate needs to be degraded to return Pi to the soil. Phytase (also named as phytate-degrading enzyme) is a generic term used to describe enzymes that initiate the sequential releasing of one or more inorganic phosphate groups from phytases. Several phytase classes are now known: histidine acid phosphatase, β-propeller phytases (BPP), cysteine phosphatase and purple acid phosphatase (Mullaney and Ullah, 2007). The terms phytic acid, phytate and phytin refer to the free acid, salt and calcium/magnesium salt, respectively. In the literature, the terms phytic acid and phytate have been used interchangeably. Phytases (myo-inositol hexakisphosphate hydrolases) are a special class of phosphatase enzymes able to catalyze the sequential hydrolysis of phosphate ester bonds of phytate (Angel et al., 2002).

Many bacteria, yeasts and fungi, isolated from a wide range of sources including soil, fermented food/feed, water and also gastrointestinal fluid of ruminants, produce phytases.

1.6.3 Biological indicators

Biological indicators include properties involeved in soil organic matter transformation such as microbial biomass and respiration rate; specific metabolic quotient (qCO_2, ratio of respired C to biomass C) and ratio of microbial biomass C to total organic C are other valid biological measurements that have been suggested as indicators for assessing long-term soil management effects on soil quality (Dilly et al., 2007; Riffaldi et al., 2002, 2006; Saviozzi et al., 2007). In particular, such parameters are sensitive to changes in soil C availability, caused by alterations in soil management practice, and can change markedly before any changes in organic matter content are detected (Haynes and Beare, 1996). Biological properties have been used as soil quality indicators because of their relationship with organic matter content, terrestrial arthropofauna, lichen, microbial community (biomass or functional groups), metabolic products as ergosterol or glomalin and soil activities as microbial respiration and enzyme production (Martinez-Salgado et al., 2010).

1.6.3.1 Soil respiration

The respiration is the biological oxidation of the organic matter to CO_2. It play a vital role in the C cycling. Soil respiration is the primary path by which CO_2 fixed by land plants returns to the atmosphere. Schlesinger and Andrews (2000) estimated this flux as equal to approximately $75 \cdot 10^{15}$ gC/yr, and this large natural flux is likely to increase due to changes in the Earth's condition, and small changes in the magnitude of soil respiration could have a large effect on the concentration of CO_2 in the atmosphere. Soil respiration is a measure of potentially mineralizable carbon in soil and reflects the global activity or energy spent by the microbial pool (Anderson and Domsch, 1990), providing an estimate of the decomposing activity of microorganisms in the soil (Kennedy and Papendick, 1995). Balogh et al. (2011) found significant influence of abiotic factors on the respiration such as soil clay content, total organic carbon (TOC), tempetature and moist. In warm climates, respiration rates are higher and vary with soil pH, moisture content, supplemental O_2 and availability of N. Soil respiration is larger near the soil surface, due to the high concentrations of organic matter and the availability of oxygen. The respiration is also influenced by organic matter and microbial activity. At an annual scale, soil respiration contributes to 60 and 80% of ecosystem respiration.

The method largely used to measure soil respiration rate is described by Alef (1995) and consists in incubating a soil sample in a closed jar (Figure 1.14) containing an alkaline trap (NaOH o KOH) and measuring the CO_2 accumulated by acid titration. CO_2 released during aerobic respiration in soils may be adsorbed by the alkaline solution according to the following reaction:

$$CO_2 + 2NaOH = Na_2CO_3 + H_2O$$

The amount of CO_2 adsorbed is equivalent to the amount of NaOH consumed. To determine this, the carbonate (CO_3^{-2}) are precipitated with $BaCl_2$ and the remaining NaOH is titrated with HCl as indicated by the following reactions:

$$Na_2CO_3 + BaCl_2 = 2NaCl + BaCO_3$$

$$NaOH + HCl = NaCl + H_2O$$

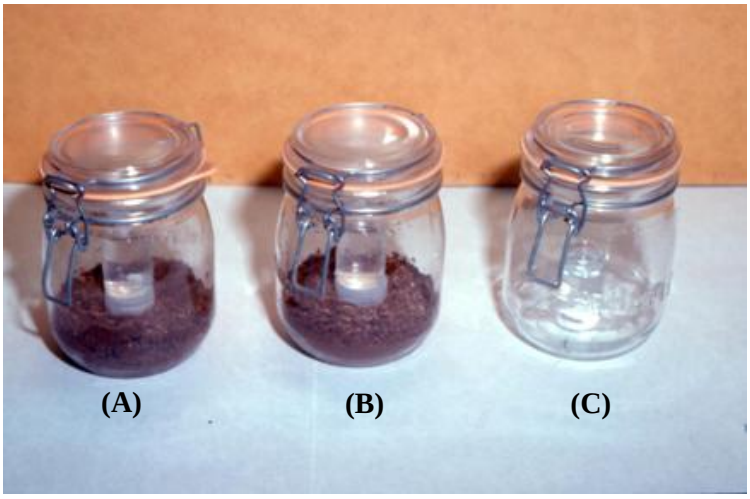

Figure 1.14 Estimation of soil respiration in closed jairs: (A) and (B) soil samples with NaOH solution in the trap; (C) Control (without soil)

Incubation periods usually lasting between 10 and 30 days, and the carrier is periodically opened to allow gas exchange with the atmosphere and maintain aerobic conditions.The extent of CO_2 may also be performed using a gas chromatograph techniques or electrical conductivity measurement of infrared spectroscopy (Alef, 1995). As mentioned before, the soil respiration is strongly influenced by temperature, soil moisture, nutrient availability and soil structure, to minimize these variables can effect a pre-conditioning of the soil prior to the measurement. Respiration measurements in the field are less frequent due to the high dependence on weather conditions, although these measures have proved capable of discriminating between different management practices in soil (Pankhurst et al., 1995).

1.6.3.2 Carbon Microbial biomass

Soil carbon microbial biomass (MB-C) is defined as the living microbial component of the soil, excluding the macrofaun and the plants roots. MB-C plays important roles in nutrient cycling, plant-pathogen suppression, decomposition of residues and degradation of pollutants; therefore, it is often regarded as a good indicator of soil quality (Kaschuk et al., 2010).

Soil microbial biomass performs the transformation of organic matter in soil. It has a much faster turnover than the rest of organic matter, for this reason is considered as soil quality indicator (Duxbury et al., 1989).

MB-C could be related to diverse soil processes, including decomposition of organic residues, nutrient cycling, solubilization of nutrients (particularly phosphates), degradation of xenobiotic compounds and pollutants, soil structuring, organic matter storage, and biological control and suppression of plant pathogens; and for that reason, it has often been indicated as an important component for maintaining soil quality and plant productivity (Nogueira et al., 2006; Roscoe et al., 2006). Sugihara et al. (2010) found that microbial biomass is highly influenced by the seasonal conditions, and texture of soil, in particular, it is high in dry season and retains nutrients when plant activity is low. By contrast, during the rainy season soil microbial biomass is low because of accelerated turnover caused by enhanced grazing by soil macro-fauna.

The N fertilization influence microbial biomass-C. Nitrogen as one of the essential components in terrestrial ecosystems, soil microorganisms play important roles in soil nutrient biogeochemical cycles, particularly in nitrogen transformation (Rich and Myrold, 2004; Shen et al., 2010). Nitrogen addition can change soil microbial communities in a relatively short time compared to plant communities (Bradley et al., 2006; Zhang et al., 2008). Li et al. (2013) reported carbon microbial biomass decreased after ammonium sulfate or urea application, and increased with soil depth.

Soil texture is also an important factor that controls soil microbial dynamics, the sandy soils are normally characterized by lower amount of soil organic matter, and clayey soil has the structure of high-clay content, protecting soil microbes from predators and dry stress, generally is belived so that soil microbial biomass is generally lower in sandy soil as compared to clayey soil. But, also is demonstrated that the sandy soil has a faster turnover rate of soil microbes compared to clayey soil (Malik et al., 2013).

MB-C has been correlated with several functional microorganisms, such as ammonifiers and nitrifiers (Andrade et al., 1995), microbial diversity (Nogueira et al., 2006), legume-nodulating bacterial populations (Pereira et al., 2007) and enzyme activities in the soil (Balota et al., 2004).

The determination of MB-C is usually performed with the fumigation method with chloroform that includes two different techniques: fumigation-incubation (FI) and fumigation-extraction method (FE) described by Vance et al. (1987). In both cases the chloroform vaporsbrakesmicrobial cells and releases cellular contents into the soil.

A widely used indicator of carbon availability for soil biota is the C_{mic}/C_{org} ratio (Sparling, 1992) that is the carbon microbial biomass respect to the total organic carbon content.

1.7 Soil organic matter

Soil organic matter (SOM) is a complex dynamic system whose chemical, microbial, and biochemical components change over time and space, depending on several abiotic and biotic factors, organic residue inputs, and on their degree of association with inorganic components. Piccolo (2012) has defined SOM as a noncovalent supramolecular association of small molecules surviving microbial degradation of plant and animal tissues. It is a carbon-rich material that includes plant, animal, and microbial residues in various stages of decomposition. Live soil organisms and plant roots are part of the carbon pool in soil but are not considered soil organic matter until they die and begin to decay. In natural and introduced crops, biogeochemical nutrient cycling is directly controlled by SOMproduction and decomposition.

The conservation of sufficient SOM levels is crucial for the biological, chemical and physical soil functioning in the farming ecosystems. The SOM content of agricultural soils usually ranges between 1% and 5% (w/w). Appropriate levels of SOM ensure soil fertility and minimize agricultural impact on the environment through carbon sequestration, reducing erosion and preserving soil biodiversity.

Organic matter plays a central role in maintaining key soil functions and is an essential determinant of soil fertility and resistance to erosion. The build-up of organic matter in soils is enhanced by such farm management techniques as conservation tillage including zero tillage, organic farming, maintenance of permanent grassland and cover crops, mulching, manuring with green legumes, application of farmyard manure and compost, strip cropping and contour farming (Lal, 2005; Roldan et al., 2005). These techniques have also proved effective in reducing erosion, increasing fertility and enhancing soil biodiversity. Among these various techniques, the transformation of organic wastes (sewage sludge, green waste, industrial and organic waste, animal manure) to compost is becoming increasingly popular across Europe, thus reducing the use of artificial fertilizers, and the amounts of waste added to landfill sites.

1.7.1 Humic substances

Humic substances (HSs) are complex and heterogeneous mixtures of polydispersed materials formed by biochemical and chemical reactions during the decay and transformation of plant and microbial remains (a process called humification). Plant lignin and its transformation products, as well as polysaccharides, melanin, cutin, proteins, lipids, nucleic acids, fine char particles, etc., are important components taking part in this process. Soil animals and microorganisms, intracellular and extracellular enzymes and inorganic surfaces are the catalysts that continuously process, modify, and bind residues into small molecules and metabolites of plants and microorganisms into humic substances (HSs) (Huang and Hardie, 2009). Because of the beneficial effects that HSs have

on the physical, chemical, and biological properties of soil, their role in the soil environment is significantly greater than that attributed to their contribution to sustaining plant growth. The HSs are recognized for their controlling both the fate of environmental pollutants and the chemistry of organic carbon in the global ecosystem (Piccolo et al., 1996). Schnitzer and Monreal (2011) defined HS as a portion of the total SOM that is extracted and solubilized with dilute alkali (0.1-0.5M NaOH or KOH). The alkaline extract is usually partitioned into three fractions which are humic acid (HA), fulvic acid (FA), and humin.

Humic substances in soils and sediments can be divided into three main fractions: humic acids (HA or HAs), fulvic acids (FA or FAs) and humin. The HA and FA are extracted from soil and other solid phase sources using a strong base (NaOH or KOH). Humic acids are insoluble at low pH, and they are precipitated by adding strong acid (adjust to pH 1 with HCl). Humin cannot be extracted with either a strong base or a strong acid.

Humic substances are highly chemically reactive yet recalcitrant with respect to biodegradation. Most of the data on HA, FA and humin refer to average properties and structure of a large ensemble of components of diverse structure and molecular weight. The precise properties and structure of a given HS sample depends on the water or soil source and the specific conditions of extraction. Nevertheless, the average properties of HA, FA and humin from different sources are remarkably similar.

1.8 Organic amendements

Organic matter (OM) supply is of great importance for soil quality and fertility, because it improve the physico-chemical structure, root penetration and water retention capacity, nutritional status, buffer capacity; moreover it improve biochemical and microbiological properties promoting metabolic activity in the rhizosphere, enhancing and maintaining an appropriate level of microbial growth, functionality and biodiversity.

The use of amendments is fundamental to improve soil quality thus resolving different limitants factors of soil, for example the increment of OM in semirarid soil, the mitigation of soil carbon losses, the increase of activities and humus (Bastida et al., 2012; Ghosh et al., 2012; Karhu et al., 2012; Peltre et al., 2012). It also improve abundance and community composition of soil organisms, promote the growth of salt-tolerant in saline soils (Bongoua-Devisme et al., 2012; Yazdanpanaha et al., 2012), suppress plant disease and nematode (Ozores-Hampton et al., 2012), improve soil chemical characteristics (Shaimaa et al., 2012), and sustain the microbial activity (Alka et al., 2013; Karhuet al., 2012; Neeru, 2012).

1.8.1 Compost as organic amendment

Composting is a biological transformation or decomposition and stabilization of organic substrates, under aerobic conditions that allow developmet of thermophilic and mesophilic conditions of temperatures as a result of biologically-produced heat, by which native microorganisms produce a final stable material, pathogens free and plant seedsand with an important concentration of humic substances that can be beneficially applied to land. Some of the advantages of incorporating organic amendments into soil, are the decrease of soil bulk density, the increase of soil water holding capacity and infiltration, the improving of soil structure, and maintenance of soil fertility for crop growth.

The composting process converts biodegradable wastes into sanitised and stable organic matter that is valuable for agriculture. The biodegradability of composts after their application on soil as amendments is correlated to their stability and depends on the biochemical characteristics of their organic matter (Zhang et al., 2012). High-quality compost, rich in biologically stable and humified organic matter, non-phytotoxic and showing low concentrations of heavy metals, should be used in reclamation of polluted soils. High-quality composts can be prepared from a wide range of residues, such as wastes generated in the agro-food industry: composting is a suitable way of recycling and adding value to them, however the application of the different organic amendments, composts obtained from widely available wastes such as sewage sludges and municipal solid wastes, is restricted since they usually contain large amounts of potentially toxic metals (Albuquerque, et al., 2011).

Compost amendments are an attractive way to incorporate organic matter in the soil as it has beneficial properties, including mobilization of mineral phosphates (Wickramatilake, 2010).

1.9 Soil microbial population

Microorganisms, mainly bacteria and fungi, play crucial roles in nutrients availability to plants. Many microbial processes are essential for the long-term sustainability of agricultural systems. The microbial population and their dynamic is high sensitive to changes in the environment and agricultural management as heavy metals (Niemeyer et al., 2012), N fertilization, amendments and compost apply (Nair and Ngouajio, 2012; Nakatani et al., 2012), pesticides application (Bastida et al, 2010), compaction (Pengthamkeerati et al., 2011), tillage, grazing (Huang et al., 2011; Vallejo et al., 2012), elevated atmospheric CO_2 in rhizosfere (Jin and Evans, 2010; Nguyen et al., 2011) mainly. Microbial populations can provide advanced evidence of light changes in soil long before it can be accurately measured by changes in such things as organic matter or other parameters. Soil microorganisms immobilize carbon and nitrogen by forming new biomass using the energy they obtain from oxidation of carbon sources through respiration or inorganic chemical reactions (Chen

et al., 2007). Biodiversity loss makes the soil vulnerable to changes and disturbances (Folke et al., 2004).

Changes in the soil physical and chemical properties resulting from management practices and soil heavy metals pollution can alter the soil environment that supports the dynamic of the microbial population, but Niemeyer et al. (2012) notified that the microbial population dynamic is highest sensitive to vegetation cover, soil organic carbon, pH, and nutrient availability, but Naira and Ngouajio (2012) found that microbial communities were more responsive to compost applications than cover crop effects. The microbial population as bacteria and fungi influences greatly macroaggregate formation. The microbial community is instead influenced by temperature, moisture content, plant diversity, plant activity, soil texture, fire regime, and nutrient availability (Nacke et al., 2011; Rasche et al., 2011; Smith et al., 2008).

In a biological sense, healthy ecosystems are generally considered to have a high biodiversity in a space in which coexist numerous taxa with many trophic levels. Cultivated soils under conventional agricultural practices often have lower microbial diversities than they had as a natural habitat (Buckley and Schmidt, 2001). By contrast, organically managed soils have been shown a higher diversity of bacteria (Drinkwater et al., 1995; Mäder et al., 2002) than conventionally managed soils; also a higher microbial activity (Workneh et al., 1993) and microbial biomass (Mäder et al., 2002; Mulder et al., 2003) were found in organic soils. Nevertheless some authors found no significant changes in bacterial biodiversity (Lawlor et al., 2000) or in fungal communities (Franke-Snyder et al., 2001) between organically or conventionally managed soils.

The two major soil microbial taxa, fungi and bacteria, to have both synergistic and antagonistic behavior (Romani et al., 2006). Both bacteria and fungi compete for nutrient; in addition, bacteria grow better together with fungi than alone and have low enzymatic activities in the absence of fungi (Romaní et al., 2006). Bacteria and fungi are considered as playing a predominant role in the production of nitrous oxide (N_2O) in arable soil. The relative amounts of C and N in the soil system also has an influence on microbial community composition by influencing the relative amounts of fungi and bacteria (Högberg et al., 2007). In soils where N levels are high, leading to a lower C:N ratio, the bacterial relative amountis higher than fungi, the oppositesituation occurs for higher C:N ratios (Högberg et al., 2007). Bacteria may also form a relationship with ectomycorrhizal colonized roots, thus changing soil bacterial community structure and diversity. In turn, it has also been noted that the bacterial community, specifically ‘mycorrhiza helper bacteria’ aid in ectomycorrhizal fungi root colonization and proliferation. Other bacterial groups located in mycorrhizal root systems may be associated with N fixation (Izumi and Finlay, 2011).

DNA fingerprinting techniques such as terminal restriction fragment length polymorfism (TRFLP) (Kim and Marsh, 2004; Liu et al., 1997) and denaturing gradient gel electrophoresis (DGGE)

(Kolwalchuk et al., 2006; Muyzer et al., 1993) can be used to follow changes in microbial populations (Muyzer et al., 1993). In particular, DGGE is applied to 18S rRNA gene or internal transcribed spacer (ITS) regions in fungi and to 16S rRNA gene in bacteria. Bacterial ribosomal DNA contains both conserved and variable regions; as sequencing of these genes has been carried out frequently in microbial ecology, there is a considerable database of known sequences. In both techniques TRFLP and DGGE, DNA is extracted from mixed populations and primers are used to amplify the sequences of a specific group of organisms, via polymerasechain reaction (PCR).

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2 Aim

The organic managements and organic management practices are strategies used to mitigate the loss of organic matter and soil degradation often as alternative to intensive farming managements and conventional agriculture. The continued increase in world population has caused necessarily the increase of food production. Historically, the farmers adopted intensified systems guaranteeing fast and high productions, but all these factors together caused a dramatic effect on soil degradation, environmental pollution by inducing erosion and water contamination, due to abundant use of agricultural chemicals, and finally the loss of soil biodiversity.

The present study was based on two hypothesis. The first was that the long-term soil cultivation under intensive management, in particular under greenhouse, could negatively affect soil fertility, chemical and biological parameters. On the other hand, the use of organic amendments is spreading as sustainable management which improves the C input and enhances productivity and quality of soil. But, the inputs of this organic material provided by sustainable agricultural practice, in general undergoes fast transformation and C loss, having as consequence shortly organic matter replenishment and the return to the starting conditions. Therefore, an advantageous condition could be achieved by regular substrate addition.

The second hypothesis was that conventional management of agricultural soils can make worse chemical and biochemical properties and microbial activity with particular effects on functionality and diversity of soil microbial population. While it is well-known that the conventional management provides constantly nutrients, which could maintain the functionality and microbial population activity, there are still unclear aspects regarding the microbial activity processes and microbial population dynamics and functionality under non-conventional agricultural systems.

Therefore the present study had the following purposes:

1. to assess soil quality in intensive farms sited in a Southern Italy region markedly devoted to under greenhouse crops that used no organic amendments for a long time.

2. to evaluate the effect of low mineralization rate organic amendments, containing wood scrapes with compost at different ratio, on soil fertility of two farms having soils with different pedological characteristics.
3. to characterize the organic matter of variously amended soils by conventional spectroscopic analyses.
4. to assess the biochemical and biological parameters in industrial tomato crop in a Southern Italy region in conventional and organic management of soils cultivated with processing tomatoes (*Solanum lycopersicum*).
5. to determinate the differences in microbial populations of soils under organic and conventional management, and the presence of a specific gene of *Bacillus* β-propeller phytase genes.

3 Wood scraps and compost to improve soil chemical properties and enzymatic activities in stressed agricultural soils under intensive farming

3.1 Introduction

Long-term performance of intensive agricultural systems is important for poverty alleviation and sustaining food production against continued population growth, but studies on long-term effects of intensive farming can provide key data for better understanding the relevant effects of the intensive agricultural managements on soil properties (Bonanomi et al., 2011).

Intensive agriculture can affect soil fertility, because it is based on crop systems such as greenhouses, monoculture, continuous tillage, excessive use of pesticides and mineral fertilization, and failed recovery of the organic matter (OM) removed by crops. In particular, use of greenhouses, due to high temperature and automatically operating irrigation systems, favors mineralization processes, determining a strong decrease in soil organic carbon (Bonanomi et al., 2011). Decline in soil OM content is a major process of soil degradation, considering the chief role of organic matter in affecting soil fertility both directly, by releasing macro and micro elements, and indirectly, by improving soil physical (Van-Camp et al., 2004) and chemical properties (Nambiar, 1997), and also reducing heavy metal toxicity (D'Ascoli et al., 2006).

Application of organic amendments is a reliable tool to improve soil health and to support sustainable agriculture systems (Bronson et al., 1997; Yadav et al., 1998; Conklin et al., 2002). Indeed, their use improves soil properties, including higher nutrient availability, higher water holding capacity and cation exchange capacity (CEC), lowers bulk density, and it can be beneficial for microorganisms too (Doran, 1995; Drinkwater et al., 1995). Moreover, if organic amendments positively affect soil chemical properties, crops grown under organic amendment can give yields comparable with those achieved under conventional farming systems (Drinkwater et al., 1995; Stamatiadis et al., 1999). For example, vegetable fields under organic amendments in California produced yields equal to those obtained under conventional production (Drinkwater et al., 1995; Stamatiadis et al., 1999).

The application of compost, as organic amendments, has been successfully proposed in many cases, to improve structure and both chemical (Magid et al., 2001; Cavigelli and Thien, 2003) and biological fertility of soils (Borken et al., 2002; Ros et al., 2003), as well as to suppress soil borne pathogens (Bonanomi et al., 2007). In addition, benefits of compost to soil include pH stabilization and higher water infiltration rate due to enhanced soil aggregation (Stamatiadis et al., 1999). Many of the previous cited studies demonstrated that the effectiveness of organic amendments in recovering soil fertility depends on the biological and biochemical quality of compost, its

application rate and on the associated agronomic practices (Ros et al., 2006). In fact, application of compost under permanent plastic tunnels gave negative results for C stock recovery (e.g. Iovieno et al., 2009). In detail, compost amendments up to 45 t ha^{-1} $year^{-1}$ resulted in no or limited C stock increase (Iovieno et al., 2009) because of the high biochemical compost quality (i.e. C/N around 13), coupled with the tunnel microclimatic conditions that boost OM microbial decomposition.

Agro-ecosystem C storage depends not only on C inputs, but also on its outflows, regulated by OM decay rate (Aerts, 1997; Swift et al., 1979). Therefore, it appears evident that it is necessary to set up experimental design aimed to identify conditions in which the use of organic amendments can maximize the humification efficiency thus allowing a stable C stock recovery in order to produce long-term agronomical advantages. Under plastic tunnel cultivation systems, temperature and water availability cannot be modified because they are crucial ecological factors to sustain a rapid crop growth. As a consequence, a strategic management of biochemical quality of organic amendment is the only suitable approach for a long-term recovery of soil OM content.

A three-years research project was dedicate to evaluate the possibility of a long-term recovery of soil quality by a combined application of high-quality (compost with a C/N ratio of 13) and low-quality (wood scraps with a C/N ratio of 375) organic substrates was investigated. The rationale of this choice was to finely tune the OM quality, defined in term of C/N ratio, to allow a long-term OM recovery and, at the same time, do not hinder short-term nitrogen mineralization that commonly occurs after soil amendment with high C/N ratio substrates. The hypothesis was tested in a highly intensive cultivation system (plastic tunnel cultivation) by a field experiment carried out in two farms located in Southern Italy and previously studied by Bonanomi et al. (2011).

The main soil chemical properties (pH, electrical conductivity, cation exchange capacity, organic carbon, nitrogen and nitrate content, available phosphorus) and enzymatic activities (FDA-hydrolytic activity, dehydrogenase, β-glucosidase, phosphatase, invertase, urease and arylsulphatase) were determined to assess the effects of tested organic mixtures. Moreover, an enzymatic soil index, AI 3, (Puglisi et al., 2006), based on three enzyme activities (β-glucosidase, phosphatase and urease) and Principal Component Analysis were also applied to better discriminate between amended and control soils. In fact the AI 3 index is able to discriminate between altered and unaltered soils, under a wide range of conditions, namely irrigation with brackish water, heavy metal contamination, intensive agricultural regimes (Puglisi et al., 2006).

Within this research project, the work performed in the present thesis regarded the second and third year, and in particular the related results are discussed in detail in this Chapter and in the following Chapter 4. For comparison and useful evaluation of the whole response of the two soils subjected to the organic amendments, results obtained in the first year of the experimentation by Scotti (2011) are summarized and briefly commented.

3.2 Materials and methods

3.2.1 Study site

Two intensive farms (named F1 and F2) were studied. They are located in the Plane of Sele river (Salerno, Southern Italy) at 40° 34' 58.362" N, 14° 59' 42.438" E and 40° 26' 4.851" N, 14° 59' 18.369" E, respectively, and they are representative of the Mediterranean area. They were selected among the farms of a previous study aiming to assess changes in soil quality due to intensive farming (Bonanomi et al., 2011). Both farms are characterized by horticultural growing and intensive cultivation (i.e. exclusive use of chemical fertilizers, no use of organic amendment, greenhouses with automatically operating irrigation systems), that has determined stressed soil conditions (Bonanomi et al., 2011). Both farms show different geopedologic characteristics, in particular, F1 farm has a clay loam soil, defined *Pachic Haploxerolls*, whereas F2 farm has a calcaric sandy loam soil defined *Lithic Haplustolls* (Regione Campania, 2004; USDA, 1998).
The main physical and chemical soil properties of F1 and F2 farms are reported in Table 3.1.

Table 3.1 Mainly physical and chemical properties of F1 and F2 farms.

Properties	**compost**	**F1 soil**	**soil**
Texture	-	clay loam	sandy loam
Sand, %	-	37±3	56±1
Silt, %	-	26±2	27±1
Clay, %	-	37±2	17±2
pH	7.9±0.1	7.74±0.12	7.65±0.12
EC, dS m^{-1}	4.37±0.12	0.08±0.02	0.17±0.03
Limestone, g kg^{-1}	-	1.55±1.03	639±75
Organic C, g kg^{-1}	280±5	10.47±0.56	16.19±0.39
Total N, g kg^{-1}	21±1	4.13±0.31	3.90±0.03
C/N	13.3	2.5±0.2	4.11±0.15
P_2O_5, mg kg^{-1}	8000	162.34±9.21	174.71±17.32
CEC, cmol(+) kg^{-1}	-	21.1±0.2	13.6±0.9
K^+, cmol(+) kg^{-1}	-	1.47±0.06	0.62±0.20
Na^+, cmol(+) kg^{-1}	-	0.74±0.04	0.40±0.05
HAs + FAs, %	14.2	-	-
Cu, mg kg^{-1}	67	-	-
Zn, mg kg^{-1}	146	-	-

The F1 soil was a clay loam soil, with sub-alkaline pH, low electrical conductivity (EC) and limestone, and high cation exchange capacity (CEC). The F2 soil, by contrast, was characterized by

sandy loam soil, with sub-alkaline pH, high EC, but in particular very high limestone, reaching over 600 g kg^{-1}. Organic carbon content of F1 soil was low (10.47 g kg^{-1}), for a clay loam soil. While F2 soil showed a good organic carbon content (16.19 g kg^{-1}), considering its sandy nature.
In both farms, the most common crops were: lettuce (*Lactica sativa*), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*) and sweet pepper (*Capsicum annuum*).

3.2.2 Organic amendments and experimental design

In this study two different organic fertilizers were used:

- compost from municipal solid waste (GeSeNuSrl, Perugia, Italy), characterized by a C/N ratio of 13.3;
- wood from scraps of poplars pruning (from "Improsta" Experimental Regional Farm), characterized by a C/N ratio of 375.

The organic fertilizers were mixed together with different ratio in order to obtain two mixtures:

- A1, with compost:wood 10:1 and a final C/N ratio of 15,
- A2, with compost:wood 2:1 and a final C/N ratio of 25.

In both the farms F1 and F2 an area under greenhouse (about 160 m^2) was selected and divided in thirty plots to carry out in triplicate all amendment treatments and controls following a randomized block design. In particular, experimental design (Figure 3.1) provided for supplying to some plots A1 and A2 mixtures at doses 30 t ha^{-1} (low-dose, indicated with L) and 60 t ha^{-1} (high-dose, indicated with H), and to other plots (named with M), besides organic amendments as described above, also a commercial mineral fertilizer, at dose 200 kg ha^{-1} (N-P-K; 14-7-17). In the experimental field three untreated plots (without organic amendments and mineral fertilizer) and three plots treated only with mineral fertilizer (i.e. control plots, named C and CM, respectively) were also designed to assess correctly the effect of the treatments.

After the first amendment occurred on February 2009, experimental plots were further amended on April 2010 (Figure 3.2).

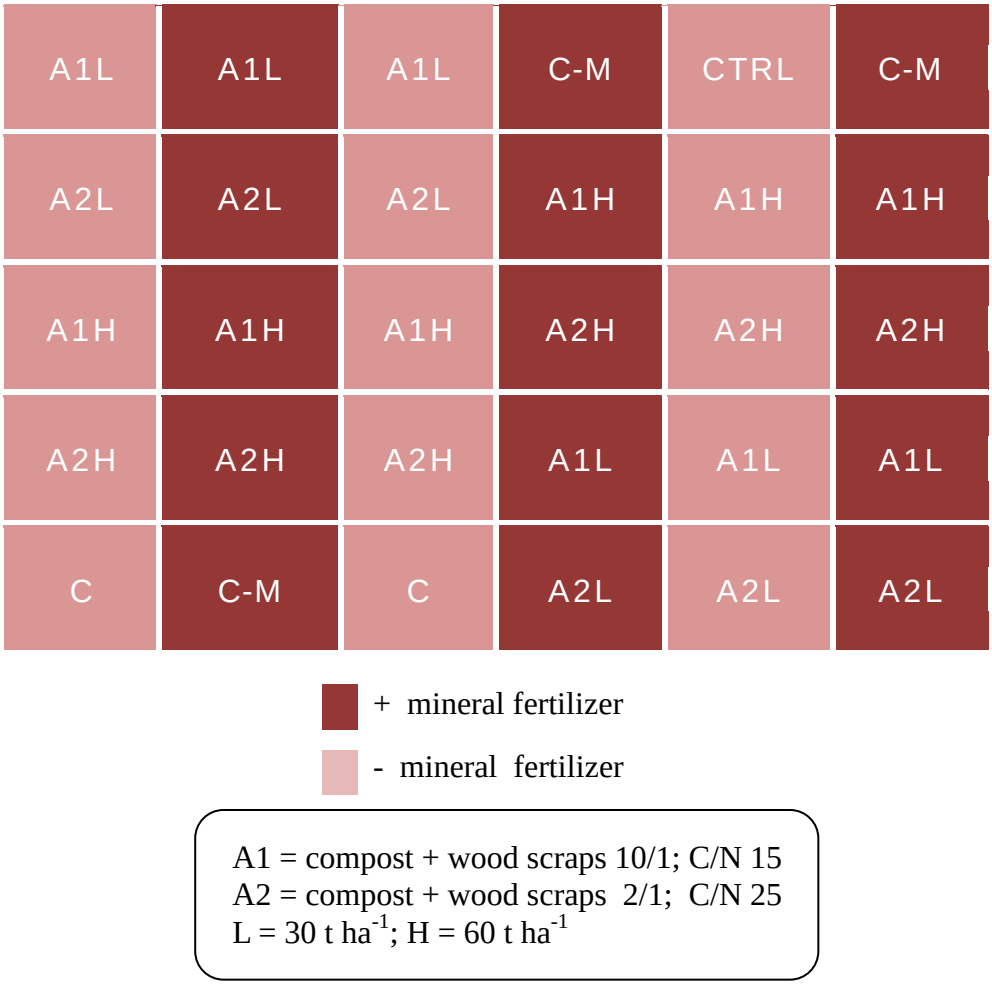

Figure 3.1 Scheme of experimental design

3.2.3 Soil sampling

Soil samples were collected in according to the schedule reported in Figure 3.2. The samplings carried out by Scotti (2011) were also listed to have the complete view of the experimental design. In fact, as already explained in the Introduction, results obtained in the second year of the experimentation (V, VI and VII samplings) are discussed in detail in the present Chapter, by also comparing them to those obtained in the first year (Scotti, 2011), instead results obtained in the third year will be reported in Chapter 4.

In each plots, five sub-samples were collected following a W scheme from the topsoil (0-20 cm), then sub-samples were mixed to form only one sample per plot. Samples were packed in polyethylene bags, sieved (< 2 mm, and air dried at room temperature (for physical and chemical analyses) or stored at 4 °C (for biochemical analyses) (Figure 3.2).

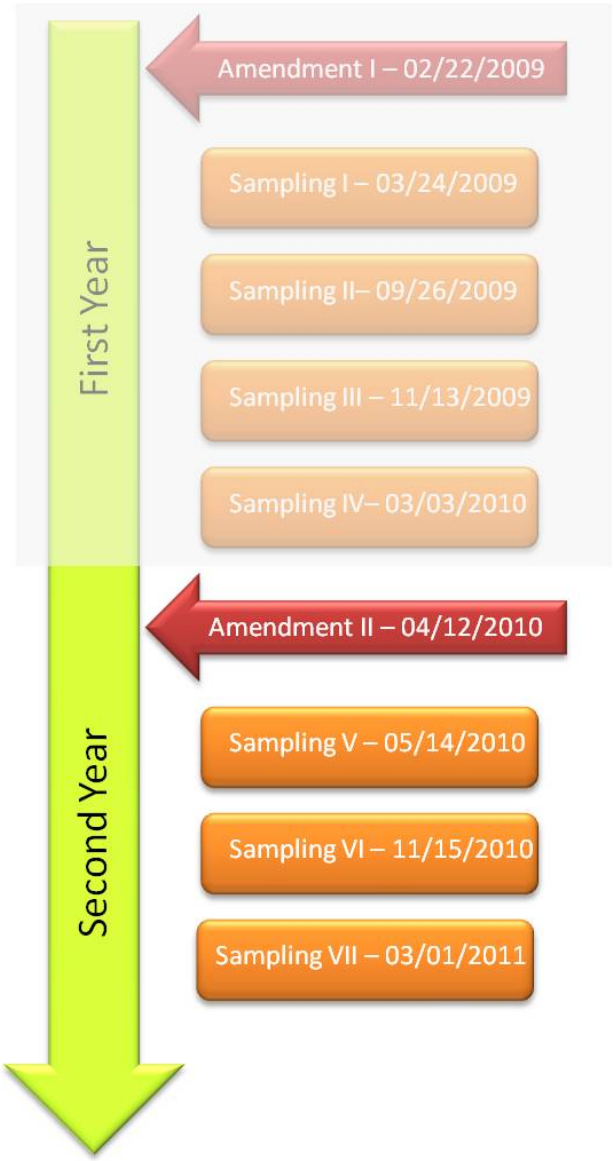

Figure 3.2 Schedule of amendment and samplings during the three years of research project.

3.2.4 Soil chemical properties

Chemical properties of soils were determined by standard methods (Sparks, 1996). Electrical conductivity (EC) and pH were measured in 1:5 and 1:2.5 soil:water suspensions, respectively; cation exchange capacity (CEC) was measured after soil treatment with a barium chloride and triethanolamine solution at pH 8.2; available phosphate was measured by bicarbonate extraction. Exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) were assayed by flame atomic absorption spectrometry. Organic C content was assayed by chromic acid titration method (Walkley and Black, 1934); total N was determined (on 30 mg pulverized soil) by flash combustion with a CNS Elemental Analyser (Thermo FlashEA 1112, Fisons).

3.2.5 Enzymatic activities

Dehydrogenase (DHY, E.C. 1.1) activity was measured with buffered tetrazolium salts solution, according to Trevors (1984). Arylsulphatase (ARYL, E.C. 3.1.6.1), phosphatase (PHO, E.C. 3.1.3.2) and β-glucosidase (GLU, E.C. 3.2.1.21) activities were determined using *p*-nitrophenylsulphate (p-NPS), *p*-nitrophenylphosphate (*p*-NPP), or *p*-nitrophenyl-β-D-glucopyranoside (*p*-NG) as the substrates, respectively. Specific buffers and pHs, and reaction stop procedures were used as reported in Gianfreda et al. (2005). Concentrations of *p*-nitrophenol (*p*-NP) were determined at 405 nm after addition of NaOH and $CaCl_2$ for PHO and ARYL, and Tris/NaOH buffer (pH 10.0) and $CaCl_2$ for GLU. Urease activity (UR, E.C. 3.5.1.5) was assayed as described by Kandeler and Gerber (1988) using urea as substrate. Invertase activity (INV, E.C. 3.2.1.26) was determined with 50 mM sucrose as substrate in 2 M acetate buffer (pH 5.5), incubating for 3 h at 50°C. The released reducing sugars were determined following the method of Nelson (1944).

One unit of enzyme activity was defined as μmoles of product released at 30 °C h^{-1} by 1 g of dried soil. Triplicates were performed for each activity assay.

3.2.6 Soil quality index

The enzymatic soil index AI 3 proposed by Puglisi et al. (2006) and based on the activity values of three enzymes (β-glucosidase, phosphatase and urease) was applied to the investigated systems to discriminate between soils subjected to different treatments.

The index was developed from datasets of the three enzyme activities and the raw canonical coefficients are:

$$AI\ 3 = 7.87\ \beta\text{-glucosidase} - 8.22\ \text{phosphatase} - 0.49\ \text{urease}$$

3.2.7 Statistical analysis

Two-way ANOVA was used to examine the effects of organic amendment in different ratio and doses (A1, A2, L, H) and addition of mineral fertilizer (MIN) on all soil properties analyzed, during the two experimental years.

The relationships among all soil chemical and biochemical properties (excluded pH and CEC), of all samples, were assessed by using Pearson correlation coefficients, and, on these results, the analysis of Principal Component (PCA) was performed.

All statistical analysis were performed by SPSS (PASW Statistics 18 - IBM SPSS Statistics).

3.3 Results

In the second year of the experimentation, almost all investigated properties showed a behavior quite similar to that exhibited in the first year (Scotti, 2011): a general increase soon after the application of the organic amendments and then a peculiar trend related to the analysed properties, such as a gradual decrease to the original values or, in some cases, to higher values than the initial ones (i.e. those registered at the beginning of the project) were reached.

3.3.1 Effect of organic amendments on soil chemical properties

A two-way statistical analysis was performed for all soil properties analyzed in the second year samplings (V, VI and VII samplings) to assess the effects of the organic amendments at different ratios and doses (A1L, A1H, A2L, A2H) and the addition of the mineral fertilizer (M) (Table 3.2).

Table 3.2 Summarized results of two-way ANOVA for all analysed parameters in the two soils in the second phase of this study that include three sampling (from second amendment 12/04/2010). Different ratio and doses of amendment and mineral fertilization were the independent variables.
P-value Duncan test; * = significant difference.

			Farm 1			Farm 2		
Parameter	**Source**	**d.f.**	**Sum of squared**	***F***	***P*-value**	**Sum of squared**	***F***	***P*-value**
pH	Amendment	4	0.02	0.05	0.994	0.272	1.731	0.151
	Mineral fertilization	1	0.11	1.11	0.296	0.009	0.240	0.626
	Interaction	4	0.33	0.81	0.524	0.066	0.419	0.794
EC	Amendment	4	0.00	0.02	0.999	0.479	3.099	0.016*
	Mineral fertilization	1	0.0004	0.06	0.800	0.038	0.996	0.319
	Interaction	4	0.00	0.11	0.980	0.101	0.655	0.624
P_2O_5	Amendment	4	39175.29	6.57	<0.0001*	67924.24	13.544	<0.0001*
	Mineral fertilization	1	0.179	0.00	0.991	1323.2	1.055	0.305
	Interaction	4	624.438	0.1051	0.981	35300.612	7.039	<0.0001*
CEC	Amendment	4	198.135	3.190	0.014*	417.99	6.048	<0.0001*
	Mineral fertilization	1	8.384	0.540	0.463	26.508	1.534	0.217
	Interaction	4	121.109	1.950	0.103	58.761	0.850	0.495
Organic C	Amendment	4	223.50	26.42	<0.0001*	293.788	40.202	<0.0001*
	Mineral fertilization	1	0.03	0.15	0.900	5.925	3.243	0.087
	Interaction	4	4.20	0.50	0.730	5.356	0.733	0.580
Total N	Amendment	4	0.04	21.20	<0.0001*	0.021	6.798	0.001*
	Mineral fertilization	1	0.0000	0.25	0.875	0.000	0.189	0.668
	Interaction	4	0.001	0.68	0.611	0.002	0.795	0.542
C/N	Amendment	4	3.95	16.70	<0.0001*	7.878	6.650	0.001*
	Mineral fertilization	1	0.00	0.01	0.940	0.148	0.499	0.488
	Interaction	4	0.27	1.16	0.359	0.860	0.726	0.585
DHY	Amendment	4	110.47	8.03	<0.0001*	160.785	57.782	<0.0001*
	Mineral fertilization	1	0.02	0.01	0.947	1.088	1.563	0.212
	Interaction	4	33.70	8.42	0.047*	3.545	1.274	0.281

			Farm 1			Farm 2		
Parameter	**Source**	**d.f.**	**Sum of squared**	***F***	**P-value**	**Sum of squared**	***F***	**P-value**
GLU	Amendment	4	3.08	9.59	<0.0001*	1.190	12.079	<0.0001*
	Mineralfertilization	1	0.042	0.53	0.469	0.003	0.106	0.745
	Interaction	4	0.110	0.34	0.850	0.122	1.234	0.297
PHO	Amendment	4	15.91	18.00	<0.0001*	24.219	18.667	<0.0001*
	Mineral fertilization	1	0.98	4.44	0.036*	0.021	0.065	0.799
	Interaction	4	1.52	1.71	0.148	0.139	0.107	0,980
ARYL	Amendment	4	0.06	22.34	<0.0001*	0.114	45.146	<0.0001*
	Mineral fertilization	1	0.0010	1.20	0.275	0.000	0.005	0.947
	Interaction	4	0.003	1.08	0.367	0.007	2.582	0.038*
INV	Amendment	4	2.20	28.04	<0.0001*	6.069	24.815	<0.0001*
	Mineral fertilization	1	0.01	0.04	0.840	0.000	0.004	0.948
	Interaction	4	0.03	0.43	0.789	1.059	4.330	0.002*
UR	Amendment	4	2.09	3.93	0.004*	5.355	9.329	<0.0001*
	Mineral fertilization	1	0.019	0.15	0.704	0.765	5.334	0.022*
	Interaction	4	0.55	1.04	0.387	2.029	3.535	0.008*
K	Amendment	1	0.568	2.928	0.021*	4.730	91.846	<0.0001*
	Mineral fertilization	4	0.002	0.035	0.852	0.037	2.904	0.090
	Interaction	1	0.130	0.670	0.614	0.105	2.033	0.090
Na	Amendment	1	0.035	1.222	0.302	10.396	14.203	<0.0001*
	Mineral fertilization	4	0.033	4.639	0.032	0.290	1.584	0.209
	Interaction	1	0.039	1.347	0.253	0.726	0.992	0.413
Ca	Amendment	1	461.413	2.732	0.030*	8.21.833	2.616	0.036
	Mineral fertilization	4	140.796	3.335	0.069	192.529	2.452	0.119
	Interaction	1	111.384	0.660	0.621	1.805	5.749	<0.0001*
Mg	Amendment	1	0.857	0.047	0.996	0.475	0.953	<0.0001*
	Mineral fertilization	4	0.992	0.020	0.887	0.004	0.300	0.584
	Interaction	1	1.719	0.094	0.984	0.394	7.431	<0.0001*

According to results obtained in the first year of the study (Scotti, 2011), the addition of whichever organic amendment mixture in the second year did not show effects on pH values in both F1 and F2 soils (Table 3.3).

While the first addition of organic amendments (02/22/2009) determined an immediate increase of EC valuesin F1 soil, particularly at one month after the addition, the second application of organic and mineral fertilizers (04/12/2010) did not determine significant increases of EC in farm F1 soil and the parameter decreased over time, returning to its initial values (Figure 3.3 and Table 3.3).

F2 soil was characterized by higher initial EC values which further increased after the first addition of organic and mineral fertilizers, especially in the A1H plot (Figure 3.3). During the first year of the experiment a continuous decrease of EC values in all plots was observed until to reach again the starting conditions. The second application of organic and mineral fertilizer showed significant effect on EC in F2 soils mainly at the Sampling V (05/14/2010) (Figure 3.3 and Table 3.4).

Table 3.3 pH values (±sd) in F1 and F2 soils in the second phase of this study that include three sampling (from second amendment 04/12/2010).

pH												
	F1						F2					
	Sampling V		Sampling VI		Sampling VII		Sampling V		Sampling VI		Sampling VII	
C	6.95	±0.06	7,91	±0.03	8.16	±0.03	7.74	±0.17	8.19	±0.19	8.13	±0.15
A1L	7.43	±0.01	7.98	±0.01	8.14	±0.03	7.91	±0.03	8.30	±0.07	7.90	±0.06
A1H	7.46	±0.06	7.84	±0.03	8.14	±0.12	7.98	±0.07	8.30	±0.12	8.09	±0.10
A2L	7.45	±0.02	7.82	±0.00	8.13	±0.03	7.98	±0.08	±8.40	±0.09	8.14	±0.10
A2H	7.29	±0.02	7.73	±0.01	8.08	±0.08	8.01	±0.05	8.32	±0.02	8.06	±0.08
CM	7.51	±0.04	8.03	±0.04	8.14	±0.04	7.85	±0.17	7.86	±0.11	8.29	±0.05
A1LM	7.47	±0.03	7.89	±0.02	7.98	±0.02	7.90	±0.02	8.14	±0.09	8.32	±0.05
A1HM	7.61	±0.11	7.75	±0.01	8.14	±0.01	8.02	±0.09	8.23	±0.21	8.30	±0.04
A2LM	7.44	±0.06	7.77	±0.02	8.11	±0.04	7.93	±0.01	8.13	±0.09	8.26	±0.08
A2HM	7.86	±0.04	7.75	±0.02	8.13	±0.05	7.92	±0.04	8.24	±0.18	8.35	±0.06

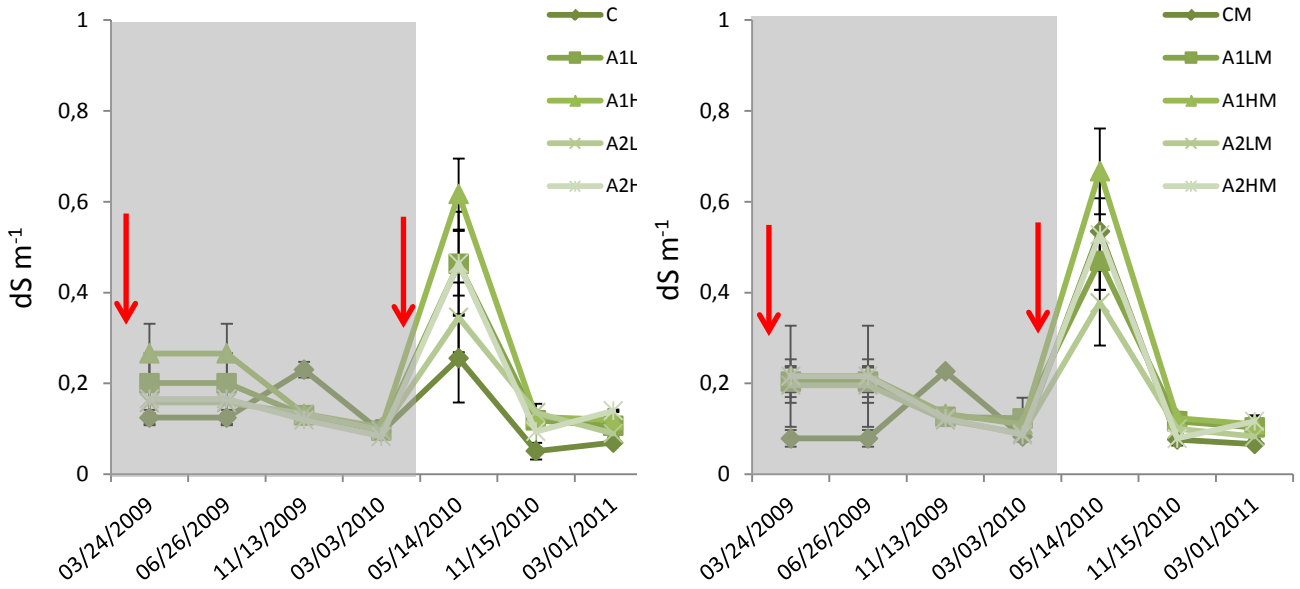

Fig. 3.3 Effect of amendment application on EC (dSm^{-1}) in F2 farm soils in the all phases of this study from first sampling of the first year of this study to Sampling VII of the second year. The red arrow indicates the amendment application.

Also P_2O_5 showed a positive response to organic amendment application (Figures 3.4 and 3.5) in both farms. In F1, at the Sampling V (i.e. after the second amendment application) the P_2O_5 values showed a substantial increment as respect to the values reached at the end of the first year (Figure 3.4). The highest increment of this nutrient (around 94% higher with respect to Control plot of Sampling I (03/24/2009) and 15% to the control of Sampling V(11/15/2010) was measured in A1L plot.

Table 3.4 Summarized results of one-way ANOVA for EC (dSm^{-1}) in F2 farm soils in the second phase of this study that include three sampling (from second amendment 04/12/2010). Different lower case letters indicate significant differences ($P \leq 0.05$ from Duncan test) between treatments and uppercase letters indicate significant differences across time.

	EC (dSm^{-1})		
Plot	**SamplingV 05/14/2010**	**SamplingVI 11/15/2010**	**SamplingVII 03/01/2011**
C	dA	B	dB
A1L	bA	B	bB
A1H	aA	B	bB
A2L	cA	B	cB
A2H	bA	C	aB
CM	bA	cB	dB
A1LM	bA	aB	bB
A1HM	aA	aB	abB
A2LM	cA	bB	cB
A2HM	bA	cB	aB

This results is confirmed by the statistical analysis (Table 3.5) that clearly indicate significant differences between treatments and samplings in the second year, and highlight that the Sampling V had values always significantly higher than those measured in the subsequent samplings.

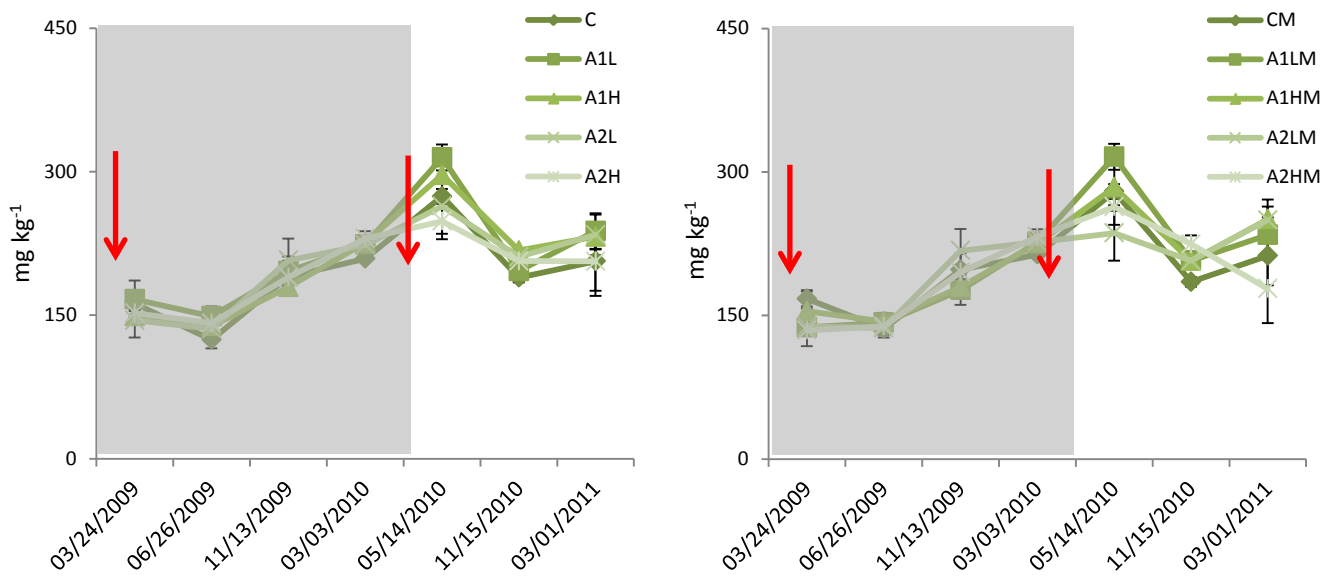

Figure 3.4 Effect of amendment application on P_2O_5 (mg kg^{-1}) in F1 farm soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

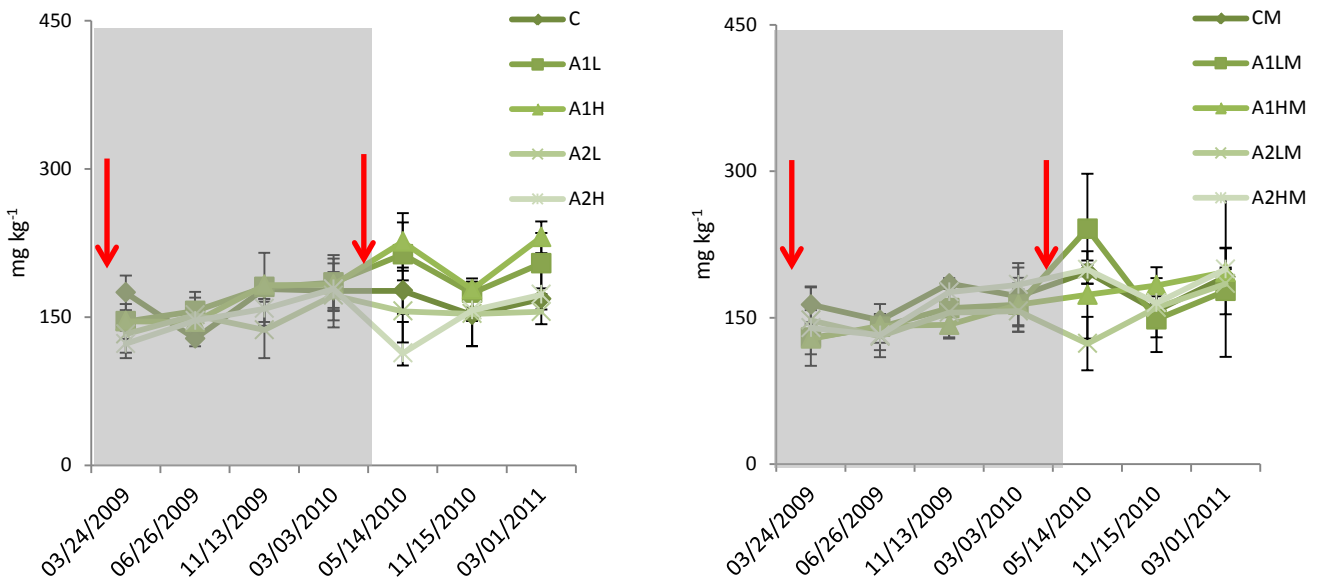

Fig. 3.5 Effect of amendment application on P_2O_5 (mg kg^{-1}) in F2 farm soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

Table 3.5 Summarized results of one-way ANOVA for P_2O_5 (mg kg^{-1}) in F1 and F2 farm soils in the second phase of this study that include three sampling (from second amendment 04/12/2010). Different lower case letters indicate significant differences ($P \leq 0.05$ from Duncan test) between treatments and upper case letters indicate significant differences across time.

	F1			F2		
Plot	**SamplingV 14/05/2010**	**SamplingVI 15/11/2010**	**Sampling VII 01/03/2011**	**Sampling V 05/14/2010**	**Sampling VI 11/15/2010**	**Sampling VII 03/01/2011**
C	aC	cC	bB	bA	bB	cA
A1L	aA	cC	aB	aA	aB	bA
A1H	bA	aB	aB	aA	aB	aA
A2L	cdA	abC	aB	Ns	b	Ns
A2H	dA	bB	bB	cB	bA	cA
CM	bA	cC	cB	b	b	A
A1LM	aA	bC	bB	aA	bB	B
A1HM	bA	bC	aB	b	a	A
A2LM	dC	bC	aA	cB	bA	A
A2HM	cA	aB	dC	bA	bB	A

In F2, at the first sampling of the second year (i.e. after the second amendment application) the P_2O_5 values also showed an increment as respect to the values reached at the end of the first year (Figure 3.5). The increment was less pronounced than that registered in F1 soils, but highest with respect to the control than F1. In A1LM and A1H plots the increments of P_2O_5 (Figure3.5) were respectively about 26% and 25% higher than the value measured in the control C. This result is confirmed by

statistical analysis (Table 3.2), that clearly highlights the significant differences between treatments and samplings in the second year.

The CEC of F1 did not present major overall differences over time (Figure 3.7), thus indicating that the application of organic amendment did not exert significant influence on this parameter, although a little increase was measured at the Sampling V of the study. Similar result was in according to Zebarth et al. (1999) that did not found significant differences in the CEC values between soils with or without amendment application and mineral fertilizer, suggesting that an immediate beneficial increase in soil CEC does not automatically follow the addition of organic amendments. By contrast, plots with the mineral fertilizer showed the highest values. Therefore CEC parameter responded more to the application of mineral fertilizer rather than to organic amendment and decreased in the later stages of the study. The major changes of this parameter were observed in F2 (Figure 3.8) that showed a gradual increase of CEC during the whole second year. This behavior could be related not only to the use of OM, but also to the high percentage of limestone in this soil that could have affected the response of this parameter (Edmeades, 1982). The greater values measured in the second year as respect to the control soil C were at Sampling VI (11/15/2010) in A1L, A2H and A1LM plots.by around 24 and 85%, respectively and around 19%, 11% and 25% with respect to Control plot of the Sampling VI (Table 3.7).

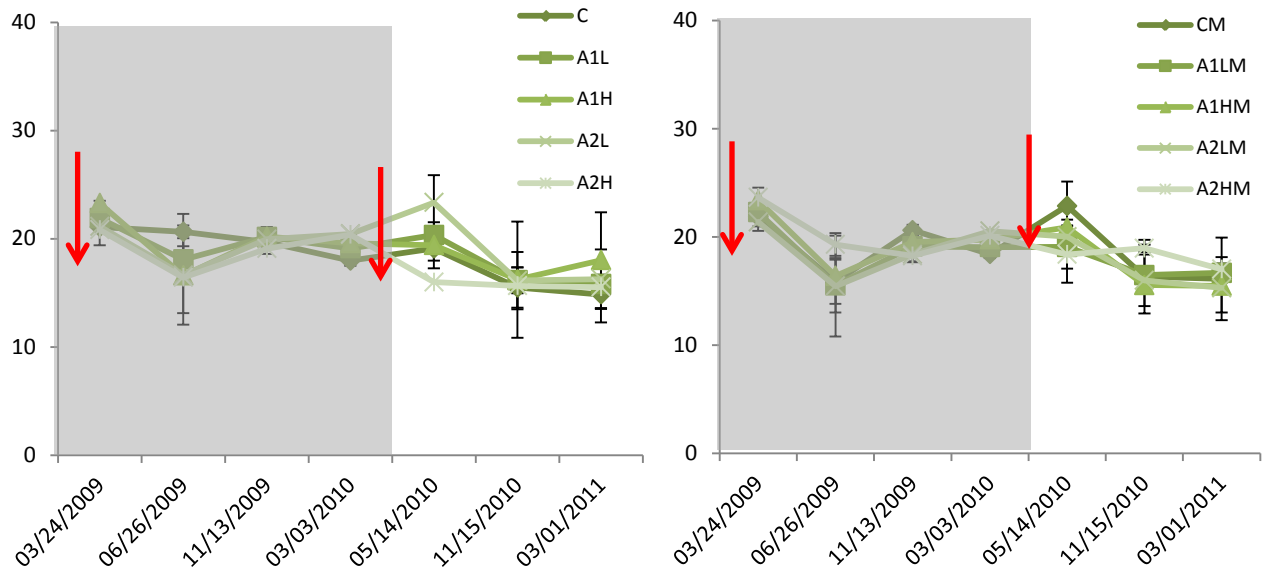

Fig. 3.6 Effect of amendment application on CEC (cmol(+) kg^{-1}) in F1 farm soils in the all phases of this study from first sampling I to Sampling VII.The red arrow indicates the amendment application.

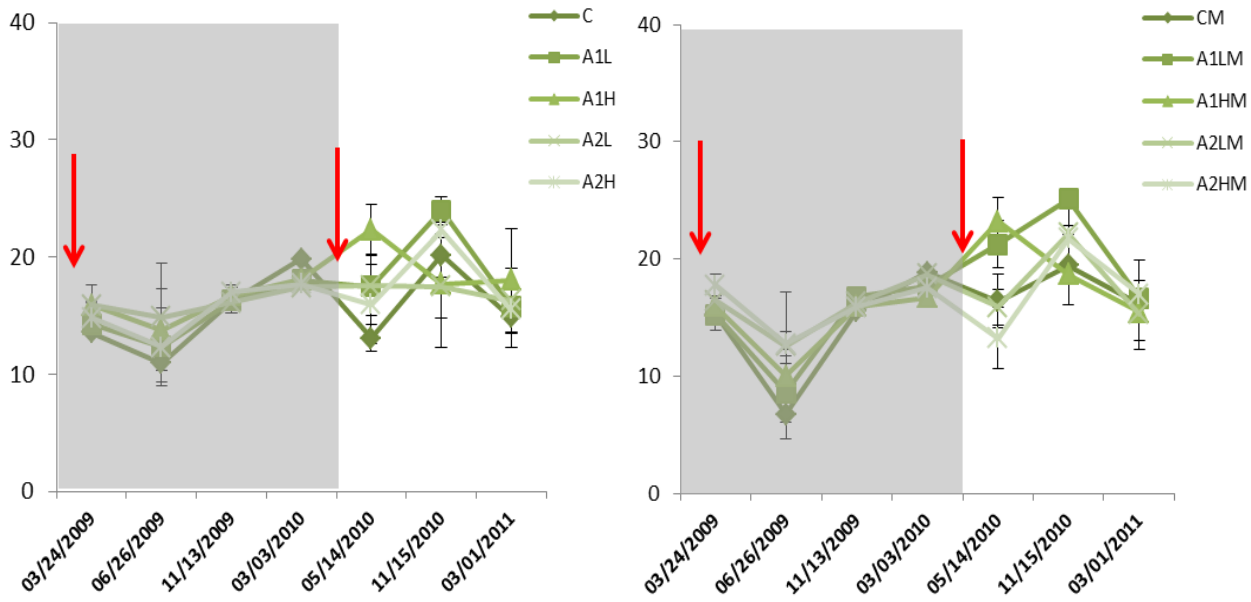

Figure 3.7 Effect of amendment application on CEC (cmol(+) kg^{-1}) in F2 farm soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

Table 3.6 Summarized results of one-way ANOVA for CEC (cmol(+) kg^{-1}) in F1 and F2 farm soils in the second phase of this study that include three sampling (from second amendment 04/12/2010). Different lower case letters indicate significant differences ($P \leq 0.05$ from Duncan test) between treatments and uppercase letters indicate significant differences across time.

	CEC (cmol(+) kg^{-1})					
	F1			F2		
Plot	**SamplingV 05/14/2010**	**SamplingVI 11/15/2010**	**SamplingVII 03/01/2011**	**Sampling V 05/14/2010**	**Sampling VI 11/15/2010**	**Sampling VII 03/01/2011**
C	bA	B	cB	cB	cA	B
A1L	bA	B	bA	bB	aA	B
A1H	b	Ns	b	a	c	Ns
A2L	bA	B	aA	b	c	Ns
A2H	bB	B	aA	bB	aA	B
CM	aA	B	cB	cB	cA	b
A1LM	bB	B	bA	bB	aA	C
A1HM	bB	c	aA	a	cB	C
A2LM	abA	B	bA	cB	bA	B
A2HM	b	Ns	b	dC	cA	B

The behavior of available P and CEC observed in this study confirms that, at least in some cases, the practices of organic fertilization by using suitable organic amendments are of importance for a sustainable agriculture. Indeed, several recent research findings have stated that organic amendments can be of great help to improving soil quality and resolving different limiting factors.

Long-term use of organic amendment improved available P and CEC in sandy soils (Ozores-Hampton et al., 2011) and in general chemical characteristics in clay soils (Shaimaa et al., 2012).

In both farm soil the addition of organic amendment mixtures had a positive effect also on the exchangeable K^+ and Ca^{+2}. In both F1 and F2 soilsthe highest values in K^+ were observed on Sampling V: in particular in A2H and A2HM plots in F2 soil and in A1H and A1HM plots in F1 soil (Table 3.7). As regarding Ca^{+2}, in F1 the highest values were obtained in Sampling V (A2HM and A2H plot), in F2 in Sampling VI (A2H and A2LM plots). Na^+ exchangeable was not influenced by treatment in F1 farm, but the in F2 farm all treatment were higher than control plot, the Sampling VI obtained highest values by A2H and A2LM plots.

Table 3.7 Effect of organic amendments on exchangeable bases (±sd) of F1 and F1 soils

F1

Exchangeable bases concentration (cmol(+) kg^{-1})										
	C	**A1L**	**A1H**	**A2L**	**A2H**	**CM**	**A1LM**	**A1HM**	**A2LM**	**A2HM**
					Sampling V					
K+	0.7 ±0.1	0.8 ±0.0	0.9 ±0.0	0.8± 0.1	0.9± 0.0	0.7±0.1	0.8± 0.0	0.8±0.0	0.7±0.0	0.7±0.1
Na^+	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.3±0.1	0.3± 0.0	0.2±0.0	0.3± 0.1	0.3±0.0	0.3±0.0	0.2±0.0
Ca^{2+}	18.6±0.8	23.5 ±1.0	22.2 ±1.1	18.7±0.7	23.7±0.3	24.5± 0.5	22.7±0.6	24.6±0.8	27.9±0.8	33.7±0.8
Mg^{2+}	6.6 ±0.5	7.8 ±0.3	8.3 ±0.4	8.2±0.2	8.6± 0.1	8.2± 0.3	6.6± 0.1	8.7±0.1	8.0±0.3	7.40. ±1
					Sampling VI					
K+	0.6 ±0.0	0.7 ±0.0	0.7 ±0.0	0.7±0.0	0.7± 0.3	0.6± 0.0	0.7± 0.0	0.7±0.0	0.6±0.0	0.7±0.0
Na+	0.2 ±0.1	0.2 ±0.0	0.2 ±0.0	0.2±0.0	0.3± 0.2	0.2± 0.0	0.2± 0.1	0.2±0.0	0.2±0.0	0.3±0.0
Ca^{2+}	25.7 ±0.9	18.4 ±0.0	18.6 ±0.7	18.2±0.8	17.6± 0.2	27.3± 0.0	18.5± 0.1	18.90.7	17.5±0.9	15.9±1.0
Mg^{2+}	5.2 ±0.3	4.7 ±0.2	4.7 ±0.0	4.6±0.1	4.6± 0.2	6.0± 0.5	4.7± 0.0	4.6±0.1	4.6±0.1	4.7±0.0
					Sampling VII					
K^+	0.4 ±0.1	0.4 ±0.0	0.5 ±0.0	0.5±0.0	0.5± 0.2	0.4± 0.0	0.4± 0.0	0.5±0.0	0.8±0.8	0.5±0.0
Na^+	0.4 ±0.0	0.4 ±0.0	0.3 ±0.0	0.3±0.0	0.3± 0.0	0.4± 0.0	0.4±0.0	0.3±0.0	0.2±0.0	0.3±0.0
Ca^{+2}	20.8 ±0.2	18.8 ±0.2	22.4 ±0.4	21.0±0.4	23.0± 0.2	23.2± 0.7	21.3±0.5	23.6±0.2	28.2±0.9	21.6±0.5
Mg^{+2}	4.8 ±0.2	4.6 ±0.3	5.1 ±0.2	4.9±0.1	5.0± 0.8	5.4± 0.8	5.2±0.6	5.0±0.1	4.3±0.5	5.0±0.5

F2

Exchangeable bases concentration (cmol(+) kg^{-1})										
	C	**A1L**	**A1H**	**A2L**	**A2H**	**CM**	**A1LM**	**A1HM**	**A2LM**	**A2HM**
					Sampling V					
K^+	0.3 ±0.1	0.6±0.1	0.8±0.0	0.5± 0.0	0.6 ± 0.1	0.3 ±0.1	0.6 ± 0.0	0.8±0.0	0.5±0.0	0.7±0.1
Na^+	0.3 ±0.0	0.6±0.1	0.6±0.1	0.3±0.1	0.3± 0.0	0.2±0.0	0.3± 0.1	0.3±0.0	0.3±0.0	0.5±0.0
Ca^{2+}	26.6±2.8	28.8±7.1	25.4±1.1	18.7±0.7	23.7±0.3	24.5± 0.5	22.7±0.6	24.6±0.8	27.9±0.8	22.44±0.
Mg^{2+}	3.5±0.3	4.3±0.6	4.5±0.6	8.2±0.2	8.6± 0.1	8.2± 0.3	6.6± 0.1	8.7±0.1	8.0±0.3	3.8±0.4
					Sampling VI					
K^+	0.2±0.0	0.7 ±0.0	0.6±0.1	0.4±0.1	0.5 ± 0.1	0.2 ± 0.0	0.5± 0.1	0.6±0.1	0.5±0.0	0.4±0.1
Na^+	0.1±0.1	0.2 ±0.0	0.3±0.2	0.7±0.2	0.8 ± 0.1	0.1 ± 0.1	0.2± 0.0	0.7±0.2	0.9±0.0	0.7±0.2
Ca^{2+}	18.3±0.5	18.4 ±0.0	22.7±4.2	30.0±1.2	41.6± 0.1	26.7± 0.7	21.1± 2.3	32.8±2.1	38.5±0.9	23.1±0.5
Mg^{2+}	5.2 ±0.2	4.7 ±0.2	5.5±0.3	3.6±0.2	4.7 ± 0.9	7.2± 0.1	5.5± 0.3	3.9 ±0.9	4.4±0.2	3.4±0.5
					Sampling VII					
K^+	0.3±0.0	0.5±0.1	0.6±0.1	0.6±0.1	0.6 ± 0.2	0.3± 0.0	0.4± 0.0	0.6 ±0.0	0.8±0.8	0.6±0.0
Na^+	0.1±0.1	0.2±0.1	0.2±0.1	0.2±0.1	0.3± 0.0	0.1± 0.0	0.4±0.0	0.2 ±0.0	0.2±0.1	0.2±0.0
Ca^{2+}	24.1±1.8	41.9±0.2	21.7±1.8	22.9±0.4	19.2 ± 0.2	25.1± 0.7	21.3±0.5	22.4 ±0.2	19.4±0.9	21.0±1.1
Mg^{2+}	3.5±0.4	6.6 ±0.2	3.2±0.3	3.8±0.1	4.0± 0.3	4.7 ± 0.8	5.2±0.6	3.4 ±0.2	5.0±0.5	3.4±0.2

Bulluck III et al. (2002) reported differences in chemical properties of the soil were more related to amendment type than to production history. Calcium, potassium, magnesium and manganese increased in the studied soils that received organic amendments, but not in those soils receiving synthetic fertilizers. Weber et al. (2007) also found highest values of exchangeable bases, in particular K^+ and Mg^{2+}, in soil amended with compost. In contrast Clark (2007) reported an enhancement of K^+ in clay sodic soil amended with chickpea residues, whereas no effect on Mg^{+2}. Moreover the same author highlighted the importance of the nature of organic amendment as a marked positive effect of chicken manure but a very slight influence of sawdust and cheak pea on exchangeable Ca^{+2} were observed.

Organic carbon content was significantly influenced by organic amendments in both the first and second phase of the study (Table 3.8). In the first year after one month from the first amendment, organic carbon content slightly increased (by about 20% as respect to the control) in all plots of F1 soil treated with organic amendments (Figure 3.8). In the following months (second and third samplings of the first year) an increase of organic carbon in all amended plots was registered with a little decrease at the fourth sampling. After the second addition of the organic amendment a further increase occurred (in particular at VI sampling in A2HM, A1HM and A1H plots, Figure 3.8) with more lasting effects. In fact, at the end of study an increase of about 80% of the carbon content was registered in the plots treated with the higher dose of amendment whit respect to control (Figure 3.8).

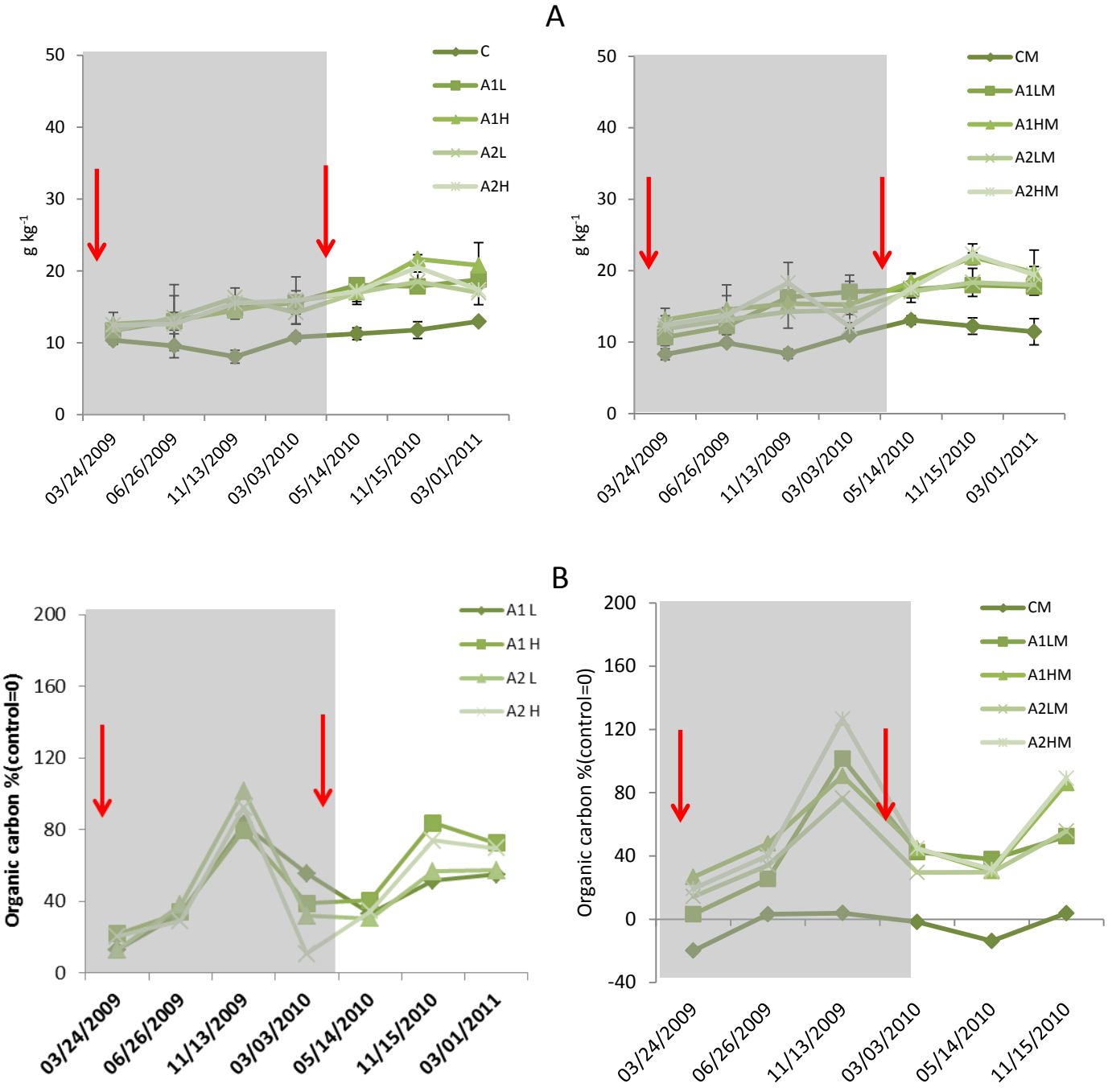

Figure 3.8 Effect of amendment application on Corg (g kg^{-1}) (A) and percentage variation (B) with respect to the control in F1 farms soils in the all phases of this study from first Sampling I to Sampling VII. The red arrow indicates the amendment application.

Similarly, in F2 soils, after one month from the first addition of organic mixtures, a slight increase in organic carbon was observed in the amended plots (Figure 3.9), but at the third sampling organic carbon in amended plots was 40% higher than control (~22 g kg^{-1} in the amended plots vs. 16.19 g kg^{-1} in control plot, Figure 3.9B). After one year, the added OM was almost completely degraded and a clear decrease in organic carbon was found. Only the addition of new organic matter (second

amendment) determined a new and lasting increase in soil organic carbon that remained higher (until 60% in A1H) as respect to control plot till the end of the second year (Table 3. 9).

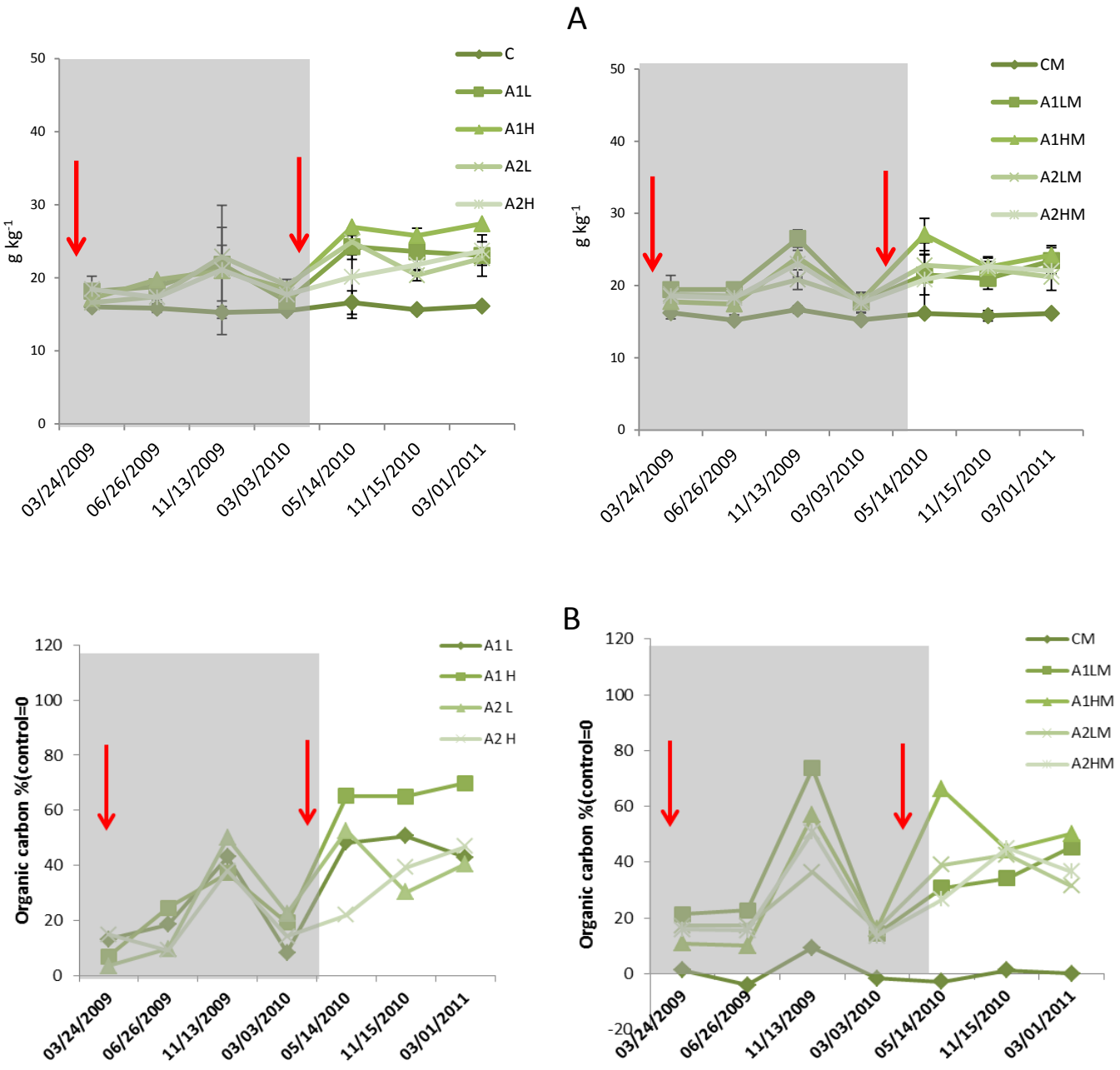

Figure 3.9 Effect of amendment application on Corg (g kg^{-1}) (A) and percentage variation (B) with respect to the control in F2 farms soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

Soils object of this study were characterized by a high total nitrogen content (Table 3.9) compared to the content of soils with similar geopedologic characteristics (Batjes, 1996).

In amended plots of both soils, a slightly increase (about 10-20%) in total N was observed compared to control of the first sampling (Figure 3.11). Moreover, after two years, the addiction of the organic amendments determined in all treated plots a maintenance of nitrogen content over time

in spite of gradual decrease of this parameter in control plots. This last effect was probably due to interruption of the constant agricultural practice of mineral fertilization in soils during the experiment.

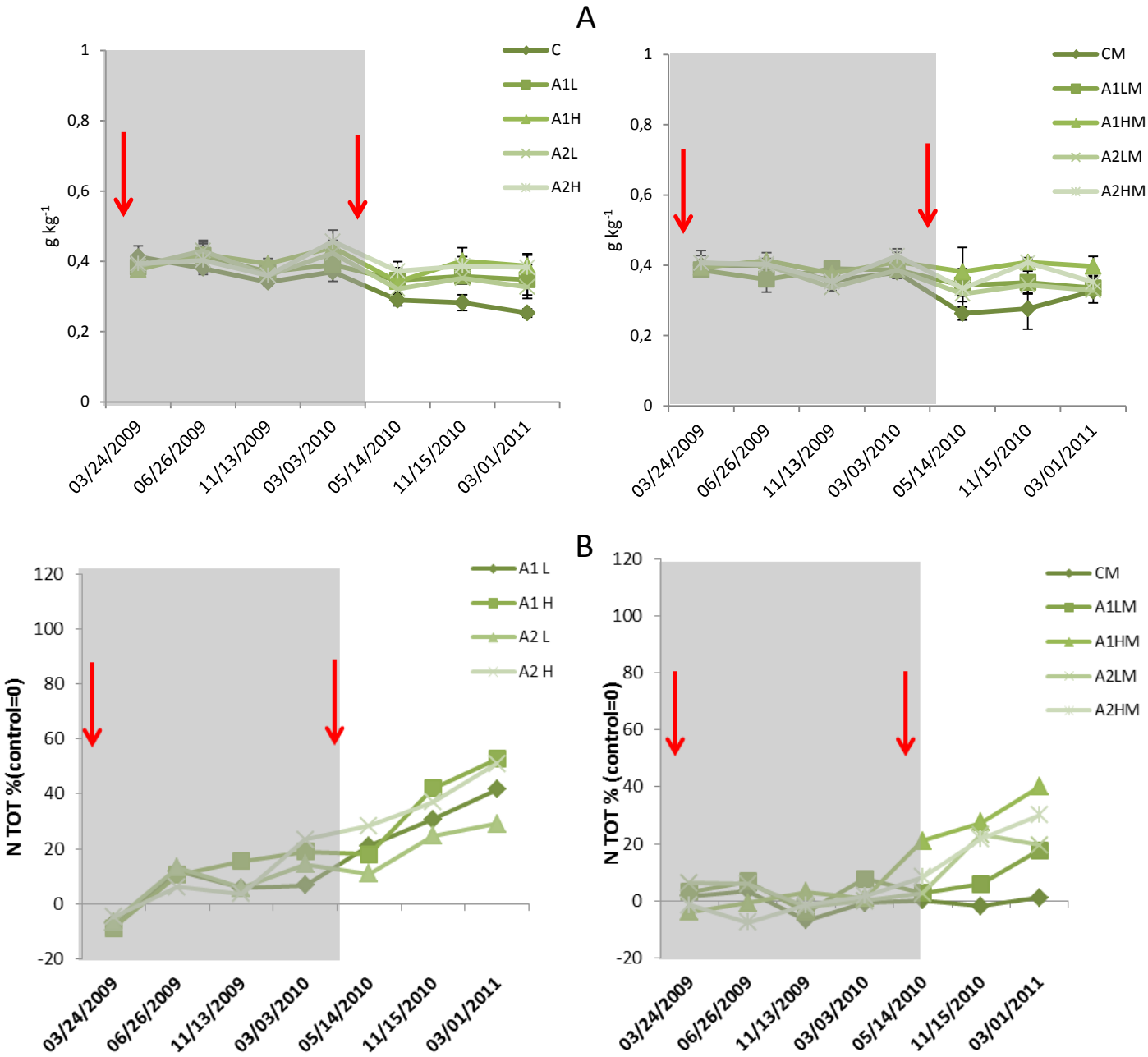

Figure 3.10 Effect of amendment application on N tot (g kg^{-1}) (A) and percentage variation (B) with respect to the control in F1 farms soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

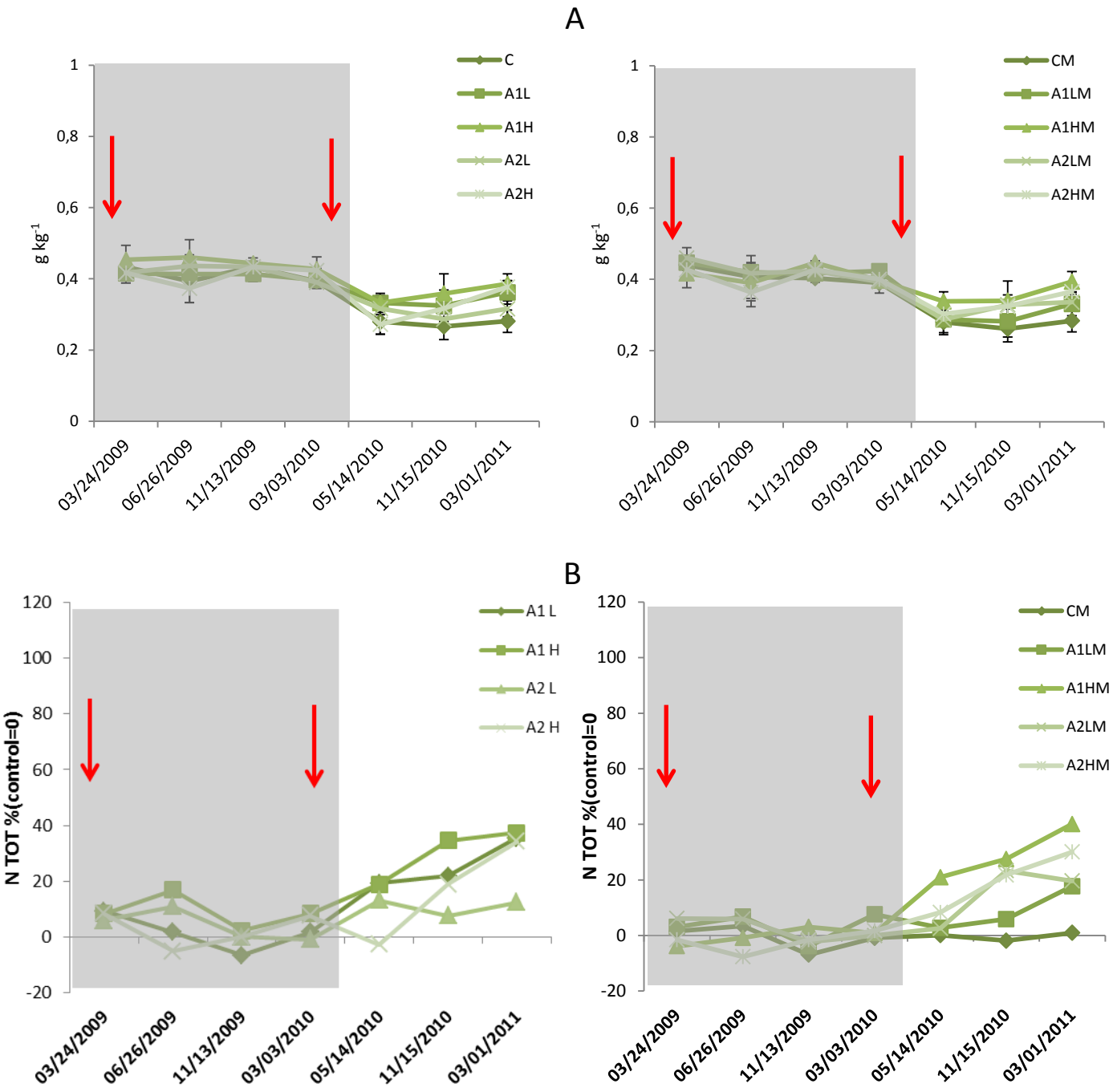

Figure 3.11 Effect of amendment application on N tot (g kg^{-1}) (A) and percentage variation (B) with respect to the control in F2 farms soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

In the first sampling of the first year all amended plots of F1 farm showed an increase of C/N ratio (until to 4.52 and 4.88 for F1 and F2 plots, respectively, compared to control plot) (Scotti, 2011). After two yearly amendments (SamplingVII), the all treated plots showed values higher than 5. In F2 soil having a starting value of C/N ratio higher than that in F1 soil (3.70 against 2.51, Scotti, 2011), only a lower increase was observed in all treated plots. After the second amendment C/N ratio showed an increase in all amended plots reaching in some plots (A1H and A2Lvalues higher than 7).

Table 3.10 Effect of soil amendment on C/N ratio (±sd) in F1 and F2 soils after two yearly amendments.

Second year-amendment li poi mettere tutti sulla stessa riga Fi e F2			
	after 1 month SamplingV	**after 6 months samplingVI**	**after 12 months samplingVII**
Farm 1			
C	4.51 ±0.10	4.18 ±0.32	4.23 ±0.11
A1L	5.06 ±0.50	5.00 ±0.32	5.17 ±0.77
A1H	5.38 ±0.44	5.43 ±0.46	5.08 ±0.41
A2L	5.31 ±0.14	5.25 ±0.62	5.51 ±0.10
A2H	4.68 ±0.80	5.31 ±0.3	5.10 ±0.37
Farm 2			
C	6.05 ±0.27	5.94 ±0.74	5.78 ±0.59
A1L	7.30 ±0.43	7.27 ±0.46	6.38 ±0.55
A1H	8.14 ±0.86	7.34 ±0.59	7.12 ±0.30
A2L	7.93 ±0.85	7.15 ±0.79	7.19 ±0.38
A2H	7.35 ±0.55	6.87 ±0.12	6.29 ±0.46

It should be remarked that in both farms also the controls plots showed an increase in C/N ratio over time. Likely this is a consequence of gradual decrease in total nitrogen values (Figure 3.10 and 3.11) due to interruption of the mineral fertilization practice of soils during the study period. 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 samplings I, samplings III and samplings VI, in plots without mineral fertilizer in sampling IV and samplings plots with mineral fertilizing of the sampling II and VII sampling, whereas in F2 soil increased values of C/N ratio were showed by in plots with mineral fertilizers in Sampling I and III and in plots without chemical fertilizer in the Sampling IV. 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), is not directly related with differences.

3.3.2 Effect of organicamendments on enzymaticactivities

Among the enzymatic activities assessed in this study the DHY activity seemed strongly enhanced by the amendment already at the first sampling, in both soils (Figure 3.12 and 3.13). In all treated plots of the F1 soil, the DHY activity was higher than in control plot. In particular, in the plots treated with the highest dose of both mixtures (A1H and A2H), after one month from the first

amendment, the DHY activity went up to 2.0 and 2.6 µg TPF g^{-1} h^{-1}, respectively. At the second sampling, a further increase of DHY in A2L and A2H plots as well as in control plot soil was observed. After that the activity values strongly felt down until to initial value of control soil. After the second amendment, the DHY activity followed the same trend of the previous year, but characterized by a greater increase, until to around 5 µg TPF g^{-1} h^{-1} in all treated plots. In the Table 3.11 the results of one-way ANOVA of the DHY activities to better understand the real effect of different treatment on this enzymatic activity: the value of the Sampling V was significantly higher than those measured in the later samplings.

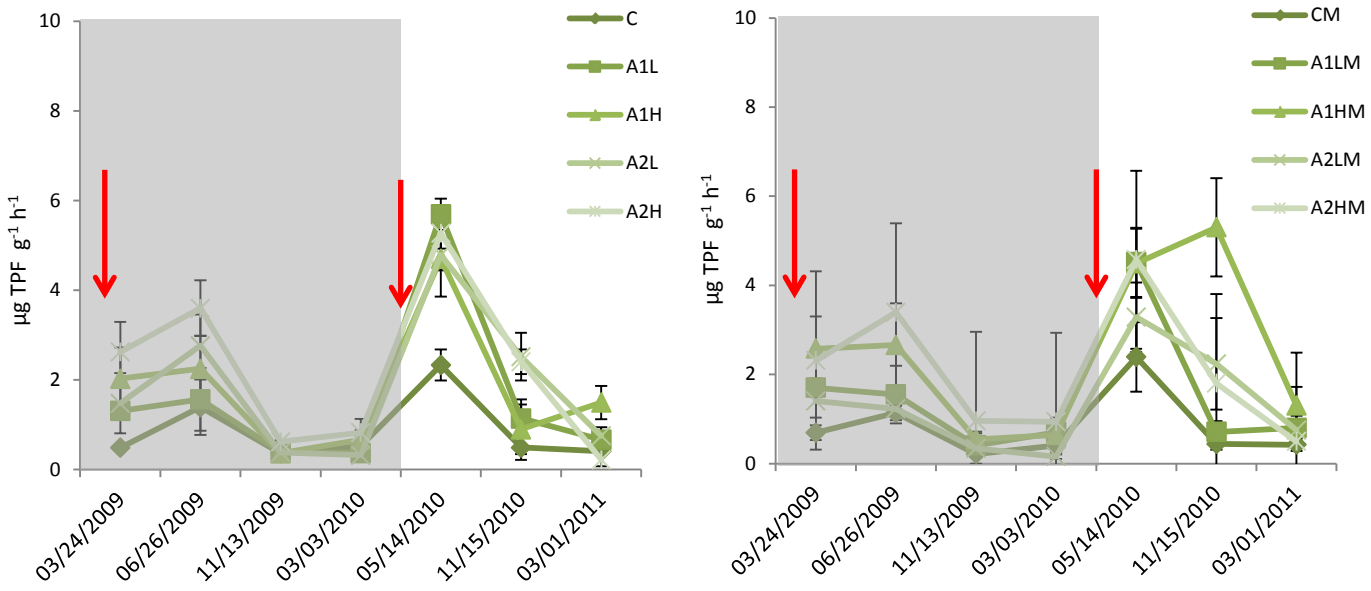

Figure 3.12 Effect of amendment application on DHY (µg TPF g^{-1} h^{-1}) in F1 farms soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

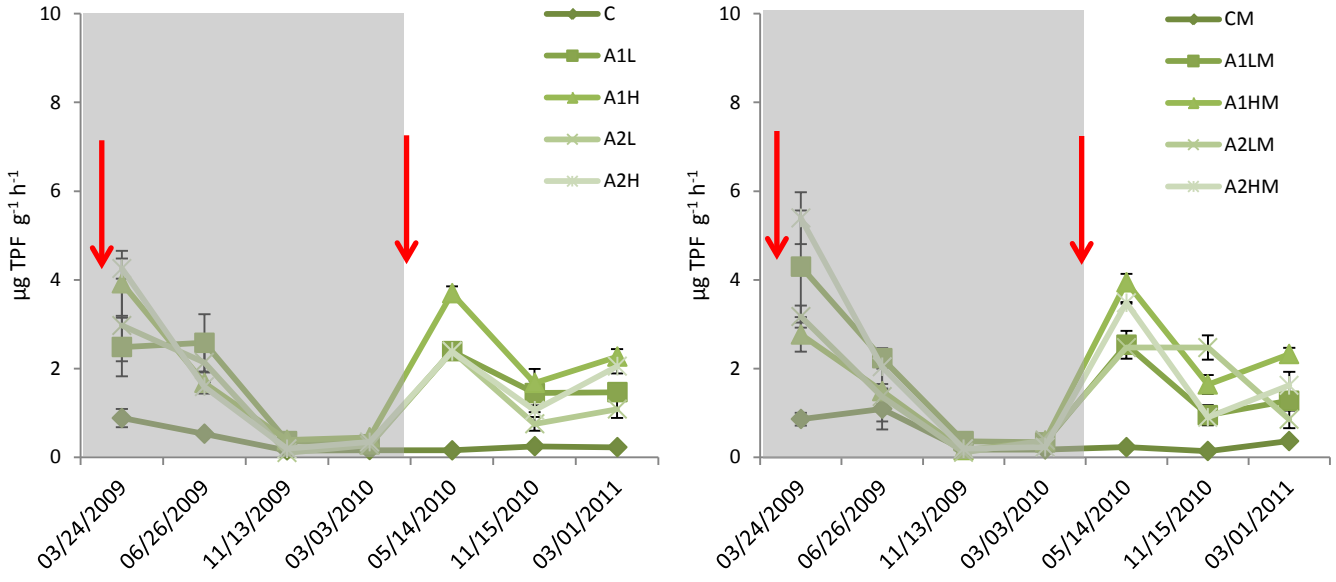

Figure 3.13 Effect of amendment application on DHY (µg TPF g^{-1} h^{-1}) in F2 farms soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

Table 3.11 Summarized results of one-way ANOVA. DHY (μgTPF g^{-1} h^{-1}) in F1 and F2 farm soils in the second phase of this study that include three sampling (from second amendment 12/04/2010). Different lower case letters indicate significant differences ($P \leq 0.05$ from Duncan test) between treatments and uppercase letters indicate significant differences across time.

Plot	F1			F2		
	SamplingV 05/14/2010	SamplingVI 11/15/2010	SamplingVII 03/01/2011	Sampling V 05/14/2010	SamplingVI 11/15/2010	SamplingVII 03/01/2011
C	bA	cB	cB	c	d	D
A1L	aA	bB	bB	bA	abB	bB
A1H	aA	bB	aB	aA	aC	aB
A2L	aA	aB	bC	bA	cB	cB
A2H	aA	aB	cC	bA	bcC	aB
CM	bA	cB	cB	dB	dB	dA
A1LM	aA	cB	bB	cA	cB	bB
A1HM	aA	aA	aB	aA	cB	aB
A2LM	aA	bB	bC	cA	aA	cB
A2HM	aA	bB	cC	bA	cC	bB

In F2, differing from F1 soil, the first addition of OM stimulated immediately the DHY activity to higher values than in F1 soils (Figure 3.12), but at the second sampling DHY already began to decrease and continued to fall down even below the control plot values, until the end of the first year, when the arrive of new OM determined a new increase. Moreover, while after two years in F1 soils DHY activity felt down until the initial value of control soil, in F2 soil the values remained higher (2.27 and 2.03 μg TPF g^{-1} h^{-1} in A1H and A2H, respectively) than control (0.23 μg TPF $g^{-1}h^{-1}$) soil (Table 3.13).

In both F1 and F2 soils the values of PHO significantly decreased after organic amendment application. The effect was much more marked after the second amendment application at the first sampling of the second year (Figure 3.14 and Figure 3.15). In F1 after the first amendment the value of PHO for the control C was 0.44 μmol ρ-$NPg^{-1}h^{-1}$. Immediately after the second application A2L and CM plots showed values of PHO significantly lower (Figure 3.14), in the order of 0.20 and 0.12 μmol *p*-$NPg^{-1}h^{-1}$, around of -54.5% and -72.7% less than that of the control in the initial conditions, respectively. During the second year (Samplings V and VII) the values of PHO remained quite stable returning to their initial values. Data of Table 3.12 indicate that this enzymatic activity is influenced by the mineral application ($P \leq 0.05$) moreover plot A2HM, which was treated also with mineral fertilizer, presented the greatest values for this activity.

Similar behavior occurred for the samplings of F2 farm, in which the significant lowest values of PHO were observed for A1LM and CM plots (Figure 3.15), evidencing that this parameter was very responsive to the mineral fertilizer application in both farms, if the initial PHO value of the control

C, 0.77 µmol ρ-NPg^{-1}h^{-1}, is considered. After the second amendment application, A2L and CM plots showed values of 0.17 and 0.14 µmol ρ-NPg^{-1}h^{-1}, about -136% and -143% lower than the control at the initial conditions, respectively. The higher values of PHO were measured for A1H, A2H, A1HM and A2HM plots but they did not show significant differences among them (Table 3.12). Moreover, phosphorus (P) species interacts with the components of calcareous soils causing both surface reactions and precipitation given especially in the presence of calcite and limestone (Von Wandruszka, 2006).

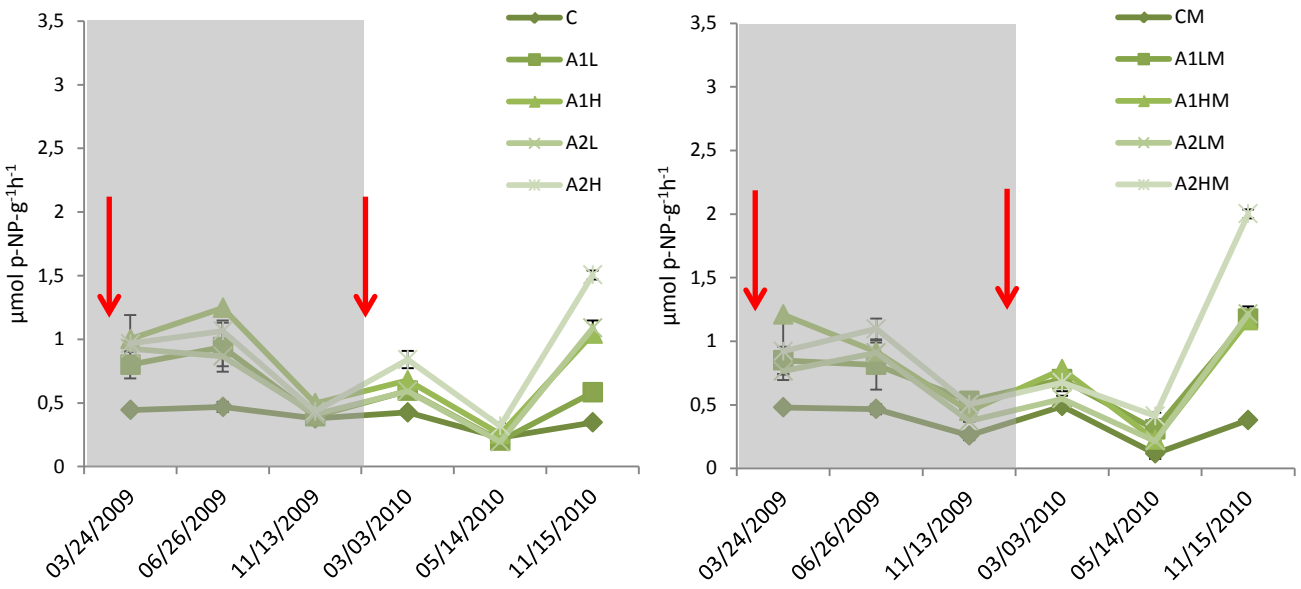

Figure 3.14 Effect of amendment application on PHO (µmolρ-NPg^{-1}h^{-1}) in F1 farms soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

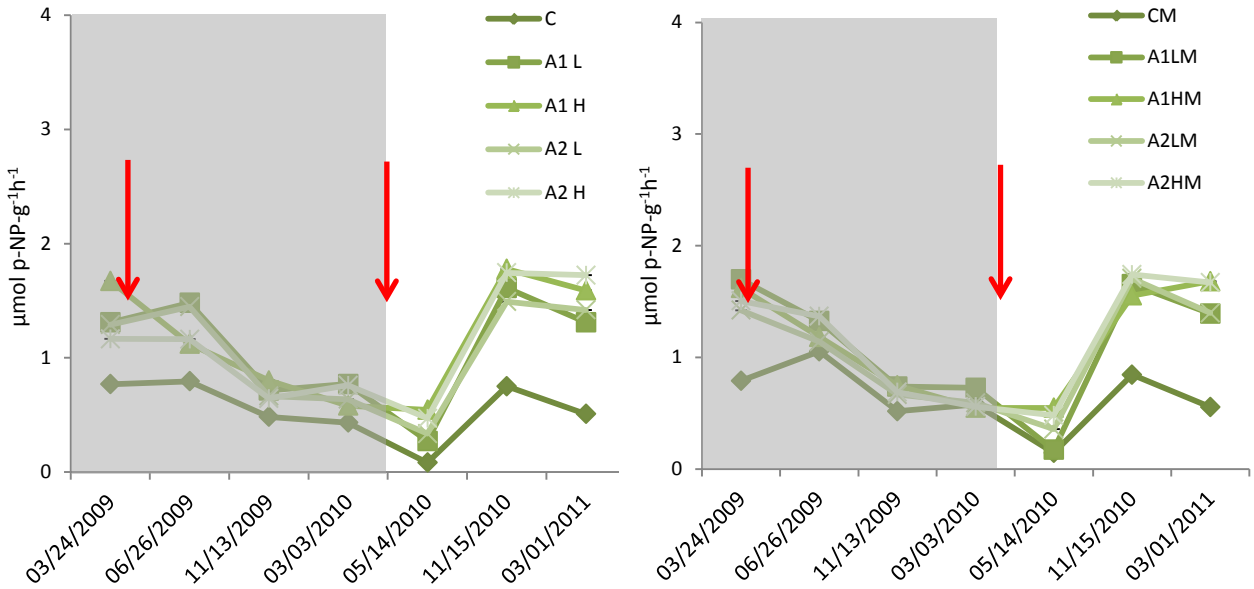

Figure 3.15 Effect of amendment application on PHO (µmolρ-NPg^{-1}h^{-1}) in F2 farms soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

Table 3.12 Summarized results of one-way ANOVA PHO (µmol *p*-NP$g^{-1}h^{-1}$) for F1 farm soils in the second phase of this study that include three sampling (from second amendment 04/12/2010). Different lower case letters indicate significant differences ($P \leq 0.05$ from Duncan test) between treatments and uppercase letters indicate significant differences across time.

	F1			F2		
Plot	**Sampling V**	**Sampling VI**	**Sampling VII**	**Sampling V**	**Sampling VI**	**Sampling V II**
C	bC	dB	dA	dC	cA	dB
A1L	bC	cB	cA	cC	bA	cB
A1H	aC	bB	aA	aB	aA	bA
A2L	bB	bA	bA	bB	bA	cA
A2H	aC	aB	bB	abB	aA	aA
CM	dC	cB	bA	cC	bA	cB
A1LM	bB	bA	bA	0B	aA	bA
A1HM	cB	bA	bA	aB	aA	aA
A2LM	cC	bA	cB	bC	aA	bB
A2HM	aC	aA	aB	aB	aA	aA

In both farm F1 and F2 the value of GLU substantially increased after the second organic amendment application performed in the second year. In F1, after the first amendment, the values of this enzymatic activity were stable and similar among them, but the second application of the organic amendment strongly stimulated the activity causing a large increase of them (Figure 3.16). The plots A2L, A2H and A2LM showed the highest values of GLU in all the three samplings of the second year. In F2 the activities of GLU related to the Sampling I (i.e. after the first amendment) were higher than F1, and showed a gradual decline over time (Figure 3.17). Similarly to F1 an increase of their values occurred after the second amendment application, showing A2L, A2LM, and A2H plots the highest values. These results seem to suggest that this enzymatic activity responded better to the A2L mixture prepared in a ratio of compost and wood of 2:1 with a C/N ratio of 25.

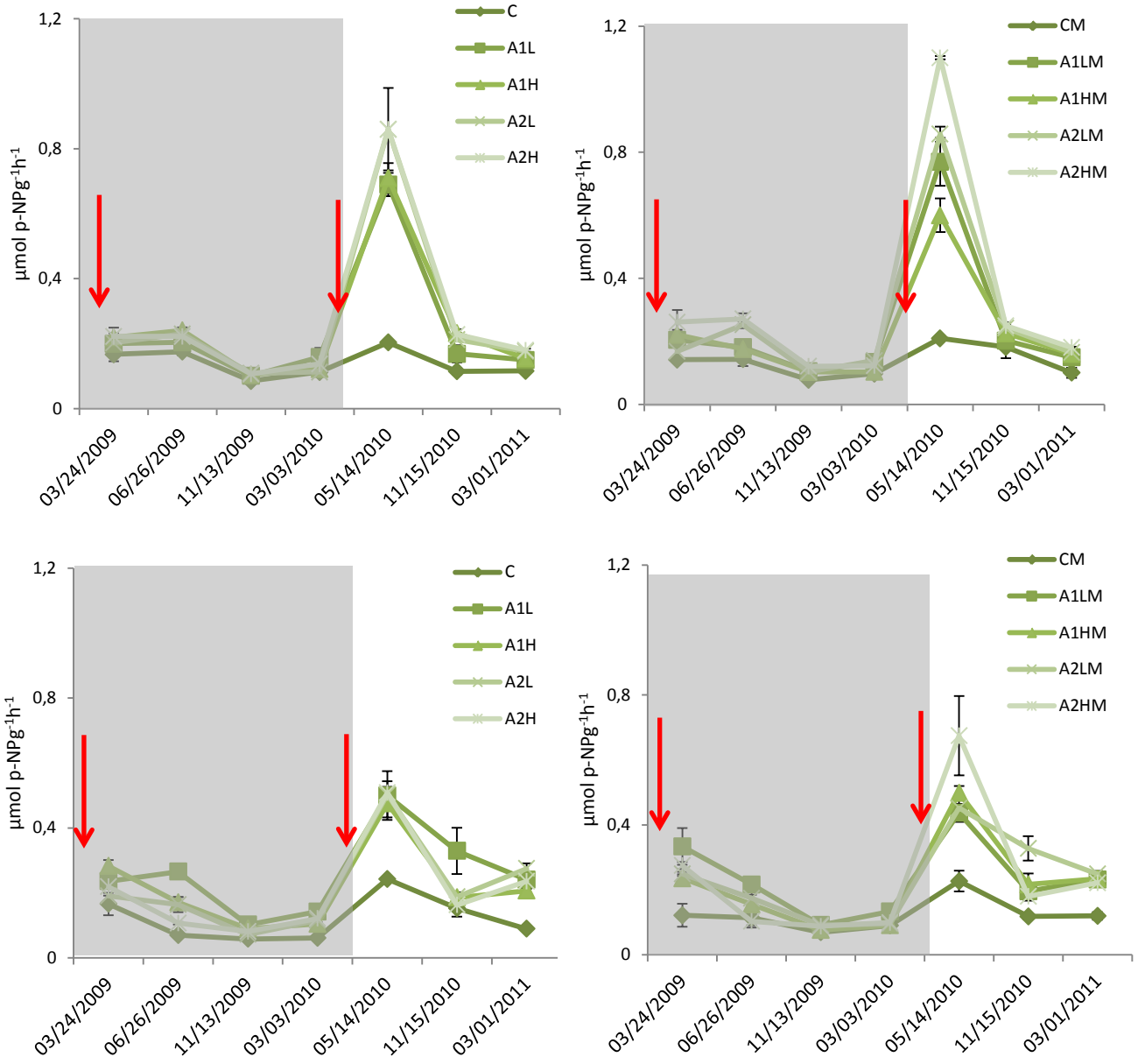

Figure 3.17 Effect of amendment application on GLU (µmol ρ-NPg^{-1}h^{-1}) in F2 farms soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

Table 3.13 Summarized results of one-way ANOVA for GLU (μmol ρ-NP$g^{-1}h^{-1}$) in F1 and F2 farm soils in the second phase of this study that include three sampling (from second amendment 04/12/2010). Different lower case letters indicate significant differences ($P \leq 0.05$ from Duncan test) between treatments and uppercase letters indicate significant differences across time.

	F1			F2		
Plot	**Sampling V**	**Sampling VI**	**Sampling VII**	**Sampling V**	**Sampling VI**	**Sampling VII**
C	cA	cB	cB	bA	bB	dC
A1L	bA	bB	bB	aA	aB	bB
A1H	bA	aB	bbC	aA	bB	cB
A2L	aA	aB	aB	aA	bC	aB
A2H	aA	aB	aB	aA	bC	bcB
CM	dA	cB	cB	cA	cB	bB
A1LM	bA	bB	bB	bA	bB	aB
A1HM	cA	bB	abC	bA	bB	aB
A2LM	bA	abB	abC	bA	aB	aC
A2HM	aA	aB	aB	aA	B	aB

After the first organic amendment application the activities of INV (Figure 3.18) in all plots of F1 showed values (in average of 0.23 μmol g^{-1} h^{-1}) similar to those measured initially in the control plot. After the first treatment only the A2L and A2H plots showed an activity that increased up to 0.44 μmol g^{-1} h^{-1}. At Sampling IV this activity for the all plots amended with organic mixturesas well as the A2LM and A2HM plots achieved values higher than 0.8 μmol g^{-1} h^{-1}, increasing by around 300% as respect to the Control at the initial condition of the first year. However, after of second organic amendment application the INV values showed a decrease and in A2H and A2HM plots (that in the first year were the highest) in the second year a reduction of about 128% in both the plots was measured. In the second year highest values were registered for A1L and A1H plots with 0.64 μmol g^{-1} h^{-1} and 0.62 μmol g^{-1} h^{-1}, respectively, and differences between treatments (Table 3.14).

In F2 farm the activity of INV showed a behavior opposite to that observed in F1 farm (Figure 3.19). The values in the first year were stable until the last sampling (I –IV) in the order of 0.10 μmol g^{-1} h^{-1} for the control C and of 0.42 μmol g^{-1} $h^{-1}$and 0.35 μmol g^{-1} h^{-1} in A2LC and A1HC plots, respectively. After the second organic amendment application the values showed a significant increase specially in A1HC and A1H plots achieving values of 1.22 μmol g^{-1} h^{-1} and 1.08 μmol g^{-1} h^{-1} (Table 3.14).

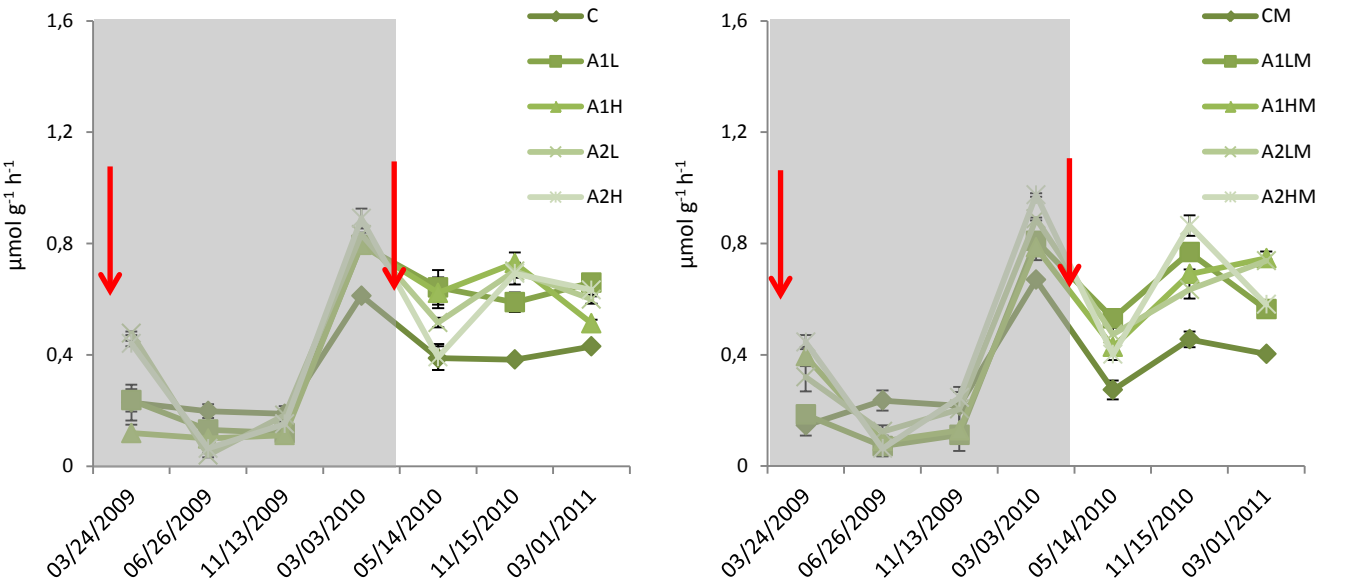

Figure 3.18 Effect of amendment application on INV (µmol g^{-1} h^{-1}) in F1 farms soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

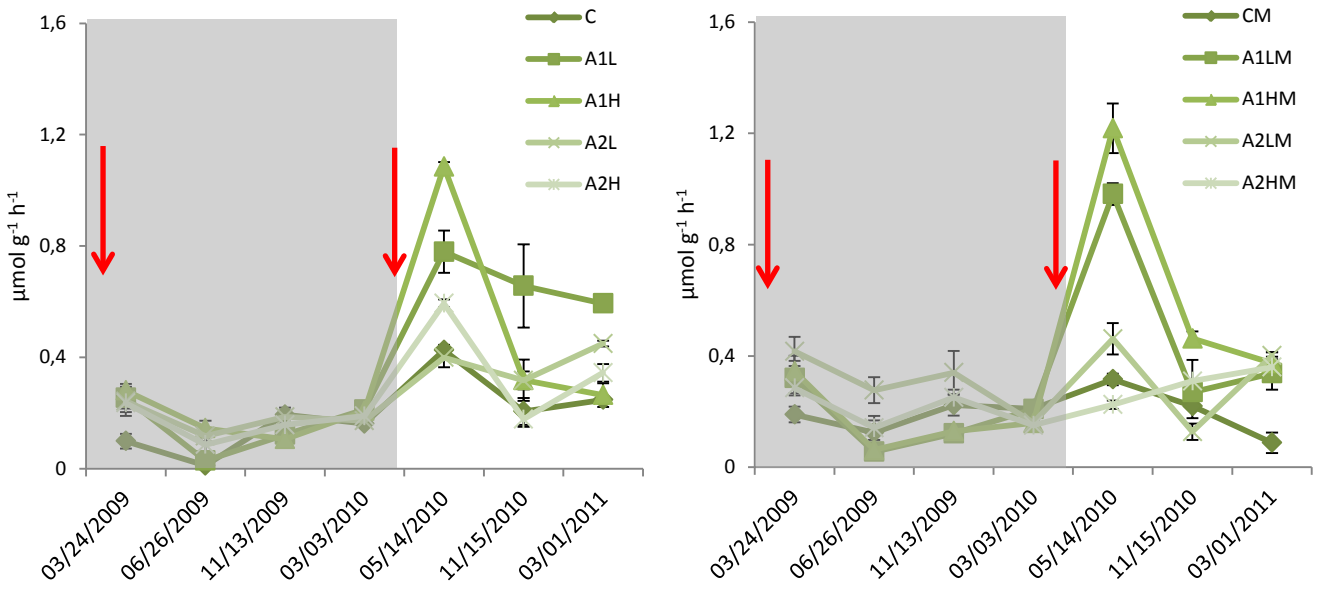

Figure 3.19 Effect of amendment application on INV (µmol g^{-1} h^{-1}) in F2 farms soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

Table 3.14 Summarized results of one-way ANOVA for INV (µmol g^{-1} h^{-1}) in F1 and F2 farm soils in the second phase of this study that include three sampling (from second amendment 04/12/2010). Different lower case letters indicate significant differences ($P \leq 0.05$ from Duncan test) between treatments and uppercase letters indicate significant differences across time.

	F1			F2		
PLOT	**SamplingV**	**Sampling VI**	**SamplingVII**	**SamplingV**	**Sampling VI**	**Sampling VII**
C	c	c	c	cA	b B	dB
A1L	a	b	a	b	a	a
A1H	aB	aA	bC	aA	bB	dB
A2L	bB	abA	aB	cAB	bB	bA
A2H	cB	bA	aA	dB	bB	cA
CM	dB	dA	cA	dA	bcB	bC
A1LM	aB	bA	bB	bA	bB	aB
A1HM	cB	bcA	aA	aA	aB	aB
A2LM	abB	cA	aA	d	cB	aA
A2HM	cC	aA	bB	cA	bB	aB

The urease activity showed great variations throughout the study (Figure 3.20 and Figure 3.21). In F1 farm the A1H plot had the highest values whereas in Sampling VII the highest values were measured for the plot A2L. In F2 farm the first organic amendment application did not caused significant variations and differences as respect the control plot (Figure 3.20) in according to Puglisi et al. (2006) and Garcia-Gil et al. (2000). By contrast, in the second year the plots A2LM, CM, A1HM with the mineral fertilization showed the highest significant values (Table 3.15). This result confirms the influence of the mineral fertilizer on the this activity in F2 (Table 3.15). Indeed, the UR enzyme is responsible for the hydrolysis of urea fertilizers applied to the soil into NH_3 and CO_2 (Andrews et al., 1989; Byrnes and Amberger, 1989), thus it is closely related to the application of this mineral fertilizer. Moreover, this activity seems to respond late to the organic amendment application, because its highest values were shown in the months following the application.

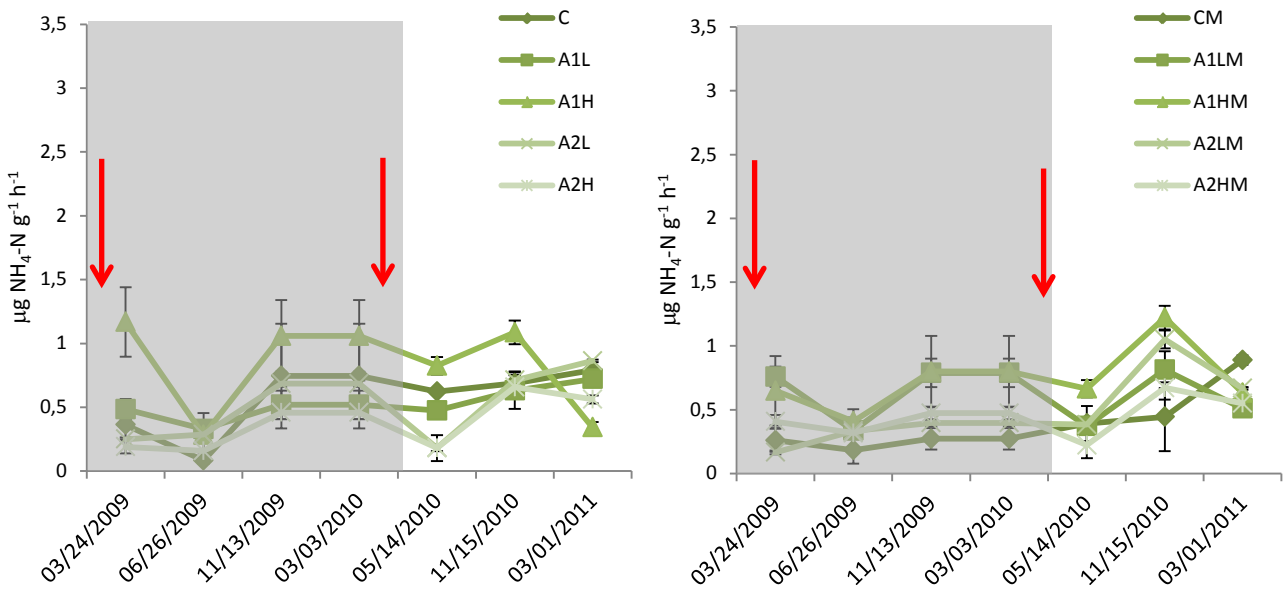

Figure. 3.20 Effect of amendment application on UR (µg NH_4-N g^{-1} h^{-1}) in F1 farms soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

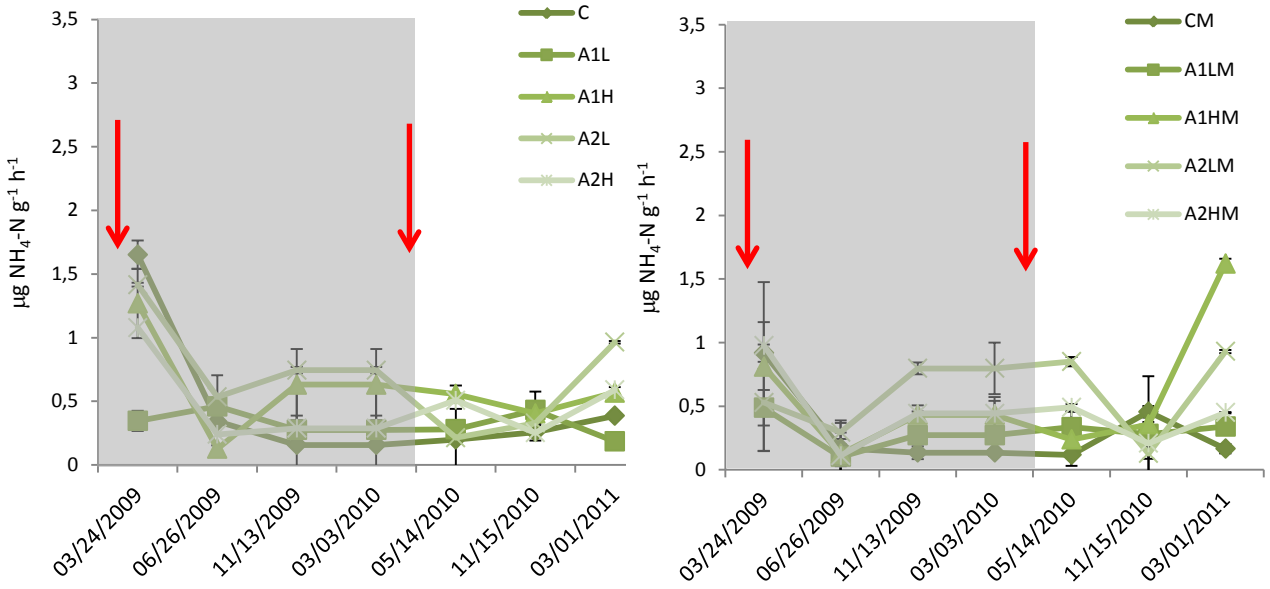

Figure. 3.21 Effect of amendment application on UR (µg NH_4-N g^{-1} h^{-1}) in F2 farms soils in the all phases of this study from first sampling I to Sampling VII. The red arrow indicates the amendment application.

Table 3.15 Summarized results of one-way ANOVA UR (μg NH4-N g^{-1} h^{-1}) in F1 and F2 farm soils in the second phase of this study that include three sampling (from second amendment 04/12/2010). Different lower case letters indicate significant differences ($P \leq 0.05$ from Duncan test) between treatments and uppercase letters indicate significant differences across time.

	F1			F2		
Plots	**SamplingV**	**SamplingVI**	**SamplingVII**	**SamplingV**	**SamplingVI**	**SamplingVII**
C	b	b	ab	b	n	ab
A1L	cb	b	ab	bAB	A	bB
A1H	aB	aA	cC	a	N.s	a
A2L	cB	bAB	aA	bB	AB	aA
A2H	dB	bA	cA	aA	B	aA
CM	b	c	aA	cB	aA	bB
A1LM	bB	bcA	B	b	ab	b
A1HM	aB	aA	B	cB	abB	aA
A2LM	bC	bA	B	aB	cC	aA
A2HM	ab	c	B	b	b	b

The activity of arylsulphatase in F1 and F2 showed gradual increases over time from the first application of compost during the first year (Figures 3.22 and 3.23). Since the second application of organic amendment the rising of ARY values was more pronounced, declining slightly at the last sampling as confirmed by the analysis shown in Table 3.16 where it is also evident that A1L, A1H and A2L plots presented the highest values of this enzymatic activity. This effect of treatment on activities is not evident in the first year in F1 until fourth sampling, which this activity showed great increasing. In F2 soils, ARYL activity was higher in organic amended plots at the first sampling, showed fluctuating trend in the time and presented a gradual increase from fourth sampling, the plot consisting in A2 mixture showed values higher respect to the others plots, which was richer in wood scraps and therefore more slowly degradable, furnished suitable substrate to this activity. also in long-term. A similar result presented A1Hm, Scotti (2011) reported that as for PHO, also for ARYL it is possible to implicate inhibition phenomenon due to sulphate releasing from OM decomposition (Burns, 1978). The stability of the data collected during the experiment shows that this enzyme. in the experimental conditions cannot be considered a good indicator for this study, due to the its sensibility to trace elements present in compost.

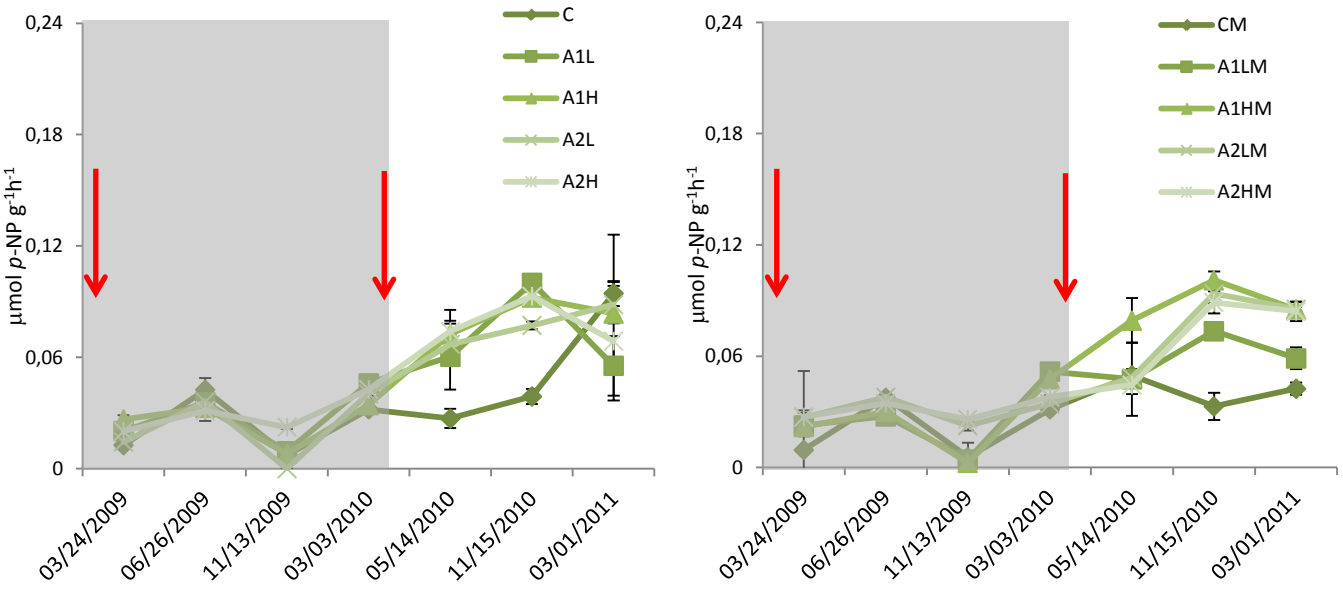

Figure 3.22 Effect of amendment application on ARYL (µmol *p*-NP g^{-1} h^{-1}) in F1 farms soils in the all phases of this study from Sampling I to Sampling VII. The red arrow indicates the amendment application.

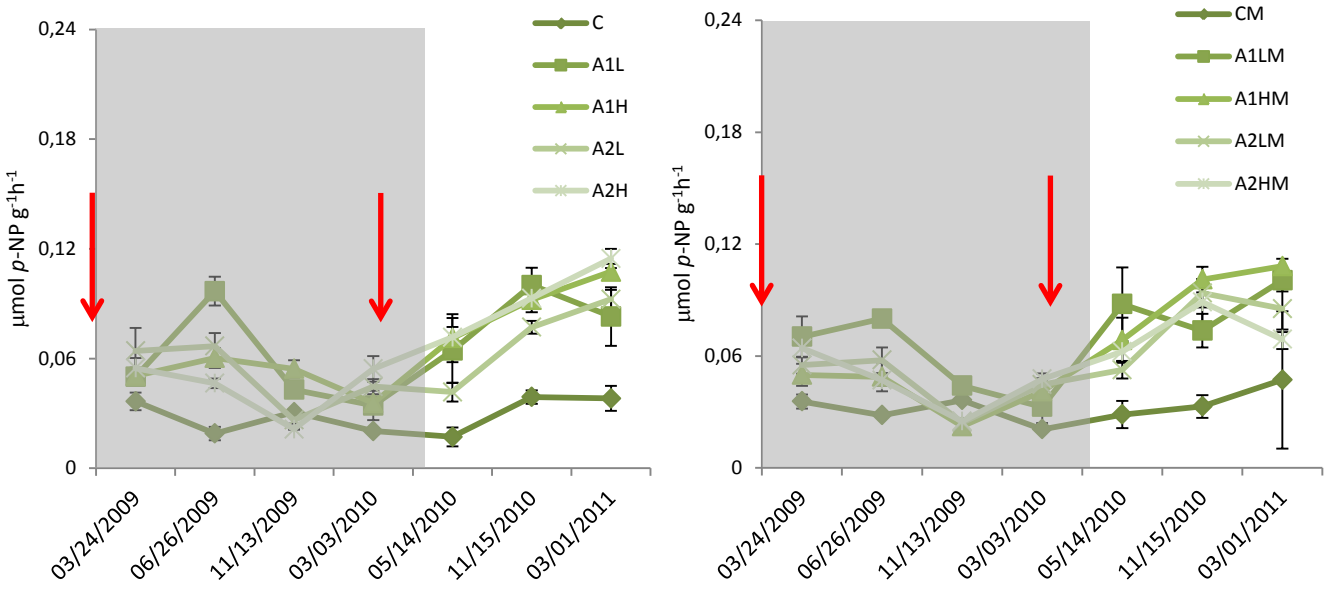

Figure 3.23 Effect of amendment application on ARYL (µmol *p*-NP g^{-1} h^{-1}) in F1farms soils in the all phases of this study from Sampling I to Sampling VII. The red arrow indicates the amendment application.

Table 3.16 Summarized results of one-way ANOVA ARYL (μmolpNP g^{-1} h^{-1}) in F1 farm soils in the second phase of this study that include three sampling (from second amendment 04/12/2010). Different lower case letters indicate significant differences ($P \leq 0.05$ from Duncan test) between treatments and uppercase letters indicate significant differences across time.

	F1			F2		
PLOTS	**Sampling V**	**Sampling VI**	**Sampling VII**	**Sampling V**	**Sampling VI**	**Sampling VII**
C	B	b	d	cB	bA	dA
A1L	bB	aA	cB	aB	aA	cB
A1H	a	a	a	aB	aAB	abA
A2L	aB	aA	aA	bB	aA	bA
A2H	aB	aA	bB	aC	aB	aA
CM	abA	cB	cA	cB	cB	dA
A1LM	ab	ab	b	a	b	a
A1HM	a	b	a	abB	aA	aA
A2LM	abB	aA	aA	bB	abA	bA
A2HM	bB	aA	aA	bB	abA	cB

3.3.3 The enzimatic index AI 3

The enzymatic index AI 3, developed by Puglisi et al. (2006) and based on three enzyme activities (GLU, PHO and UR) was able to identify altered soils among large soil series. Lower values of this index indicate less altered soils; vice versa higher values indicate more altered soils.

The application of AI3 index to the enzymatic activities measured in the studied soils (Figure 3.24) gavefor all treated soilsvalues lower than control soils in both farmsthroughout the two years experiment, thus indicating reduced altered situations in the amended soils.

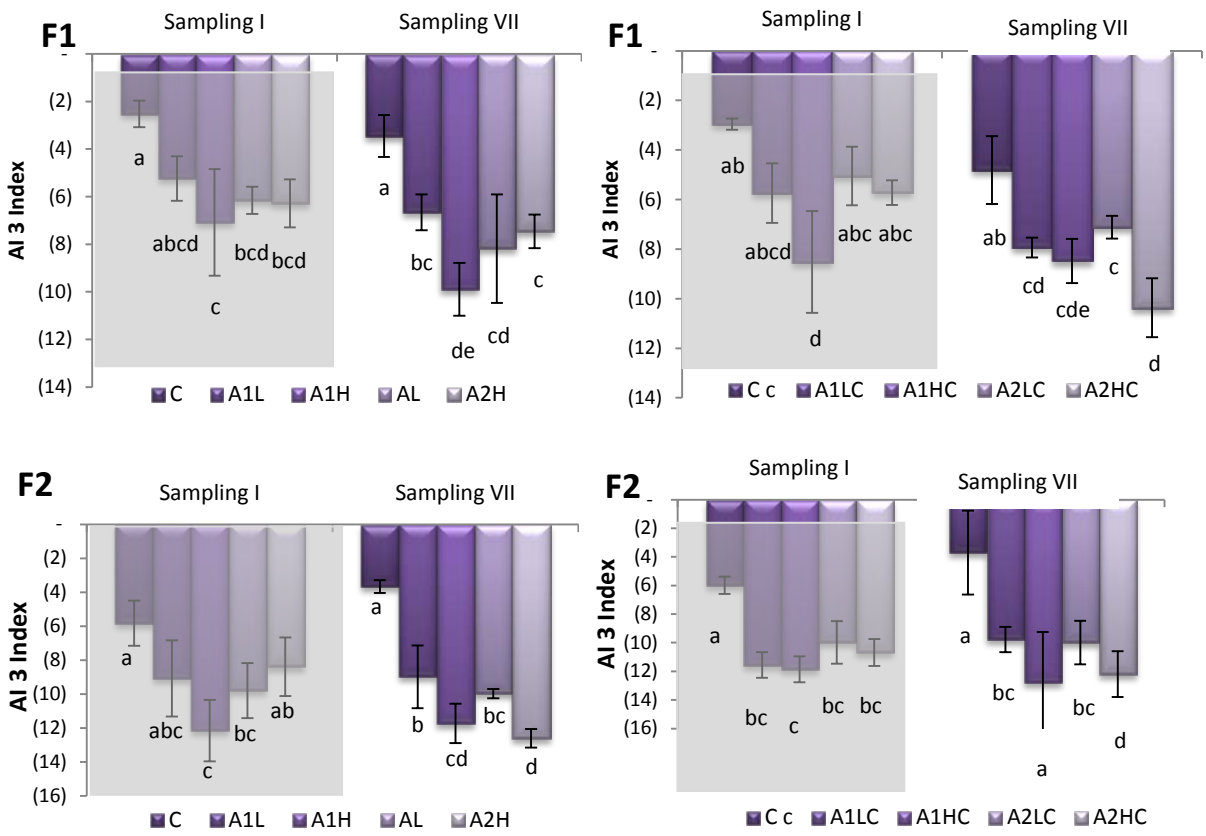

Figure 3.24 AI3 index in F1 farm referred to data of Samplings I and VII in F1 and F2 farms. Different lower case letters indicate significant differences ($P \leq 0.05$ from Duncan test) between treatments and uppercase letters indicate significant differences across time.

3.3.4 Multivariate analysis of chemical and biochemical properties

Differences among the non-treated. control soils, and the amended plots, in both farms, were determined by PCA considering all chemical and biochemical properties determined in this study, and chemical and biochemical data produced in the first year, excluding pH and CEC that showed no effects of organic amendments and mineral fertilizerin the overall context of the study.

The main purpose of PCA was to reduce variables and to identify more readily explainable derived factors (principal components), best elucidating data variation (Jongman et al., 1995). The factorial map of the principal component analysisthat encompasses the entire study (Figure 3.25A) of F1 soil, accounting for 60.38% of the variation in the data, showed two distinct clusters of variables. The first (PC 1, 35.98%) was positively correlated with available phosphorus, organic carbon, C/N ratio, ARYL and INV activities. The second (PC 2, 25.38%) was correlated with EC, DHY and GLU activities, and was opposed to PHO and UR activities. The score plot indicated that samples could be divided in three clusters (Figure 3.25B).

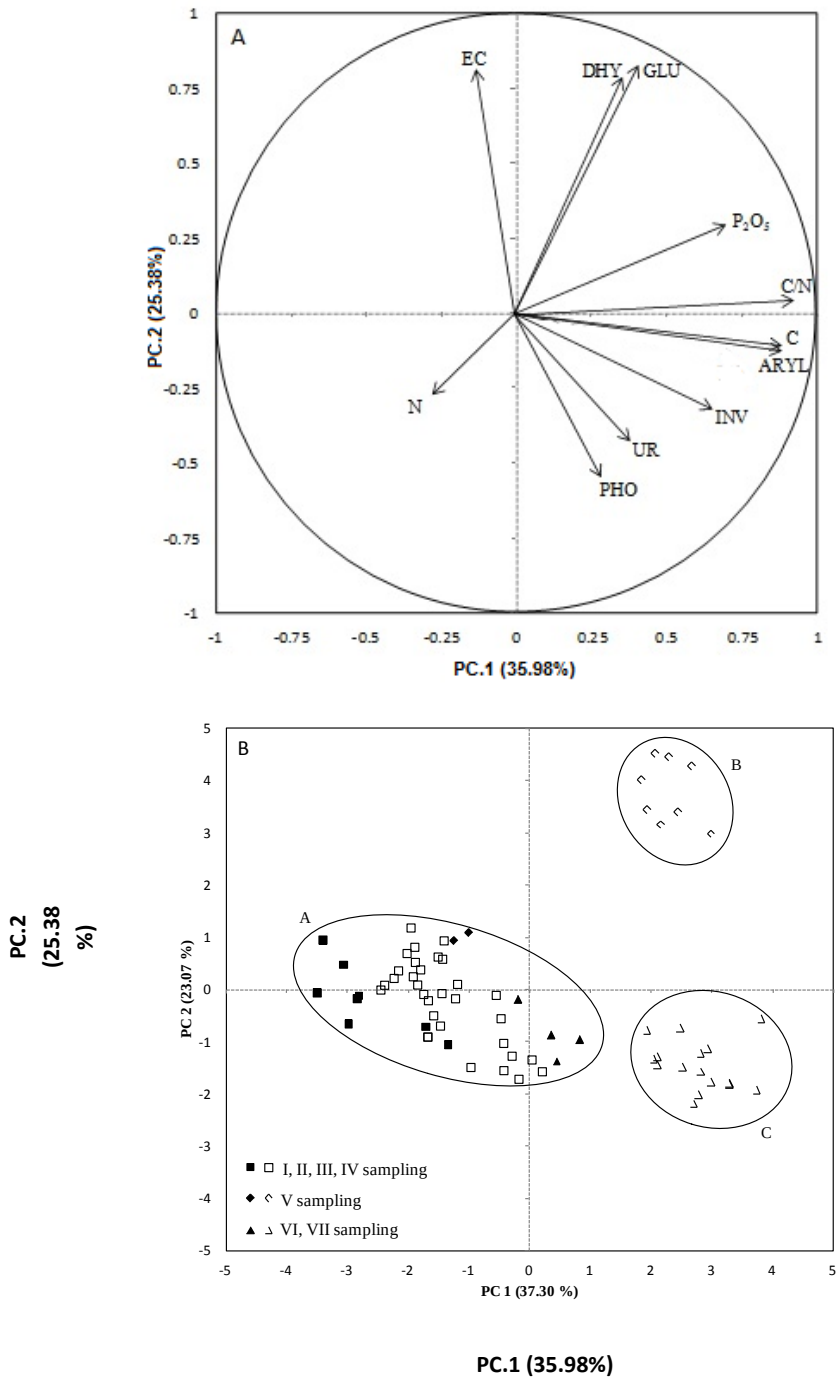

Figure 3.25 The factorial map of the principal component analysis of the two years in F1 soil.

In the first cluster, named A, all control samples (non-amended) and all amended samples of the first year of the study were clustered, whereas, the other two clusters represented only amended samples of the sampling after one month from the second amendment (the fifth sampling, cluster named B) and sixth and seventh sampling (cluster named C). Within each cluster, control samples were always distinct from treated samples. indicating that important changes in the analyzed soil properties upon amendments occurred.

The PCA of F2 soils showed that the first and second clusters of variables explained 56.72% of the total variance (Figure 3.26A).

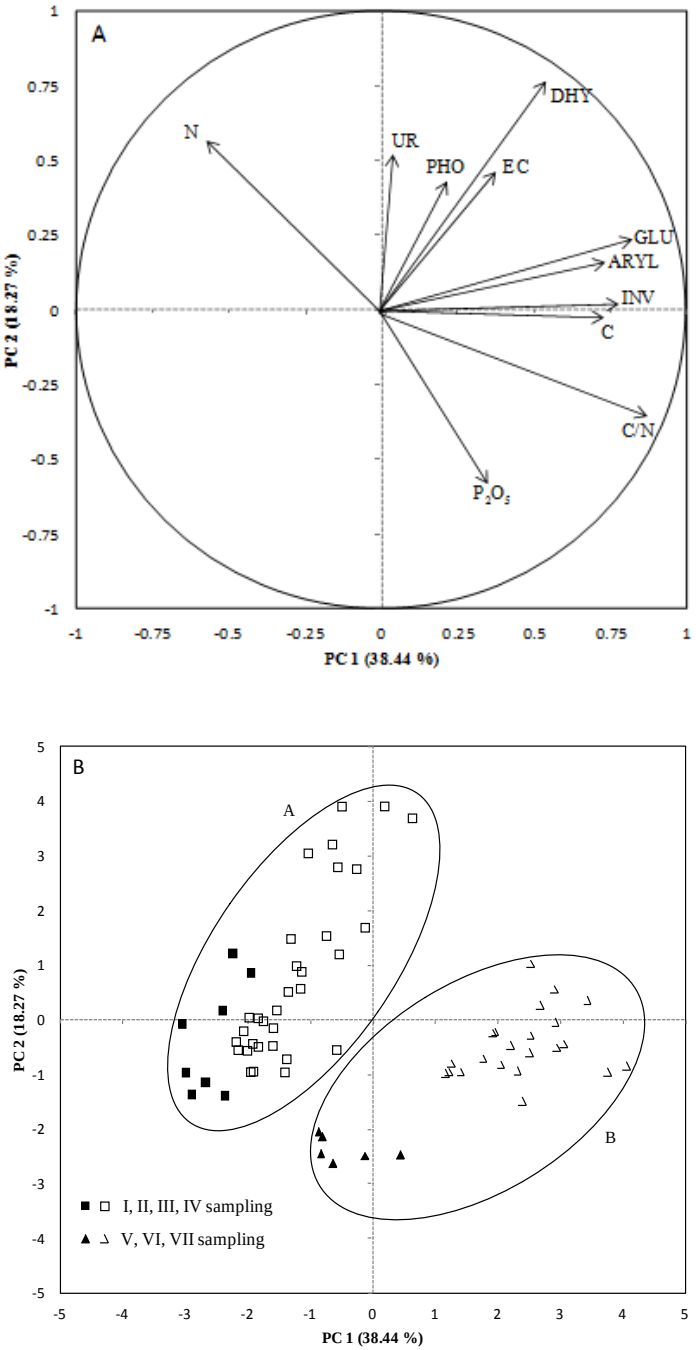

Figure 3.26 The factorial map of the principal component analysis of the two years in F2 soil.

The first one (PC 1, 38.44%) was positively correlated with organic carbon and nitrate content, C/N ratio, GLU, ARYL and INV activities, whereas the second one (PC 2, 18.27%) was positively correlated with electrical conductivity. DHY and UR activity and negatively correlated with available phosphorus content.

Unlike F1 soil samples, F2 score plot showed two clusters (Figure 3.26 B), representing samples collected during the first year of study, after only one amendment (cluster named A), and the samples collected during the second year, after two yearly amendment (cluster named B). As occurred in F1 soil, also in this farm control samples were always distinct from treated samples.

3.4 Discussion

In this research, different kinds of amendments were tested for their effects on soil chemical and biochemical soil properties. In particular, the use of wood scraps of poplar in addition to compost represented an innovative way to improve organic carbon and determined a recovery over time of carbon stock in soils. Application of tested organic mixtures, characterized by the presence of a source of slow-mineralization carbon, determined important changes in the properties of studied soils.

Some soil properties, as pH and EC in clay soil at the second yearwere not affected by different organic amendments, because principally related to the geopedological characteristics of soils. Others, as organic carbon content, were influenced by the kind and dose of tested amendment mixtures. In the treated plots of both farms, after one month from the amendment in the first year the organic carbon slightly changed. The ready response could have been missed because both the compost was still mostly in pellet form and the wood scraps were not yet degraded, causing a separation of amendments from soil during sieving phase. However, in F1 soils, at the end of the two study years and after two amendments, the percentage of organic carbon recovery was of 70%, respect to control soil. These results testified the positive effect of tested organic mixtures in terms of OM recovery, also if the clay nature of F1 soils had an important role in this result. In fact, clay particles are involved in biophysical and chemical processes of carbon stabilization (Christensen, 1996), by forming organo-mineral complexes that protect soil OM and delay its mineralization. Moreover, the lower porosity and therefore the less aired environment could slow down soil microbial activity and, consequently, OM degradation processes by microorganism (Amato and Ladd, 1980; Schulten and Leinweber, 2000).

Differently from F1 soils, in F2 organic carbon increase. after two years. was around 40% compared to control plot. The sandy loam nature of F2 soil, and consequently its low clay content, probably favored leaching process of OM and determined aired conditions that increased biological activity and oxidative processes, contributing to a faster degradation of added OM, as also reported by other authors (Christensen, 1996; Hassink et al., 1997).

Total nitrogen content, in the amended plots. remained constant in the F1 soils and showed a slight decrease in F2 soils respect to control plots during the first year. While chemical fertilizations determine a rapid N release, organic amendments determine a slow N release, but extended over time (Claassen and Carey, 2006). In both farms, during the two years of trial, the constant agricultural practice of mineral fertilization of soils was interrupted. Therefore, considering the uptake from crop cycles during two years of study (about 6 cycles in each farm), the leach process favored by the agricultural practice, such as solarization, and the microbial activity under greenhouse, it is clearly understandable the progressive decline in nitrogen content in soils of

control plots. On the contrary, organic amendments determining a slow release of mineral N, favored the maintenance of N content in soils of amended plots, in spite of leach process, crop cycles, and mineralization processes.

The C/N ratio generally falls within well-defined limit, usually from about 10 to 12 for a agricultural soils (Batjes, 1996). The decay of organic residues by soil microorganisms leads to incorporation of part of the C into microbial tissue with the remainder being liberated as CO_2. The decay process is accompanied by conversion of organic N to mineral nitrogen (NH_4^+ and NO_3^-), but soil microorganisms utilize part of this N for synthesis of new biomass. The gradual transformation of organic material into stable OM (humus) leads to the establishment of consistent relationship between C and N.

In this study, although the used compost mixtures were characterized by a C/N ratio of 15 and 25 (A1 and A2, respectively), only a slight increase of C/N ratio in the studied soils was observed.

When OM is incorporated into soils, microorganisms start to decompose it through enzymatic hydrolysis. Nutrients released in this process can be used by bacteria and fungi, and, if they are in excess, also by other soil organisms, as plants (Borken et al., 2002). However, although the choice to mix wood scrapes to compost in amendments used in this research was done in order to have material with a high C/N ratio, more resilient to decomposition, the resultant C/N ratios were still too low to have the nitrogen deficiency risk.

A ready enhance of microbial activity could be taken in account and the direct consequence could be the increase in organic carbon and nutrients in soil solution (EC increase) observed only from the second sampling, due to the degradation of pelleted amendment. Also the missed increase of nitrogen leads to believe that soil microorganism, in their metabolic activity utilized nitrogen liberated from decomposed OM.

Our study on the use of organic fertilizers in stressed agricultural soils revealed increased enzymatic activities, due to a greater amount of organic materials and nutrients available at the soil surface according with results of other authors (Fernandes et al., 2005). In general, enzymaticactivity values were higher with organic amendments, due to the high organic matter content, despite there were different effects between the treatments.

The amendments used in this study were a mixture of compost and wood which have different behavior and fate. The A1H and A2H, which were the plots receiving the larger amount of compost (54 and 40 t ha^{-1} respectively), showed the higher increase of DHY after the treatment with organic amendments, in both soils. As expected, DHY values were correlated to the other parameters closely related to this enzyme, in particular to organic carbon (Aon and Colanieri, 2001). The activity of this enzyme is closely related to the activity of soil microbial biomass and directly

reflects the conditions of the biological activity in the soil. Therefore, its performance is strongly affected by those factors that modify the growth and the activity of the soil biomass (Dick, 1994).
GLU activity showed a major response (highest values) in A2L, A2LM A2H plots, suggesting that this enzymatic activity responds better to the A2L mixture prepared in a ratio of compost and wood of 2:1 with a C/N ratio of 25. β-glucosidase is one of the enzymatic activities involved in C cycling in soil, gives an indication of the activity of enzymes involved in cellulose degradation (Puglisi and Trevisan, 2012), for this reason the ratio 2:1 compost and wood and the high C/N ratio could have stimulated this activity.
To explain the behavior of the studied soils, seasonal effects should be also taken in account. In fact samplings occurred in different seasons. As the third and fourth samplings, also the sixth and seventh were carried out in autumn and winter, when, as reported in Ros et al. (2003), soil biological activity is reduced compared to the spring and summer (first, second and fifth samplings). However also the reduced amount of labile organic C fraction over time should be also taken in account. Microbial biomass is able to consume for its metabolic needs above all more available compounds, like polysaccharides, lipids etc. (Kandeler et al., 1999), but the mixtures used were rich in more recalcitrant compounds, as lignin, so when the few available carbon compounds were finished, the microbial activity decreased (Trasar-Cepeda et al., 2008).
The great diminution of phosphatase activity after each amendment application could to be explained for the inhibition process of this enzymatic activity in the presence of high level of phosphate reported firstly in 1979 by Spiers and McGill. When there is a signal indicating P deficiency in the soil, acid and alkaline phosphatase secretion from plant roots is increased to enhance the solubilization and remobilization of phosphate, thus influencing the ability of the plant to cope with P-stressed conditions (Karthikeyan et al., 2002; Mudge et al., 2002; Versaw and Harrison, 2002). Oshima (1997) reported that in several soil microorganisms (i.e Saccharomyces) the transcription of genes encoding acid and alkaline phosphatases and the inorganic phosphate (Pi) transporter depends on the Pi concentration in the culture medium. Dick et al. (2011) pointed out that the codification of PHO is controlled under feedback system, and that in high inorganic phosphato medium the activation of PHO genes is inhibited by hyperphosphorylation; the gene hyperphosphorylated remains in the cytoplasm and is unable to activate the transcription of the PHO genes. Kandeler et al. (2002) reported an inverse and significant correlation between the acid or the alkaline phosphomonoesterase activity and the content of organic P in the rhizosphere soil sampled from *Triticum aestivum* and *Trifolium alexandrium*, whereas the content of inorganic P increased towards the rhizoplane. Garcia-Gil et al. (2000) also reported significant reduction in sandy soil amended with MSW compost.

The behavior of invertase activities, i.e. a decrease of their values in F1and an increase in F2 after the second organic amendment application in the second year, are in agreement with the results of Puglisi et al. (2006) which found that in a sandy loam soil (similar condition of F2) amended with 25 ton ha $^{-1}$ of MSW compost. INV was significantly enhanced, while according to Nayak et al. (2007) INV activity was instead reduced in a sandy clay loam soil. The presence of clays might as well played a role in adsorbing the enzyme and thus reducing its activity (Gianfreda et al., 1991), situation that may have occurred in F1 soil for its clay texture. INV activity in F2 showed a significant influencing response in their values of o the mineral fertilizer (Table 8), which could explain why the values of the plot with fertilizer and without fertilizer of A2H mixtures are similar. In this context, Hoffmann et al. (2002) did not found significant differences between the use of mineral fertilizers and organic fertilizers and their association on INV activity in sandy loam conditions.

As already cited, the activity of urease showed a delayed response to the organic amendment application, because its values were highest in the months following the amendment application. This could to be explained by a seasonal effect, Sardans et al. (2008) and Akmal et al. (2012) observed seasonal changes in soil UR activity and found higher urease activity in winter when the soil temperatures were low than in summer. This seasonal effect could explain the behavior of this activity observed in both farms in the two years of the experiments: i.e. the lowest values of UR were measured in both farms at second sampling of the first year (06/26/2009) in the summer season and lightly greater at fifth sampling in the second year (05/14/2010). By contrast, the highest values of UR were measured in F1 at March and November of the first and second year. and in F2 at March of both years. The organic amendment application did not influenced soon the UR activity possibly because urease extracted from plants or microorganisms is rapidly degraded in soil by proteolytic enzymes (Pettit et al., 1976; Zantua and Bremner, 1977). However, in the plots A1H and A2L with and without the mineral fertilizer this activity was positively influenced in the cold season. Several studies have testified that compost application has shown improving effects on UR activity in clay soil (Crecchio et al., 2004; Pramanik et al., 2010) and more specifically in clay loamy soil (Abdelbasset et al., 2011) and in sandy silty loam soil (Albiach et al., 2000). Similar im proving effects by compost were also demonstrated for ARYL activity in silty clay loam soil (Elfstrand et al., 2007), clay loam soil (Abdelbasset et al., 2011), in sandy - silty loam (Albiach et al., 2000) and sandy loam soil (Puglisi et al., 2006).

To better highlight the altered conditions of the studied agricultural soils, it is important to observe the results obtained by applying the AI3 index. The higher values of control plots. compared to those of amended plots, highlighted a stress condition of soils in these farms, recuperated by the use of the kind of organic amendments utilized in the present study. The use of compost, enriched with

scraps of poplar, determined an improvement of conditions of amended plots, due to the enhancement of organic carbon content and, consequently, of microbial activity.

By the analysis of principal component of F1 soils (Figure 3.25) it is clear the effect of the amendment over time. The cluster A, representing the samples of the first year of study, had negative values of PC 1, in particular in the control soils not treated with organic amendment. This result suggested and confirmed that the addition of organic amendments have determined an increase of organic carbon. Important soil properties, as C/N ratio, as well as important nutrient, as phosphorus and nitrate, and enzymatic activities involved in their cycle, as phosphomonoesterase and urease activity, increased with the use of organic amendments. Microbial activity, stimulated by added OM, determined a degradation of organic phosphorus and nitrogen, led to an increase of available phosphorus and nitrate, very important for plants physiological needs (Criquet and Braud, 2008; De Wever et al., 2002).

The cluster B, formed by all treated samples collected after one month from the second amendment, was the only one with positive values of PC 1 and PC 2.

A further increase of organic carbon content was observed in these samples, and consequently, the C/N ratio was higher than control soils. Moreover, these samples showed high values of EC. The second addition of OM, determined an increase of electrical conductivity, due to the nature of compost utilized. The compost product by municipal solid waste is characterized by a high salinity (Zhang et al., 2006), but in our case the values of EC decreased over time, until to values of control soils, as shown by the cluster C.

The second cluster was also characterized by high DHY and GLU activity, in this case both the arrive of new OM and the non complete degradation of residues from the previous amendment, can have determined this result. The nature of amendment. enriched with a matrix recalcitrant to degradation, as scraps of poplar, had represented an important substrate for this class of enzymes (Dilly and Nannipieri, 2001).

The last cluster represented, in the score plot of F1 soil, soils samples collected during the last two samplings (VI and VII). These samples were characterized by negative values of PC 2 and the same PC1 score of the cluster B. Treated samples had higher values of PC 1 than respective control samples. highlighting the highest values of total organic carbon and nitrate content over time, after two yearly amendments. Also in this cluster the results confirm the positive effect of the amendment used, in particular, in terms of organic carbon recovery in the stressed agricultural soils object of this work.

Therefore, the separation of all samples in three clusters, related to time, indicated an evolution of analyzed soil properties over time, that could be related to the seasonal factor and the maturity of added OM.

Also the analysis of principal component of F2 soils showed an important effect of amendment and of time on the samples (Figure 3.26). Also in this case a clear separation of amended soils, respect to control soils, was observed. The two formed clusters are different. The cluster A formed by the samples collected during the first year was heterogeneous, whereas, the second, represented by the samples collected during the second year and after two amendments, was more homogenous. In this last cluster, samples were characterized by higher organic carbon and nitrate content respect to the first cluster, as occurred in F1 soils. Differences between F1 and F2 soils observed in the principal component analysis were determined by the geopedological differences of the two farm soils and different responses to various organic amendments were obtained.

3.5 Conclusions

This study confirmed that a compost enriched with wood scraps of poplar can be an important tool to improve soil fertility over time. The soils of both farms, after two yearly amendments, showed an improvement of soil chemical properties, in particular in terms of organic carbon content. The use of compost mixed with wood scraps. as low mineralization rate material, determined a stable increase of OM over time, in particular in F1 soils, due to its geopedologic characteristics. Organic amendments provided both positive effects on organic C content and nutrients especially as available phosphorus and nitrate, so demonstrating to be a good alternative to conventional fertilizers and consequently improve crop yield, but above all to guarantee an OM recovery that kept stable over time.

Biochemical properties, such as enzymatic activities, were positively influenced by organic amendments. though after the first year strongly decreased. The seasonal effect and changes in substrate availability could determine a decrease of DHY and GLU activities, whereas the degraded OM into simpler carbohydrates, as disaccharides, caused the increase of INV activities.

In conclusion. no clear different response to different amendment doses and to the kind of mixture supplied was observed. At the light of these results, already the use of the lower amount (30 t ha^{-1}) of A1 mixture led to significant advantages, appearing the most adequate to maintain the level of soil organic C, sustain biological activity, and to guarantee vegetable crop productivity. Results suggest that in Mediterranean agricultural soils repeated annual addition of compost with scraps of poplar is an advisable practice to restore and to preserve soil fertility.

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4 Assessing of long time amendment on soil quality and humic substances under greenhouse condition in Southern Italy

4.1 Introduction

The great challenge of sustainable agriculture is to contrast the declining soil quality and water resources available, maintaining the productivity and resilience. The decline in organic matter content of many soils is becoming a major process of soil degradation, particularly in European semi-arid Mediterranean regions.

It is proposed that soil health is dependent on the maintenance of four major functions: carbon transformations; nutrient cycles; soil structure maintenance; and the regulation of pests and diseases. Each of these functions is determined by different biological processes provided by the interacting soil organisms under the influence of environment (Kibblewhite et al., 2008). The soil quality is the outcome of interactions between biochemical and biological characteristics, and its assessment requires the determination of several parameters (Bloem et al., 2006; Marzaioli et al., 2010).

Amendment, as the use of compost, have a higher influence in the microbial activity than another strategy sustainable agriculture as cover crops (Nair and Ngouajio, 2012). Nevertheless, the effects of additions of organic matter (OM) on the soils properties depend on climate, soil characteristics, crop management, and the rate and type of organic amendments (Herencia et al., 2011). Composting represents a strategy of organic waste treatment that is fully compatible with sustainable agriculture given compost term application may counteract depletion of organic matter in soils (Albrecht et al., 2011).

Diacono and Montemurro (2010) reported, in a long-term experimental study (3–60 years), that the use of organic amendments have effects in the both for organic matter replenishment and to avoid the high levels of chemical fertilizers application, moreover, repeated organic matter supply to cropland led to an improvement in soil biological functions (i. e microbial biomass and enzymatic activity), moreover, improve level nutrients as total nitrogen and organic carbon, enhance the soil and physical conditions by improving aggregate stability and decreasing soil bulk density. Repeated application of composted materials enhances soil organic nitrogen content by up to 90%, storing it for mineralization in future cropping seasons, often without inducing nitrate leaching to groundwater.

In general, recent studies show the effect of additions of several compost type the properties of soil (Yupeng et al., 2013; Hasse et al., 2013; Mondini, et al., 2012; Cellier et al., 2012; Tian et al., 2012;

Pant et al., 2012; Phuong-Thi Ngo et al., 2012), but few are the studies have been conducted on long-term, under greenhouses (Herencia et al., 2011).

On other hands, the greenhouse production crop is affect by several adverse conditions, Huaiying Yao et al., (2006) reported that continuous plastic-greenhouse cultivation and management can cause the reduction in the species diversity of the biota, and that the reduction in diversity of microbial communities found in continuous cultivation soils as compared with rotation soils might be due to the differences in the quantity, quality and distribution of soil organic matter. The greenhouse coltivation presents problems such as nutrient accumulation induced by excessive fertilization, acidification, salinization and continuous cultivation and monocultivation (Li et al., 2004; Fei Ying-heng et al., 2008).

The organic matter of soils can be divided into non-humic and humic substances. Carbohydrates, amino acids, protein, lipids, nucleic acids, and lignins are non-humic substances that originate from plants and other organisms. However, humic substances (HSs) are materials originated by the decomposition of plants and animal residues with or without the assistance of micro-organisms (humification) (Xavier et al., 2012).

Aim of this work was to study the fertility recover in agricultural soils, under intensive farming for long time, by supplying compost from municipal solid waste enriched with green waste as wood scraps applied in different dose and C/N ratio with the purpose of to have a material that undergoes a slower mineralization process.

The hypothesis was that when less easily degradable OM arrives in soil, more complex chemical and biological processes occurred that could lead to well know increase of OM, but also to retard mineralization process or at least to improve the quality and stability of soil OM. All these expected results would guarantee beneficial effects on soil fertility in long-term.

This study is the previous study continuation described in the *Chapter 3* and was based on the comparison of biochemical parameters and production yield of the first stage of amendment use (February 2009) with the last stage and end of this research (January, 2012 and June, 2012) and include humic substances characterization of soils collected during the last years. The study was conducted in two farms located in Southern Italy with different soil types. In the farms selected for this subsequent study, different amount of a mixture having compost from municipal solid waste, as a source of easily degradable OM, and wood (scraps of poplars pruning), as a slow degradation source as amendment elaborated at different ratio C/N and doses as described in *Chapter 3*, after amendment supply time were determined enzymatic activities as (dehydrogenase, β-glucosidase, phosphatase, invertase); and main soil chemical properties (pH, electrical conductibility, CEC, organic carbon content, total nitrogen, available phosphorus) were determined to assess the effects of organic amendments on soil fertility, during the three years.

The present work research is the study accomplished full within the framework "Monitoraggio e recupero della Fertilità dei suoli in sistemi agricoli intensivi" a research project funded by CCIAA of Salerno (Italy) and Campania Region, Settore Sesirca, in collaboration with the research groups of Prof. Astolfo Zoina, Dipartimento di Agraria, Università degli Studi di Napoli Federico II, and Dr. Rosaria D'Ascoli, Dipartimento di Scienze Ambientali, Seconda Università di Napoli.

4.2 Materials and Methods

4.2.1 Study site description and selection of farms

Two intensive farms, named F1 and F2, were selected among the farms of the previous assessing soil quality study (Bonanomi et al., 2011) in the Plain of Sele River (Salerno, Southern Italy). The two selected farms, characterized by different geopedologic characteristics (F1: clay loam; F2: sandy loam), have provided a field, under greenhouse, of 160 m^2 that was divided in thirty plots, to allow the execution of the experimental design.in agricultural farms of Salerno district (Southern Italy). 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 of 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; Battipaglia meteorological station located near the study area).

On February 2009, the field plots of F1 and F2 were treated with different organic amendments and then they were cultivated. During the study, in particular, three crop cycles were performed:

- First cycle: Water melon (*Citrullus lanatus*) (Thunb.) in both farms in the Springtime (year 2009).
- Two last crop cycles of lettuce (*Lactuca sativa*) in F1 and kohlrabi (*Brassica oleracea* L. var. gongylodes) in F2, during the Wintertime and the springtime 2012. Respectively.

Soil samples were collected after one month from the amendment (24/03/2009), the Sampling VIII was collected approximately three years following the initiation (01/18/2012) and six month after Sampling IX was collected (15/06/2012).

In each plots, five sub-samples were collected following a W scheme from the topsoil (0-20 cm), then sub-samples were mixed to form only one sample per plot. Samples were packed in polyethylene bags, air dried at room temperature and sieved (< 2 mm).

4.2.2 Organic amendments

In this study two different organic fertilizers were used: (i) compost from municipal solid waste (GeSeNu Srl, Perugia, Italy), whose properties are reported in Table 3.1 (*See Chapter*) and (ii)

wood from scraps of poplars pruning (Experimental Regional Farm Improsta), characterized by a C/N ratio of 375.

Two amendments were obtained by mixing compost and wood at different two ratios:

- A1 amendment with compost: wood 10:1 and C/N ratio of 15;
- A2 amendment with compost: wood 2:1 and C/N ratio of 25.

The two amendments, A1 and A2, were supplied in two doses: 30 and 60 t ha^{-1}, named L and H, respectively.

Figure 4.1 Chronologic line of amendment and samplings into the three years of research project.

Samples collected, in each sampling, have been characterized for the main chemical properties (pH, EC, CEC, exchangeable basis and available phosphorus). Chemical properties of soils were determined by standard methods (Sparks, 1996). pH and electrical conductivity were measured in 1:2.5 soil : water suspensions and 1:5 soil : water extracts. Organic C content was performed on 1 g of pulverized soil by using a chromic acid titration method; total N was determined (on 30 mg pulverized soil) by flash combustion with a CNS Elemental Analyser (Thermo FlashEA 1112) available phosphate was measured by bicarbonate extraction; cation exchange capacity was

measured after soil treatment with a barium chloride and triethanolamine solution at pH 8.2; and exchangeable bases (Ca_2^+, Mg_2^+, K^+, Na^+) were assayed by flame atomic absorption spectrometry.

4.2.3 Soil Enzymatic activities

The activity of the following enzymes was measured: dehydrogenase, arylsulphatase, phosphatase, β-glucosidase and urease. Dehydrogenase (DHY, E.C. 1.1.) activity was measured with buffered tetrazolium salts solution, according to Trevors (1984). Phosphatase (PHO, E.C. 3.1.3.2) and β-glucosidase (GLU, E.C. 3.2.1.21) activities were determined using p-nitrophenyl sulphate (p-NPS), p-nitrophenyl phosphate (p-NPP) or p-nitrophenyl-ρ-D-glucopyranoside (p-NG) as the substrates, respectively. Specific buffers and pHs and reaction stop procedures were used as reported in Gianfreda et al. (2005). Concentrations of p-nitrophenol (p-NP) were determined at 405 nm after addition of NaOH and $CaCl_2$ for PHO and ARYL, and Tris/NaOH buffer (pH 10.0) and CaCl2 for GLU. Urease activity (UR, E.C. 3.5.1.5) was assayed as described by Kandeler and Gerber (1988) using urea as substrate. One unit of enzyme activity was defined as μmoles of product released at 30 °C h^{-1} by 1 g of dried soil. Triplicates were performed for each activity assay. One unit of enzyme activity was defined as μmoles of product released at 30 °C h^{-1} by 1 g of dried soil. Triplicates were performed for each activity assay.

4.2.4 Yield crops

The yields of melon crop were measured by summing the weight of all melons produced in each plot during the complete crop cycle. The average weights of lettuces and kohlrabies were assessed by recording the weights of 15 plants collected per plot, the values were expressed in percentage with respect the control.

4.2.5 Extraction of soil organic matter

OM was extracted from all samples collected in Sampling VIII (01/18/2012) and Sampling IX (06/15/2012). Soil (150 g) was shaken for 24 h with 750 ml of 1M NaOH - 0.1 M $Na_4P_2O_7$ (1:1 v/v) solution under N_2 atmosphere. After shaking, samples were centrifuged at 7000 rpm for 20 minutes. The supernatants were filtered on a quartz filter (Whatman GF/C), and acidified to pH 1 with concentrated HCl. The solution was centrifuged at 7000 rpm for 20 minutes, the precipitated (humic acid, HA) was separated by supernatant (fulvic acids, FA + non-humified fractions, NH). The pellet, formed by HA, was purified by a 48 h shaking with 0.1 M HCl/0.3 M HF solution (1:50 w/v). The solution was centrifuged at 7000 rpm for 20 minutes and the final residue was resuspended in deionized water, dialyzed against deionized water, frozen and lyophilized, subsequently were weighed and ground in an agate stone mortar.

The supernatans, formed by FA + NH, was pour in on small columns packed with Superlite DAX 8 resine (Supelco, USA) previously washed with 0.5 M NaOH and distilled water and then equilibrated with 0.1 M HCl.

The non-retained materials (non-humified fractions, NH) was discarded. The fractions retained on the column (Fulvic acids, FAs) was eluted with 0.5 M NaOH and collected in a beaker, and then acidified to pH 1 with concentrated HCl. The fulvic acids was dialyzed against deionized water, frozen and lyophilized, subsequently were weighed and ground in an agate stone mortar.

4.2.5.1 Elemental analyses

The elemental composition (C and N) of HAs and FAs, of the samples of HA and Fas after one year from amendment, was determined by the ash combustion procedure with a Fisons 1108 Elemental Analyzer. Calibration of the Fisons instrument with appropriate standard (acetanilide) was carried out. Accuracy (<0.05%) and recovery of C and N (for both instrument detection limit 10 mg kg^{-1}) were checked, analyzing a sample of the standard material after each set of eight sample analyses. The percentage of C and N were obtained directly from analysis. Were performed by Dr. Rosaria D'Ascoli at the Department of Environmental Sciences, Second University of Naples.

4.2.5.2 FT-IR analyses

Diffuse reflectance infrared Fourier transform spectroscopy (FTIR) spectra of HA and FA was recorded with a Perkin Elmer Spectrum-One spectrometer, equipped with a diffuse reflectance accessory (DRIFT), and by accumulating up to 100 scans with a resolution of 4 cm^{-1}. Before DRIFT analysis, dry samples was finely ground in an agate mortar while diluting with oven-dried powder (1/100, w/w) potassium bromide (KBr) as described by Adani and Spagnol (2008).

4.2.5.3 Thermogravimetrical analyses

The TG, DTG and DSC analysis were carried out simultaneously using a Simultaneous Perkin Elmer STA 6000 instrument controlled by Pyris Software, (Perkin Elmer, 2009) 30°C for 2 min, and subsequently headed from 30 to 700°C in a dynamic air atmosphere (air flow 5 L h^{-1}). The heating rate was 10°C min^{-1}, as describred by Montecchio et al. (2006).

4.2.6 Statistical analysis

The relationships among chemical and biochemical properties were assessed by using Pearson correlation coefficients, and on these results the analysis of Principal Component (PCA) was performed.

Analysis of variance (ANOVA-two way) was used by to evaluate the effect of different organic amendments in the all parameters of soil and humic substances, (ANOVA-one way with replication) was used to evaluate the effect of different organic amendments on crop yields. The significance between means with $P < 0.05$ was determined using the Duncan test. All statistical analysis were performed by SPSS (PASW Statistics 18 - IBM SPSS Statistics).

4.3 Results

4.3.1 Effect of organic amendments on soil chemical properties

For all soil properties analyzed was performed a two-way ANOVAs statistical analysis to assess the effects of organic amendment in different ratio and doses (A1, A2, L, H) (Table 4.1). As reported in Table 4.1, it was clear the effect, significantly statistically, of organic amendment on all studied parameters, for sampling VIII (18/01/2012) and IX (15/06/2012). After three years, in F1, chemical parameters showed influence of treatment at exception P_2O_5 EC, CEC and Mg exchangeable, also in F2, the treatment showed significant effect, at exception C/N ratio, Ca and Mg exchangeable.

Table 4.1 Summarized results of two-way ANOVAs for all chemical parameters analyzed in the two soils. Different ratio and doses of amendment were the independent variables. P-value from Duncan test; Significant difference=*

		F1				F2			
Source		**Sum of Squares**	**df**	**F**	**Sig.**	**Sum of Squares**	**df**	**F**	**Sig.**
pH	Trat	0.35	4.00	10.04	<0.0001*	0.57	4	10.054	<0.0001*
	Time	2.34	2.00	133.37	<0.0001*	1.49	2	52.465	<0.0001*
	Interaction	0.31	8.00	4.44	<0.0001*	1.35	8	11.879	<0.0001*
P_2O_5	Trat	850.21	4	1.393	0.260	10933.22	4	15.74168	<0.0001*
	Time	2122.45	2	6.955	0.003	10045.89	2	28.92819	<0.0001*
	Interaction	2765.25	8	2.265	0.050	3644.27	8	2.623516	0.026*
EC	Trat	8.12	4	1.018	0.414	0.88	4	22.9072	<0.0001*
	Time	3.27	2	.819	0.451	2.39	2	125.3494	<0.0001*
	Interaction	16.14	8	1.011	0.448	0.56	8	7.310577	<0.0001*
CEC	Trat	115.32	4	2.139	0.100	47.14	4	3.404462	0.021*
	Time	817.52	2	30.323	<0.0001*	538.28	2	77.75607	<0.0001*
	Interaction	176.26	8	1.634	0.157	103.89	8	3.751648	0.004*
C	Trat	430.16	4	42.51	<0.0001*	200.57	4	39.70	<0.0001*
	Time	722.98	2	142.88	<0.0001*	57.22	2	22.65	<0.0001*
	Interaction	151.50	8	7.49	<0.0001*	75.08	8	7.43	<0.0001*
N	Trat	0.00	4	2.35	0.077	0.03	4	18.10	<0.0001*
	Time	0.23	2	244.94	<0.0001*	0.37	2	434.97	<0.0001*
	Interaction	0.02	8	4.96	0.001	0.01	8	4.02	0.002*
C/N	Trat	35.51	4	10.37	<0.0001*	1.41	4	0.66	0.625
	Time	269.17	2	157.14	<0.0001*	180.48	2	168.17	<0.0001*
	Interaction	29.27	8	4.27	0.002*	10.08	8	2.35	0.043*

		F1				F2			
Source		**Sum of Squares**	**df**	**F**	**Sig.**	**Sum of Squares**	**df**	**F**	**Sig.**
K	Trat	0.60	4.00	7.406	<0.0001*	1.88	4	6.71	0.001*
	Time	12.55	2.00	307.834	<0.0001*	6.32	2	45.05	<0.0001*
	Interaction	0.15	8.00	0.943	0.497	1.14	8	2.03	0.077
Na	Trat	0.24	4.00	10.689	<0.0001*	0.85	4	8.14	<0.0001*
	Time	0.24	2.00	21.298	<0.0001*	1.81	2	34.63	<0.0001*
	Interaction	0.07	8.00	1.593	0.169	0.42	8	2.03	0.076
Ca	Trat	3.48	4.00	4.990	0.003*	3.39	4	1.79	0.158
	Time	1279.40	2.00	3665.452	<0.0001*	877.08	2	923.35	<0.0001*
	Interaction	6.10	8.00	4.366	0.001*	5.30	8	1.40	0.238
Mg	Trat	0.71	4.00	1.922	0.133	9576.02	4	1.00	0.423
	Time	98.40	2.00	536.275	<0.0001*	4419.87	2	0.92	0.408
	Interaction	1.81	8.00	2.469	0.035	19121.26	8	1.00	0.457

In the F1 and F2 soils, pH was influenced positively for the treatments and showed increment from Sampling I (03/24/2009) to Sampling VIII and IX (01/18/2012) (Table 4.2).

Table 4.2 Effect of amendment application on pH values in F1 and F2 farm soils in Sampling I (one month after first amendment), Sampling VIII and Sampling VIII (one and six months after third amendment). Different letters indicate significant difference ($P \leq 0.05$) lowercase indicate differences between treatments and uppercase indicate differences across time.

	pH F1			pH F2		
Plots	**Sampling I 03/24/2009**	**Sampling VIII 01/18/2012**	**Sampling IX 06/15/2012**	**Sampling I 03/24/2009**	**Sampling VIII 01/18/2012**	**Sampling IX 06/15/2012**
C	7.74dC	7.97bB	8.27aA	7.65bC	8.35aA	8.11aB
A1L	8.0aB	8.25aA	8.24abA	7.52bB	7.98cA	8.07aA
A2L	7.95abB	8.27aA	8.28aA	7.87a	7.87d	7.94c
A1H	7.83cB	8.28aA	8.24abA	7.87aB	8.11bA	8.09aA
A2H	7.88bC	8.19aA	8.22bA	8.04a	8.09b	8.00b

P_2O_5, EC and CEC not show significant differences in F1 (data not shown). The available phosphorus, in F2, in the first year, showed highest values respect to last sampling (Table 4.3), moreover, the Control plot show the highest values. After three years, at Sampling IX, there were d differences between Control and amended plots. The CEC values were not influenced by organic amendments in F1 (Table 4.1) and remained unchanged during the study, while in F2 soils, a general increase of CEC values in all plots until to achieve the maximum values in Sampling IX (Table 4.3), was observed. EC showed increase in the Sampling IX in F2 specially in A1H, A2L and A2H plot.

Table 4.3 Effect of amendment application on P_2O_5 (mg kg $^{-1}$), EC (dS m^{-1}) CEC (cmol $_{(+)}$ kg^{-1}) in F2 farm soils in Sampling I (I month after first amendment), Sampling VIII and Sampling VIII (one and six months after third amendment). Different letters indicate significant difference (P≤0.05) lowercase indicate differences between treatments and uppercase indicate differences across time.

	P_2O_5 (mg kg $^{-1}$) F2 Soil			EC (dS m^{-1}) F2 soil			CEC (cmol $_{(+)}$ kg^{-1}) F2 soil		
	Sampling I	Sampling VIII	Sampling IX	Sampling I	Sampling VIII	Sampling IX	Sampling I	Sampling VIII	Sampling IX
Plots	03/24/2009	01/18/2012	15/06/2012	24/03/2009	01/18/2012	06/15/2012	03/24/2009	01/18/2012	06/15/2012
C	174.71aA	122.37bB	119.05aB	0.17bB	0.09bB	0.35cA	13.55bB	24.10aA	21.39A
A1L	145.52ab	141.41a	123.60a	0.31abB	0.17abB	0.58bcC	14.24abB	17.49bB	22.58A
A1H	145.88a	127.45b	120.90a	0.41aB	0.25aB	1.23aA	15.92aB	24.34aA	23.26A
A2L	132.64bA	83.66cB	76.87bB	0.27bAB	0.10bC	0.58bcA	15.88aC	19.48abB	24.65A
A2H	123.06b	115.27b	105.48A	0.26abB	0.14bB	0.72bA	14.79abC	18.76bB	23.49A

Organic carbon content was significantly influenced by organic amendments (Table 4.1) The of percent of variation in F1 increase strongly over time (Figure 4.2), all treatment showed a significant increment in each sampling confirmed by table 4.4, but after of three years the plot A1H and A2H showed 112% and 109% Corg content respect to the control plot at Sampling IX. The percent variation in F2 of Corg (Figure 4.3), is more pronounced in F2 farm, the increment across the time is strong achieving values in A1H and A2H around 141.85 and 123.5% respectively respect to control plot.

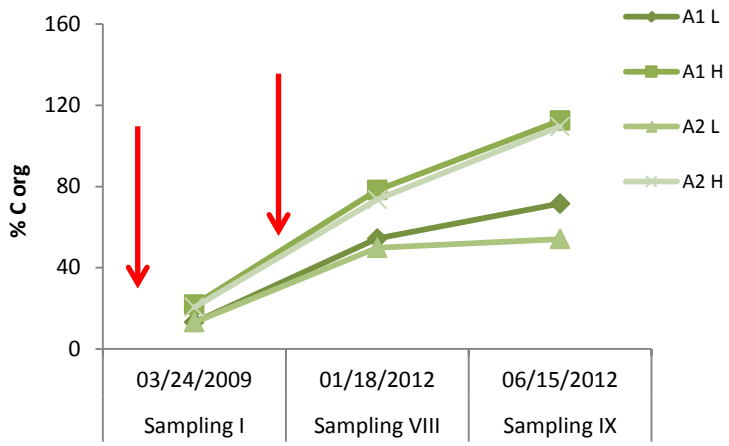

Figure 4.2 Effect of amendment application on Corg percentage in F1 farm soils in the all three sampling. The red arrow indicates the amendment application. Values are percentages compared with unamended control soils (0%, base line).

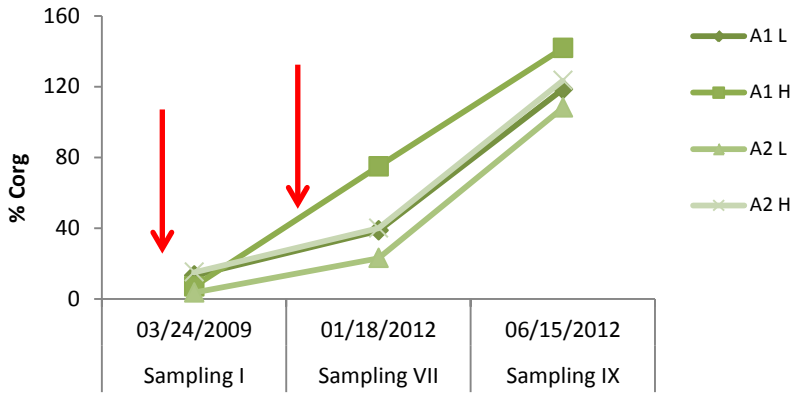

Figure 4.3 Effect of amendment application on Corg percentage in F1 farm soils in the all three sampling. The red arrow indicates the amendment application. Values are percentages compared with unamended control soils (0%, base line).

Table 4.4 Effect of amendment application on Corg (g kg^{-1}) in F1 and F2 farm soils. Different letters indicate significant difference (P≤0.05) lowercase indicate differences between treatments and uppercase indicate differences across time. Letters from Duncan test (P≤0.05).

	Corg (mg kg^{-1}) F1 soil			**Corg (mg kg^{-1}) F2 soil**		
Plots	**Sampling I 03/24/2009**	**Sampling VIII 01/18/2012**	**Sampling IX 06/15/2012**	**Sampling I 03/24/2009**	**Sampling VIII 01/18/2012**	**Sampling IX 06/15/2012**
C	10.36	11.78c	12.68c	16.02A	13.97dA	16.91dA
A1L	11.71C	18.18abB	21.75bA	18.14	19.37b	20.05bc
A1H	12.61	20.99aB	26.94aA	17.16B	24.44aA	23.98aA
A2L	11.71B	17.64bA	19.55bA	16.6	17.20c	18.29d
A2H	12.49C	20.45abB	26.58aA	18.44	19.56b	20.89b

In F1, total nitrogen content was not influenced by the organic amendment addition (Table 4.1), but by the time. The first sampling showed significant differences in the values of nitrogen content respect to others samplings but not between treatment into the each samplings (Table 4.5 and Figure 4.4). In F2, total nitrogen was influenced by treatment and the time A1H and A2H plots achieved highest percent values at Sampling IX respect to the control. The amount of nitrogen decreased with the time in both farms (Table 4.5 and Figure 4.5)

Table 4.5 Effect of amendment application on total nitrogen content (g kg^{-1}) in F1 and F2 farm soils in the three Samplings. Different letters indicate significant difference (P≤0.05) lowercase indicate differences between treatments and uppercase indicate differences across time. Letters from Duncan test (P≤0.05).

	N (g kg^{-1}) F1			N (g kg^{-1}) F2		
Plots	**Sampling I**	**Sampling VIII**	**Sampling IX**	**Sampling I**	**Sampling VIII**	**Sampling IX**
C	0.41A	0.17cC	0.24B	0.43A	0.19cB	0.19bB
A1L	0.38A	0.26aB	0.24B	0.42A	0.26abB	0.23bC
A1H	0.38A	0.26aB	0.25B	0.45A	0.28bA	0.30bA
A2L	0.39A	0.22bB	0.25B	0.42A	0.19cB	0.22bB
A2H	0.39A	0.25aB	0.26B	0.42A	0.25bB	0.27aB

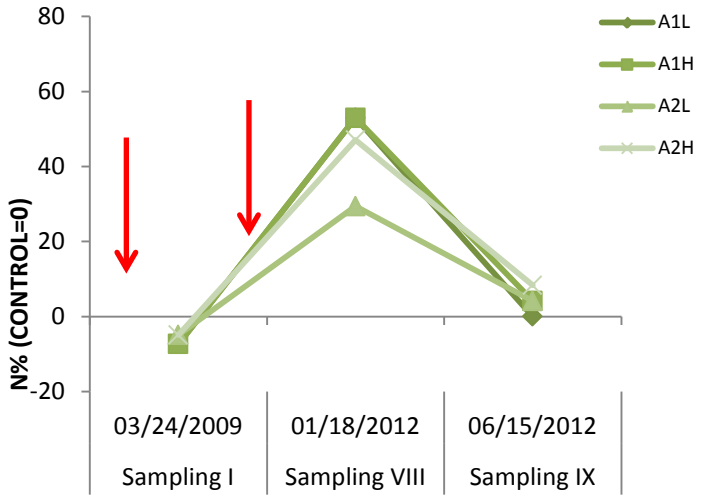

Figure 4.4 Effect of amendment application on N tot percentage in F1 farm soils in the all three sampling. The red arrow indicates the amendment application. Values are percentages compared with unamended control soils (0%, base line).

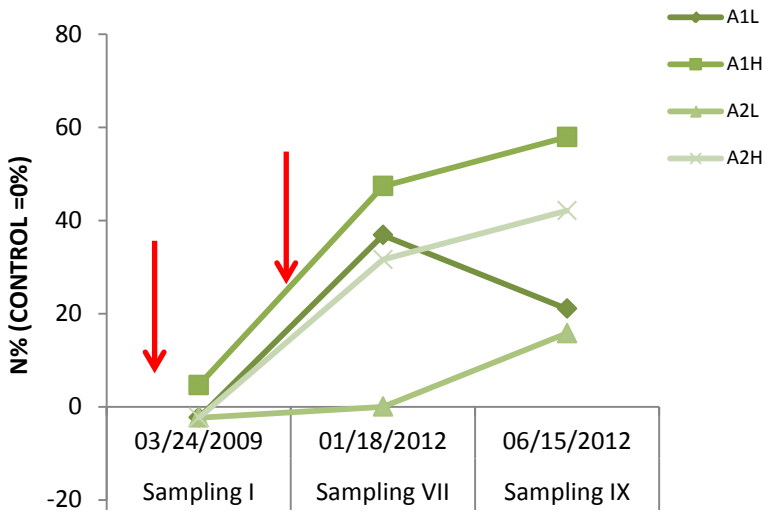

Figure 4.5 Effect of amendment application on N tot percentage in F2 farm soils in the all three sampling. The red arrow indicates the amendment application. Values are percentages compared with unamended control soils (0%, base line).

The C/N In the first sampling, all amended plots of F1 farm showed an increase of C/N ratio (Table 4.6) compared to control plot at exception to A1L in Sampling VIII. From Sampling I until to Sampling VII, the values of C/N increased strongly. From Sampling VIII until Sampling IX the increase of values of C/N were less pronounced. The highest values were achieving by A1H presenting an increasing around of 94.64% with respect to the Control Plot in the Sampling IX.

In F2 soil, having starting value of C/N ratio higher than that in F1 soil (Figure 4.7), only a slight increase was observed, in all treated plots. After the third amendment, C/N ratio showed an increase in all amended plots, over 8.

It should be remarked that in both farms the controls plots showed an increase in C/N ratio over time. Likely this is a consequence of gradual decrease in total nitrogen values (Table 4.6) due to interruption of the mineral fertilization practice of soils during the study period (except for the annual experimental addition).

Table 4.6 Effect of amendment application on C/N ratio in F1 and F2 farm soils in Sampling I (03/24/2012) until Sampling III (06/15/2012), Different letters indicate significant difference (P≤0.05) lowercase indicate differences between treatments and uppercase indicate differences across time. Letters from Duncan test (P≤0.05).

	C/N ratio F1 soils			C/N ratio F2 soils		
Plots	**Sampling I**	**Sampling VIII**	**Sampling IX**	**Sampling I**	**Sampling VIII**	**Sampling IX**
C	C	A	B	B	bA	A
A1L	C	B	abA	C	bA	A
A1H	C	B	aA	C	aA	B
A2L	B	A	bA	B	aA	A
A2H	B	A	abA	B	bA	A

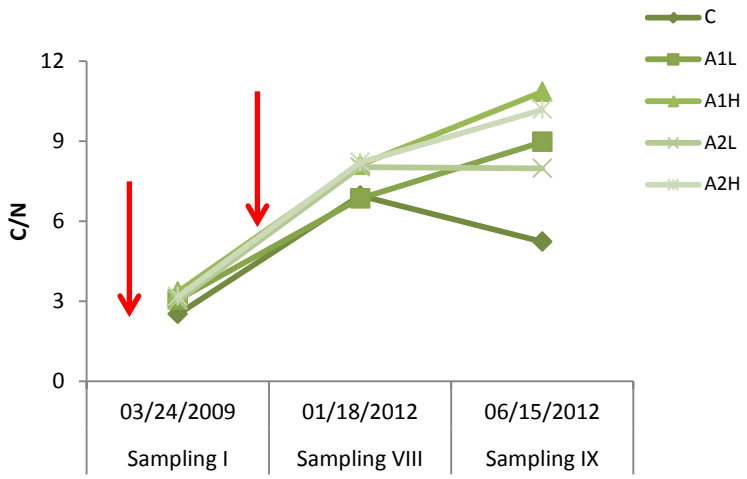

Figure 4.6 Effect of amendment application on C/N in F1 farm soils in the all three sampling the red arrow indicates the amendment application.

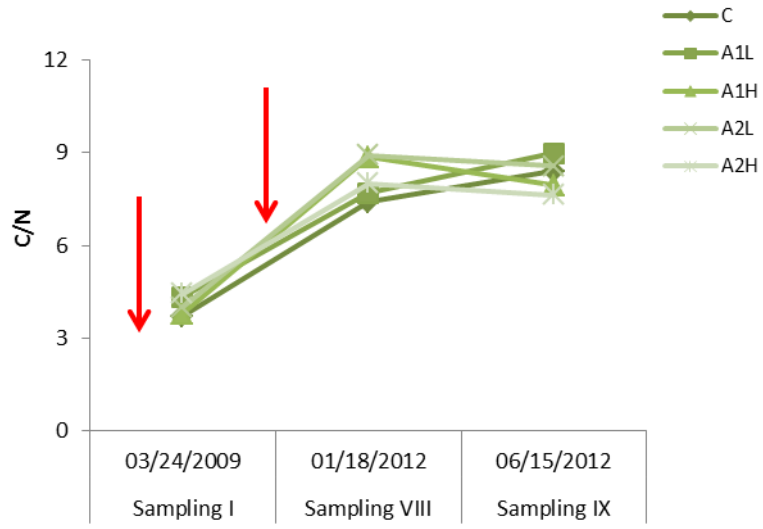

Figure 4.7 Effect of amendment application on C/N in F2 farm soils in the all three sampling the red arrow indicates the amendment application.

4.3.2 Effect of organic amendments on soil enzymatic activities

For all soil enzymatic activities analyzed was performed a two-way ANOVAs statistical analysis to assess the effects of organic amendment in different ratio and doses (A1, A2, L, H) (Table 4.7). As reported in table 4.3.2, it was clear the effect, significantly statistically, of organic amendment on enzymatic activities studied, at exception INV of F1 that not had statistical significance by the treatment and was only influenced by over time (Table 4.7).

Table 4.7 Summarized results of two-way ANOVAs for all enzymatic activities analyzed in the two soils. Different ratio and doses of amendment were the independent variables. P-value from Duncan test; Significant difference=*

		F1				F2			
Source		**Sum of Squares**	**df**	**F**	**Sig.**	**Su of Squares**	**df**	**F**	**Sig.**
DHY	Treatmentt	75,13	4	33,89	<0.0001*	180,03	4	44,20	<0.0001*
	Time	3,42	2	3,09	0,049*	38,51	2	18,91	<0.0001*
	Interaction	7,56	8	1,71	0,104	28,00	8	3,44	0,001*
PHO	Treatmentt	8,20	4	51,63	<0.0001*	14,04	4	56,85	<0.0001*
	Time	0,14	2	1,80	0,170	2,52	2	20,43	<0.0001*
	Interaction	0,59	8	1,86	0,072	3,85	8	7,79	<0.0001*
GLU	Treatmentt	0,08	2	38,97	<0.0001*	0,26	4	33,08	<0.0001*
	Time	0,04	4	9,73	<0.0001*	0,19	2	47,78	<0.0001*
	Interaction	0,00	8	0,56	0,808	0,07	8	4,23	<0.0001*
INV	Treatmentt	0.064	4	0,773	0,552	0,521	4	10,224	<0.0001*
	Time	2.135	2	51,933	<0.0001*	0,303	2	11,882	<0.0001*
	Interaction	0.769	8	4,678	<0.0001*	0,384	8	3,762	0,004*
UR	Treatmentt	8,71	4	6,54	<0.0001*	11,83	4	14,06	<0.0001*
	Time	46,59	2	69,90	<0.0001*	24,21	2	57,56	<0.0001*
	Interaction	19,36	8	7,26	<0.0001*	5,44	8	3,24	0,002*

In F1 (Figure 4.8) dehydrogenase activity (DHY) showed a trend of gradually increase, evidenced mainly by A1H plot, however all the treatments showed higher values than control. In Sampling VIII after one year of amendment addition, A1H, A2L and A2H plot showed high values and shows no statistically significant difference between them (Table 4.7) and responds to organic amendment addition in the same way, similar situation was showed in the sampling I by A1H and A2H plot, the A2H plot showed high values soil dehydrogenase activity in the three sampling, suggesting that the microbial activity remains constant over time.

In F2, as happened in F1, (Figure 4.9) dehydrogenase activity responded positively to amendment addition in the treatments, but this enzyme is higher in Sampling VIII than Sampling I and Sampling IX which A1H plot showed a significant highest values of enzymatic activity (Table 4.8), in the three samplings highest values by A1H and A2H were showed. The values of dehydrogenase activity showed a trend to decrease of values over time, but the plot with highest values was A1H at the Sampling VIII.

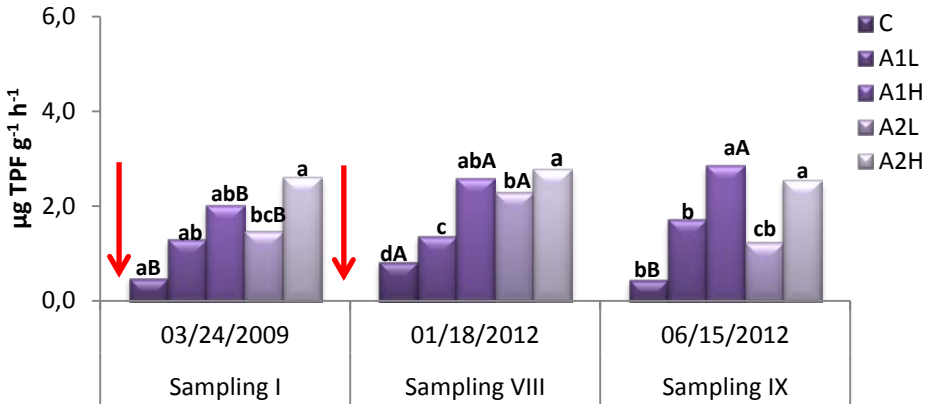

Figure 4.8 Effect of amendment application on Dehydrogenase activity (µg TPF g^{-1} h^{-1}) in F1farm soils in the all three sampling the red arrow indicates the amendment application.

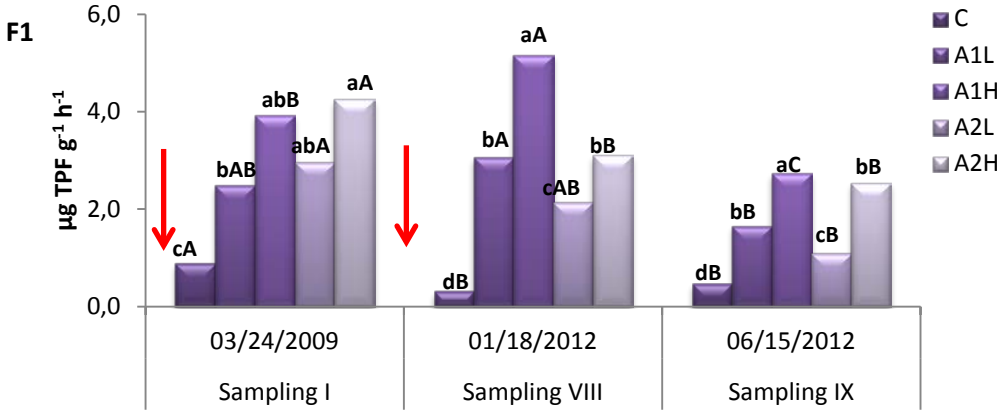

Figure 4.9 Effect of amendment application on Dehydrogenase activity (µg TPF g^{-1} h^{-1}) in farm soils in the all three sampling. The red arrow indicates the amendment application.

Table 4.8 Effect of amendment application on Dehydrogenase activity (µg TPF g^{-1} h^{-1}) in F1 and F2 farm soils in Sampling, Sampling VIII and Sampling IX. Different letters indicate significant difference (P≤0.05) lowercase indicate differences between treatments and uppercase indicate differences across time. Letters from Duncan test (P≤0.05).

	DHY (µg TPF g^{-1} h^{-1})					
	F1			F2		
PLOTS	**Sampling I**	**Sampling VIII**	**Sampling IX**	**Sampling I**	**Sampling VIII**	**Sampling IX**
C	aB	dA	dB	cA	dB	dB
A1L	ab	c	b	AB	bA	bB
A1H	abB	abA	aA	abB	aA	aC
A2L	bcB	bA	cb	abA	AB	cB
A2H	a	a	a	aA	bB	bB

The PHO in F1 activity showed a similar behavior in all sampling (Figures 4.10), the treatment not show great changes in the time, but showed significant highest values respect to control plot, mainly A1H and A2H (Table 4.9).

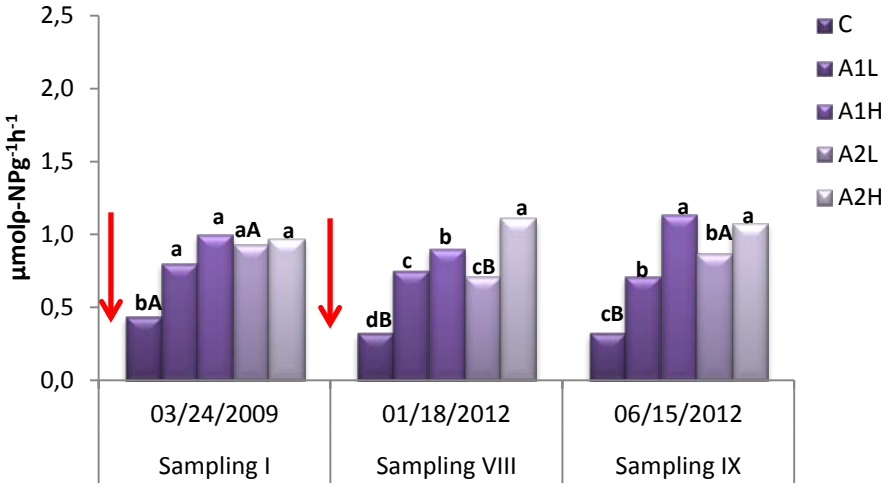

Figure 4.10 Effect of amendment application on Phosphatase activity (µmol ρ-NPg^{-1}h^{-1}) in F1 farm soils in the all three sampling. The red arrow indicates the amendment application.

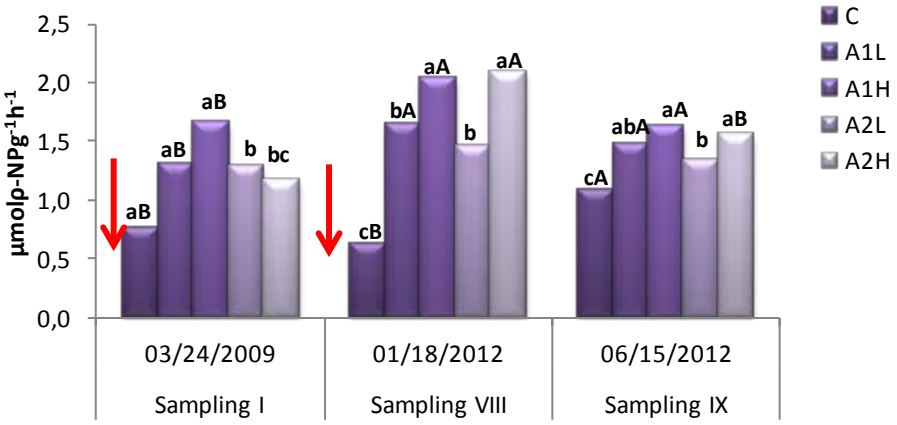

Figure 4.11 Effect of amendment application on Phosphatase activity in F2 (µmol ρ-NPg^{-1}h^{-1}) farm soils in the all three sampling. The red arrow indicates the amendment application.

In F2 the treatment and the time had significant effect of organic amendment addition on the PHO (Figure 4.11) , all treatments the PHO level was highest at Sampling VIII, the control plot in Sampling IX showed highest values in comparison with the previous Sampling. The plot with highest values were, as in F1, A1H and A2H plot.

Table 4.9 Effect of amendment application on Phosphatase activity (µmol ρ-NPg^{-1}h^{-1}) in F1 and F2 farm soils in Sampling I, Sampling VIII and Sampling IX. Different letters indicate significant difference (P≤0.05) lowercase indicate differences between treatments and uppercase indicate differences across time. Letters from Duncan test (P≤0.05).

	PHO (µmolρ-NPg^{-1}h^{-1})					
	F1			F2		
PLOTS	Sampling I	Sampling VIII	Sampling IX	Sampling I	Sampling VIII	Sampling IX
	03/24/2009	01/18/2012	06/15/2012	03/24/2009	01/18/2012	06/15/2012
C	bA	dB	dB	aB	cB	cA
A1L	a	c	b	aB	bA	abA
A1H	a	b	a	aB	aA	aA
A2L	aA	cB	bA	b	b	b
A2H	a	a	a	bC	aA	aB

The organic amendment addition had effects on β-glucosidase activity in F1 and F2 (Table 4.10), all treatment showed values highest with respect to control plot (Figures 4.12 and Figures 4.13), however, not there are differences between the treatment of different mixture and dose, in F1 the sampling I has similar behavior with Sampling IX.

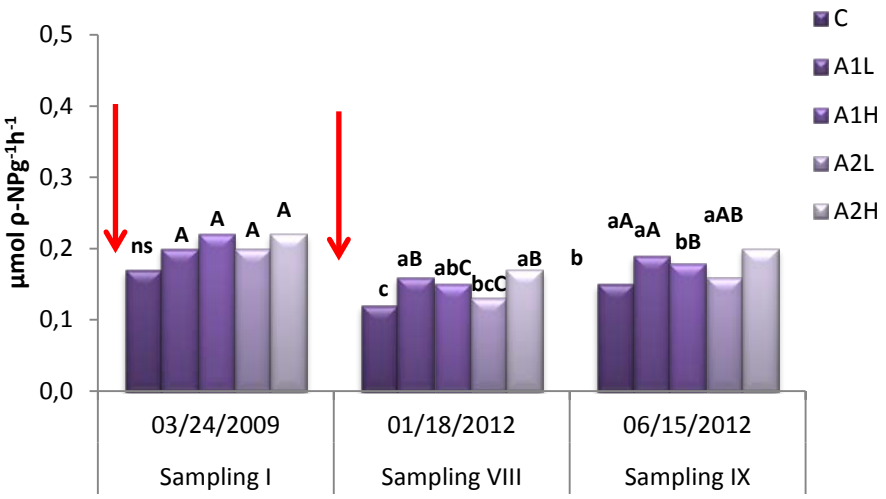

Figure 4.12 Effect of amendment application on β - glucosidase activity (µmol ρ-NPg^{-1}h^{-1}) in F1 farm soils in the all three sampling. The red arrow indicates the amendment application.

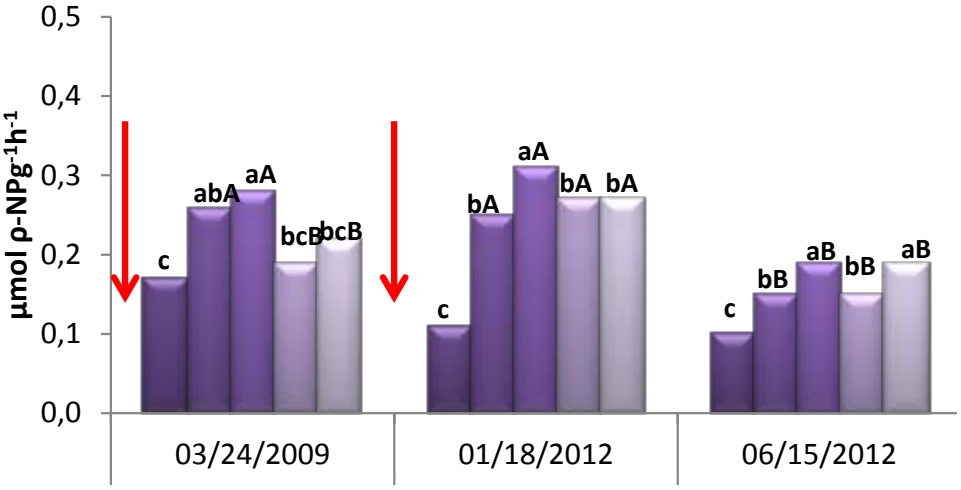

Figure 4.13 Effect of amendment application on β - glucosidase activity (µmol *p*-NPg^{-1}h^{-1}) in F2 farm soils in the all three sampling the red arrow indicates the amendment application.

In F2 the values of GLU were higher after the organic amendment addition, the highest values were obtained in A1H plot at Sampling I, but three years after the two follow Sampling A1H and A2H not present significant differences, maintain relatively constant level of activity.

Table 4.10 Effect of amendment application on β - Glucosidase activity (µmol ρ-NPg^{-1}h^{-}1) in F1 and F2 farm soils in Sampling I, Sampling VIII and Sampling IX. Different letters indicate significant difference (P≤0.05) lowercase indicate differences between treatments and uppercase indicate differences across time. Letters from Duncan test (P≤0.05).

	GLU (µmol ρ-NPg^{-1}h^{-1})					
	F1			**F2**		
PLOTS	**Sampling I**	**Sampling VIII**	**Sampling IX**	**Sampling I**	**Sampling VIII**	**Sampling IX**
C	Ns	c	b	c	c	c
A1L	A	aB	A	abA	bA	bB
A1H	A	abC	aB	aA	aA	aB
A2L	A	bcC	bB	bcB	bA	bB
A2H	A	aB	aAB	bcB	bA	aB

ANOVA two way analysis (Table 4.8) not showed significant effect of organic amendment addition on the Invertase activity in F1, information confirmed by Table 4.11, which the statistical differences were given by the time. In F2 (Figure 4.14) the differences were expressed in the Sampling VIII which A1H and A2L plot revealed the highest values for this enzyme, being stimulated by amendment addition.

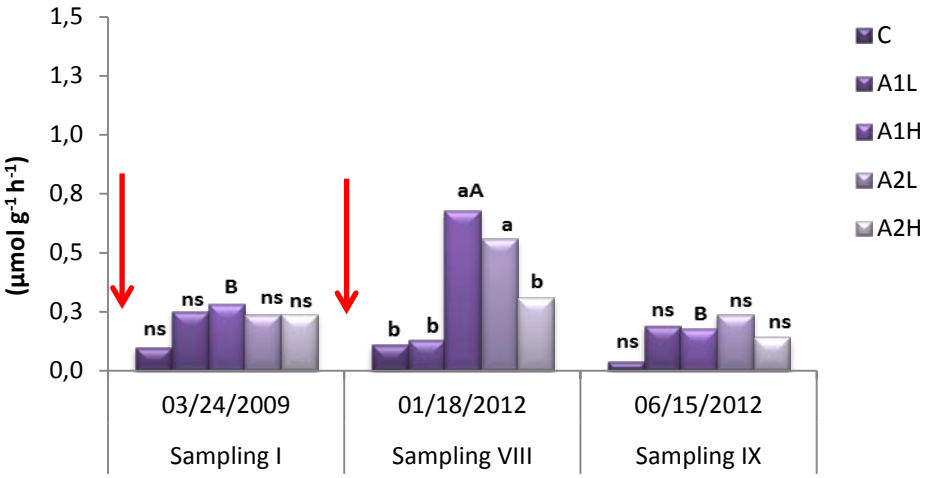

Figure 4.14 Effect of amendment application on Invertase activity INV (µmol g^{-1} h^{-1}) in F2 farm soils in the all three sampling. The red arrow indicates the amendment application.

Table 4.11 Effect of amendment application on Invertase activity (µmol g^{-1} h^{-1}) in F1 and F2 farm soils in Sampling I, Sampling VIII and Sampling IX. Different letters indicate significant difference (P≤0.05) lowercase indicate differences between treatments and uppercase indicate differences across time. Letters from Duncan test (P≤0.05).

	INV (µmol g-1 h-1)					
	F1			**F2**		
PLOTS	**Sampling I**	**Sampling VIII**	**Sampling IX**	**Sampling I**	**Sampling VIII**	**Sampling IX**
C	0.23B	0.73A	0.85aA	Ns	b	Ns
A1L	0.24B	0.70A	0.65abA	Ns	b	Ns
A1H	0.12C	1.08A	0.74abB	B	aA	B
A2L	0.48	0.8	0.46d	Ns	a	Ns
A2H	0.44	0.81	0.54cd	Ns	b	Ns

The Urease activity was influenced positively for admendment addition (table 4.12), precisally after of amendment application this enzymatic activity showed an increment in F1 and F2 farm (Figures 4.15 and 4.16), this high was not maintained in Sampling IX, but the decline of this activity was lees in Sampling IX of F2 farm, the treatments with highest values in this activity were A1L and A1H in F1, and A2H, A1H and A2L in F2.

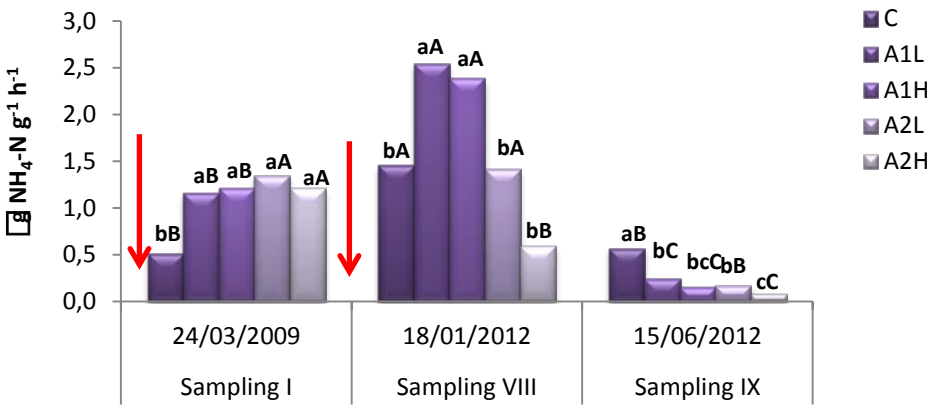

Figure 4.15 Effect of amendment application on Urease activity (µg NH4-N g^{-1} h^{-1}) in F1 farm soils in the all three sampling the red arrow indicates the amendment application.

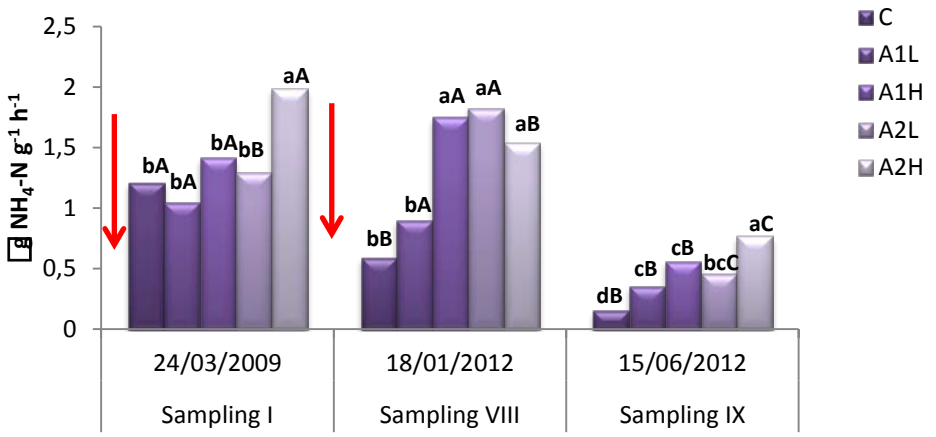

Figure 4.16 Effect of amendment application on Urease activity (µg NH4-N g^{-1} h^{-1}) in F2 farm soils in the all three sampling the red arrow indicates the amendment application.

Table 4.12 Effect of amendment application on Urease activity (µg NH4-N g^{-1} h^{-1}) in F1 and F2 farm soils in Sampling I, Sampling VIII and Sampling IX. Different letters indicate significant difference (P≤0.05) lowercase indicate differences between treatments and uppercase indicate differences across time. Letters from Duncan test (P≤0.05).

	UR (µg NH4-N g^{-1} h^{-1})					
	F1			**F2**		
PLOTS	**Sampling I**	**Sampling VIII**	**Sampling IX**	**Sampling I**	**Sampling VIII**	**Sampling IX**
C	bB	bA	aB	bA	bB	dB
A1L	aB	aA	bC	bA	bA	cB
A1H	aB	aA	bcC	bA	aA	bB
A2L	aA	bA	bB	bB	aA	bcC
A2H	aA	bB	cC	aA	aB	aC

4.3.3 Multivariate analysis of chemical and biochemical properties

Differences among the control (unamended soils) and the amended plots, in both farms, were determined by PCA considering all chemical and biochemical properties determined in this study in the three sampling, in F1 were excludes P_2O_5, EC, CEC, N, Mg^{+2} and Invertase which not showed effects of organic amendments (Table 4.7) and mineral fertilizer. In F2 C/N ,Mg^{+2} and Ca^{+2} were excludes.

The main purpose of PCA was to reduce variables and to identify more readily explainable derived factors (principal components), best elucidating data variation (Jongman et al., 1995). The factorial map of the principal component analysis (Figure 4.13 A) of F1 soil, accounting for 82.58% of the variation in the data, showed two distinct clusters of variables. The first (PC 1, 51.24%) was positively correlated with C org, C/N ratio, pH, Na exchangeable, DHY and negatively with Ca^{+} and K^{+} exchangeable. The second (PC 2, 31.35%) was correlated positively with GLU, PHO activities, and was opposed to UR activity. The score plot indicated that samples could be divided in three clusters (Figure 4.13 A1). In the first cluster, named A, the control plot samples (no amended) a second cluster named B with all amendment samples of the Sampling I (03/24/ 2009), and the third cluster represented only amended samples of the Sampling VIII (01/18/2012) and Sampling IX (06/15/2012). Within each cluster, control samples were always distinct from treated samples, indicating that important changes in the analyzed soil properties upon amendments occurred.

The PCA of F2 soils showed that the first and second clusters of variables explained 67.0% of the total variance (Figure 4.14 A). The first one (PC 1, 38.15%) was positively correlated with GLU, DHY, PHO, INV and UR activities and Organic carbon, whereas the second one (PC 2, 32.9%) was positively correlated with N tot, K exchangeable, Na exchangeable, P_2O_5 and electrical conductivity (EC), and negatively correlated with cation exchange capacity (CEC) and pH.

F2 score plot showed also three clusters (Figure 4.14 A1). The cluster named A represented control plot of Sampling I, VIII and IX, cluster named B the amended plot collected during the Sampling I and cluster named C correspond to amended plot of Sampling IX. As occur in F1 soil, also in this farm control samples were always distinct from treated samples, however the Sampling I in F2 showed some chemical parameters highest including control in the case of P_2O_5. Moreover, these samples showed high values of EC. The second addition of OM, determined an increase of electrical conductivity, due to the nature of compost utilized. The compost product by municipal solid waste is characterized by an high salinity (Zhang et al., 2006), but in our case the values of EC decrease over time, until values of control soils, as showed by the cluster C.

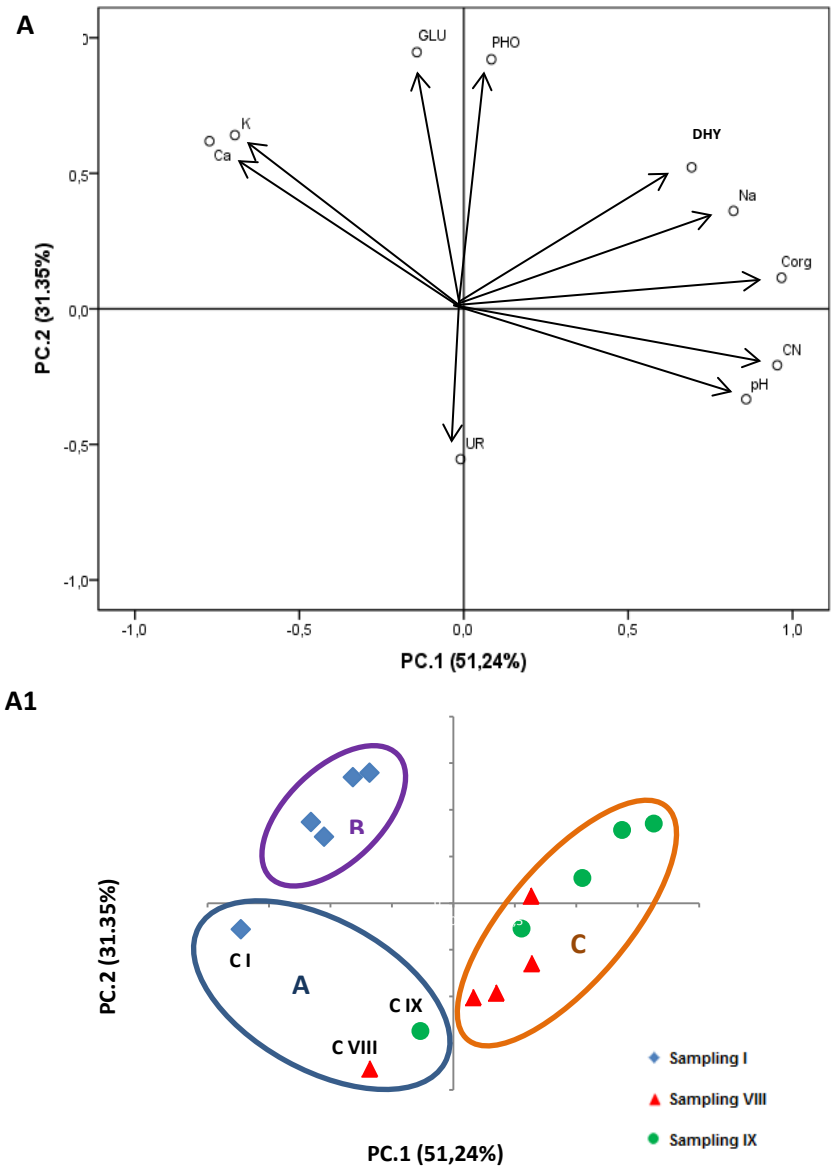

Figure 4.13 Principal Component Analysis (PCA) of analyzed soil properties of F1 soil (A), and Score Cluster (A1) of the treatments of the Samplings (I, VIII and IX).

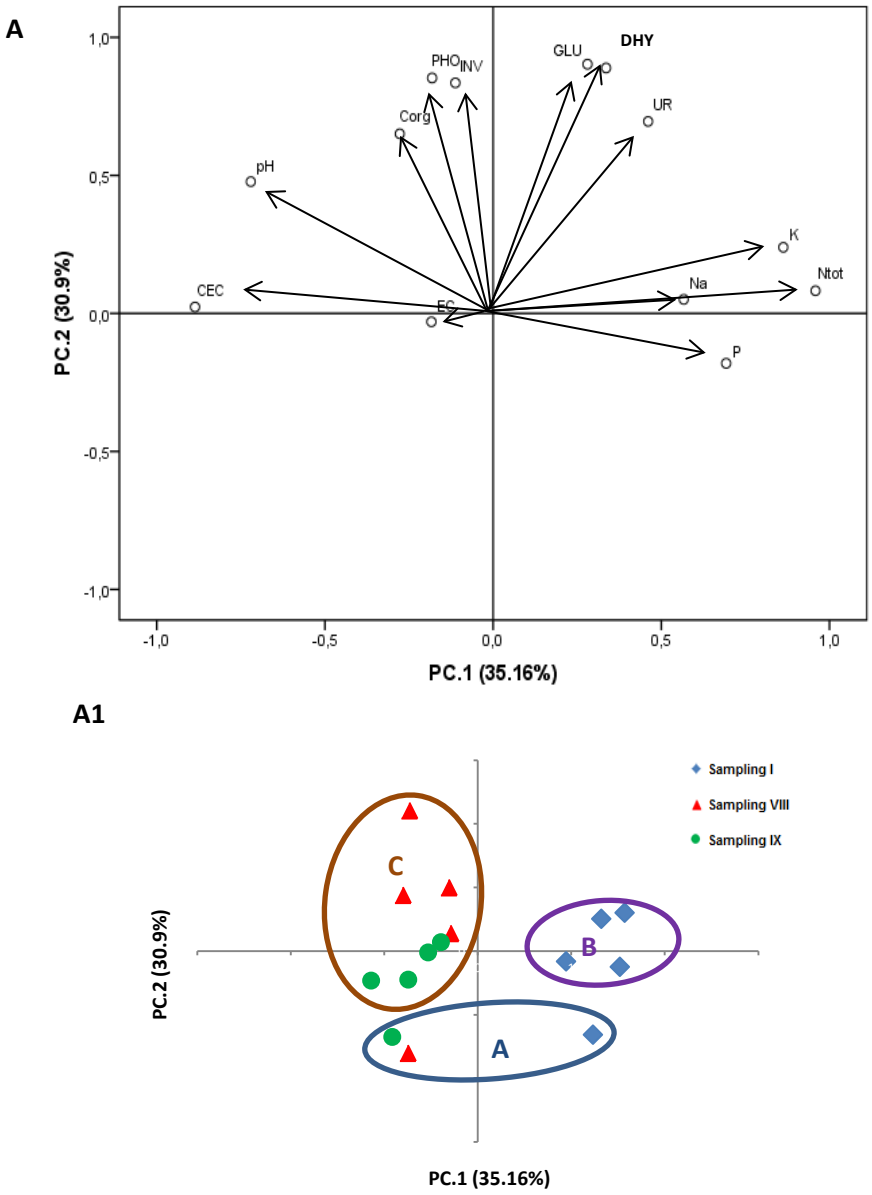

Figure 4.14 Principal Component Analysis (PCA) of analyzed soil properties of F2 soils (A), and Score Cluster (A1) of the treatments of the Samplings (I, VIII and IX).

4.3.4 Yield crop

Figure 4.15 and 4.16 show the crop productions percentage with respect to the control obtained in F1(A) and F2 (B) farms. During the first crop cycle (year 2009) a significantly difference among soils under organic amendment of water melon production was observed (data not showed). All treated plots produced lower melon yields than in both farm F1 and F2 with respect to Control plot. A2H was presented the lower values with a crop production around 33% less than control.

Crop production of lettuce was significantly enhanced by organic amendments in the seventh crop cycles (year 2012) in F1 and F2, specially A1H plot which showed in F1 32.8% and 68.6% in F2.

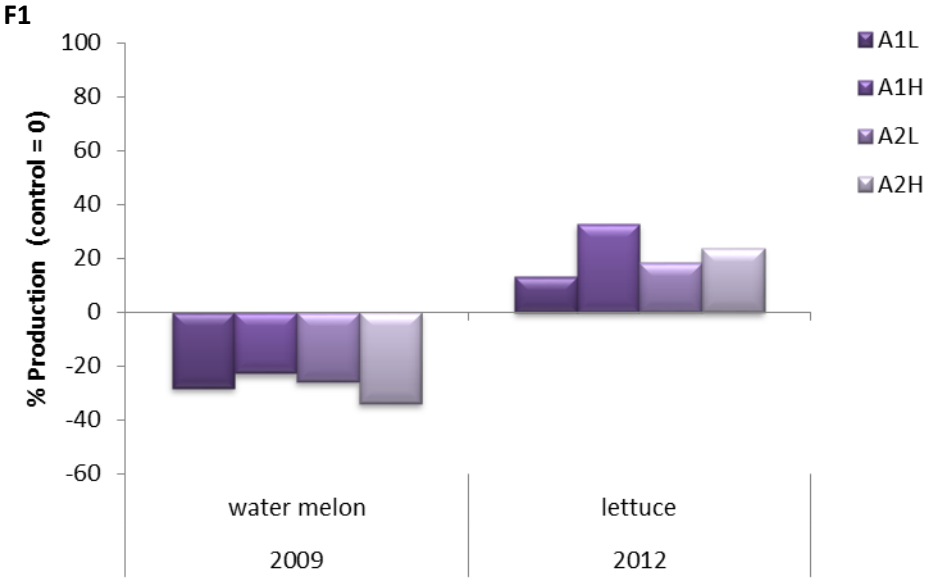

Figure 4.17 Effect of amendment application on yield crop percentage in the first year and second year in F1farm soil. Values are percentages compared with unamended control soils (0%, base line).

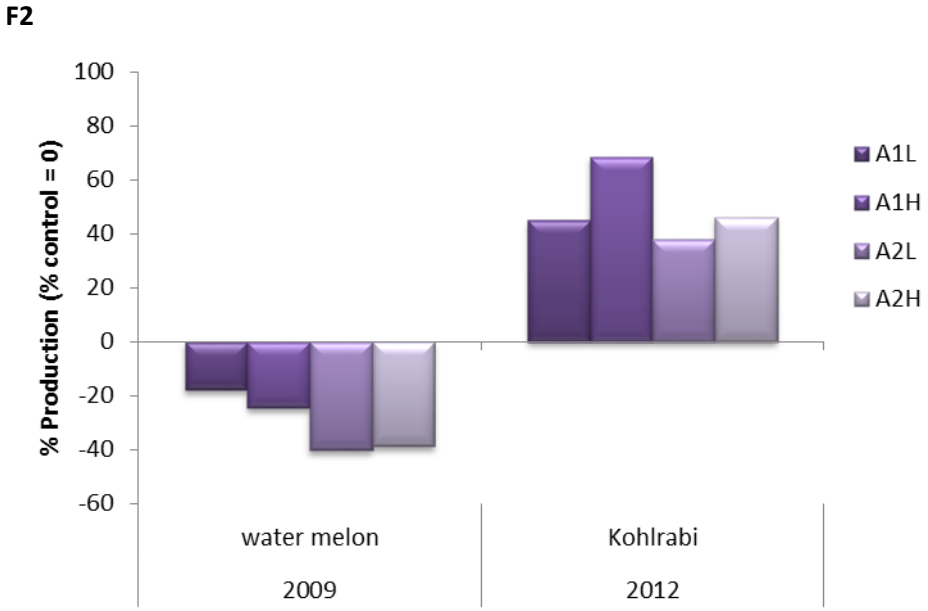

Figure 4.18 Effect of amendment application on yield crop percentage in the first year and second year in F2 farm soil. Values are percentages compared with unamended control soils (0%, base line).

4.3.5 Yields and elemental content of organic fractions in amended soils

Organic fractions were extracted from the soils in Sampling VIII and Sampling IX, at the end of the experimental session, one month and six month after the third organic amendment. HA and FA yields of extraction from soils F1 and F2 soils are reported in Table 4.17. In general, the Humic subtances (Has) extracted, in the amended soils, were higher with respect to the control in F1, around 81% and F2 around 87%. In both organic fractions an increment was observed as a consequence of the organic amendment, with the exception of Fulvic Acid in Sampling IX for both soil, which decrease with respect to the control. However, Sampling VIII and Sampling IX showed

some plot in F1 with absolute values of HAs extraction highest respect to F2, as A1H and A2H particularly in HA. The HA percentage extraction in Sampling VIII with respect to the control was higher in F2 soil, in contrast in Sampling IX the HA percentage extraction with respect to control was higher in F1, the FA percentage extraction was higher in F1 soil in Sampling VIII but in Sampling IX both soil farms showed values lowest respect to the control.

According to ANOVAs Two way analysis (data not show) in both sampling and farms, HA were not influenced by the treatment and samplings, however, were influenced by different farm soils given by the geopedologic differences between the two soils. FA not were influenced by treatment, but were influenced by farms and samplings time.

The Elemental content in HA not was influenced by the treatments, A2H plot show slightly highest values with respect to control plot (Table 4.18) in HA fraction, however, in F2 soil in the Sampling IX, the C% and N% showed increase of values. FA fraction showed higher values of C% in Sampling IX in F1 (Table 4.19) and a slightly increase of this element in some plot in F2 in Sampling IX, as A1L, A1H and A2H.

Table 4.17 Extraction yields (g kg^{-1} of soil), and % respect to Control, of HAs and FAs from soils of F1 and F2 farms, collected after one (Sampling VIII) and six months (Sampling IX) of third amendment.

Yield Humic Substances Extraction								
	F1							
	HA				**FA**			
PLOTS	**Sampling VIII**		**Sampling IX**		**Sampling VIII**		**Sampling IX**	
	g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}	%
C	2.11	0	2.08	0	1.42	0	2.47	0
A1L	2.81	33.12	4.12	97.76	1.73	21.6	2.09	-15.41
A1H	6.5	207.57	7.06	239.01	1.7	19.72	1.65	-32.97
A2L	4.45	110.41	3.02	45.29	1.39	-2.35	3.08	24.86
A2H	6.71	217.67	8.14	291.03	2.58	81.69	1.87	-24.32

	F2							
	HA				**FA**			
PLOTS	**Sampling VIII**		**Sampling IX**		**Sampling VIII**		**Sampling IX**	
	g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}	%
C	1.27	0	1.33	0	1.49	0	0.99	0
A1L	4.31	240	2.93	119.75	2.17	45.09	0.51	-48.32
A1H	5.17	307.89	4.37	227.75	1.76	17.86	0.77	-22.82
A2L	2.22	75.26	2.8	110	1.47	-1.79	0.87	-12.75
A2H	2.89	127.89	3.44	158	1.87	25.45	0.47	-52.35

Table 4.18 Elemental analyses of Humic acid fraction of soils treated with different amendments after one month (Sampling VIII) and six month (Sampling IX) of third amendment.

HUMIC ACID ELEMENTAL ANALYSIS												
	Sampling VIII 01/18/2012						Sampling IX 06/15/2012					
	SOIL F1			SOIL F2			SOIL F1			SOIL F2		
	Mass% of Humic acids		Atomic ratio	Mass% of Humic acids		Atomic ratio	Mass% of Humic acids		atomic ratio	Mass% of Humic acids		Atomic ratio
	N%	C%	C/N	N%	C%	C/N	N%	C%	C/N	N%	C%	C/N
C	4.98	50.01	11.71	5.37	48.48	10.54	4.71	48.37	11.96	3.76	33.31	10.29
A1L	6.48	50.96	9.24	5.76	48.37	9.79	5.39	49.00	10.61	4.22	37.12	10.33
A1H	6.90	47.02	8.34	8.55	47.33	7.27	5.49	47.81	10.18	5.63	48.63	10.07
A2L	5.54	49.59	10.44	4.11	47.13	14.92	5.49	50.05	10.63	5.85	49.69	9.90
A2H	5.41	48.82	10.52	5.69	49.54	10.15	5.50	48.85	10.36	5.63	49.74	10.31

Table 4.19 Elemental analyses of HAs fraction of soils treated with different amendments after one month (Sampling VIII) and six month (Sampling IX) of third amendment.

FULVIC ACID ELEMENTAL ANALYSIS												
	Sampling VIII 01/18/2012						Sampling IX 06/15/2012					
	SOIL F1			SOIL F2			SOIL F1			SOIL F2		
	Mass% of Humic acids		Atomic ratio	Mass% of Humic acids		Atomic ratio	Mass% of Humic acids		Atomic ratio	Mass% of Humic acids		Atomic ratio
	N%	C%	C/N	N%	C%	C/N	N%	C%	C/N	N%	C%	C/N
C	4.07	33.35	9.60	5.16	35.48	8.02	1.53	13.39	10.19	5.02	36.49	8.49
A1L	3.74	30.51	9.52	3.53	26.68	8.82	2.18	17.50	9.38	5.28	38.50	8.51
A1H	4.20	32.25	8.95	6.85	52.62	8.96	2.37	19.61	9.63	5.21	38.62	8.64
A2L	3.79	31.93	9.83	4.99	37.32	8.72	2.92	15.55	6.21	5.17	37.05	8.36
A2H	2.96	24.36	9.59	2.93	23.87	9.50	2.43	20.07	9.65	5.35	36.84	8.03

4.3.6 Infrared Spectroscopy (DRIFT)

The DRIFT spectra of HAs extracted from soils sampling VIII and IX a are shown in Figures 4.20 , 4.21, 4.22 and 4.23,

The main absorption bands and corresponding assignments are summarized in Table 4.19**.**

All the spectra feature common and distinctive absorption bands, which slightly differed in their relative intensity. The peak at 3300 cm-1 is characteristic of N-H stretching of several functional groups and that at 2920-2850 cm^{-1} is characteristic of asymmetric and symmetric C-H stretching of CH_2 in long alkyl chains of lipid compounds (Silverstein et al., 2005), in HAs Sampling VIII and Sampling IX in both farm soil, on the shoulder of the broad O-H stretching vibration band (3700-2600 cm^{-1}) were observed. The CH_2 band is stable in intensity and in frequency for a given concentration regardless of the chemical environment of the CH_2 group (Tremblay, 2002), this signal were keeping relatively constant in the HA in both sampling and farms.

Table 4.19 Main IR absorption bands and assignments for analyzed HAs.

Wavenumber (cm^{-1})	**Assignment**
3300	N-H stretching
2920-2850	Aliphatic asymmetric and symmetric C-H stretching
1716	C=O stretching of -COOH
1657	C=O stretching of amide I groups
1540	N-H deformation and C=N stretching of amide II groups
1450	C-H asymmetric banding of $-CH_3$ groups
1420	O-H deformation and C-O stretching of phenolic groups
1250	C-O stretching and -OH deformation of -COOH
1030-1070	C-O stretching of polysaccharides or polysaccharides-like substances

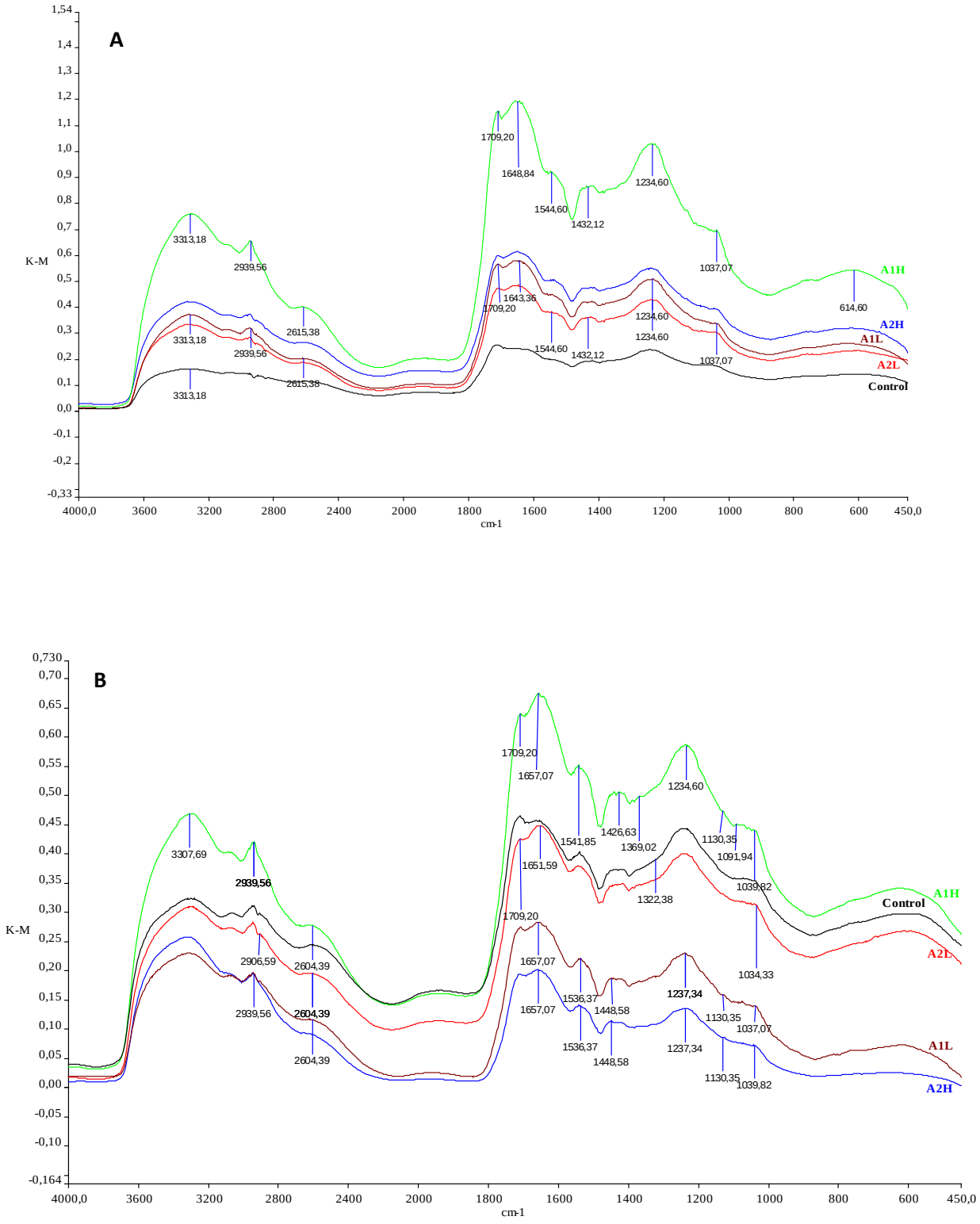

Figure 4.20 DRIFT spectra of Humic Acid in Sampling VIII, F1 farm soil (A) F2 farm soil (B).

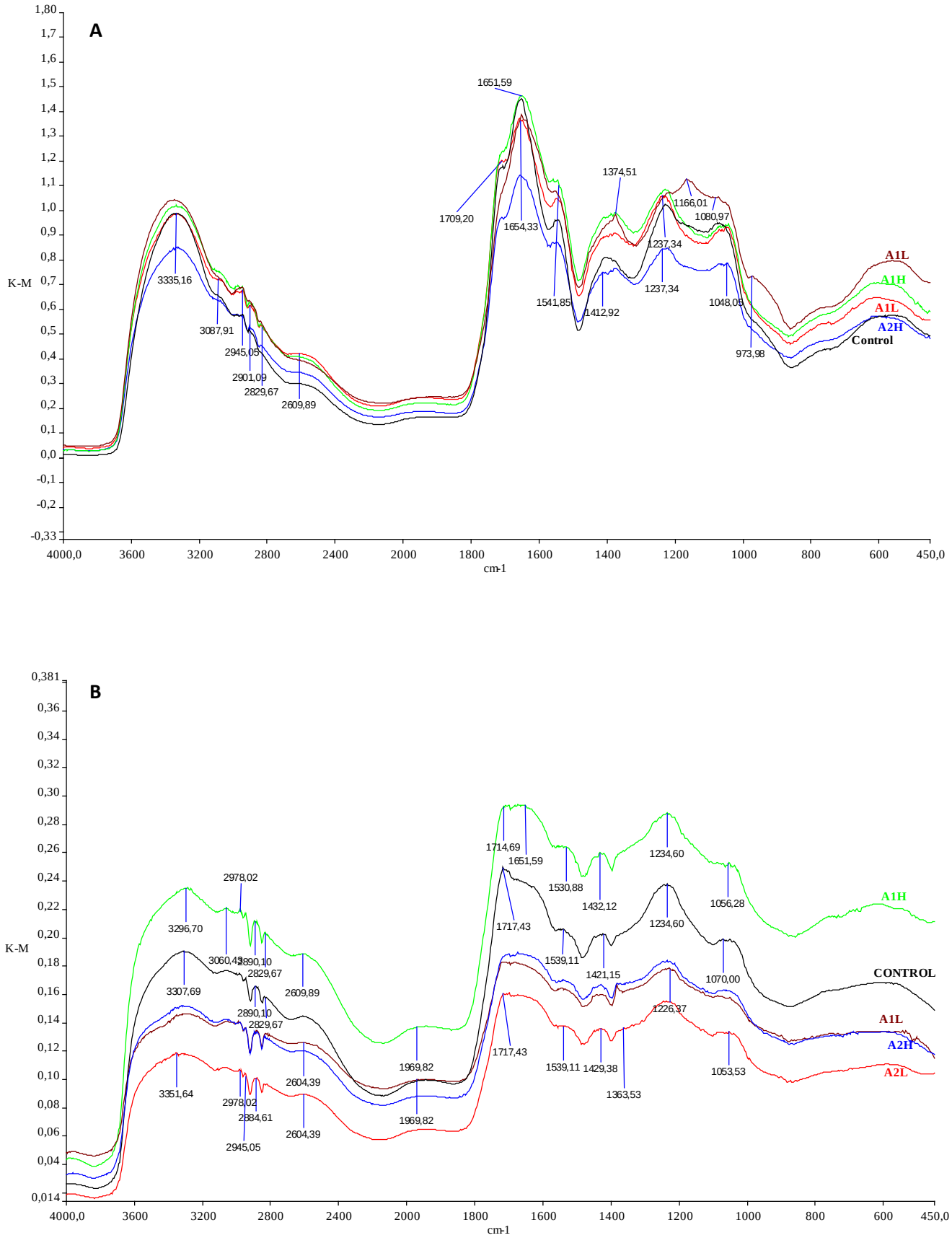

Figure 4.21 DRIFT spectra of Humic Acid in Sampling IX, F1 farm soil (A) F2 farm soil (B).

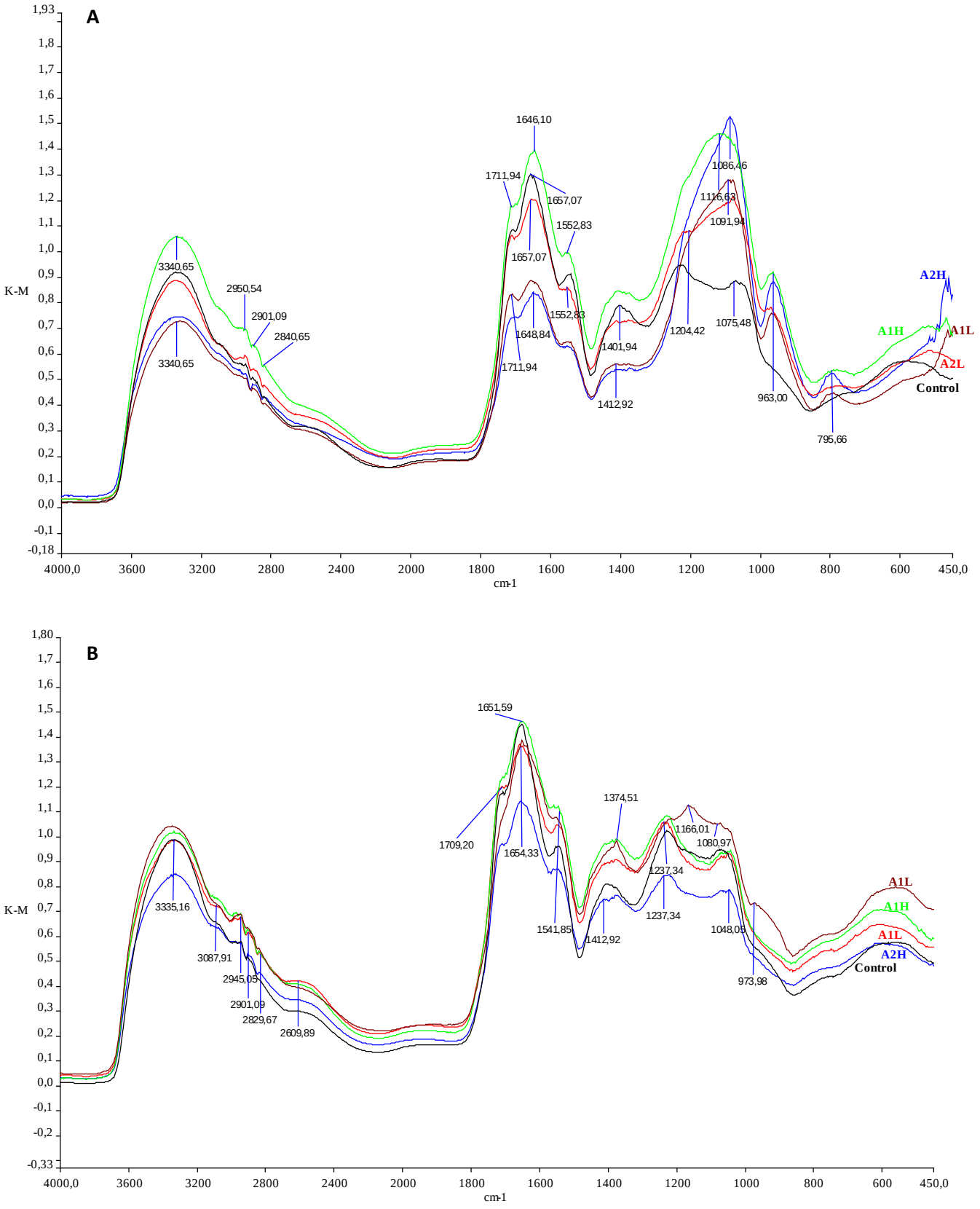

Figure 4.22 DRIFT spectra of Fulvic acid Acid in Sampling VIII, F1 farm soil (A) F2 farm soil (B).

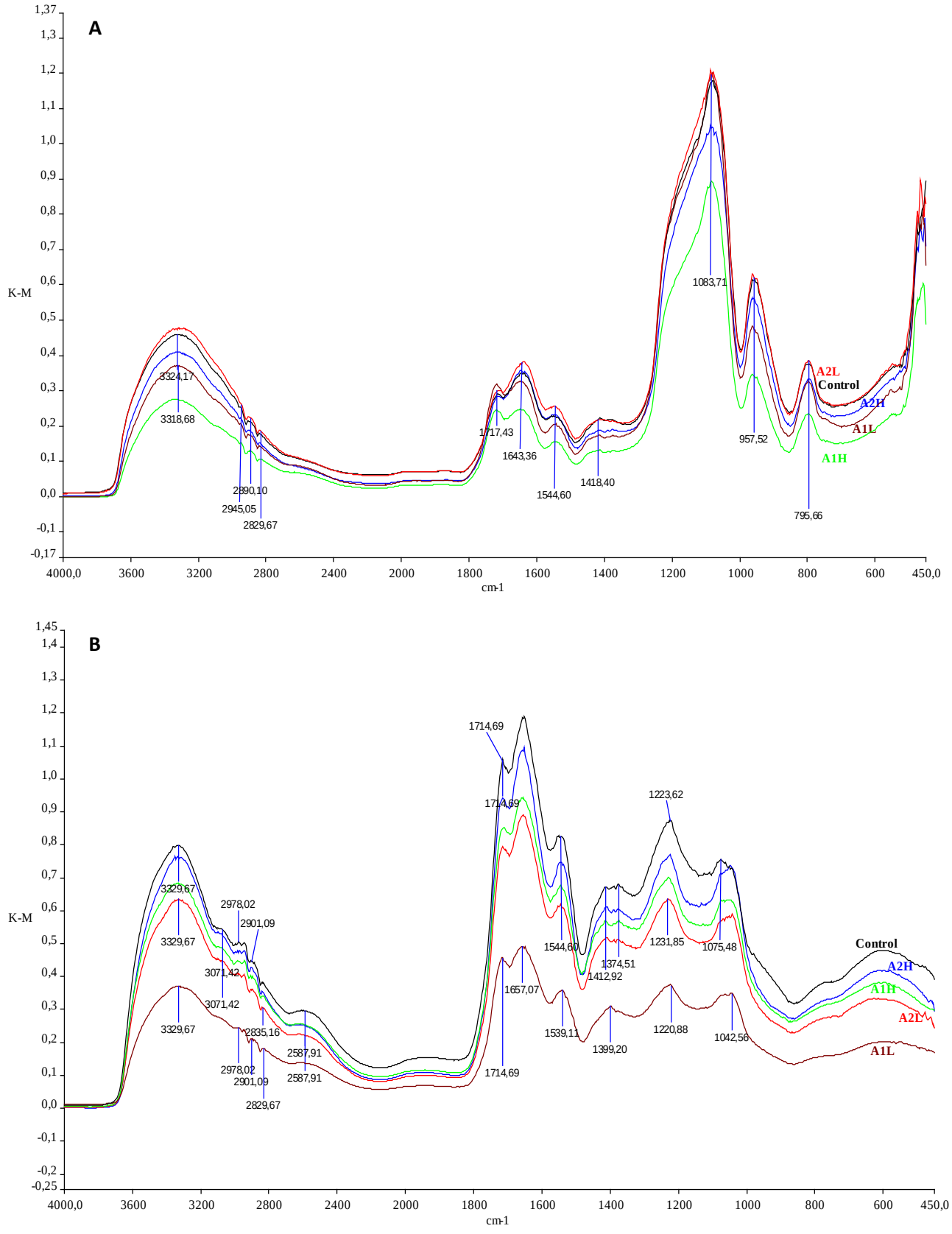

Figure 4.23 DRIFT spectra of Fulvic Acid in Sampling IX , F1 farm soil (A) F2 farm soil (B).

The signal at 1709 cm^{-1} and 1240 cm^{-1} may be assigned to the C=O and C-O bonds, respectively, in protonated carboxylic groups of alkyl chains in fatty acids (Silverstein et al., 2005). Those at 1657 cm-1 and 1540 cm-1 are, respectively, identified how the C=O stretching of amide I groups, and N-H deformation and C=N stretching of amide II groups, respectively (Silverstein et al., 2005). The signal at 1450 cm^{-1} is characteristic of CH_3 bending vibrations ever in long alkyl chains of lipid compounds (Droussi et al., 2009 ; Spaccini and Piccolo, 2008), and that at 1420 cm^{-1} is typical of O-H deformation and C-O stretching of phenolic groups, such as lignin and their derivates (Droussi et al., 2009). A common broad band centred around 1234 cm^{-1} is generally ascribed to C-O stretching and O-H deformation of carboxyls and C-O stretching of aryl ethers (Ferrari et al, 2011). The band in 1160 registered by A2L plot may be assigned to C-O bonds in both polyalcoholic and ether functional groups, such as those of simple carbohydrates and polysaccharides, such as those of simple carbohydrates and polysaccharides (Spaccini and Piccolo, 2008) .Finally, the presence of carbohydrates is confirmed by peaks at 1030 and 1070 cm-1, usually attributed to C-O bonds in both polyalcoholic and ether functional groups, such as those in oligo- and polysaccharides (Zaccheo et al., 2002; Tatzber et al., 2007).

4.3.7 Thermogravimetric analysis

The TGA (% loss weight) of HA of samples showed two peak loss weight in all treatment at around 90ºC, the second peak is at 350ºC, DSC and DGT showed more specifically first endothermic peak at 70ºC. The second TGA peak of 350ºC showed the most important loss weight, after of this temperature the main losses of organic matter occurs, chemically indicate more detailed by DSC and DTG. Three DSC exothermal peak were indicates for the all treatments, however the release of energy in the control is less with respect to all treatments. Thermal degradation of HA proceeds in three exothermic peaks, (two peaks TGA asassociated with rapid weight loss), the first peak could to be atribuited to chemical analysis showed that the two first peaks (∼200 and ∼ 450ºC) are associated with recombination and decomposition reaction of aliphatic parts and a majority of the functional groups (biodegradable components), whereas in the third peak at the high temperature range (∼500–600◦C) to decomposition of aromatic structures (humified components). This peaks temperature are expressed in several peaks of DTG curves into the same range of temperature for the different sampling. The first peak DTG in Control and A1L plots is at between 230 -250 ºC and 250-370 ºC in the A1L, A1H and A2H plot may be attributed to aliphatic structures combustion (Dell'Abate et al. 2002) and aromatic structures and cleavage of C-C bonds (Peuraviour et al., 1999).

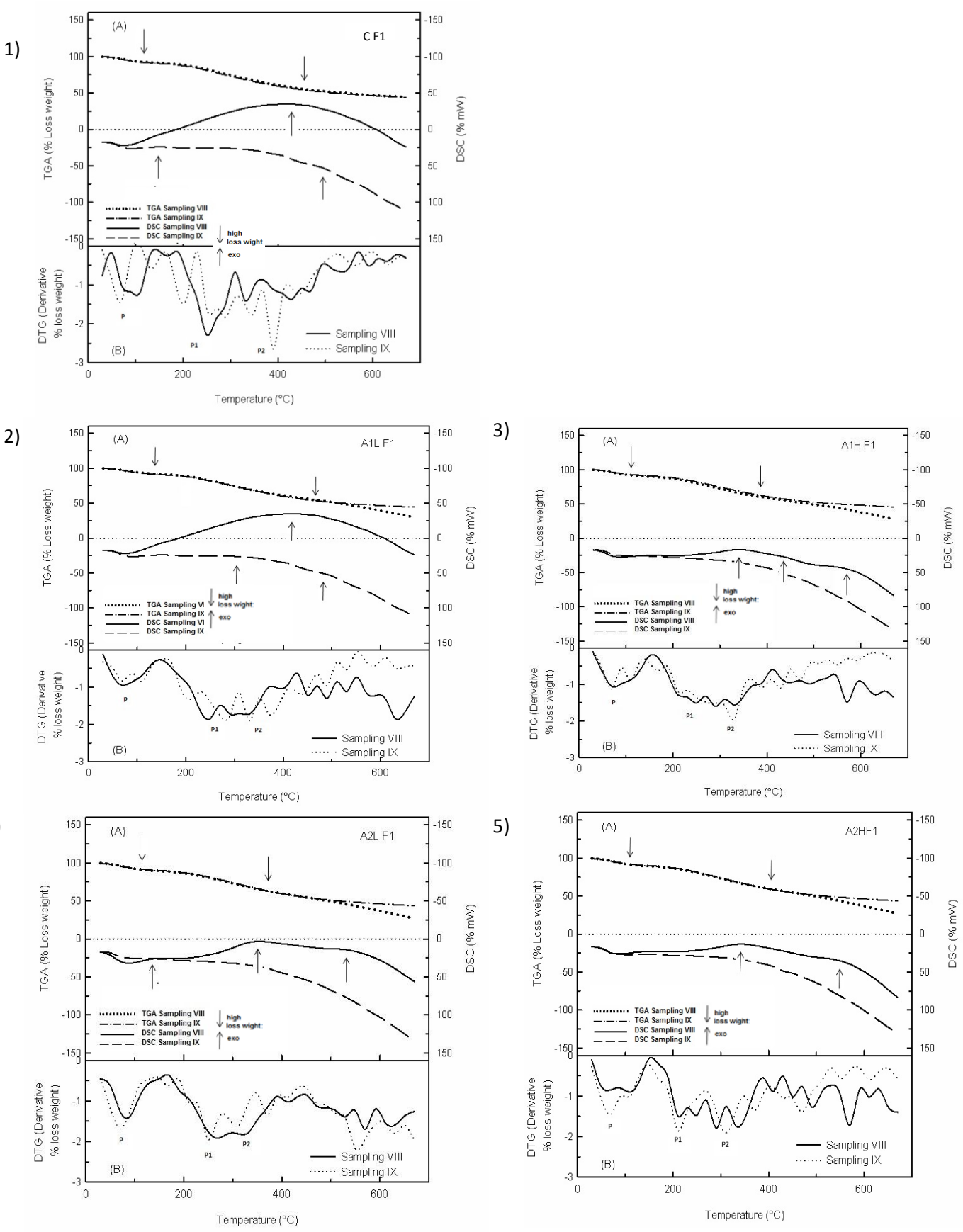

Figure 4.24. (A) TGA: Thermogravimetric curve (Weight %) and DSC: Differential scanning calorimetry curve (mW= mJ/sec); B) DTG: Derivative of weight %. Tº= Celcius Grade. P= Dehidratation peak; P1= First peak of loss organic matter; P2= Second Peak of organic matter. Measured in HA with treatments. 1) Control; 2) A1L; 3) A1H; 4) A2L; 5) A2H.

The second peaks Control and A2L plot in the sampling IX is reflected in DTG curve around 390 ºC for. A1H and A2H plot showed prominent second peak between 470-570ºC ,which is slightly in A1L.

In F1 and F2 the DSC curve are similar, the exotherm peaks showed in medium temperature range between 200 ºC - 450ºC and between 500 and 600ºC which may be assigned to decarboxylation reactions. With sampling time, this exotherm gradually becomes reduced and disappears at the end of the process, the thermograms of the treatments then becoming very similar to those of control soil humic acids, situation that is more evident in F2.

Usually, during heating of HS a first exothermic reaction (≈300 ◦C) is produced by the decomposition of proteins and carboxyl groups, while the exothermic reaction at higher temperatures (≈450 ◦C) is originated by decomposition of refractory C such as aromatic rings and saturated aliphatic chains.

DTG curves in F2, (Figure 4.18) showed 3 peaks in the different plots for organic matter combustion. The first between 200-300ºC given for the combustion of polysaccharides, decarboxylation, acid groups, and dehydratation of hydroxylate aliphatic structures (Fernández et al. 2008; Dell'Abate et a. 2002; Sheppard and Foregon, 1987) , second around of 390-400ºC (given by combustion of aromatic structures and clavage of C-C bonds (Peuravuori et al., 1999) and third between 500-600ºC related to the thermal breakdown of more aromatic and stable moieties, such as lignin (Dell'Abate et al. 2000; López-Capel et al., 2005) The thirds peaks around 600 ºC would to be given by oxidation of refractory C as well as the decomposition of both mineral and biogenic salts, such as carbonates and mainly composed by inorganic nutrients (N, P or S) which decomposed at higher temperature. described by Baffi et al., 2007 and Carballo et al. 2008 and as described in F1.

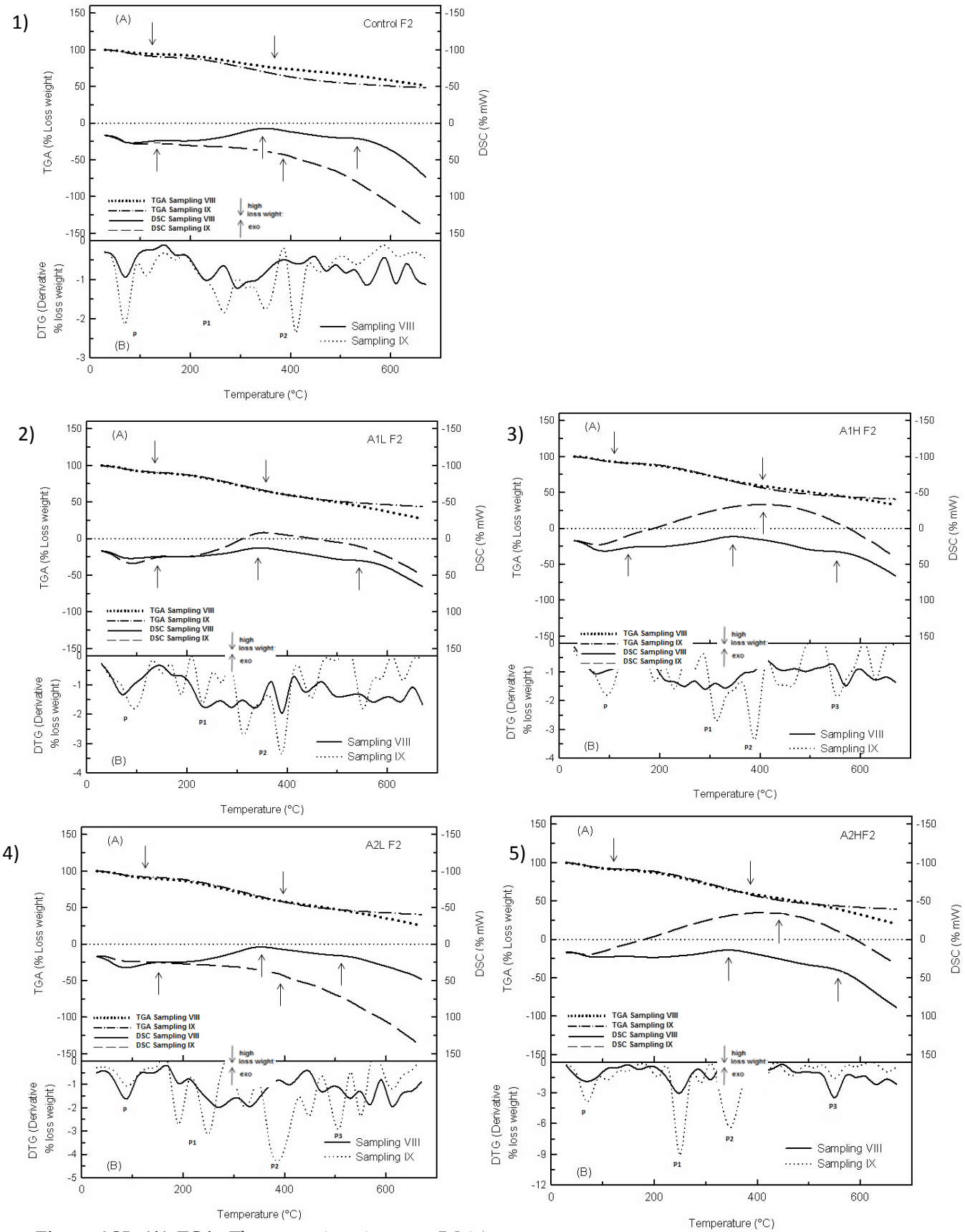

Figure 4.25. (A) TGA: Thermogravimetric curve (Weight %) and DSC: Differential scanning calorimetry curve (mW= mJ/sec); B) DTG: Derivative of weight %. Tº= Celcius Grade. P= Dehidratation peak; P1= First peak of loss organic matter; P2= Second Peak of organic matter. Measured in HA with treatments. 1)Control; 2) A1L; 3) A1H; 4) A2L; 5) A2H.

4.4 Discussion

Soil pH increase can be caused by decarboxylation of organic anions applied with manure or plant residues (Yan et al. 1996; Yan and Schubert 2000) this could to associate at the wood scraps and compost mixture. In addition, ammonification of urea or decarboxylation of organic anions can also cause a relatively rapid soil pH increase within a few weeks (Yan et al. 1996; Watson et al. 1990). On the other hand, soil pH increase due to nitrate uptake and assimilation by plants from soil is an ongoing process and may last as long as the active plant growing period. Darmody, et al. 1983 found increase of pH, Ca, K in silty loam soil amended with sewage sludge compost.

The P_2O_5 in F2 values showed the first year achieve highest values respect to last sampling, moreover, the Control plot showed the highest values, however the values in Sampling VIII of the control plot decreased presenting the highest values the plot A1L and A1H, in the Sampling IX control plot not show differences with amended plot as A1L and A1H, these plot showed high values of P2O5. Wandruszka (2006) reported that organic amendments serves both as a source of subsurface P and an effective mobilizing agent in calcareous soil, as is the case of F2, the organic matter of amendment blockage of P sorption sites by organic acids, as well as complexation of exchangeable Al and Fe in the soil. Humic materials, both native and added, appear to increase recovery of Olsen P. In the presence of metal cations, strong complexes between inorganic P and humates are formed. Moreover, Whalen and Chi Chang (2001) reported lower values in soil with organic amendment irrigated than those not irrigated, this could to be rationed with the greenhouse conditions where the leached level are very high, in other study Wei-Ming Shi, et al., 2009, found one seasonal effect of P olsen under greenhouse conditions in sandy clay loam soil, where pointed an increase of this nutrient at winter and decrease at summer indicates by an increase in mineralization of organic substances of soil, arguing that the uptake of nitrate by plants compensates the nitrate release by mineralization with mineralization of organic substances not only nitrogen but also sulfur can be release, suggesting, thus, the release of phosphate, this could, also explain the lowest values at third sampling, because the data of collected soil is at summer (06/15/2012). K+ and Ca2+ exchangeable showed highest values in Sampling I by A2H and A1H plot, this similar behavior at P2O5 could to be explained this seasonal effect in these Basis.

The EC increase by organic amendment addition in F2, Kavdir and Killi (2008) reported increment of EC in the time using olive oil solid waste as amendment in sandy soil.

The CEC values were not influenced by organic amendments en F1 (Table 4.1) and remained unchanged during the study, except for F2 soils, that showed a general increase of CEC values in all plots until to achieve the maximum values in Sampling IX. This behavior could be related to not only the use of OM, but also the high percentage of limestone in this soil that could have affected the measurement of this parameter (Edmeades, 1982).

The organic amendment addition increase strongly Organic carbon level, Shrestha, et al. (2013) out pout that long time of continue application of organic amendment in alfisol increment the pH, EC and enhance soil C pool, similar results were reported by Herencia et al. 2007 and Subhadip Gosh et al., 2012. Clark et al. (2007) reported in an study of organic amendmet using, that sawdust presented less microbial activity and residue breakdown(C low soluble), and therefore produced less bacterial by-products, resulting in less protection from aggregate formation, and higher C/N ratio of the mature residues, together with the accessibility of the residue, will favour a slow degradation by fungal hyphae and other associated microorganisms, thus, remaining residue C with very slow loss of this element in the time. This authors reported slow mineralization of N in compost with less microbial activity (most recalcitrant compost), showing an augmentation of N two months after of amendment, as in A1H and A2H plot in our study that presented highest dose of both mixture in Sampling VIII (one month after of amendment addition). Zarabi and Jalali (2012) found that the course of N mineralization differed according to the type of amendment and soil. The increase was slower for clay soil than sandy loam soil. This indicates greater nitrification (conversion of NH4 +to NO3-) in sandy loam soil compared with clay soil. Moreover reported mineralization rates highest in Municipal waste in sandy soil than clay soil, and claimed that municipal waste compost is less leachable, this could to explain the lowest values of A1L plot respect to the other treatment with high dose of compost and C/N ratio in F1 and F2. In F2 A1H and A2H showed the highest values with 57% and 42% respect to the control (Figure 4.3). Shao-Jun et al., (2013) reported that the addition of C substrates to the intensively managed agricultural calcareous soils (similar to F2 farm) could effectively promote the transformation of accumulated excessive soil NO3-N to Soil organic Nitrogen (SON). Increasing availability of the C substrate increased the immobilization of accumulated soil nitrate and also greatly stimulated the mineralization of native SON. The SON abundance in the SON abundance in the C substrates of slow mineralization treatment increased gradually in the time. This phenomenon may be dependent on the contribution of di fferent mic availabilities. The return to the soil of the this kind of biomass as wood, straw or another green manure would increase the interception of NO3-N before it could be leached to the subsoil, as is the case of greenhouse conditions.

All enzyme activities tested appeared to be positively affected by the organic amendment addition in F1 and F2, at exception Invertase of F1. The plot with major responses to soil enzymatic activities are the higher application rates (A1H and A2H).

Dehydrogenase (DHY) is considered to be a measure of a soil microbiological activity (Moreno et al., 2009; Nannipieri et al., 2003). A significant increase in DHY activity, and was present constant values, similar result were obtained by López-Piñeiro et al., 2011; Roig et al., 2012 and Saha, et al.

2008 both notified studies in long term amendment use in clay soil and sandy loam soil respectively. Compared to the control, DHY activity showed great increment in A2H and A1H plot in the three sampling. This effect can be attributed to greater microbial biomass due to the addition of available organic substrates that can promote the growth of indigenous microorganisms (Benitez et al., 2000).

The PHO (P cycle) and β-glucosidase (C cycle) activity trend to increase in F1 and F2, especially after organic amendment addition, similar situation was pointed by Bastida et al., (2008) in Mediterranean conditions, and Ge et al, (2009) on Fluvo Acqui soil, both in long term studies. The F2 soil showed higher phosphatase activity than F1, which could be to explain by the lower values of P2O5 in F2 than F1, as reported by Dick et al. (2011). In F2, as occurred for PHO, β - glucosidase showed lowest values respect to F2, this could be explained by Yan et al., 2010 which was reported immobilization of β - glucosidase by soil colloids as clays associated to organic matter for interaction reaction, moreover F2 had higher values of Corg than F1, until started stage, this parameters is positively correlated with β-glucosidase and closely related by several authors, in F2 the values of GLU were higher after the organic amendment addition, the highest values were obtained by A1H in Sampling I, but three years after the two follow Sampling A1H and A2H not present significant differences, maintain relatively constant level of activity, this due to the labile C continued in the fresh organic matter and slow mineralization of compost mixture (García-Gil et al., 2000).

In F1, Invertase activity not was affect by organic amendment addition, similar result were found by Saha et al. (2008). In others hand Hu et al., (2010) found positively effect of organic amendment application in long term on Invertase activity in sandy loam soil , as in the case of F2.

The Urease activity in F1decrease in the treatments achieving values lowest at starter conditions, this situation was also reported by Pasqual, et al. (2002) which described immobilization phenomena of urease activity in a clay loam soil amended with organic wastes. The Urease activity in F2 increase with organic matter application similar result were pointed out by Roig, et al (2012).

By the analysis of principal component of F1 soils (Figure 4.3.4 A), it is clear the effect of amendment over time. The cluster A, representing the samples of the Sampling I, had negative values of PC 1, in particular in the control soils not treated with organic amendment, similar behavior had the control plot of Sampling VIII and Sampling IX. This result suggested and confirmed that the addition of organic amendments have determined an increase of organic carbon A. Important soil properties, as Corg, C/N ratio, pH and Ca, and enzymatic activities, as DH activity, PHO activity and GLU activity, increased with the use of organic amendments. The opposed way, the UR activity decrease with the organic amendment addition immobilization phenomena of urease activity in a clay loam soil amended with organic wastes. Nayak et al., 2007

reported Soil organic C (Corg) content showed significant positive correlation with dehydrogenase, urease, β-glucosidase and invertase. C/N ratio which was lowest in unamended control plots showed a significant positive relationship with only the enzymes involved in C cycle. PC1 concerns all enzymatic activities in relationship with Corg. The PCA score of F2 4.3.4 B1 show at Sampling VIII and IX with the highest values in the biochemical parameters, and show at Sampling I with highest values in some chemicals properties, as mentioned above.

Therefore, the separation of all samples in three cluster, related to time, indicated an evolution of analyzed soil properties over time, that could be related to the seasonal factor and the maturity of added OM.

Differences between F1 and F2 soils observed in the principal component analysis were determined by the geopedologic differences of the two farm soil and different responses to various organic amendments, as explained in detail the in previous paragraphs.

In the yield crops, Scotti, 2011 reported in the previous study enhance of crop production from second cycle in with amendment plot, this confirm the positive effects of all used organic amendment in this study was highlighted only from the second crop cycle, suggesting as in short-term a negative effect attributable to initial phytotoxicity of supplied OM was observed (Singh and Agrawal, 2007). In the long-term the disappearance of phytotoxicity phenomena, the complete integration of added compost, and the contribute also from wood scraps degradation worked all together to improve the OM content and quality, as well as soil physical, chemical and biological properties (Bulluck III et al., 2002).

The Humic subtances (HAs) extracted from the treated plots were higher respect to the control in F1 around 81% and F2 around 87%, in both organic fractions an increment was observed as a consequence of the organic amendment, the organic amendment supplied new OM rich in labile carbon fractions (promptly used by soil biomass) humified matter and wood scrapes which contribute either straightaway or over the time by degradation processes to increase soil humic fractions (Kiem and Kandeler, 1997; Liu et al., 2010). The F2 soil was a calcaric sandy loam soil and its sand nature determined aired conditions that favoured the biomass activity and OM oxidative processes, thereby leading to faster OM degradation, as confirmed by the reduction of organic carbon in these soils, see figure 4.4, that too, was reflected in the C% of elemental analyses of HA and FA of Sampling IX of F2. The HA extraction was highest in the sampling IX in F1 y highest in Sampling VIII in F2, The FA extracted in F1 soil was highest than F2, evidencing highest values in Sampling IX, this two situation also could to be explain by the amount of Corg in the soil (Table 4.4) that showed values significant higher expressed in mgkg-1 in the last stage corresponding perhaps C recalcitrant by low microbial activity in F1 and associated at clay amount, in contrast with sandy soil in F2 which the loss of C is very fast.

The thermogravimetrical analysis was performed by TGA, DSC and DTG. Thermogravimetry is a technique in which the weight change (increase or decrease) is measured during the incremental heating of the sample. DSC is related to energy, measurement of the thermal behavior of organic matter under oxidative conditions allows separating thermally labile and stable compounds by means of measuring peak temperatures, Differential scanning calorimetry measures the differential heat flow of a sample (endo- or exotherm) Leifeld et. al., (2006), the sample energy change during a transformation is more directly measured than DTA. The first derivative of the TG trace (DTG) on time permits a better resolution of the steps by which the reactions take place: it does not contain any new information, however it clearly identifies the temperatures at which mass loss is at a maximum, as well as superimposed transformations appear more clearly shown as DTG peaks (Dell'Abate, 1998).

The TGA (% loss weight) of HA of samples showed two peak loss weight in all treatment at around 90ºC, the second peak is at 350ºC, DSC and DGT showed more specifically first endothermic peak at 70ºC, generally representative of dehydration reactions loss of peripheral polysaccharide chains (Provenzano and Senesi, 1999), since it is unlikely that a large quantity of organic volatile groups were lost in such a temperature range (Dell'Abate, et al. 2000; Smidt and Lecher, 2005; Melis and Castaldi, 2004). Thermal degradation of HA proceeds in three exothermic peaks, (two peaks TGA associated with rapid weight loss), the first peak could to be attributed to chemical analysis showed that the two first peaks (around 200 and 450ºC) are associated with recombination and decomposition reaction of aliphatic parts and a majority of the functional groups (biodegradable components), whereas in the third peak at the high temperature range (500–600◦C) to decomposition of aromatic structures (humified components). This peaks temperature are expressed in several peaks of DTG curves into the same range of temperature for the different sampling. The first peak DTG in Control and A1L plots is at between 230 -250 ºC and 250-370 ºC in the A1L, A1H and A2H plot may be attributed to aliphatic structures combustion (Dell'Abate et al. 2002) and aromatic structures and cleavage of C-C bonds (Peuraviour et al., 1999). The second peaks Control and A2L plot in the sampling IX is reflected in DTG curve around 390 ºC for. A1H and A2H plot showed prominent second peak between 470-570ºC ,which is slightly in A1L. The second peaks is reflected in DTG curve around 390 ºC for Control and A2L plot in the sampling III, this thermical profile is influenced by the accumulation of C as cellulose and lignin structures (Francioso, et al. 2005), The Plot is slightly in A1L expressed by Sampling I and II and prominent at A1H, and A2H showed them second peak between 470-570ºC expressed by Sampling II and III to the thermal breakdown of more aromatic and stable moieties, such as lignin (Dell'Abate et al. 2000; López-Capel et al. 2005) due to the dissociation and decomposition of aromatic structures and polynuclear systems of higher molecular weight, Ranalli et al., 2001; P. Melis, P. Castaldi. (2004) Aggarwal et

al. 1997 assigned this thermal pattern to cellulose and lingo cellulosic substances, that are a main component of plant materials. The control and A2H plot showed one gradual loss of weight around 600ºC, that could indicates oxidation of refractory C as well as the decomposition of both mineral and biogenic salts, such as carbonates (Baffi et al., 2007).

In F1 and F2 the DSC curve are similar, the exotherm peaks showed in medium temperature range between 200 ºC - 450ºC and between 500 and 600ºC which may be assigned to decarboxylation reactions. With composting time, this exotherm gradually becomes reduced and disappears at the end of the process, the thermograms of the treatments then becoming very similar to those of native soil humic acids, situation that is more evident in In F2 in the plot A1H and A2H which was present their exothermic peaks very slightly, Smidt and Tintner (2007) found that compost with high humic acid content loss the exothermic heat flow peak and present the only one exothermic peak at 550ºC, this situation would to be related with the analyses of Humic acid DSC of this study, similar results were observed during the composting of municipal solid wastes and farmyard manure in previous work by Ouatmane et al. 2000 and by Provenzano and Senesi, 2006, Senesi et al. 2007. Specially for sampling II in the Control plot in F2 and A1H and A2H in the Sampling III in F2. however, Senesi, et al. 2007 found in one study of DSC curves in organic wastes, i.e., sawdust of cedrus minor changes in DSC curves observed for sample that may be attributed to the limited extent of transformations occurred, which is possibly related to the dominant presence of recalcitrant lignin structure in this substrate. In DSC, usually, during heating of HS a first exothermic reaction (around 300 ºC) is produced by the decomposition of proteins and carboxyl groups, while the exothermic reaction at higher temperatures (≈450 ◦C) is originated by decomposition of refractory C such as aromatic rings and saturated aliphatic chains.

The HA F1 spectra (Figures 4.20 (A)and 4.21(A)), showed that after of one month of admendment the differences between Control and amended plot seemed were given by an increase of aliphatic portion, due probably to the lipidic portion contained in the amendment mixture, showed by the principal band around to 2940 cm-1, moreover, the polypeptide portion, also showed an increase in the treated plots, likely by the protein inputs of amendment, band 1648 cm-1 (I amide), and the band around 1530 cm-1(II amide band). In each case, the differences between the amendment dose of treatment not show influences on the HA characteristics. After six months, from the third amendment, the differences between control and treatment were annulled, likely by high microbial activity spring season (I and II amide bands). In the control spectra, the band showed an increase by comparison with Sampling VIII (one month after amendment), at the same time the aliphatic portion of amended soils showed a decrease, resembling to the control spectran.

The band in 1160 cm-1 registered by A2L in plot may be assigned to C-O bonds in both polyalcoholic and ether functional groups, such as those of simple carbohydrates and

polysaccharides, such as those of simple carbohydrates and polysaccharides (Spaccini and Piccolo, 2008) .The intensity of the bands 2939cm-1 decrease (alkyl signal) in HA Sampling IX of F1 soil observed in compost with highest maturity stages, suggesting a decomposition of bioavailable lipids content, the treatments showed highest intensity of this bands with respect to the control (band around to 2940 cm-1).

In F2, (Figures 4.20 (B)and 4.21(B)), also the polypeptide substances contribute increase in the treatment in comparison by control plot (band 1650 and 1530 cm-1, I and II amide band), this would to be explained for a quick organic matter transformation in the sandy soil than clay soil as F1 farm soil. After six months of amendment (Sampling IX), the polypeptide portion showed decrease trend in amended soils, contrary to clay soil, given probably by aerobic conditions and soil granulometry.

In FA context, F1 soil, also were influenced by treatment (Figures 4.22 (A)and 4.23(A)), however, this influence is presented only after one month of amendment, which the carbohydrate content is more evident in amended soil, determined by band around to 1100 cm-1 and 963 cm-1. In sampling IX, the FA composition was similar, the control not show differences in comparison bay treatment, in contrast to the previous sampling, in this sampling, in this case too, the plant residues would may have helped in the carbohydrate increment.

In F2 soil, FA in Sampling VIII, as well, Sampling IX, the carbohydrate content was not maintained (Figures 4.22 (B) and 4.23(B)), in contrast with clay soil, in all treatment including control plot, which would to be influenced, also by humus maturation aerobic conditions which the labile substance accumulation, in sandy soil the protein and polysaccharides presented faster mineralization than clays soils, this would to explain the lowest extraction content of this fraction in yields.

The HA and FA fractions indicate that after one month of amendment showed an increase aliphatic, proteins, and carbohydrate characteristics of humus, likely, because the amendment addition provided high Has fractions, that still are not transformed in presence of low temperature and the short permanence time in the soil. After six month, the soil process activation, as well, the amendment transformation, given by enough time permanence in the soil in one stage of rise temperature, which determinate humus homogenization characteristic that have resulted in a decrease of amendment aliphatic portion and increase of carbohydrate portion to the control plot, also by residues plant or roots provides.

4.5 Conclusions

The organic amendment with low degradation material as wood scraps improved chemical and biochemical characteristics of amended soils and keeping the nutritional level over time. Amendment ameliorated generally chemical properties showing a significant high response.

The organic amendment addition increased strongly organic carbon level, thanks to recalcitrant C pool of amendment. A consequent effect was observed on the C/N ratio that was significantly improved by enhancing in turn enzymatic activities, especially those enzymes involved in the carbon cycle. All enzyme activities tested in F1 and F2 soils appeared to be positively affected by the organic amendment addition, except for invertase in F1 soil. The plots with better response in terms of soil enzymatic activities were those amended with the highest application rates (60 t ha^{-1}, A1H and A2H). Conversely the urease activity decreased, probably due to inhibition phenomena by ammoniac forms deriving from amendment. The humic fractions extracted from soils showed differences in the structure, likely due to different amendment mixtures and doses, as revealed by thermal and FT-IR analyses.

The organic amendment practice increased greatly the crop production over time especially in the plot A1H of the sandy soil; this texture made more available the labile fraction of amendment, providing fast nutrient inputs for plants.

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5 Biochemical properties and bacterial diversity in soils under organic farming

5.1 Introduction

The adoption of organic farming systems is an important sustainable agriculture strategy to mitigate the organic matter reduction and pollution of water and soil ecosystem caused by conventional agriculture, and at the same time to improve fertility and quality of agricultural soils. In this context, after the recent use of policy by the European Union to implement best farming practices environmental awareness is taking great importance in the accreditation of organic farming.

Italy is among the first countries of the world that present crop lands with conservational methods, and among these Italy presents a high percentage (28%) of the total usable agricultural area that makes it second after Spain (FiBL-IFOAM Survey 2012).

No synthetic fertilizers and pesticides are used in organic farming system, improving the environmental protection, restoring the natural ecological balance, and enhancing beneficial biological interactions and processes with a development of significant synergies between them (Vandermer, 1995). Compared to conventional agriculture, organic farming tries to increase ecological processes that promoting plant nutrition, conserving soil and water resources. Organic systems eliminate agrichemicals and reduce other external inputs to improve the environment as well as farm economics (Hiroki and Ashok, 2012).

Over the last decade, organic farming has become one of the most thriving segments in the USA farm sector, mainly due to growing demand for healthy food products (Kuminoff and Wossink, 2010). Given the importance of SOM for soil quality, organic fertilization is indispensable in sustainable crop production. Many diverse organic materials, e.g. crop residues, manures, peat and composts, are used, but each of them have specific effects on SOM stock, soil functioning and the soil microbial community. However, there is little knowledge about these specific effects (Moeskops, et al. 2012).

The determination of the quality-related properties of soil (which are sensitive to changes caused by management practices and environmental stress) may help to monitor the changes in its sustainability and environmental quality. This is especially true for the agricultural management and recovery of soil, and to assist into the establishment of policies for the land use.

The identification of biological indicators of soil quality is important because soil quality is strongly influenced by microorganism mediated processes (nutrient cycling, nutrient capacity, aggregate stability), whereby the key is to identifying those components that rapidly respond to changes in

soil quality (Doran and Parkin, 1994). Nevertheless, there is the problem of knowing which indicator responds to a specific soil treatment or contaminant.

Soil enzymes activities have been suggested as appropriate indicators of soil quality since their measurement allows an indirect measure of the soil microbial activity. In fact enzymatic activities are strictly related to the nutrient cycles and transformations and rapidly may respond to the changes caused by both natural and anthropogenic factors (Calderon et al., 2000; Colombo et al., 2002; Drijber et al., 2000; Gianfreda and Bollag, 1996; Nannipieri et al., 2002). The response of soil enzyme activities to specific soil practices has been used to compare organic agricultural systems versus conventional farming (Benitez et al., 2006; Bulluck et al., 2002; Edmeades, 2003; Melero et al., 2006; Van Diepeningen et al., 2006).

In a biological sense, healthy, thriving ecosystems are generally considered to be highly diverse with numerous taxa, which form a complex food web with many trophic levels (Metting and Blaine, 1993). Therefore, taxonomic and functional diversity indices are often used as an index for the health conditions of soils (Brussaard et al., 2004; Van Bruggen and Semenov, 2000). A healthy soil is defined as a stable system with resilience to stress, high biological diversity, dynamical and functionality, and high levels of internal nutrient cycling (Van Bruggen and Semenov, 2000). Cultivated soils under conventional agricultural practices often have lower microbial diversities than they had as a natural habitat (Buckley and Schmidt, 2001). In contrast, organically managed soils show a higher diversity of microorganism (Drinkwater et al., 1995; Mäder et al., 2002) than conventionally managed soils. Also, a higher microbial activity (Workneh et al., 1993) and microbial biomass (Mäder et al., 2002; Mulder et al., 2003) were found in organic soils. However, some authors did not find significant changes in bacterial biodiversity (Lawlor et al., 2000) or in fungal population (Franke-Snyder et al., 2001) between organically or conventionally managed soils.

The study of soils characteristics could help to highlight the behavior and reduced availability in the environment of some nutrients such as phosphorous. Phosphorous is a nutrient largely applied to soil through mineral fertilizers that, in according to soil properties, yet rapidly becomes unavailable to plants, accumulating in inorganic P fractions that are fixed by chemical adsorption and precipitation, and organic P fractions that are immobilized in soil organic matter (Sanyal and De Datta, 1991). In alkaline soils, P fertilizers react with calcium to form insoluble calcium phosphates (Mullen, 2005) and accumulate as organic phosphates, primarily as phytate, which can include from 10% to 50% of the total P in both acid and alkaline soils (Mullen, 2005; Turner et al., 2002). Phytic acid (myo-inositol 1,2,3,4,5,6–hexakis dihydrogen phosphate) and mixed cation salts of phytic acid, named as phytates, are a group of organic phosphorus compounds found widely in nature. In terrestrial ecosystems Phytates are synthesized by plants, accumulate in seeds during the ripening

period and are regarded as the primary storage form of both phosphatase and inositol in plant seed an grains (Lott et al., 2000; Tuner al., 2003). The importance of soil organic P as a source of plant available P depends on the rate of its solubilization and the inorganic P release. Several types of phosphatases, such as phytases, can to increase the rate of the dephosphorylation (hydrolysis) of organic P (Hayes et al., 1999; Hubel and Beck, 1993). Several phytase classes have been studied: histidine acid phosphatase, β-propeller phytases (BPP), cysteine phosphatase and purple acid phosphatase (Mullaney & Ullah, 2007).

Many different bacteria carry genes encoding phytases (Jorquera et al., 2008; Lim et al., 2007), but little is known about their ecology or activity in soils. One important group of phytate-mineralizing rhizobacteria (PMR) includes endospore-forming bacteria such as *Bacillus*, which have been widely studied for their ability to solubilize phytate and for their potential use as biofertilizers in agriculture (McSpadden Gardener, 2004; Richardson and Simpson, 2011). Various *Bacillus sp*. are known to possess BPP, which are effective for the dephosphorylation or mineralization of phytates (Hill and Richardson, 2007; Lim et al., 2007).

Quantitative real-time PCR (*q*PCR) is a sensitive method for the detection and quantification of specific genes in DNA extracts from various environments (Heid, et al., 1996). Here *q*PCR was applied to study the prevalence population of phytate- mineralizing bacteria based on quantification of the β-propeller phytase gene in both organic and conventional farms.

The aim of this study, carried out within a national research project Italian addressed to identify the effects of organic management on the quality of two cultivars of processing tomatoes *Solanum lycopersicum*, Docet and Faraday, in two soils selected in Mediterranean environment, precisely in the South Italy, was to evaluate the impact of organic and conventional production practices on soil chemical, biochemical and biological properties. The changes in the bacterial community with particular attention to the analysis of the phylogenetic bacterial community structure were also analysed. The gene quantification of *Bacillus* Phytase gen using *q*PCR Real Time to quantify the relative abundance of this gen in soils under different management was also included.

5.2 Material and Methods

5.2.1 Experimental fields

The experiment was carried out in two farms sited in the Sele River Plain (Campania Region, South Italy) less than 5 km distant from each other and with similar soil and climatic conditions: the farm “Cra-ORT” (Research Centre for Agriculture – Horticulture, Figure 3.1) and the organic farm "La Morella" (Figure 3.2) for the cultivation of processing tomatoes in organic and conventional management, respectively, with *Solanum lycopersicum* L 1753 crop with two cultivars: Docet (elongate for peeled tomatoes) and Faraday (orbed for tomato puree).

Two cycles of tomato cultivation were carried out, in 2011 and 2012, in areas made available by each farm, different every year due to crop rotation plan,. A randomized block scheme with three replicates, each consisting of a 44 m^2 plot, with a plantation density of 3,3 plants/m^2 was adopted (Figure 3.3).

Figure 5.1. Randomized block scheme of experimental plots.

The fertilization under biological management was based on a N:P organic fertilizer (Fertbase, 4% N and 5% P_2O_5), distributed as basal fertilization and at initial flowering, and a fertirrigation (Azobios 7% N) repeated more times during the crop cycle.

The fertilization of the conventional management was based on standard fertilization: N:P:K 40:100:100, ammonium sulfate (21%), simple superphosphate (19%) and potassium sulfate (50%) as basal fertilization. After seedling transplanting nitrogen like ammonium nitrate (34%) in fertirrigation until to 20 days before harvesting (80 kg ha^{-1} N as total amount) was supplied.

5.2.2 Soil samplings

During each year two samplings occurred in different times:

1. before the seedling transplanting (April 2011 and April 2012)
2. immediately after the tomato harvest (September 2011 and September 2012).

In each plots, five sub-samples were collected following a W scheme from the topsoil (0-20 cm), then sub-samples were mixed to form only one sample per plot. Samples were packed in polyethylene bags, sieved (< 2 mm, and air dried at room temperature (for physical and chemical analyses) or stored at 4 °C (for biochemical analyses).

5.2.3 Soil chemical properties

Chemical properties of soils were determined by standard methods (Sparks, 1996). Electrical conductivity (EC) and pH were measured in 1:5 and 1:2.5 soil:water suspensions, respectively; cation exchange capacity (CEC) was measured after soil treatment with a barium chloride and triethanolamine solution at pH 8.2; available phosphate was measured by bicarbonate extraction. Organic C content was assayed by chromic acid titration method (Walkley and Black, 1934); total N with Kjeldahl method; exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) were determined by were assayed by flame atomic absorption spectrometry.

5.2.4 Enzymatic activities

The activities of dehydrogenase, β-glucosidase, invertase, phosphatase, and urease were detected as described in detail in Chapter 3, Par. 3.2.5. The activity of FDA hydrolase was determined by following the method reported by Green et al., 2006.

5.2.5 Biomass carbon

The determination of the microbial biomass carbon (Cmic) was performed using fumigation-extraction method with chloroform as described by Vance et al. (1987). The extractable C was converted to microbial C dividing it for a standard factor 0.45.

5.2.6 Soil respiration

Microbial soil respiration (MSR) was determined by an alkaline absorption method named *respiration of soil in closed jard method*, in according to Anderson (1989). Sub-samples of sieved soil equivalent to 25.0 g dry weight were adjusted to moisture of about 60% of water holding capacity were placed in mason jars with a suspended beaker containing 25 mL of 0.5 M NaOH and other beaker with 25 mL of distilled water. The jars were incubated in the dark at 25 ºC, immediately after sealing. The beaker was replaced with one containing fresh NaOH solution for five times after 1, 2, 6, 8 and 18 days from the start, in the first year, and after 1, 3, 5, 7 and 13 days from the start, in the second year. The CO_2 trapped in NaOH was titrated with 0.5M HCl. Microbial respiration was estimated as CO_2 mg h^{-1}100 g^{-1} dry soil by averaging the data.

5.2.7 Bacterial community composition

The bacterial community composition was evaluated in soils collected in post-harvest of the first year of study (2011), by Denaturing Gradient Gel Electrophoresis (PCR–DGGE). The total DNA was extracted from a 0.25 g of soil sample using UltraClean Soil DNA Isolation Kit (Mo Bio Inc., USA). Fragments of 454 bp of bacterial 16S rRNA genes were amplified by using the primers EUBf933 (CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCA CAA GCG GTG GAG CAT GTG G) and EUBr1387 (GCC CGG GAA CGT ATT CAC CG) as described by Iwamoto et al. (2000). A touchdown PCR was performed in which the annealing temperature was decreased from 65 to 55 °C at a rate of 0.5 °C every cycle followed by 10 additional annealing cycles at 55 °C. A denaturation step at 94 °C for 1 min was used, and extension was performed at 72 °C for 3 min; a final extension step at 72 °C for 7 min ended the amplification cycle. The PCR amplification was carried out using the GoTaq® Flexi DNA Polymerase kit (Promega, Co.).

The DGGE analysis was performed using a DCode Universal Mutation System (Bio-Rad Laboratories, Inc.). 20 μL of PCR products were loaded in a 6% (w/v) polyacrylamide gel by using a denaturant gradient from 35 to 65% (7 M urea and 40% formamide). The electrophoresis was performed at 100 V for 10 h. The gel was stained with SYBR Gold (Molecular Probes, Invitrogen Co.) for 30 min and photographed on a UV transilluminator. Twenty one dominants bands in the DGGE gels were analyzed and clustered using a dendrogram ~~their volume fluorescence~~ by image analysis using Phoretix 1D v10.3 (TotalLab Ltd.) software package.

5.2.7.1 Realtive qPCR Real Time of β-propeller phytase gene

The primer set MQHf (5'–TTC CTA TCC TAC CGG GAA GC–3') and MQHr (5'–TGC TTT GTA ATG TGC CGT TT–3') was designed to target the β–propeller phytase gene based on

sequences of *Bacillus* sp. MQH–15 and *Bacillus* sp. MQH–19 isolated from the rhizosphere of pastures grown in a Chilean Andisol (Jorquera et al., 2011). This primer set amplified a DNA fragment of 158 bp. All PCR reactions were performed in a 7300 Real Time PCR System (Applied Biosystems) using Maxima® SYBR Green/ROX qPCR Master Mix (Fermentas Life Sciences) following the manufacturer instructions. The PCR conditions were as follows: an enzyme activation step at 95ºC for 10 min followed of 40 cycles of 15 sec at 95ºC and 1 min of annealing plus extension at 60°C. The universal primer set targeting 16S rRNA gene, Bac1369F (5'– CGG TGA ATA CGT TCY CGG–3') and Prok1492R (5'–GGW TAC CTT GTT ACG ACT T–3') (Suzuki et al., 2009) was used to evaluate the relative abundance of BBP gene in relation to total bacterial DNA.

5.2.8 Data analysis and statistical analysis

Two-way ANOVA was used to examine the effects of organic management on all soil properties analysed, during the two experimental years.

The relationships among all soil chemical and biochemical properties (EC and INV), of all samples, were assessed by using Pearson correlation coefficients.

Multiple ANOVA (Duncan's multiple range tests) was performed to evaluate significant differences between means at the 95% level of probability using comparisons each pair.

All statistical analyses were performed using SPSS software (PASW Statistics 18, 2009 - IBM SPSS Statistics).

DGGE banding profiles were subject to a Principal Component Analysis (PCA) for two component using JMP 8 software (SAS Institute, USA).

5.3 Results

5.3.1 Soil chemical properties

Table 5.3.1 shows the physical and chemical properties of soils under organic and conventional management collected before seedling transplanting and after harvest of tomatoes in 2011 and 2012. In according to USDA (http://soils.usda.gov/technical/aids/investigations/texture/) the soils of both the farms presented the same texture: in all cases they can be defined clay loam soils.

The pH was sub-alkaline, but slightly more alkaline in soils under organic management (pH > 8), due to the higher content in limestone. Because of the calcaric nature of these latter soils, the available P was lower (about 26 g kg^{-1} in the first year and 34 g kg^{-1} in the second year) than in non-calcaric soils under conventional management (about 43 g kg^{-1} in both the years) (Table 5.3.1). Phosphorous fixation phenomena could be claimed to explain this behavior of organic soils.

The larger amount of organic carbon measured in the soils under organic farming could confirm, especially in the soil cultivated in the second year (around 14 g kg^{-1}), the beneficial effect of organic fertilizers on the continuous recovery of organic matter over time though the crop uptake. Conversely, as further confirm to previous assumption, the value of organic carbon remained around 8 and 9 g kg^{-1} in soils under conventional farming in the first and second year respectively (Table 5.3.1).

These soil characteristics determined also higher CEC of the soils under organic farming as organic colloids generally make available larger exchange surfaces. A role of clay fraction could not be exclude as the amount of clay was higher in soil of La Morella (organic farm). Consequently a positive repercussion on all exchange bases was observed in soils under organic management (Table 5.3.1).

For all soil properties analyzed, a two-way ANOVAs statistical analysis was performed to assess the effect of organic management (Table 5.3.2). A statistically significant effect of organic management on all studied parameters was observed, while no effect due to cultivars (Docet or Faraday) was highlighted so hereafter the parameters were not compared by cultivars.

Table 5. 1. Chemical properties of soils under organic and conventional management for two year in the Pre transplanting and Postharvest. Different letters indicate significant difference (P≤0.05) lowercase indicate differences between treatments and uppercase indicate differences across time. Letters from Duncan test (P≤0.05).

	Organic farm soils				**Conventional farm soils**			
	I year		**II year**		**I year**		**II year**	
Parameters	**Apr 2011**	**Sept 2011**	**Apr 2012**	**Sept 2012**	**Apr 2011**	**Sept 2011**	**Apr 2012**	**Sept 2012**
Texture	Clay Loam		Clay Loam		Clay Loam		Clay Loam	
Sand (g kg^{-1})	419		395		424		452	
Silt (g kg^{-1})	222		290		271		256	
Clay (g kg^{-1})	351		315		283		295	
pH (H_2O)	8.09abAB	7.97cBC	8.26aA	8.28aA	7.99BC	7.87C	7.83C	7.92BC
C.E. (ds m^{-1})	0.088bBC	0.092bBC	0.065cD	0.11aA	0.090bBC	0.098aA	0.055dD	0.085cC
Limestone (g kg^{-1})	34.31aA	23.84bB	9.67dD	13.94cC	6.68aE	3.98bF	4.48bF	6.77aE
CEC ($cmo_{(+)}kg^{-1}$)	20.44bBC	22.00bB	23.41bB	33.55aA	14.52cE	14.80cE	18.57aCD	16.63bDE
P olsen (g kg^{-1})	26.68cC	26.42cC	30.51bC	38.85aB	43.06A	45.13A	42.04AB	43.55A
O. M. (g kg^{-1})	21.94cC	21.69cC	24.34bB	25.16aA	14.77bE	14.35bE	16.21aD	14.66bE
Organic C (g kg^{-1})	12.41bC	12.58bC	14.12aB	14.59aA	8.57bC	8.32bE	9.40aD	8.50bE
Total N (g kg^{-1})	1.87bB	2.03aA	1.36cC	1.40cC	1.07aD	1.05aD	1.00abD	0.94bE
Ratio C/N	9.43aAB	6.20bC	10.36aA	10.41aA	6.25bC	8.01bA	9.34aAB	9.07aAB
Ca exc (meq $100g^{-1}$)	16.66cC	15.04cD	26.00bB	30.30aA	10.29bE	10.62bE	18.01aC	17.26aC
Mg exc (meq $100g^{-1}$)	4.21cC	4.31cC	6.20bB	7.04aA	3.12bE	3.22bE	3.91aD	3.67aD
K exc (meq $100g^{-1}$)	0.95cC	0.9cC	1.24bB	1.39aA	0.96aC	0.79bD	0.89bC	0.79bD
Na exc (meq $100g^{-1}$)	0.18bB	0.19bB	0.19bB	0.34aA	0.14bBC	0.17aB	0.10cC	0.18aB

Table 5.2 Summarized results of two-way ANOVAs for all analyzed parameters in the two soils. Different ratio and doses of amendment and mineral fertilization were the independent variables. P-value from Duncan test; *=significant difference.

Parameter	Source	Sum of Squares	df	F	P values
pH	Treatment	0.73	1	20.32	<0.0001*
	Sampling	0.01	1	0.40	0.156
	Interaction	0.00	1	0.11	0.736
EC	Treatment	0.00	1	2,17	0.149
	Sampling	0.01	1	21.19	0.000*
	Interaction	0.00	1	0.14	0.715
Limestone	Treatment	5372.44	1	102.66	<0.0001*
	Sampling	65.44	1	1.25	0.266
	Interaction	50.49	1	0.96	0.329
CEC	Treatment	1825.53	1	77.94	<0.0001*
	Sampling	71.88	1	3.07	0.083
	Interaction	157.06	1	6.71	0.011*
P olsen	Treatment	3949.72	1	116.68	<0.0001*
	Sampling	203.38	1	6.01	0.016*
	Interaction	30.54	1	0.90	0.345
Ca	Treatment	1519.25	1	48.90	<0.0001*
	Sampling	7.88	1	0.25	0.616
	Interaction	14.65	1	0.47	0.494
Mg	Treatment	92.04	1	103.61	<0.0001*
	Sampling	0.95	1	1.07	0.304
	Interaction	1.77	1	1.99	0.162
K	Treatment	1.64	1	53.32	<0.0001*
	Sampling	0.05	1	1.59	0.210
	Interaction	0.20	1	6.64	0.012*
Na	Treatment	0.02	1	9.46	0.010*
	Sampling	0.02	1	7.38	0.019*
	Interaction	0.00	1	0.17	0.687
C org	Treatment	554.69	1	926.96	0.000*
	Sampling	0.99	1	1.65	0.202
	Interaction	3.26	1	5.44	0.022*
OM	Treatment	499.09	1	44.86	<0.0001*
	Sampling	2.96	1	0.27	0.607
	Interaction	7.63	1	0.69	0.410
Cmic	Treatment	6456.571	1	41.57	<0.0001*
	Sampling	511.15	1	3.29	0.08*
	Interaction	829.643	1	5.34	0.03*

5.3.2 Enzymatic activities

Figures 5.2 and 5.3 show the activities of the soil enzymes measured in the soils collected in both the farms under organic and conventional management.

The activity of DHY, GLU and PHO appeared higher in the soil plots of organic farm in both the years of experiment respect to soil plots of conventional farm (Figures 5.2 and 5.3). The activity of DHY was markedly affected by the organic carbon content of soils in organic farming (in average 13 g kg^{-1}, Table 5.1) above described. The large difference between the organic carbon of these soils with that measured in soils under conventional farming (in average 9 g kg^{-1}, Table 5.1) determined in these latter a reduced activity of DHY, being these enzymes strictly related to organic material including also microbial biomass. A reduction of the activity levels was detected in conventional soils after the crop production Such a phenomenon was not observed in organic soils as the sink of organic matter, present in organic soils, could have guaranteed the constant presence of necessary substrates to be degraded.

The activity level of GLU was slightly higher in the plots cultivated under organic system in the second year (Figure 5.2a), to indicate the important role, also for this soil enzyme, of organic carbon, more abundant in these plots than in the plots cultivated in the first year (Table 5.1). In fact GLU is enzyme able to degrade cellulose and when higher organic matter higher substrate is available for this enzyme.

Even he PHO enzymes were more active in soil plots under organic farming. The determinant factor, maybe more than the influence of organic matter, was the lower content in available phosphorous, since high level of this anion could inhibit the activity of PHO, as it could be happened in soils under conventional farming (phosphorous was in average 43 g kg^{-1}, Table 5.1) . Being reduced the phosphorous content in organic soils (Table 5.1) higher the PHO activity was. It is interesting to note that in the second sampling of the second year in organic farm soil, when a higher phosphorous content was measured (38.85 g kg^{-1}, Table 5.1), the PHO activity strongly decreased, as evidence of the phenomenon hypothesized above.

The activity of INV showed a similar trend in both soils (Figure 5.3): the values of glucose formed during the enzymatic reaction did not differ because of the agricultural management, but a common decrease was observed in all soils collected after tomato harvest respect to those sampled in pre-transplanting (even until 89%).

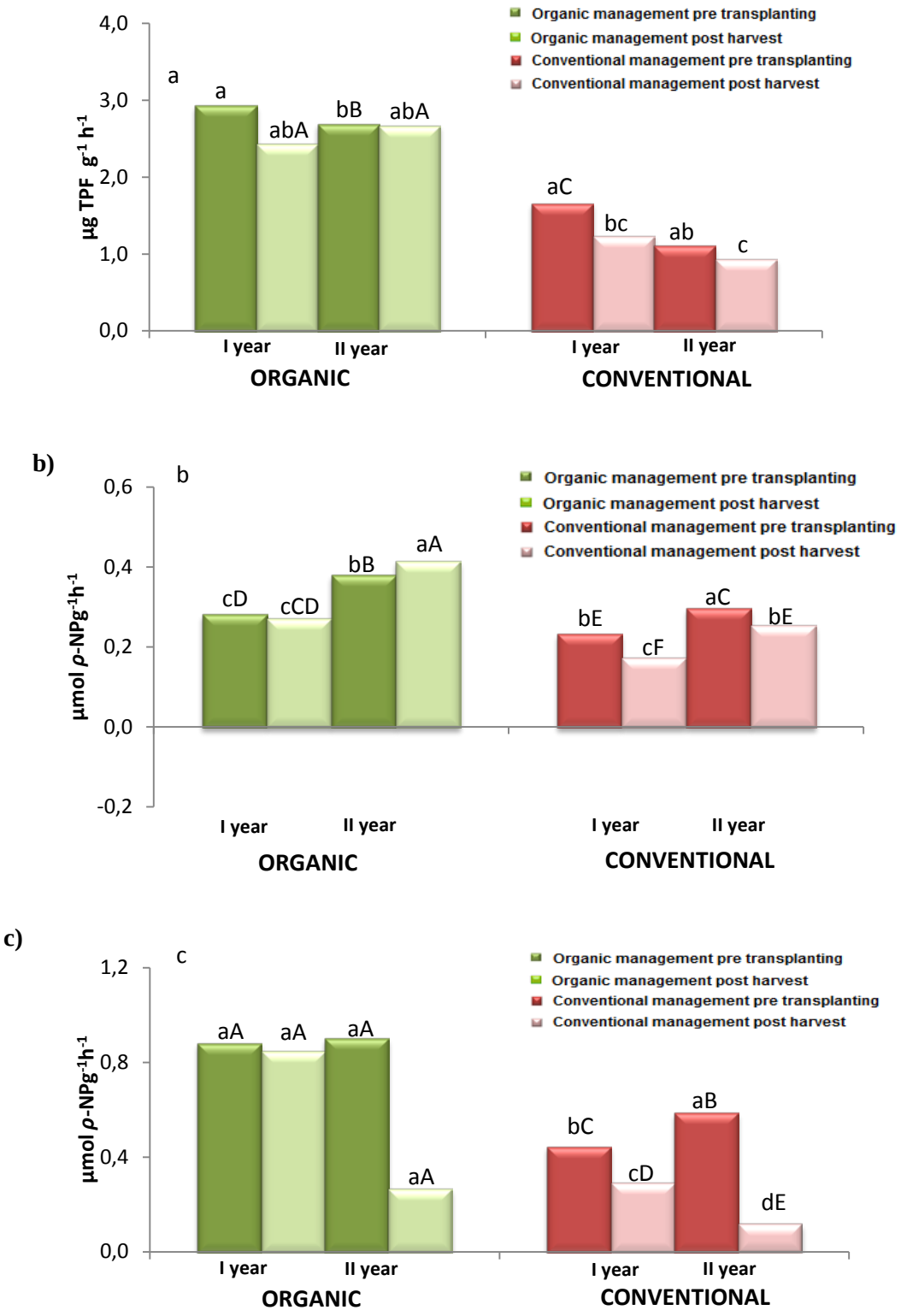

Figure 5.2 Soil enzyme activities in cultivated soil with tomatoes crop affected by conventional and organic treatments. a) DHY= Deshidrogenase activity; b) GLU= β-glucosidase activity; c) PHO= Phosphatase activity. Different lowercase letters indicate significant differences (P≤0.05 from Duncan test) between treatments and uppercase letters indicate significant differences across time.

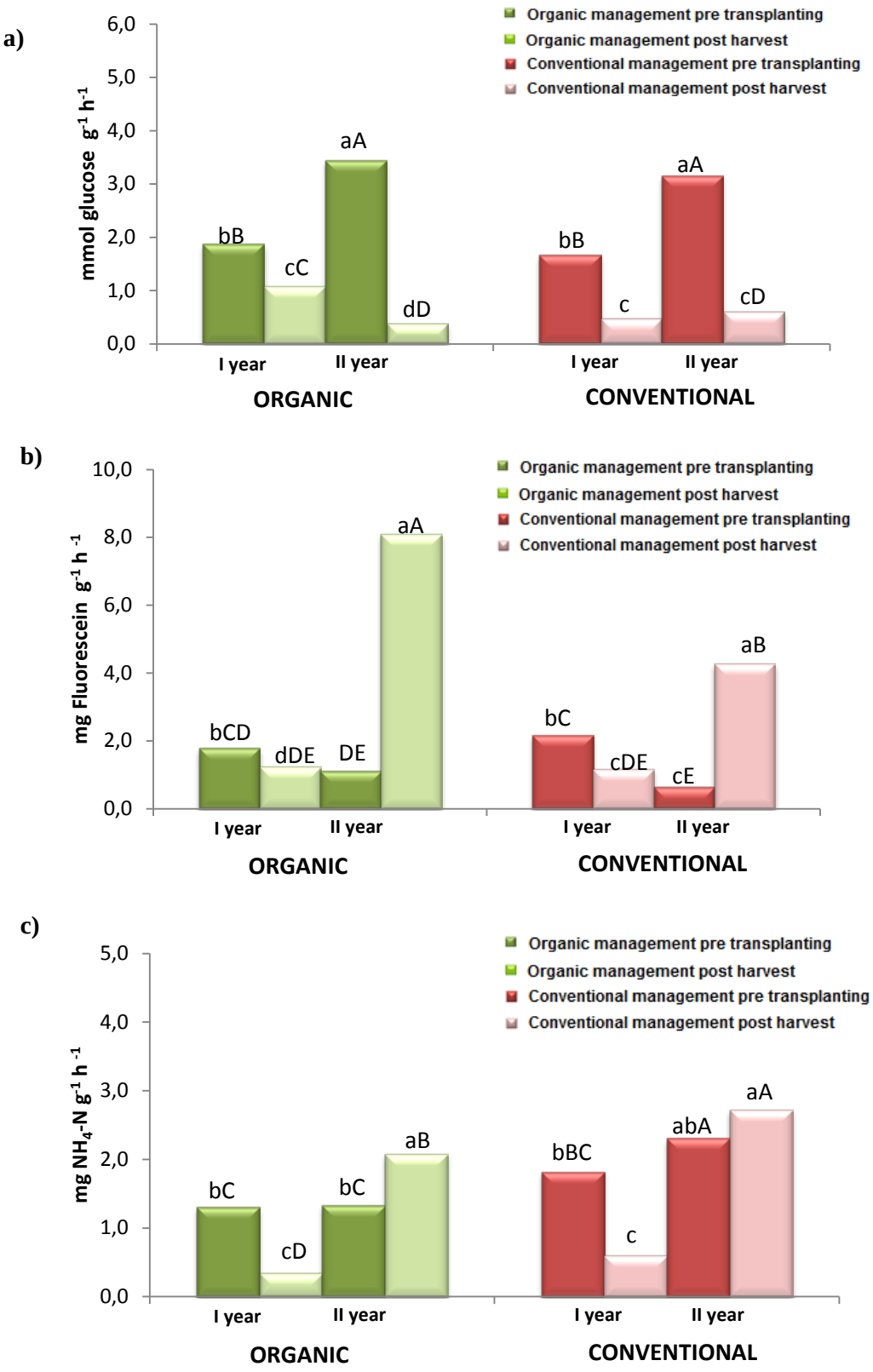

Figure 5.3 Soil enzyme activities in cultivated soil with tomatoes crop affected by conventional and organic treatments. a) INV= Invertase activity ; b) FDAH= Fluorescein diacetate hydrolases activity;. c) UR= Urease activity. Different lowercase letters indicate significant differences (P≤0.05 from Duncan test) between treatments and uppercase letters indicate significant differences across time.

The activity of FDA hydrolases remained unchanged during the first tomato cultivation cycle in both farms, whereas a marked increase was observed after harvest, growing until 8 and 4 mg fluorescein g^{-1} h^{-1} in organic and conventional soils, respectively, corresponding to a seven-fold increase.

Also the UR activity showed a similar trend in soils of both the farms (Figure 5.3). Similar activity levels in the first year with a strong depletion in post-harvest were registered, whereas in the second year a significant increase only in the organic farm was observed.

Statistical analysis by two-way ANOVAs showed that all enzymatic activities were influenced by treatment (Table 5.3) exclusive of INV.

Table 5.3 Summarized results of two-way ANOVAs for enzymatic activities analyzed parameters in the two soils. Different ratio and doses of amendment and mineral fertilization were the independent variables. P-value from Duncan test; Significant difference=*

Parameter	Source	Sum of Squares	df	F	P values
DHY	Treatment	75.34	1	355.80	<0.0001*
	Sampling	1.64	1	7.75	0.006*
	Interaction	1.64	1	7.74	0.006*
PHO	Treatment	4.75	1	115.70	<0.0001*
	Sampling	3.73	1	90.97	<0.0001*
	Interaction	0.01	1	0.13	0.718
GLU	Treatment	0.34	1	75.22	<0.0001*
	Sampling	0.01	1	3.10	0.081
	Interaction	0.04	1	7.88	0.006*
FDA	Treatment	35.68	1	6.49	0.012*
	Sampling	185.08	1	33.65	<0.0001*
	Interaction	32.46	1	5.90	0.016*
INV	Treatment	1.70	1	3.35	0.069
	Sampling	130.59	1	256.72	<0.0001*
	Interaction	0.02	1	0.04	0.846
UR	Treatment	12.69	1	10.42	0.002*
	Sampling	2.85	1	2.34	0.128
	Interaction	0.83	1	0.68	0.412

The enzymes involved in the C cycle and in relationship with both organic matter evolution and Cmic, such as DHY, GLU (Figure 5.2a and b) and INV (5.3a), and the PHO (5.2c), involved in P mineralization, showed values significant highest in organic management respect to conventional management ($P < 0.05$). In contrast, the UR activity (5.3c) showed highest values under conventional management and the FDAH activity (Figure 5.3b) reached the highest values in the second year plots, in post-harvest, under organic management, but no significant differences due to treatments was highlighted.

5.3.3 Microbial carbon biomass

The microbial biomass C (Cmic) showed significant higher values under organic management than under conventional farming (Figure 5.4). The Cmic content in soil plots under organic management, collected at pre-transplanting in the first year, was 74,18 mg 100 g^{-1} of dry soil (6% of soil organic carbon) and for conventional management at the same sampling Cmic content was 19 mg 100g^{-1} (2% of soil organic carbon) denoting a content around three-fold higher in soils under organic management than those under conventional regime.

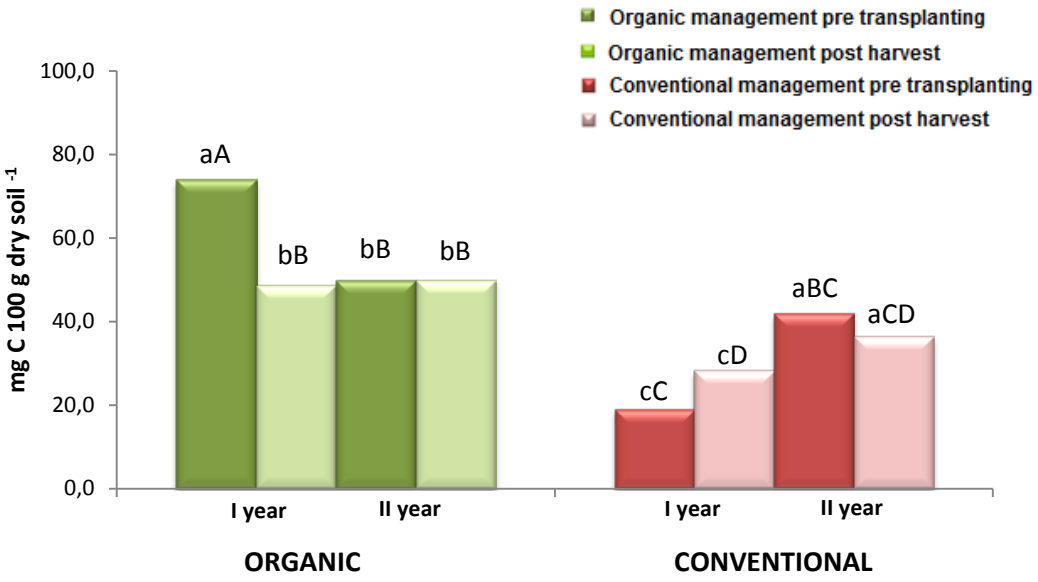

Figure 5.4 Soil microbial biomass C in soils under organic and conventional management collected at pre-trasplanting and post-harvest in two subsequent years. Different lowercase letters indicate significant differences (P≤0.05 from Duncan test) between treatments and uppercase letters indicate significant differences across time.

In the further samplings the difference between soils under different management reduced. The values of Cmic for organic management decreased down to 3.87%, 3.54% and 3.05% of organic carbon for plot soils collected at post-harvest in the first year, at pre-transplanting and post-harvest of second year, respectively (Figure 5.4). These behavior is in agreement with findings that state that the microbial biomass carbon in most soils represents about 1-4% of total soil organic carbon (Anderson and Domsch 1989). The value of Cmic equal to 6% of organic carbon represents an exception, but supported by the higher DHY activity observed in the same soil samples (Figure 5.2). Cmic is considered to be a more sensitive indicator of soil changes than total organic carbon because it is strictly related to soil microorganisms that in turn are very sensitive to soil disturbs (Liu et al. 2003; Wang and Gong 1994).

Microbial activity was measured as soil potential respiration and the results were reported in Figure 5.5. Very similar respiration levels were observed in both sets of soils and also a little and

significant increase at post-harvest sampling was registered in any case and in both the years. A seasonal effect, related to summer temperature, higher than those in spring time, likely influenced the microbial activity intensifying the mineralization process and therefore the resultant soil respiration.

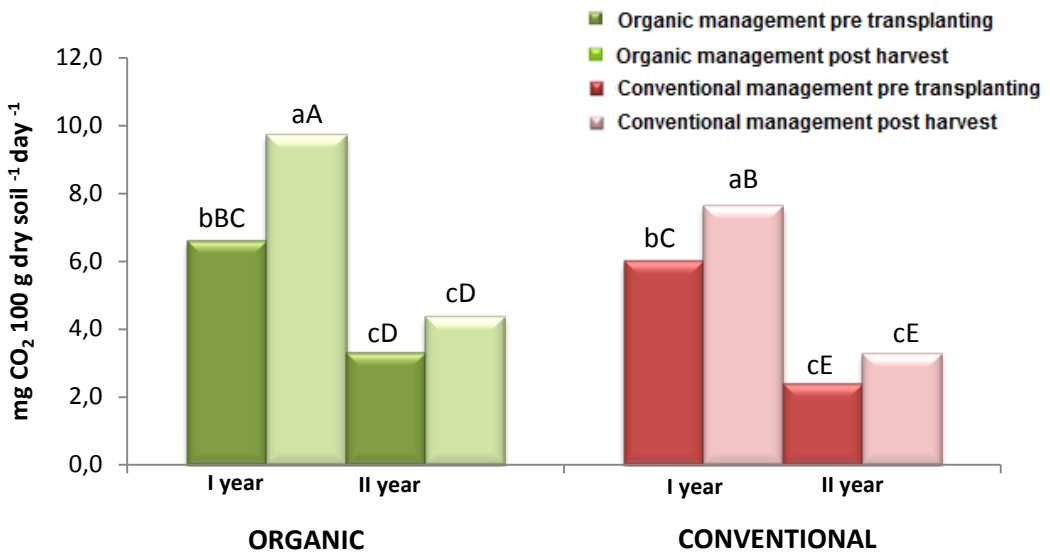

Figure 5.5 Soil respiration in soils under organic and conventional management collected at pre-trasplanting and post-harvest in two subsequent years. Different lowercase letters indicate significant differences (P≤0.05 from Duncan test) between treatments and uppercase letters indicate significant differences across time.

5.3.4 Microbial community composition

The designed primers were tested using template DNA from soil samples. PCR products were loaded in DGGE gel (Figure 5.6). The gel showed the lines 1 to 6 corresponding to the samples of DNA extracted from the soils under conventional management, and the lines 7 to 12 corresponding to the samples of DNA extracted from the soils under organic management.

The composition of the bacterial community of soils under both regimes was determined by image analysis of 16S rRNA gene by Phoretix 1D Software Advanced Package (Non Linear Dynamics, Newcastle, UK).

The UPGMA dendrogram (Figure 5.6 panel a) and Bray-Curtis measure (Figure 5.6 panel c) show two distinct clusters corresponding to the two agricultural management types (conventional and organic). In fact, the dendrogram showed a great difference between the bacterial communities of soil under conventional management and those extracted from soil under organic management (p<0.03), in both framing the cluster are grouped together showing more similarity.

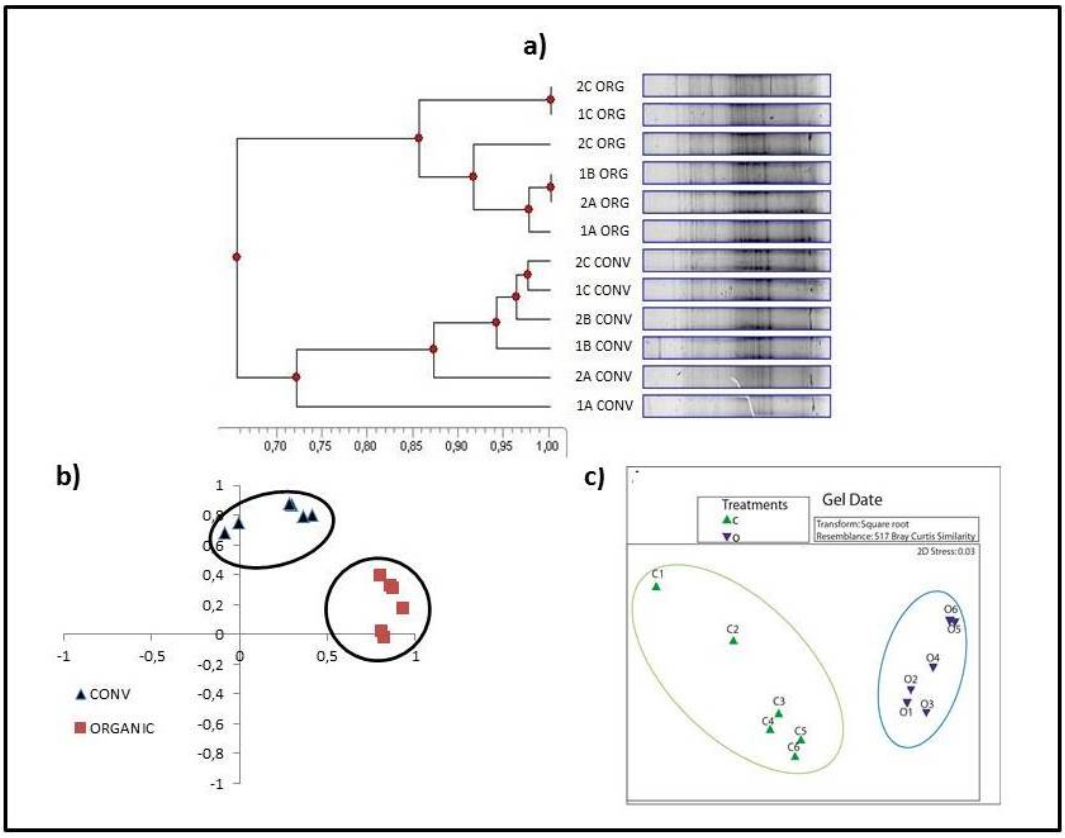

Figure 5.6 Denaturing gradient gel electrophoresis (DGGE) analysis; a) dendrogram of bacterial communities under organic and conventional management; b) Principal Component Analysis (PCA); and; c) multidimensional scaling of DGGE profiles by Bray Curtis measure.

Similarly, the Bray-Curtis index (Figure 5.6 panel c) showed that the communities C (from conventional soils) were clearly separated from the communities O (from organic soils), and each cluster, O or C, was grouped together showing more similarity within themselves.

Principal Component Analysis (PCA) of all results related to microbial structure was reported in Figure 5.6 panel b. The percentage of the data variations is explained by the components: the conventional management of soil samples were influenced by axis or component 1 (40.436%), and all organic management of soil samples were influenced by axis or component 2 (35,142%); in addition the agricultural management type explain the 75,58% of the variation of soil microbial communities. This data variation in the banding patterns and dendrogram revealed that the bacterial communities of soil under organic management differed from those of soil under conventional management.

The component 1 was associated to organic amendment (explained 40% of variation), whereas the component 2 was associated to mineral fertilization (explained 35% of the data variation). Therefore, the data strongly suggest that the principal drivers of the changes in bacterial community composition were the agricultural management, which explained until 75% of the data variation.

5.3.5 Realtive abundance of β - propeller phytase gene

Soil chemical analysis showed significant differences soil under different management (Table 5.1). Soil under organic management had lower concentrations of available P (26 mg kg^{-1}) than soils under conventional management (45 mg kg^{-1}). Also the total limestone showed significant differences between the soils as those under organic management showed 23.8 g kg^{-1} whereas those under conventional management 4.0 g kg^{-1}, denoting a calcaric nature of conventional farm soil.

The Real Time PCR of BPP gene showed the presence of this gene in all DNA samples. The relative abundance based on the double delta Method (Livak and Schmittgen, 2001) was calculated (Figure 5.3.5), using the efficiency of the primers described by Jorquera et al., (2012). The lowest signal ($p \leq 0.05$) of BPP (2.43 was the value of ratio abundance BPP/16S rRNA) was observed in conventional management. In contrast, the highest abundance ($p \leq 0.05$) of the *Bacillus sp.* BPP signal gene was observed in soils under organic management than in soils collected from conventional farm (14.95 was the value of the BPP/16S rRNA ratio abundance).

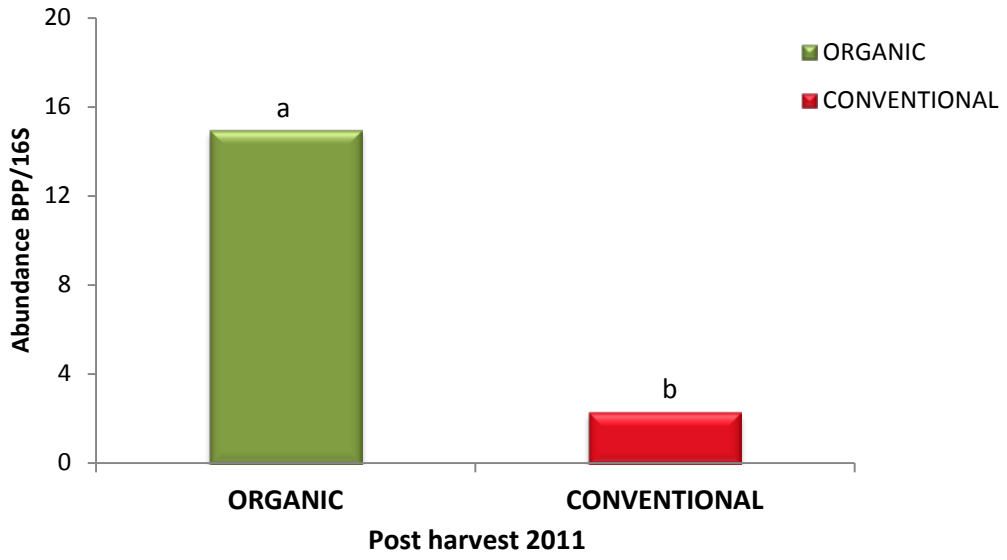

Figure 5.7 Quantification of BPP gene relative to total 16S rDNA gene of the organic and conventional managements. Different letters denote statistical difference ($p \leq 0.05$).

5.4 Discussion

Conventional agriculture with intensive tillage and high inputs of synthetic chemicals has critically depleted the soil C pools. Alternative practices such as no-tillage and organic inputs have been shown to increase soil C content. This study showed a general increment of soil quality due to a general significant increasing of chemical and biochemical indicators, as well as differences in the microbial populations of soil under organic management and the abundance of the phytase gen from *Bacillus sp.* under organic management.

Organic agricultural management is expected to have a higher C sequestration potential, which explains the high values of Corg in organic plot registered in both the experiment years. Highest C/N ratio was also observed for organic management in according to Santo et al. (2012) leading to positive and beneficial effects on the microbial growth and activity. The organic plots also had a higher soil N in according to Melero (2006) and Moeskops et al. (2010). Application of organic fertilizer reduced nitrate leaching from soil as the N release from finished composts is relatively slow (Shiralipour, 1992).

Soil salinity was affected by the agricultural management: electrical conductivity (CE) was greater when soil was organically managed than conventionally as Melero (2006) and Shiralipour (1992) also found. Soil exchangeable Ca^{+2}, K^{+}, Mg^{+2} contents were higher in the organic farm that showed significant positively correlation with Corg. Soil chemical properties, except for exchangeable Na (García- Ruíz, 2009) and pH (Melero, et al., 2006) were different among farm soils. In fact they vary as a function of soil type and agricultural management. Melero et al. (2006) and Shiralipour (1992) reported higher CEC values in organic managed soil and a significant positively correlation with Corg, Similarly Bulluck III et al. (2002) found significant positively correlation between Corg and Ca^{+2}, K^{+}, Mg^{2+}, Mn, and CEC evaluating organic systems.

Among nutrients available P was significantly affected by conventional farming as explained by Reganold, et al. (2010), in according to those this nutrient could widely response to mineral fertilization, and thus to present highest values in conventional plots. Nevertheless the soil response is related also to intrinsic soil characteristics, in particular texture and lime content. Van Diepeningen et al, (2006) found that the phosphate content in organically cultivated soils was lower in the clay soils than in the sandy soils. The presence of calcaric soil could also affect the availability of phosphorous that remained immobilized as calcium phosphate. The clay mature of sol could determine the innersphere interactions between clay minerals and phosphate anion, leading to their very strong fixation.

The organic management respect to conventional one highlighted significant differences in the values of biomass carbon (Cmic) and enzymatic activities of the studied soil. In general organic system produced a positive effect on oxidoreductases such as the intracellular DHY and hydrolases

such as the extracellular PHO and GLU. They are enzymes related to the cycling of the main biologically important elements, C and P. Numerous authors have reported that the adoption of organic norms for soil cultivation enhances microorganisms to produce enzymes related with the cycle of the most important nutrients (Madejón et al., 2001; Marschner et al., 2003; Dinesh et al., 2004, Crecchio et al., 2001 e 2004) and that these enzymatic activities are positively correlated with biomass carbon.

The microbial biomass contained in the organic fertilizers and the addition of substrate-C could account for the increase of biomass carbon in organically fertilized soils. This dual effect of organic fertilization has been also reported by several other authors in different conditions (Masciandaro et al., 1997; Schjonning et al., 2002). The increase of microbial biomass was mainly due to the addition of substrate-C, which stimulates the indigenous soil microbiota, as confirmed by prior analyses. Other authors have reported a similar dual effect on soil biomass (Diaz et al., 1994; García et al., 1998; Perucci, 1993; Santos et al., 2012).

Melero et al. (2006) reported higher values of respiration under organic management in a silt loam soil in Mediterranean conditions, indicating higher soil microbial activity, related also to seasonal differences. This explanation could be accounted for the highest values of respiration registered at post-harvest, at end of august, in the two years under organic management. Contosta et al. (2011) found higher values of soil respiration in summer associated with higher levels of nitrogen in soil, a fine loamy soil from forest. The season effect could help to understand the high values of total nitrogen at post-harvest in the first year, compared with the other sampling stage.

Microbial biomass and soil enzyme activities had been successfully applied to evaluate the effects of organic and mineral fertilization on the microbiological status of different soil types and climates (Giacometti et al., 2013; Melero et al., 2011; Pajares et al., 2009). These authors demonstrated that organically managed soils exhibited higher enzymatic activity levels than the conventionally managed soils, in agreement with other several researchers (Benitez et al., 2006; Diepeningen et al., 2006; Mäder et al., 2002; van Melero et al., 2006).

In particular, in this study soil enzymes as dehydrogenase and β-glucosidase, that are involved in the C-cycle, showed higher values due to organic amendments, as corroborated by ANOVAs two way (Table 5.3.3) and one way (Table 5.3) analysis. Similar results about of DHY and GLU were also reported by Bandik and Dick (1999) who observed higher enzymatic activities in compost amended plots referring to higher organic matter content and relatively higher biomass carbon. In this study both these enzymatic activities were significant positively correlated with Corg, and DH was significant positively correlated also with Cmic (Table 5.4). Dehydrogenase, an valid indicator of microbiological activity involved in oxidoreductive processes (Alef and Nannipieri, 1995; Wlodarczyk et al., 2002), has been found to decrease in soils treated with agrochemicals (Reinecke

et al., 2002). In fact the possible negative effect that herbicides or pesticides could exhibit on soil enzymes as inhibitors should be also taken into account. These synthetic molecules usually enter on the list of treatments, permitted under conventional farming, so their possible influence on soil biochemical and biological properties could be not excluded.

ANOVAs two ways indicated that INV was the only enzymatic activity not affected by treatment, organic and conventional management (Figure 5.3). Differences found by ANOVA one way analysis can be related only to the sampling stage: in fact this activity was higher at the pre-transplanting in both two years. Higher values of INV, in April, in paddy soil under organic management were also registered by Lopes et al. (2011).

Aseri and Tarafdar (2006) defined FDAH a good indicator of soil quality being correlated with soil physicochemical, biochemical, and microbiological properties. In the present study a particular behavior of FDA hydrolases was observed as in the post-harvest a very high activity level was observed in both organic and conventional soils (Figure 5.3). At the same stage some chemical parameters such as Corg, C/N ratio, exchange basis, CEC, pH, EC showed higher levels compared with the others samplings. Enhanced availability of labile C and others nutrients in post-harvest, due possibly to crop residues, could promote higher activity by microorganisms growing near roots where carbohydrates, amino acids and organic acids are in root exudates (Kong et al., 2008). Although associated with microbial activity, FDA hydrolysis is carried out by a range of enzymes including extracellular enzymes that can persist in the soil as part of inorganic complexes or when associated with organic colloids (Nannipieri et al. 2002).

Table 5.4 Pearson correlation coefficients between chemical and biochemical parameters in soil samples.

Parameters	pH	EC	Corg	N	C/N	M.O.	Pols	LMS	CEC	Ca	Mg	K	Na	Cmic	Resp	DHY	PHO	GLU	INV	FDAH
pH																				
EC	0,25																			
Corg	0,886**	0,21																		
N tot	0,34	0,26	0,66																	
C/N	0,51	-0,27	0,44	-0,17																
M.O.	0,862**	0,13	0,984**	0,65	0,45															
P olsen	-0,46	0,02	-0,74	-0,93	-0,04	-0,73														
LMS	0,30	0,20	0,57	0,912**	-0,01	0,58	-0,85													
CEC	0,836**	0,37	0,874**	0,36	0,58	0,858**	-0,37	0,35												
Ca^{2+}	0,802*	0,01	0,786*	0,10	0,772*	0,781*	-0,25	0,08	0,904**											
Mg^{2+}	0,896**	0,16	0,887**	0,26	0,64	0,879**	-0,36	0,18	0,939**	0,962**										
K^{+}	0,961**	0,29	0,815*	0,17	0,51	0,792*	-0,26	0,11	0,876**	,864**	0,933**									
Na^{+}	-0,18	0,31	0,04	0,39	-0,23	0,15	-0,12	0,47	0,14	-0,15	-0,08	-0,18								
Cmic	0,43	-0,04	0,68	0,724*	0,47	0,67	-0,81	0,835**	0,50	0,41	0,41	0,25	0,14							
Resp	-0,57	-0,54	-0,40	-0,15	-0,10	-0,25	0,18	-0,04	-0,35	-0,26	-0,36	-0,52	0,56	-0,15						
DHY	0,755*	0,35	0,897**	0,865**	0,12	0,879**	-0,86	0,815*	0,69	0,47	0,62	0,62	0,20	0,718*	-0,40					
PHO	0,37	-0,06	0,54	0,730*	-0,05	0,46	-0,87	0,61	0,11	0,07	0,17	0,16	-0,32	0,64	-0,48	0,67				
GLU	0,864**	0,05	0,837**	0,21	0,65	0,810*	-0,36	0,17	0,895**	0,962**	0,947**	0,906**	-0,24	0,44	-0,39	0,59	0,23			
INV	0,26	-0,38	0,17	0,00	0,24	0,07	-0,29	-0,05	-0,06	0,16	0,11	0,16	-0,90	0,26	-0,54	0,11	0,66	0,30		
FDAH	0,41	0,28	0,33	-0,12	0,38	0,40	0,22	0,00	0,69	0,60	0,57	0,56	0,46	-0,02	0,19	0,17	-0,58	0,52	-0,58	
UR	-0,02	-0,36	-0,21	-0,62	0,46	-0,18	0,50	-0,37	0,11	0,32	0,09	0,13	-0,24	-0,15	0,23	-0,38	-0,51	0,30	0,10	0,47

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

EC = Electrical conductivity (dS m^{-1}); Corg = Organic Carbon (g kg^{-1}); N= Nitrogen (g kg^{-1}); C/N= Carbon Nitrogen Ratio; M.O.=Organic matter (g kg^{-1}); Pols= Phosophorus olsen (mg kg^{-1}); LMS= Limestone (g kg^{-1}); CEC= Cation exchange capacity (cmol $(_{+})$ kg^{-1}); Ca^{+2}= Calcio (meq 100 g^{-1}); Mg^{+2}= Magnesio (meq 100g^{-1}); K^{+}= Potassium (meq 100 g^{-1}); Na^{+}= Sodium (meq 100 g^{-1}); Cmic= Microbic Carbon (C mg 100g dry $soil^{-1}$); Resp= Microbial soil Respiration (CO_2 mg 100 g dry $soil^{-1}$ d^{-1}; DHY= Dehydrogenase activity (µg TPF g^{-1} h^{-1}); PHO= Phosphatase activity (µmol ρ-$NPg^{-1}h^{-1}$); GLU= β- Glucosidase activity (µmol ρ-$NPg^{-1}h^{-1}$); INV= Invertase activity (mmol glucose g^{-1} h^{-1}); FDAH= Fluoresceine Diacetate Hydrolase activity (mg Fluorescein g-1 h -1); UR= Urease

The values of UR were higher in soils collected in the conventional farm (Figure 5.3) The urease activity plays an important role in the mineralization of nitrogen. García-Ruiz et al. (2008) reported that the significantly higher potential nitrification in the conventional farms is related with the long-term application of ammonium or urea as the main fertilizer source, and they take into account the presence of an important community of autotrophic nitrifying bacteria to explain the higher values in the conventional management. Urease activity is often considered as indicator of organic N mineralization although the enzyme is involved in urea hydrolysis and urea is not a component of soil organic N, (Nannipieri, 2011). Garcia-Gil et al. 2000, reported the diminution of urease activity of soil under organic management released by large content of NH_4^+ (a urease inhibitor) produced by organic fertilizers.

In both management soils, population diversity was detected. Conversely, the diversity may help support the resulting ecosystem function or biogeochemical process in a broader range of environmental conditions (Zak et al., 2006) or in changing environments (Loreau et al., 2001). Our findings of greater enzyme activities in organically managed soils indicate a greater functional capacity. Greater functional gene abundance in organically managed soils indicates a larger functional population. Greater functional gene diversity in organically managed soils suggests that organic systems may also support more stable or resilient ecosystem functions.

This study demonstrated through DGGE of 16S rRNA gene that the organically managed soils exhibited differences than the conventionally managed soils. In particular, this may be explained for the Shannon index (Shannon et al. 2002) that point out on the greater physiological diversity of microorganisms in organic soil.

According to van Diepeningen (2006) the change in the biodiversity detected by DGGE analysis between conventional soil management and organic soil management was observed. The sequence of bands will be of great importance to determinate the bacterial species present in the soil under different fertilization type.

The organic management respect to conventional produced significant differences on the bacteria populations including some specifics groups as B*acillus sp* evidenced by phytase. In this study, we adopted Real Time PCR to evaluate the occurrence of BPP genes in soils under organic and conventional management in South Italy. The method was used to examine the relative abundance of BPP in the both agricultural systems of soil. Our results are generally in agreement with findings reported in the literature that demonstrate that organic soil management enhance the abundance and diversity in the rhizosphere and bulk soil of organic management (Shu, et al. 2012). In addition these authors also reported analysis of variance and canonical correspondence analysis (CCA) demonstrating that C/N, C and N are important factors influencing the abundance and community structure of bacterial populations.

Similar results were found by Reganoldet al. (2010) in a study of soil quality in an agrosystem for strawberry crop, where 11 functional groups were involved in the main biogeochemical cycling, but functional groups of phosphatase and phytase did not found.

The effect of organic fertilizer additions on diversity or activity of soil bacterial communities has received less attention, specially the bacterial groups involved in the P mineralization process. This study demonstrated that the soil cultivated under organic farming had a marked relative abundance of phytase respect to the soil cultivated under conventional management; thus suggesting that the soil collected in organic farm have abundance of microorganism able to increase the concentrations of soluble P, either by degrading phytate or by producing organic acids that solubilize mineral phosphates. This is a real problem above all for P availability in alkaline soils, frequent among Mediterranean soils, as P fertilizers react with calcium to form insoluble calcium phosphates (Mullen, 2005) and accumulate as organic phosphates, primarily as phytate, which can comprise from 10% to 50% of the total P in both acid and alkaline soils (Mullen, 2005; Turner et al., 2002).

The large differences in soil microbial properties and soil functional gene abundance and diversity between the organically and conventionally farmed soils are most likely due to a combination of factors: chemical fumigation of the conventionally farmed soils, increasing level of organic matter in organic farming and lack of synthetic pesticide use on the organic fields. Several studies have documented changes in microbial diversity due to fumigants and pesticides (Zelles et al., 1997; Engelen et al.,1998; Ibekwe et al., 2001) and have shown that the use of pesticides could alter some microbial properties and enzymatic functions.

5.5 Conclusions

The soils collected in the organic farming La Morella e in the conventional farm CRA-Ort showed mainly a significant difference in organic carbon content and in enzymatic activity level. The organic farming led over time to stock and recover organic matter, after the crop uptake. The low content in organic carbon in soil from conventional farm demonstrated that the almost exclusive use of chemical fertilizer was not able to solve this problem.

All soil enzymes showed the positive influence of the organic farming in particular those involved in organic matter degradation through oxidoreductive and hydrolytic processes. Seasonal effect, due to temperature and moinsture changes, was also higlighted.

The organically farmed soils had more biomass carbon, greater microbial biomass and activity, and greater functional gene abundance and diversity which indicate an improvement of soil fertility through organic fertilization. This study demonstrates that soil DNA analyses using microarray technology can be used as an additional measurement of soil quality.

The different agricultural management of soils influenced the diversity of microbial communities and the microorganism amount related to process of nutrients cycle.

The results obtained in this study hold the importance of the incorporation of organic management and to improve the quantity and quality of organic matter in Mediterranean soil, especially with high levels of total calcareous, which are characterized by low organic matter content.

The Phytase gene form *Bacillus sp.* amount in the plots under fertilization organic soil is major, this suggest that the soil under organic management would to have the ability of to increase the concentrations of soluble P, situation of real importance for P availability in alkaline soils in the case of Mediterranean Soils. This study also revealed that Bacillus BPP gene is cosmopolitan in southern Italy soils. Future research should evaluate changes in the prevalence and expression of genes encoding other known bacterial phytases and should develop more accurate approaches for determining phytase activity in soil, including the design of degenerate primers for detection and characterization of unknown or native b-propeller phytases.

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6 Concluding remarks

The sustainable agriculture strategies could provide relevant advantages for the environment and human health. The reduced and careful use of fertilizers and chemicals in agricultural management would be already the first step in the road-map leading to more sustainable anthropic activities. Having the higher purposes in order to ameliorate environmental compartments such as soil water and air, over time, the change of conventional practices with more innovative and low-impact strategies could be helpful.

The first study carried out on the effect of organic amendments enriched with low-degradation material, such as wood scraps, on the fertility of agricultural soils under intensive management and greenhouse system gave interesting results on the long-term effect of these agricultural practices. Control soils that represent the usual management of intensive agriculture, showed a substantial loss of fertility, in terms of available nutrients, reduction of organic matter and depletion of enzymatic activities, whereas amended soils kept a suitable nutritional level over time, characterized by a suitable amount of organic carbon, nutrients and biochemical activity in terms of enzymatic activities. Specific enzymatic index supported analytic results demonstrating the real improvement of agricultural soils treated with these different mixtures of compost and wood scraps.

The second study regarding the assessment of possible advantages due to organic management of agricultural soils cultivated with processing tomatoes, was a part of a main research project having as the principal purpose to assess the nutritional and qualitative characteristics of tomatoes and processed products. It was mandatory to check the production chain starting from the first step and consequently from the first environment compartment involved, that is soil. Organic management of these soils allowed to highlighted the higher fertility of organically managed soils considering chemical, biochemical as well as biological properties. Higher organic carbon content, higher C/N ratio, higher activity level of the main soil enzymes involved in making available plant nutrients, higher biodiversity of microbial biomass, contributed to guarantee all those chemical and biochemical processes necessary to keep soil fertility.

Therefore it is clear that changes in agricultural managements toward more sustainable practices can allow to reach interesting results about environmental protection and also about agronomic aspects strictly related to crop yields and cost management, especially through a long-term view.