University of Naples Federico II
Faculty of Agriculture

Joint international Ph.D. Italy - Chile in
Environmental Resources Sciences
XXIII Cycle (2007-2010)

Effects of sustanaible soil management on soil quality

Ph.D. Dissertation
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"Ci sono soltanto due possibili conclusioni:
Se il risultato conferma le ipotesi, allora hai appena fatto una misura.
Se il risultato è contrario alle ipotesi, allora hai fatto una scoperta"
Enrico Fermi

"There is still a difference between something and nothing,
but it is purely geometrical and there is nothing behind the geometry"
Martin Gardner

All'unica persona che attraverso il suo impegno
ed il suo lavoro ha reso possibile tutto questo.

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Chapter 1
Introduction

The continuous growth of global food needs, the necessity to maintain low food prices, the reduction of tillable lands, the need to till also areas clearly unfavourable has resulted in decisive increase of agriculture intensive type. Nevertheless, the intensification of agriculture and the introduction of some irresponsible agricultural practices have caused severe damage to the environment such as degradation of soils due to the depletion of organic matter (OM), pollution of water by the massive use of chemical fertilizers and pesticides, reduction of biodiversity, etc. (Tilman et al., 2002).

One of the most worrying aspects of intensive agriculture is the gradual loss of OM in soil. Currently, in agricultural soils the cycle of OM is clearly unbalanced towards consumption and mineralization of organic carbon, thus penalizing OM accumulation and humification. However, the balance between accumulation and consumption of OM, which is essential to not compromise the conditions of soil fertility, must be maintained in an agricultural system.

The condition is alarming and without a strategy to restore the content of OM the desertification of arable land could result. Fortunately, the community agricultural policy has become more sensitive to this problem even under the pressure of the public opinion, indeed it is promoting alternatives to conventional agriculture such as organic, eco-friendly and biodynamic farming, and more respectful of environmental not renewable resources. For example in Campania region, Italy, policies have been promoted concerning the development and maintenance of OM levels in soils, with the aim to encourage farmers to adopt management techniques for the conservation of soil organic matrix by using of organic amendments, like compost.

Traditionally, the use of amendments, such as manure and other materials, in agriculture is finalized to improve the OM content in the agro-ecosystem. The aim is to ensure, through the reintegration of humic components progressively mineralized:

a) the conservation of natural fertility in terms of as workability, porosity, aeration, drainage; b) chemical fertility, in terms of as trophic environment suitable for plants, and c) biological fertility, in terms of as the variety and intensity of biogeochemical processes occurring in soil.

1.1 Agricultural soil managements

Soil management concerns all operations, practices and treatments used to protect an agricultural soil and to enhance its performance.

Modern agriculture now feeds 6,000 million people. Global cereal production has doubled in the past 40 years (Fig. 1.1a), mainly from the increased yields resulting from greater inputs of fertilizer, water and pesticides, new crop strains, and other technologies of the 'Green Revolution' (FAO, 2001; Tilman et al., 2001).

This has increased the global food supply per capita (FAO, 2001), reducing hunger, improving nutrition (and thus the ability of people to better reach their mental and physical potential) and sparing natural ecosystems from conversion to agriculture (Waggoner, 1995).

During the last century, intensive agriculture has been the most common soil agricultural management, even if, in the last decades, with the growing environmental awareness in the people, sustainable agriculture rapidly extended.

1.1.1 Intensive agriculture

Intensive agricultural practices determine the level of food production and, to a great extent, the state of the global environment. Agriculturalists are the chief managers of terrestrial 'useable' lands, which can be broadly define as all land that is not desert, tundra, rock or boreal. About half of global usable land is already in pastoral or intensive agriculture (Tilman et al., 2001). In addition, intensive agriculture adds globally significant and environmentally detrimental amounts of nitrogen and phosphorus to terrestrial ecosystems (Vitousek et al., 1997; Carpenter, 1998) at rates

that may triple if past practices are used to achieve another doubling in food production (Cassman and Pingali, 1995; Cassman, 1999).

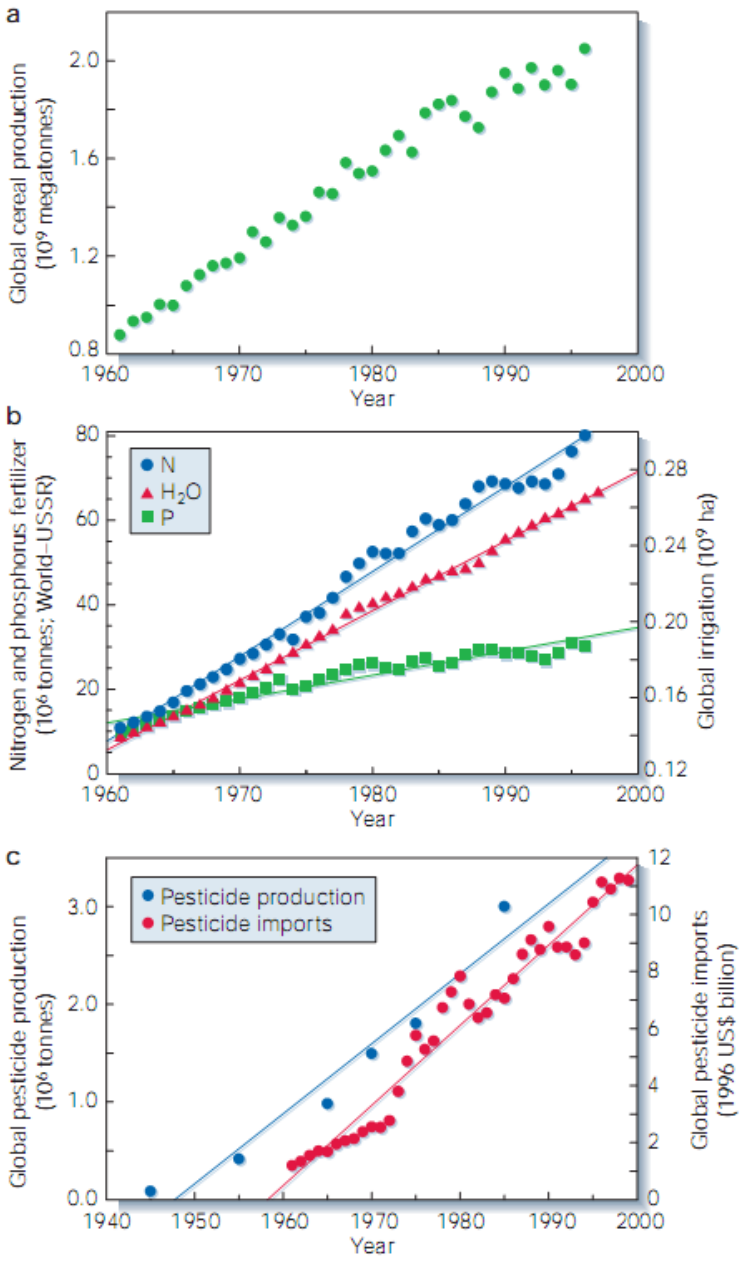

Figure 1.1 Agricultural trends over the past 40 years. a) Total global cereal production. b) Total global use of nitrogen and phosphorus fertilizers and area of global irrigated land. c) Total global pesticide production and global pesticide imports (summed across all countries). (Adapted from Tilman et al., 2002).

Intensive agricultural practices can reduce the ability of ecosystems to provide goods and services. For example, high applications of fertilizers and pesticides (Fig. 1.1b and c) can increase nutrients and toxins in groundwater and surface waters, increasing health and water purification costs (Tilman et al., 2002).

Intensive agricultural practices, moreover can contribute to eutrophication of aquatic habitats thus determining further costs increased fertilization, irrigation, and energy to maintain productivity of the degraded soils (Cassman, 1999). Practices that change species composition or reduce biodiversity in non-agricultural systems, may also diminish goods and services, 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).

The main environmental impacts of intensive agriculture come from the conversion of natural ecosystems to agriculture, from agricultural nutrients that pollute aquatic and terrestrial habitats and groundwater, and from pesticides, especially bioaccumulating or persistent agricultural organic pollutants. Agricultural nutrients enter other ecosystems through leaching, volatilization. Pesticides can also harm human health, as well as pathogens, including antibiotic-resistant pathogens associated with certain animal production practices.

About the use of mineral fertilizers and pesticides, intensive high-yield agriculture is dependent on their addition. In some regions of the world, crop production is still constrained by too little application of fertilizers (Pinstrup-Andersen and Pandya-Lorch, 1996). Without the use of synthetic fertilizers, world food production could not have increased at the rate it did and more natural ecosystems would have been converted to agriculture. Between 1960 and 1995, global use of nitrogen fertilizer increased seven fold, and phosphorus use increased (WHO, 1990; Waggoner, 1995) 3.5 fold (Fig. 1.1b); both are expected to increase another three fold up to 2050 unless there is a substantial increase in fertilizer efficiency (Cassman and Pingali, 1995; Tilman et al., 2001).

This excessive fertilization can cause eutrophication, loss of diversity, dominance by weedy species and increased nitrate leaching or NOx fluxes (Vitousek et al., 1997). Finally, nitrose inputs to agricultural systems contribute to emissions of the greenhouse gas nitrous oxide. Rice paddy agriculture and livestock production are the

most important anthropogenic sources of the greenhouse gas methane (Prather et al., 2001).

Improvements in the control of weedy competitors of crops, crop diseases and pathogens, and herbivores could significantly increase yields. Three cereals — wheat, rice and corn — provide 60% of human food. These crops, derived from once-rare weedy species, have become the three most abundant plants on Earth (Tilman et al., 2002). A central conclusion of epidemiology is that both the number of diseases and the disease incidence should increase proportional to host abundance, and this disconcerting possibility illustrates the potential instability of a global strategy of food production in which just three crops account for a so high proportion of production. The relative scarcity of outbreaks of diseases on these crops is a testament to plant breeding and cultivation practices (Tilman et al., 2002).

Another serious problem of intensive agriculture is frequently tillage. Tillage, for the production of annual crops, is the major problem in agriculture, causing soil erosion and loss of soil quality (ATTRA, 2004). Ever since mankind started agriculture, erosion of topsoil has been the single largest threat to a soil's productivity and, consequently, to farm profitability. This is still true today. In the U.S., the average acre of cropland is eroding at a rate of 7 tons per year (Edward and Burrows, 1988). To sustain agriculture means to sustain soil resources, because that is the source of a farmer's livelihood. The major productivity costs to the farm associated with soil erosion come from the replacement of lost nutrients and reduced water holding ability, accounting for 50 to 75% of productivity loss (Edward and Burrows, 1988). Soil that is removed by erosion typically contains about three times more nutrients than the soil left behind and is 1.5 to 5 times richer in OM (Edward and Burrows, 1988). This OM loss not only results in reduced water holding capacity and degraded soil aggregation (Fig. 1.2), but also in loss of plant nutrients, which must be replaced with mineral fertilizations (ATTRA, 2004).

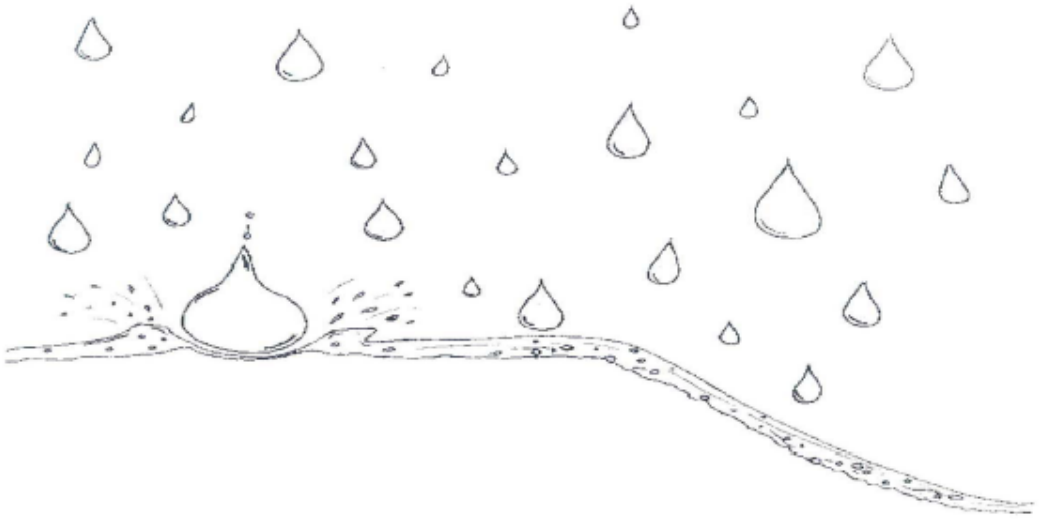

Figure 1.2 Raindrops falling on bare ground initiate erosion. (Adapted from Preston, 1998).

1.1.2 Sustainable agriculture

Sustainable agriculture is a philosophy: it is a system of farming. It empowers the farmer to work with natural processes to conserve resources such as soil, air and water, whilst minimising waste and environmental impact (Mason, 2003).

Sustainable agriculture is defined as the whole practices that meet current and future societal needs for food and fibre, for ecosystem services, and for healthy lives, and that do so by maximizing the net benefit to society when all costs and benefits of the practices are considered (Tilman et al., 2002).

Additionally, the development of sustainable agriculture must accompany advances in the sustainability of energy use, manufacturing, transportation and other economic sectors that also have significant environmental impacts.

Sustainable production practices involve a variety of approaches. Specific strategies must take into account topography, soil characteristics, climate, pests, local availability of inputs, and the individual grower's goals. Despite the site-specific and individual nature of sustainable agriculture, several general principles can be applied to help growers to select appropriate management practices:

- selection of site, species and varieties;
- diversity;

- efficient use of inputs;
- practices of soil management.

1.1.2.1 Selection of site, species and variety

Site, species and varieties must be well suited to conditions existing in the farm. Preventive strategies can reduce inputs and help to establish a sustainable production system. When possible, pest-resistant crops which are tolerant to existing soil or site conditions should be selected. When site selection is an option, factors such as soil type and depth, previous crop history, and location (e.g. climate, topography) should be taken into account before planting.

For these reasons, a previous monitoring of the crop area is fundamental. Monitoring data can provide feedback to assess the effectiveness of natural resource policies, determine the success of land management systems, and diagnose the general health of lands.

Monitoring represent just one component of several physical, chemical and biological basic informations, necessary for natural soil resource management (Fig. 1.3). On the other hand, monitoring programs must be considered with the mutually beneficial activities of mapping and modelling, and all of them should, then, be set within the context of environmental history - the latter provides an understanding of rates of change on much longer time scales (decades, centuries and millennia). Singularly, each activity fails to provide appropriate information for land management and planning. In combination, they provide a powerful and synergistic means for transforming the quality of land management.

When a monitoring of soil quality has to be performed for a relatively unknown situation, several properties must be measured. Usually, a minimum data set (MDS) of soil properties or indicators are selected in order to be responsive, affordable, interpretable, internationally accepted, and ecologically significant (Doran and Parkin 1994).

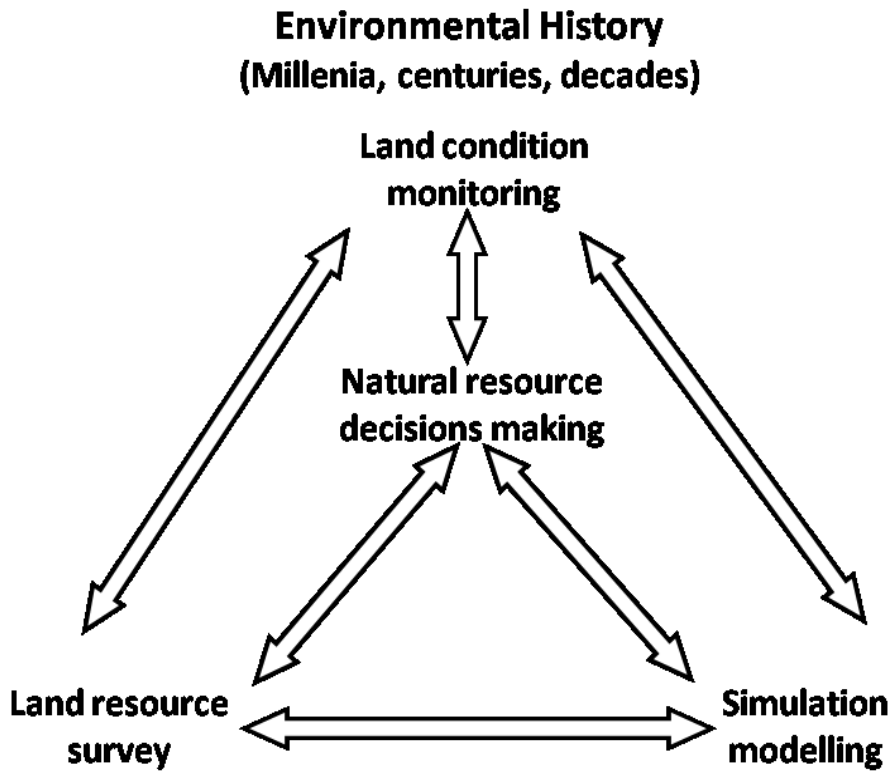

Figure 1.3 Mapping, monitoring and modelling as complementary activities for natural resource management in the context of the environmental history of events and processes for a given landscape.

Criteria for including particular soil properties in the MDS depend on their relevance to the soil under examination. The choice can be made from international literature, and preliminary studies are needed to validate the selected MDS and to standardize the sampling method. According to the concept of soil quality, soil attributes that influence the capacity of soil to perform crop production or environmental functions and are sensitive to change in land use, management, or conservation practices must be included in MDS and evaluated simultaneously, using statistical procedures that account for correlation among soil attributes (Andreoni and Gianfreda, 2007).

1.1.2.2 Diversity

Diversified farms are usually more economically and ecologically resilient. While monoculture farming has advantages in terms of efficiency and ease of management, the loss of crops in any one year could put a farm out of business and/or seriously disrupt the stability of a community dependent on that crop. By growing a variety of

crops, farmers spread economic risks and are less susceptible to the radical price fluctuations, associated with changes in supply and demand.

Properly managed, diversity can also buffer a farm in a biological sense. For example, in annual cropping systems, crop rotation can be used to suppress weeds, pathogens and insect pests. Also, cover crops can have stabilizing effects on the agro ecosystem by holding soil and nutrients in place, conserving soil moisture with mowed or standing dead mulches, and by increasing the water infiltration rate and soil water holding capacity. Cover crops in orchards and vineyards can buffer the system against pest infestations by increasing beneficial arthropod populations and can therefore reduce the need for chemical inputs. Using a variety of cover crops is also important in order to protect against the failure of a particular species to grow and to attract and sustain a wide range of beneficial arthropods.

Optimum diversity may be obtained by integrating both crops and livestock in the same farming operation. This was the common practice for centuries until the mid-1900s, when technology, government policy and economics compelled farms to become more specialized. Mixed crop and livestock operations have several advantages. First, growing row crops only on more level land and pasture or forages on steeper slopes will reduce soil erosion. Second, pasture and forage crops in rotation enhance soil quality and reduce erosion; livestock manure, in turn, contributes to soil fertility. Third, livestock can buffer the negative impacts of low rainfall periods by consuming crop residues that in "plant only" systems would have been considered crop failures. Finally, feeding and marketing are flexible in animal production systems. This can help to cushion farmers against trade and price fluctuations and, in conjunction with cropping operations, make more efficient use of farm labour.

1.1.2.3 Efficient use of inputs

Many inputs and practices used by conventional farmers are also used in sustainable agriculture. Sustainable farmers, however, maximize reliance on natural, renewable,

and on-farm inputs. Equally important are the environmental, social, and economic impacts of a particular strategy. Converting to sustainable practices does not mean simple input substitution. Frequently, it substitutes enhanced management and scientific knowledge for conventional inputs, especially chemical inputs that harm the environment in farms and in rural communities. The goal is to develop efficient, biological systems which do not need high levels of material inputs.

Growers frequently ask if synthetic chemicals are appropriate in a sustainable farming system. Sustainable approaches are those that are the least toxic and least energy intensive, and yet maintain productivity and profitability. Preventive strategies and other alternatives should be employed before using chemical inputs from any source. However, there may be situations where the use of synthetic chemicals would be more "sustainable" than a strictly nonchemical approach or an approach using toxic "organic" chemicals. For example, one grape grower switched from tillage to a few applications of a broad spectrum of contact herbicides in the vine row. This approach may use less energy and may compact the soil less than numerous passes with a cultivator or mower.

1.1.2.4 Practices of soil management

A common philosophy among sustainable agriculture practitioners is that a "healthy" soil is a key component of sustainability; that is a healthy soil will produce healthy crop plants that have optimum vigor and are less susceptible to pests. While many crops have key pests that attack even the healthiest plants, proper soil, water and nutrient management can help prevent some pest problems brought on by crop stress or nutrient imbalance. Furthermore, crop management systems that impair soil quality often result in greater inputs of water, nutrients, pesticides, and/or energy for tillage to maintain yields.

In sustainable systems, the soil is viewed as a fragile and living medium that must be protected and nurtured to ensure its long-term productivity and stability. Methods to protect and enhance the productivity of soil include use of cover crops, compost

and/or manures, reduction of tillage, avoiding traffic on wet soils, and maintenance soil cover with plants and/or mulches. Conditions in most Italian soils (warm, irrigated, and tilled) do not favour the build up of OM. Regular additions of OM or the use of cover crops can increase soil aggregate stability, soil tilth, and diversity of soil microbial life. The main method to apply OM to agricultural soil is the use of organic amendments, such as compost.

1.2 Compost

Although most has been discussed about wastes, little is still known the opportunities that may result from a correct evaluation of the residual resources. Composting is an ancient theme and actual at the same time. More generally, to address and resolve the problem, it is of help to give to urban solid wastes the status of resource, and try to enter them again in the production cycle. This is true when the compost is used in anaerobic digestion to produce biogas, or the use of compost as an amendment is taken in account.

Of the total organic wastes, delivered to composting plant, as much as 73% appears to be composed of the urban waste that are commonly identified as the "wet and green." The remainder is made up of sludge (17%) and other organic wastes of agro industrial origin (10%). Compost is the product obtained by composting the organic fraction of municipal wastes in according with appropriate technical standards, aimed at defining the content and uses compatible with environmental protection and health, and in particular to define the degree of quality (Art. 6 letter q, D.L. February 5, 1997, No. 22, Law Ronchi). It can have a granular appearance, moisture of about 30%, reaction around neutrality (pH values ranging between 6 and 8.5) and good stability, maintained without corrective actions. Due to these properties it can be packaged and stored for a long time.

Compost is ultimately an odourless, stable product, more or less rich in humus, capable to improve soil fertility in respect of environmental and human health standards.

Its use allows the implementation of a sustainable farming model, which achieves a balance between withdrawal and return of organic material to the biosphere. Agricultural soils can benefit from adequate and systematic organic amendments by use of compost, to meet the continuous depletion of OM due to intensive farming and non-optimal use of mineral fertilizers, herbicides and pesticides.
To obtain a product with the desired characteristics, it is necessary to mix the different substrates in appropriate ratio to ensure optimal C/N ratio and the presence of nutrients and metabolic activity for the growth of microorganisms. Indeed, sources of compost are of different origin (agricultural, urban, industrial) and do not always have the optimal characteristics for an efficient processing. For example, if the starting matrix is rich in nitrogen and water, bulking agents (such as straw, paper, etc.) are added. These materials, which provide carbon, mitigate the excess of moisture, provide structure to the overlapping creating interstitial spaces between the particles of the substrate, and are essential for gas exchange.

1.2.1 Effects of compost on soil

In nature, compost, as organic material, undergoes a process of decomposition which is the result of enzymatic reactions, highly dependent on the composition and complexity of the material. The process makes compost more or less likely to become a source of energy and nutrients for the microorganisms through metabolic pathways of respiration and fermentation.
During this process, minerals, mainly nitrogen, phosphorus, calcium, potassium and magnesium, are released into the soil. In addition, organic molecules, if not completely degraded, can be used as such for new synthesis, or further degraded by other soil organisms.
The release of nitrogen is particularly important being this element essential for plant growth. This is often limited by the lack of availability of soil nitrogen forms, to plants (ammonia and nitrate nitrogen). In the organic substances, when released into

the soil, nitrogen can undergo mineralization or immobilization as needed for biosynthesis of microorganisms (Choi and Chang, 2005).

Amlinger et al. (2003) identified the percentage of nitrogen release from composted amendment: up to 8% of the total in the first 2 years and 3-5% of the total from the third year onwards.

Tabaglio et al. (2008) carried out a study of four years on the influence of the input of composted amendments and manures on soil physical and chemical characteristics by distributing the same quantity of OM (10 t ha^{-1}). They observed higher concentrations of nitrates in the thesis treated with manure. This confirms that the process of manure maturation, usually conducted in static piles, is not always adequate to produce a degree of organic nitrogen with the same efficiency of a controlled aerobic process.

Compost should be considered either as a direct source of nitrogen or a source of OM, stimulating soil microbial activity. It can change the balance of mineralization-immobilization processes in the soil occurring between the nitrogen supplied with fertilizer and the nitrogen already present in soil OM.

A study on the combined effect of compost and mineral fertilizers showed that the interaction of mineral fertilizers and organic manure determined mainly an increase of immobilization of mineral nitrogen, following the stimulation of microbial activity by organic substances (Crippa and Zaccheo, 1995). OM limits immediately the efficiency of nitrogen fertilizers, but it can also reduces the losses of nitrogen, that are always conspicuous. It follows therefore that the application of OM along with nitrogen fertilizers can be an aid in maintaining the nitrogen fertilizer, ensuring its continued availability to succeeding crops. In fact biomass is a source of potentially available nitrogen in the short term, as humic substances may constitute a reservoir of slowly transferable nitrogen.

Compost can, in the short term, favour an increase in aggregate stability to water (associated with a larger diameter of aggregates), proportionally to the organic carbon content of soil (Leroy et al., 2008), especially in the absence of tillage (Whalen, 2003).

Compost of different origin and production can lead to different variations of the aggregates stability. For instance, compost produced by processes of rapid composting can improve stability by increasing the water repellence of aggregates, induced by increased microbial activity. Amendments with greater maturity, however, may increase stability by improving the cohesion between soil particles into aggregates, due to the diffusion of OM within them. The more mature products can improve the stability by increasing the OM humified into aggregates, which are more difficult to degrade (Annabi et al., 2007).

In addition, the use of compost can also help in the short term to increase the proportion of larger macroaggregates (> 2 mm), probably because of the stabilization of smaller macroaggregates. This effect can be greater than that measured with manure (Wortman and Shapiro, 2008).

Compost, because of its influence on the aggregates stability, on the carbon content and on the main mineral nutrients, seems to have an effect on soil humus, particularly on the concentration of humic carbon and on humic fractions which can be extracted. Compost appears to be able not only to improve the composition of soil humic substances by increasing humic acids (Weber et al., 2007), but also to increase, in the short term, the humified carbon content, by increasing the ratio of humified (given by the set of humic and fulvic acids, and humin) and non-humified carbon (Adani et al., 2007).

Furthermore compost, through a continuous supply of OM to the soil, is able to promote an increase of the degradation rate of native humic acids in (Kawasaki et al., 2008).

Compost, promoting the whole biological activity of soil, can also contribute to prevent colonization of pathogens by mechanisms of antibiosis and competition by saprophytic organisms present in it (Elsgaard et al., 2001). In this regard, in literature is reported a positive effect of compost on developed arbuscular mycorrhizal fungi (AMF). AMF of the *Phylum Glomeromycota* (Schüssler et al., 2001) are the most important in agriculture because they form a symbiotic association with many crop

species. AMF favour their host principally by increasing uptake of relatively immobile phosphate ions, (Sanders and Tinker, 1971; Koide, 1991; George et al., 1995; Smith and Read, 1997). In return, the fungi receive carbon from the host plant. Other benefits to the host that have been identified include increased uptake of macronutrients other than phosphorus. Indeed, uptake of nitrogen, potassium and magnesium has also been measured (Smith and Read, 1997; Clark and Zeto, 2000; Hodge et al., 2001) as well as increased uptake of some micronutrients (Faber et al., 1990; Kothari et al., 1991a; Azaizeh et al., 1995).

1.2.2 Compost as amendment

In the Mediterranean countries the lack of OM due to the use of land for production purposes implies the need to raise large amounts of organic materials of different origin. Among these materials, compost is an amendment, easily available in sufficient quantities and with relatively low prices.

The quality of compost produced in Italy has improved during the time, reaching optimal agro-environmental levels, due to the better selection of scraps (accurate differentiation, selection of appropriate arrays) and the abandonment of the use of compost from undifferentiated wastes (Centemero and Corti, 2000). Compost, when added to the soil, becomes a growth factor for crops and source of production of new OM (Gallardo-Lara and Nogales, 1987). In addition, compost improves biodiversity and activity of microbial populations in soil, thus influencing their structure, nutrient cycling and many other physical, chemical and biological properties (Albiac et al. 2000).

Chemical characterization of compost is generally based on the value for cropping and on the heavy metals content. Regarding the value for cropping it is necessary to measure the content of N, P, K, and microelements (Cu, Zn, Mn, Fe, Co, Mo). Instead, for the heavy metals content, many countries have introduced various laws relating to the specifications of compost (Soumaré et al., 2003). The quality of a compost is defined by several characteristics such as moisture, OM, carbon content,

concentration and composition of humus, nitrogen, phosphorus and potassium levels, heavy metals content, salinity, cation exchange capacity, water holding capacity, porosity and density of the mass, pathogens presence, degree of maturity and stability. However, the most important parameters to ensure the protection of public health, soil and environment in general, are those relating to pathogens, inorganic and organic compounds potentially toxic, and stability.

The degree of stability of a compost and its nitrogen content are particularly important for its use in farming. The stability of compost is not defined only by the C/N ratio, but also by its microbial activity through the respiration index. It is possible to have a "good quality" compost by using appropriate starting materials and by operating with proper techniques and an adequate treatment plant equipment. The quality of the starting matrix is decisive to have a great quality compost.

The production and use of compost derived from organic wastes seems to be able to provide a joint solution to two problems: firstly, the need to give priority to those forms of wastes, covering the recovery of materials and energy, and to minimize environmental impact; secondly, the need to supply organic fertilizers to soil to compensate for the shortage of OM, maintaining the fertility of agricultural soil, and preserving the environmental equilibrium (Felipò, 1996; Ozores-Hampton et al., 1998). These principles are clearly confirmed by the recent EU legislation, at national and regional level, together with the need to reduce drastically the amount of organic wastes for disposal in a landfill, separating the organic fraction of waste already being collected and reclaimed.

In commerce, it is possible to find three types of commercial composts usable as amendments for agriculture:

- *composted green amendment*, a product obtained through a process of transformation and stabilization of controlled wastes, consisting of wastes from the maintenance of ornamental plants, crop residues and other wastes of plant origin with the exception of algae and other marine plants;

- *composted mixed amendments*, a product obtained through a process of transformation and stabilization of controlled wastes, that can be made from the organic fraction of municipal solid waste from recycling, from animal wastes, including manure, waste-processing and agro-industrial activities, wood and natural untreated textiles, sewage and sludge, and also from all matrices required for the composted green amendment;
- *amendment composted peat*, a product obtained by blending of peat, at least 50%, with green composted amendments and/or mixed.

The disappearance of small farms and the subsequent concentration in restricted areas of production, as well as the development of an agricultural model based on monoculture and intensive agriculture, have led to a deficit of OM of numerous farmlands. The need for high levels of OM in the Italian soils, emerges very clearly in all sectors and in all areas of high agricultural vocation. More than 50% of cultivated soils are classified as low in OM, with variation from area to area depending on local realities (Bonanomi et al., 2010). In this context, the use of compost in agriculture helps to ensure that organic wastes, resulting from the different human activities, return to the ground, while maintaining, at the same time, adequate levels of fertility that can not be achieved with the exclusive use of chemical fertilizers (Ciccotti et al., 1988). In his direct agricultural use, compost should be essentially a fertilizer. Its main agronomic value is given by the budget of OM humus contained, while the presence of nutrients is limited (Scagliarini, 1999).

1.3 Soil quality

An important aim of modern agriculture is the preservation of the environmental and natural resources in the long-term.

The quality of the environment can be assessed by the quality of its main tasks, such as air, water and soil. Soil quality is usually defined as the capacity of soil to interact with the ecosystem in order to maintain the biological productivity, the quality of

other environmental compartments, thus promoting the health of plants and animals, including humans (Doran and Parkin , 1994).

Curiously, the impulse to define and assess soil quality has not departed from the scientific community, but from civil society concerned about the health of the environment. Soil quality may deteriorate quickly due to bad land management, stabilize with time under proper management, vary slightly because of the weather and growing conditions, and improve in the long time for the supply of OM.

The assessment of soil quality has many implications, such as the attitude of soil to a specific use, and the assessment of the human activities and management practices carried out on it. To ensure a correct approach, it is firstly necessary to make the distinction between intrinsic and dynamic quality (Karlen et al., 2001). The first depends on the intrinsic characteristics of a soil, defined as those that are not modifiable in the short term. The dynamic quality is rather determined by all properties that change in the short term. For example, when a study aims to assess the impact on soil quality of specific farming techniques or different methods of cultivation, the intrinsic properties of soil must be considered. Therefore, it is not useful to evaluate the texture, cation exchange capacity and pH, which are mostly determined by the characteristics of the parent rock and the pedogenic processes that formed that land. It is be appropriate, instead, to focus the attention on the organic components of land, assessing the amount (total content of a soil) and quality (properties and relative importance of different fractions) or the properties of its vital components (meso and microorganisms).

Two concepts are useful to assess changes in soil quality: the resistance to degradation, and the resilience, that is the ability of a soil to recover its functional and structural integrity after an external disturbance (Griffith et al, 2001).

Soil quality can be assessed by estimating changes in its characteristics as defined by indicators capable of being synthesized in harmonious, complex and interrelated phenomena.

1.4 Soil quality indicators

Soil quality and soil health are very often used interchangeably (Doran and Safley, 1997). Doran et al. (1996) gave a definition of soil health very similar to that previously proposed for “soil quality”. Indeed these authors defined “soil health” as 'the continued capacity of soil to function as a vital living system, within natural or managed ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant, animal and human health (Doran and Parkin, 1994).

Soil quality is the end product of soil degradative or conserving processes and is a combination of the physical, chemical and biological properties (Fig. 1.4) that are essential for plant growth, regulation and partitioning of surface to ground water, and buffering, detoxifying and scrubbing of hazardous chemicals. It is rather dynamic and can affect the sustainability and productivity of land use. It is increasingly proposed as an integrative indicator of environmental quality (National Research Council, 1993; Monreal et al., 1998), food security (Lal, 1999) and economic viability (Hillel, 1991).

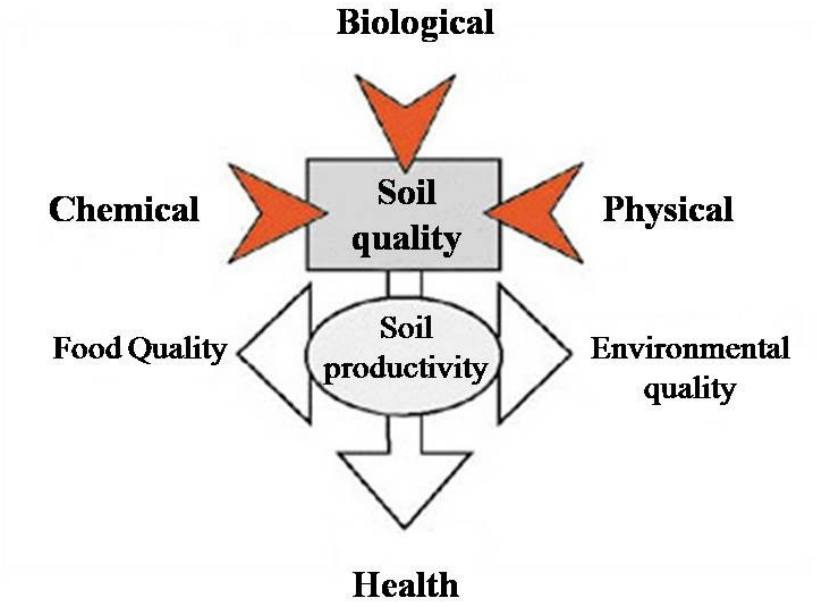

Figure 1.4 Soil quality as affected by soil properties.

Basic soil quality indicators should: a) well correlate with ecosystem functions such as C and N cycling (Visser and Parkinson, 1992); b) integrate soil physical, chemical, and biological properties and processes, and serve as basic inputs, needed for the estimation of soil properties or functions which are more difficult to measure directly; c) be measurable by as many users as possible and not limited to a select group of research scientists; d) be applicable to field conditions, *i.e.* they should describe the major ecological processes in soil and ensure that measurements reflect conditions as they exist in the field under a given management system; e) be sensitive to variations in management and climate; and f) be components of existing soil data bases where possible (Doran and Parkin, 1994). Quantifying these variables through long-term monitoring may lead to an understanding about the effects of land management practices and natural or human-caused disturbances on the soil component of ecosystems (Knoepp et al., 2000).

It is often difficult to separate soil functions into chemical, physical, and biological processes because of the dynamic, interactive nature of these processes (Schoenholtz et al., 2000). Because of these interactions, soil indices are extremely variable.

Many soil chemical properties directly influence microbiological processes (*e.g.* via nutrient and carbon supply), and these processes, together with soil physical-chemical processes, determine the capacity of soils to hold and supply nutrients cycles (including carbon), and the movement and availability of water. Therefore, soil chemical indicators are used mostly in the context of nutrient relations and may also be referred to as "indices of nutrient supply" (Powers et al., 1998) (Table 1.1).

By contrast, biological and biochemical properties (Table 1.1), including soil respiration, microbial biomass and the activities of soil enzymes, are most useful for detecting the deterioration of soil quality (Visser and Parkinson, 1992) because of their importance in cycling of OM and regulating active nutrient pools in soils (Caravaca and Roldán, 2003).

The identification of biological indicators of soil quality is important because soil quality is strongly influenced by microorganism mediated processes (nutrient cycling,

Table 1.1 Main soil quality indicators.

Indicator	Method	Considerations
Physical properties		
Aggregates stability	Strong sieving	It is useful to evaluate soil erosion resistance
Porosity	Volumetric techniques, drying	It indicates the level of compaction
Chemical properties		
Total organic carbon	Hot oxidation in acid	It indicates the soil organic matter content
Carbon humic and fulvic acids	Precipitation of humic acids in acid solution and purification of fulvic acids by polyvinylpyrrolidone	They give information on the humification of organic matter
pH	pHmeter	It indicates the potential availability of micro and macro nutrients
Cation exchange capacity	Extractions by ammonium acetate or barium chloride	It indicates the potential availability of cationic nutrients
Biological and biochemical properties		
Biomass carbon	Extractions and fumigations by chloroform	It indicates the dimensions of microbial population
Mineral nitrogen	Aerobic and anaerobic incubations for more weeks	It gives information about potential available nitrogen
Soil respiration	Incubation at 30 °C for 21-28 days	It indicates the microbial activities
Biomass carbon and organic carbon content ratio		They indicate the increasing of microbial population on total organic matter
Metabolic quotient	Soil respiration and biomass carbon ratio	High levels correspond to a stress condition of microbial biomass
Mycorrhizal fungi	Grid line intersect	They indicate the influence of soil management on the development of mycorrhizal fungi
Soil enzymes	Colorimetric methods	They give information on activity and changes of microbial biomass

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.

Therefore, the use of multiple biological and biochemical properties is often suggested (Ros et al., 2006). General biochemical properties such as microbial biomass carbon (Brookes, 1995), or ecophysiological quotients such as qCO_2 and qD (Anderson and Domsch, 1993), as well as specific biochemical properties such as hydrolytic soil enzymes related to C, N and P cycles (Nannipieri et al., 1990) are suggested.

One limitation in using biological assays for soil quality indication is the lack of standard methodologies. Considerable variation exists among assay procedures used by various researchers, making actual activity comparison between sites difficult. It was thus emphasized that if bioassays have to be used as soil quality indicators, soil sample pre-treatment, assay procedures and units of measurement must be standardized (Dick, 1994).

1.4.1 Soil physical and chemical properties

Many soil physical properties as they depend on the nature of parent material and pedogenic processes, have to be considered inherent. Others, however, are influenced by non-living OM content and the activity of living fraction. This second group of properties, having a living nature, can be used to assess soil quality. This category includes the stability of aggregates, which reflects the ability of soil to ensure the physical stability and resistance against erosion and compaction, and the porosity, which determines the "fitness for habitation" of soil to the microorganisms and influences the balance of chemical and biochemical processes, affecting the relationship between the gas and the liquid phases (Table 1.1).

Chemical properties of soils give information on the quantity of available nutrients in complexed and free form, and, consequently, the adaptability of land to support and promote the growth of plant and microorganisms (Table 1.1).

Since many chemical properties are influenced by soil OM (regulation of the availability of nutrients for plants and microorganisms, exchange capacity, pH, buffering capacity, adsorption of organic and inorganic xenobiotics), the assessment of its total content and its fractions always constitutes a landmark in the study of soil quality (Table 1.1).

1.4.2 Soil biological and biochemical properties

Soil biological and biochemical properties react more quickly to agricultural practices and, in general, to any kind of alteration affecting soil.

Microorganisms (mainly bacteria and fungi) govern the processes of digestion and nutrient cycling through decomposition of OM. Total organic carbon and microbial biomass are key factors in assessing soil quality and are correlated (Elliott, 1997) by the "microbial quotient (ratio of microbial biomass C and total organic C), considered an indicator of both biological activity and accumulation of organic carbon in soil (Elliott, 1994). The respiration of a soil as a measure of the amount of CO_2 produced from the oxidation of OM, is an indicator of microbial mineralization process.

In addition, soil nitrogen content is correlated to its availability to crops and microbial components, and to the risks of air pollution and water.

Soil enzymes are considered good indicators of soil quality, because they are involved in nutrient cycling and decomposition of OM, and respond quickly to any form of change occurring in the system (Table 1.1).

1.4.3 Soil quality indexes

A large number of physical, chemical and biochemical properties, that influence biogeochemical processes and their spatial and temporal variations, contribute to define soil quality. Consequently, individual soil properties may fail to give an

appropriate estimation of soil quality. Moreover, since a number of these properties are difficult to measure, it is useful to condense the information in a numerical value, an index. To define a soil quality indexes it is important: i) the choice of the quality criteria, to which the index values refer; ii) the selection of reliable properties, sensitive to changes in management practices and environmental stresses, to build up the index; and iii) the application of a proper numerical analysis to the development of the index (Puglisi et al., 2006).

A very important step in using soil quality to assess management practices is the availability of tools which producers or managers can use to interpret the technical soil measures and provide guidance for their management. These tools can take the form of an interpretive guide or a computer program that calculates an index using measured soil properties. So, the calculated index can be used to identify the problems present in the study areas and evaluate management practices over time (Wienhold et al., 2004).

A lot of studies have been done on soil quality indicators at point to regional scales (Karlen et al., 1999; Liebig and Doran, 1999; Brejda et al., 2000 a,b,c; Gomez et al., 1996) developed an on-farm index for assessing sustainability based on productivity, profitability, stability, and social acceptability. This measurement used soil processes (crop yield and frequency of crop failure) or properties (soil depth, organic C) as indicators and identified threshold values that are based on local conditions to generate an index of sustainability. This example is only one of several approaches that have been used to perform an index which uses soil quality as an indicator for sustainability (Karlen et al., 1998; Hussain et al., 1999; Jaenicke and Lengnick, 1999; Wander and Bollero, 1999; Andrews et al., 2002a,b; Andrews and Carroll, 2001).

To compare soil quality indicators among sites or among treatments it is useful to normalize the values of indicators using scoring curves. These are mathematical equations developed to describe the relationship between an indicator value and a specific soil process. Indicator selection for a particular process or function can be

done using expert opinion or a statistical procedure such as principle component analysis (Andrews et al., 2002b).

Scoring curves generally have one-of-three forms: more is better (a sigmoid shaped curve with an upper asymptote), less is better (a sigmoid shaped curve with a lower asymptote), and an optimum value with higher or lower values being less desirable (a bell shaped curve) (Fig. 1.5). Once scoring curves are developed for a soil or group of soils, indicators for a minimum data set can be quantified for soils under a range of management systems and the indicators can be scored using the curves. The scored values are then combined in some way (additive, multiplied, or weighted) to form an index value for that management system (Fig. 1.5). Index values created in a similar way can then be compared among management systems or overtime for a particular management system (Wienhold et al., 2004).

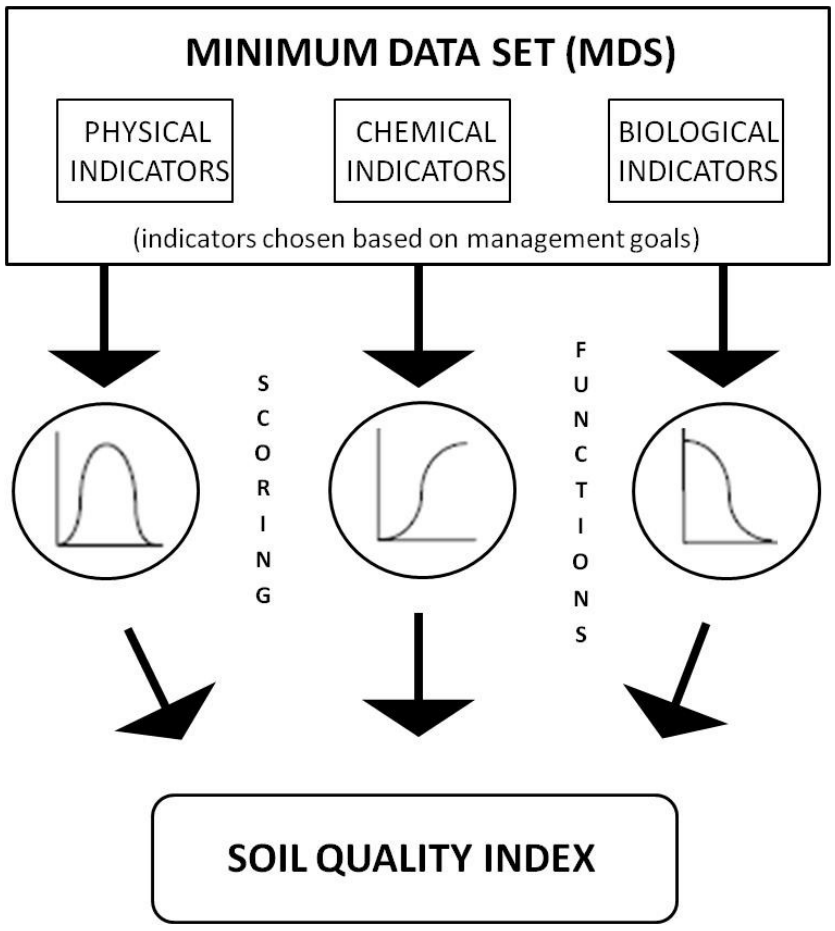

Figure 1.5 Conceptual relationship between soil quality minimum data sets, scoring functions, and index values (Adapted from Wienhold et al., 2004).

1.5 Soil organic matter

The importance of OM in soil has been overshadowed in the last years due to the wide availability of synthetic fertilizers, the introduction of monoculture systems, and large number of tillages. For a long time it was believed that the introduction in agro-ecosystems of technical factors of production could provide fertile conditions to ensure optimum productivity in terms of quantity and satisfactory quality, although remaining constant over time. Today, however, the belief that fertility is restored only with the help of technical means has been abandoned. OM is one of the main factors of soil fertility; it has significant influence on all aspects of life in soil, on its evolution and on the organisms living there.

It has a crucial role in soil structure due to its contributes to the aggregation of mineral particles, promoting a correct balance among the components of soil, air, water and solid phase, which is essential for the containment measures for erosion, compaction and crusting (Fig. 1.6).

In agriculture, a significant content of OM in soil determines the most efficient response to tillage as well as contributes to improve the culture conditions for crops.

OM is the source of energy for all soil microorganisms. These are very important for their role in the demolition and transformation of organic materials and, indirectly, in the cycle of nutrients, useful for the growth of crops.

OM explicates a defensive action against plants for several reasons: the balanced intake of nutrients that strengthen the plant; the diversification and multiplication of soil microorganisms that depend on the quality and quantity of OM added to the soil; the capability of performing actions against the colonization and specialization of pathogen strains (Vizioli, 1997). The contribution of OM to plant nutrition is direct, as a sink of nutrients, and indirectly, by multiple actions, such as availability and solubility of elements and functions of plant root absorption.

As carbon is the most important element for living organisms, OM is the main source of CO_2 in nature. OM is, then, an abundant reserve of all the nutrients that may be fully assimilated by plants.

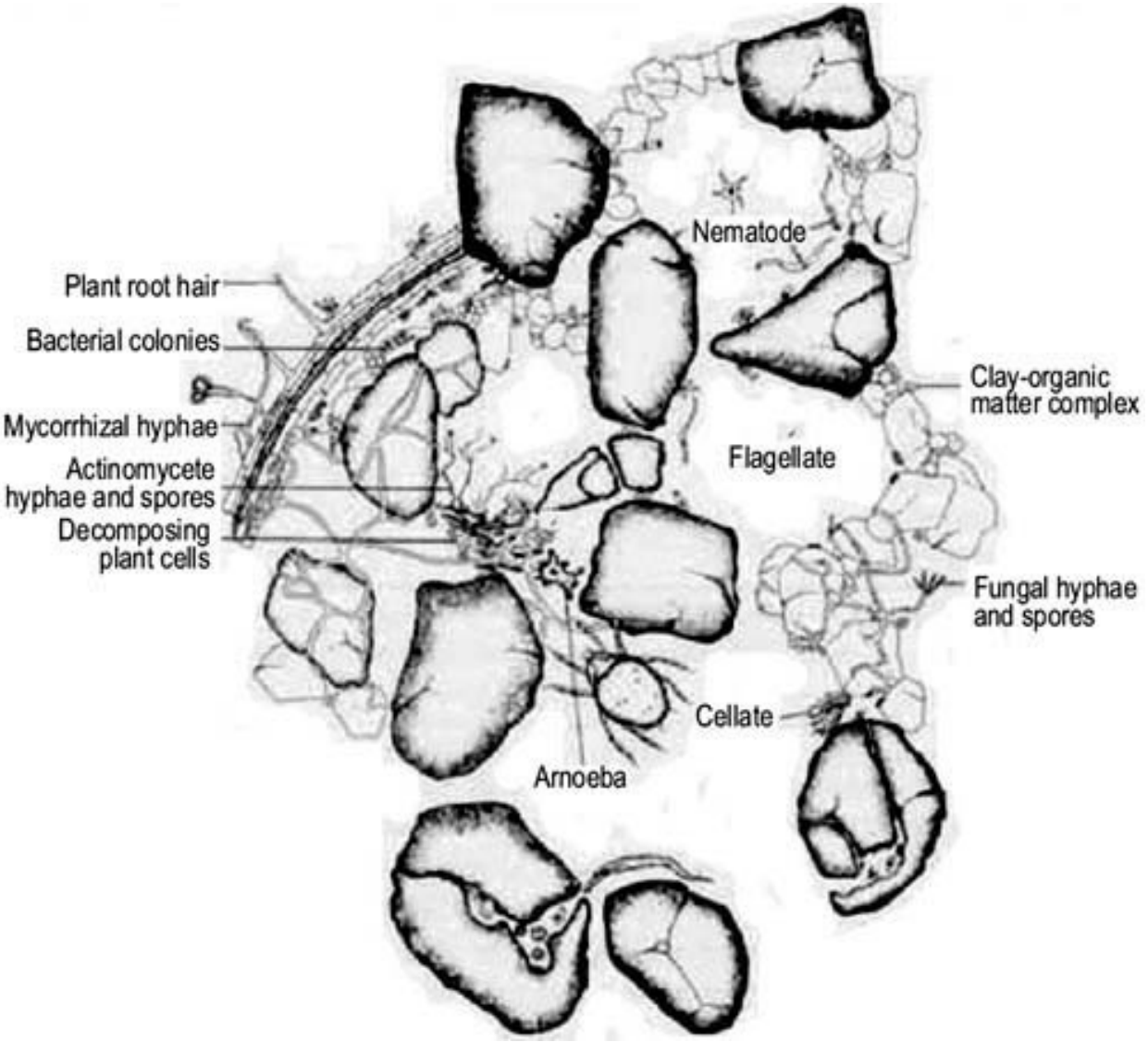

Figure 1.6 Interactions of clay-organic matter complex with the others soil components, such as roots, decomposting plant cells, microorganisms.

The drastic increase in atmospheric CO_2 concentration, mainly due to change of land use since the industrial revolution, necessitates identification of strategies for offsetting the threat of global climate change (Lal, 2004).

Afforestation of agricultural land has been recognized to be an effective tool to mitigate elevated atmospheric CO_2 concentration (IPCC, 2000; Lal, 2005; Laganière et al., 2010). A change in land use from agriculture to forestry can enable the development of a larger biomass with a longer rotation and sequester C in growing biomass (Vesterdal et al. 2002). However, compared with C sequestered in biomass, C sequestered in soils is difficult to be quantified (Teklay and Chang, 2006) and may

vary with many factors such as previous land use, tree species planted, soil properties, preplanting disturbance, stand age, climate zone and methodological approaches (Richter et al., 1999; Guo and Gifford, 2002; Paul et al., 2002; Vesterdal et al., 2002; Peichl and Arain, 2006; Morris et al., 2007; Ritter, 2007; Hu et al., 2008; Berthrong et al., 2009; Laganière et al., 2010). As carbon stored in soils accounts for over two-thirds of the C in forest ecosystems (Dixon et al., 1994) and is more stable than that stored in living plant biomass (Vesterdal et al., 2002; Chen et al., 2005; Laganière et al., 2010), a better knowledge of dynamics of soil organic carbon (SOC) stock following afforestation of agricultural land is needed.

High CO_2 levels in the atmosphere determine serious atmospheric changes, while the increase of soil OM stocks in agroecosystems could potentially help mitigate the atmospheric changes by increasing soil C sequestration, while simultaneously improving soil productivity (Nissen and Wander, 2003). Increased sequestration in an annual crop system would be mainly achieved through stabilization of increased plant inputs in soil OM (Lal, 2004; Six et al., 2002). There is gathering evidence that increased inputs will not always result in soil OM accrual, and that the influence of elevated [CO_2] on soil organic carbon stocks varies as a consequence of plant type and soil nutrient status. According to van Groenigen et al. (2006), increases in soil OM levels are restricted when soil nitrogen (N) supply is limiting. When nutrients are limiting, plant-microbe competition for N can accelerate soil OM decay and ultimately degrade soils even if as plant growth increases under elevated [CO_2] (Barron-Gafford et al., 2005).

Equally important, is the OM capability of improving the cation exchange capacity (CEC). In fact, OM is characterized by several functional groups, and phenolic acids, which can hold and then slowly release nutritional reserves, readily assimilated by plants. About the availability of elements, it cannot be neglected the chelating action explicated by OM with the reversible sequestration of some elements by organic molecules, useful to overcome the risks associated with lack of antagonism or reaction of the soil.

An important example of protection by OM, useful for cultivation, is the link-bridge between iron and aluminium, created by organic substances with phosphates. In phosphorus deficiency, plants increase the concentration of chelating agents in their exudates. These latter remove the metals from OM and make up bridges with phosphates which go into soil solution, thus becoming available.

The microbial activity is strongly influenced by the presence of OM in soils. The concomitant presence of microorganisms and OM explicates a positive action against the solubilization of some poorly soluble, mineral elements.

An usual case in many soils of central Italy, characterized by alkaline pH and presence of calcium, is the lack of availability of important elements such as phosphorus and potassium. The reaction between carbon dioxide, produced by respiration of microorganisms that attack the OM, with soil water and limestone shifts the balance toward calcium carbonate, which is made less stable. A greater amount of OM in soil increases the microbial activity, and then the production of carbon dioxide thus shifting to right the reaction and favouring the solubility of some nutrients. By contrast, a smaller amount of OM leads to less microbial activity and more likely to the insolubility of some elements.

Another important contribution of OM derives from humic acids. They are the main and rich components of soil OM and their contribution to the functions of OM is essential. Consequently, the amount, the characteristics, and especially, the quality of humic acids are considered as important indicators of the of soil OM quality.

Humic acids explicate an hormone-like action, more commonly known as bio-stimulating action. Basically, these actions can relate to the influence of:

1. humic acids on enzymatic activities and therefore, once again, on the availability of nutrients, as many macromolecules could not be treated if they were not rapidly hydrolysed by enzymes present in OM;
2. OM on all the physiological activities of plants such as germination, rooting, root growth, etc..

Equally important is the ability of humic acids to hold water. Humic substances in fact, hold water up to 20 times their weight, are capable to stabilize the soil pH by controlling the delicate balance of chemical and biological agents, and have the ability to interact with pesticides and xenobiotic substances thus influencing their bioactivity, persistence, and biodegradability.

Because of OM important functions, it is clear that its decrease can have a significant impact on the fertility and quality of soil (Costantini, 1995). Therefore, it is essential to maintain an adequate level of OM through the use of organic amendments such as manure (Bastian and Ryan, 1986).

For these reasons, the understanding of the mechanisms involved in the interaction between OM and the other chemicals in the environment is of great importance.

Therefore, a better knowledge of the chemical and physicochemical properties of OM, and in particular of humic acids, will contribute to understand the mechanisms that control an optimum supply of nutrients for crops, as well as many soil physical and chemical characteristics as affected by the organic amendment application.

The key methods to study soil OM, and its humic fractions, are based on the characterization of its elemental and functional composition by spectroscopic techniques. They are modern non-destructive analytical methods. Only small sample amounts are required for the analysis. Most of them are experimental, simple and do not require special manipulative skills. Main techniques are: ultraviolet-visible absorption spectroscopy, fluorescence spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy.

1.6 Soil enzymes

Nutrient cycling in soils involves biochemical, chemical and physico-chemical reactions, with biochemical processes being mediated by microorganisms, plant roots, and soil animals. It is well known that all biochemical reactions are catalysed by enzymes, which are proteins that act as catalysts without undergoing permanent alteration and causing chemical reactions to proceed at faster rates. In addition, they

are specific for the types of chemical reactions in which they participate (Tabatabai, 1994).

Burns (1982) classified soil enzymes according to their location in soil (Fig. 1.7). Three main enzyme categories (termed *biotic enzymes*) are associated with viable proliferating cells. They are located: i) intracellularly in cell cytoplasm, ii) in the periplasmic space, and iii) at the outer cell surfaces. Enzymes in the soil solution are generally short-lived because they are readily inactivated by physical adsorption, denaturation or degradation (Burns, 1986).

The remaining categories are broadly characterized as *abiontic* (Skujinš, 1976). Abiontic enzymes are those exclusive of live cells that include enzymes excreted by living cells during cell growth and division from extant or lysed cells but whose original functional location was on or within the cell. Additionally, abiontic enzymes can exist as stabilized enzymes in two locations: adsorbed to internal or external clay surfaces, and complexed with humic colloids through adsorption, entrapment, or copolymerization during humic matter genesis (Boyd and Mortland, 1990). Enzymes associated with humic substances and, to a lesser extent, with clay particulates are protected against thermal denaturation, proteolysis, dehydration or decomposition. They are part of a persistent extracellular enzyme pool that is independent of the existing microbiota (Burns, 1982; Sarkar and Burns, 1984; Miller and Dick, 1995). The humic-enzyme fractions retain the original properties of the enzymes (Busto and Perez-Mateos, 1995) as stable enzyme-organic matter complexes and they were found to allow diffusion of substrates to the active enzyme site (Burns, 1982). Therefore, soil can be considered as a sink and source of indigenous and persistent enzymatic capacity, which is independent of current or recent microbial and plant activity (Galstian, 1974; Burns, 1986; Lähdesmäki and Piispanen, 1992; Busto and Perez-Mateos, 1995). Moreover, the enzymatic activity of a soil is conditioned by land use history, since enzymes are produced by living organisms which contribute to the biological soil formation.

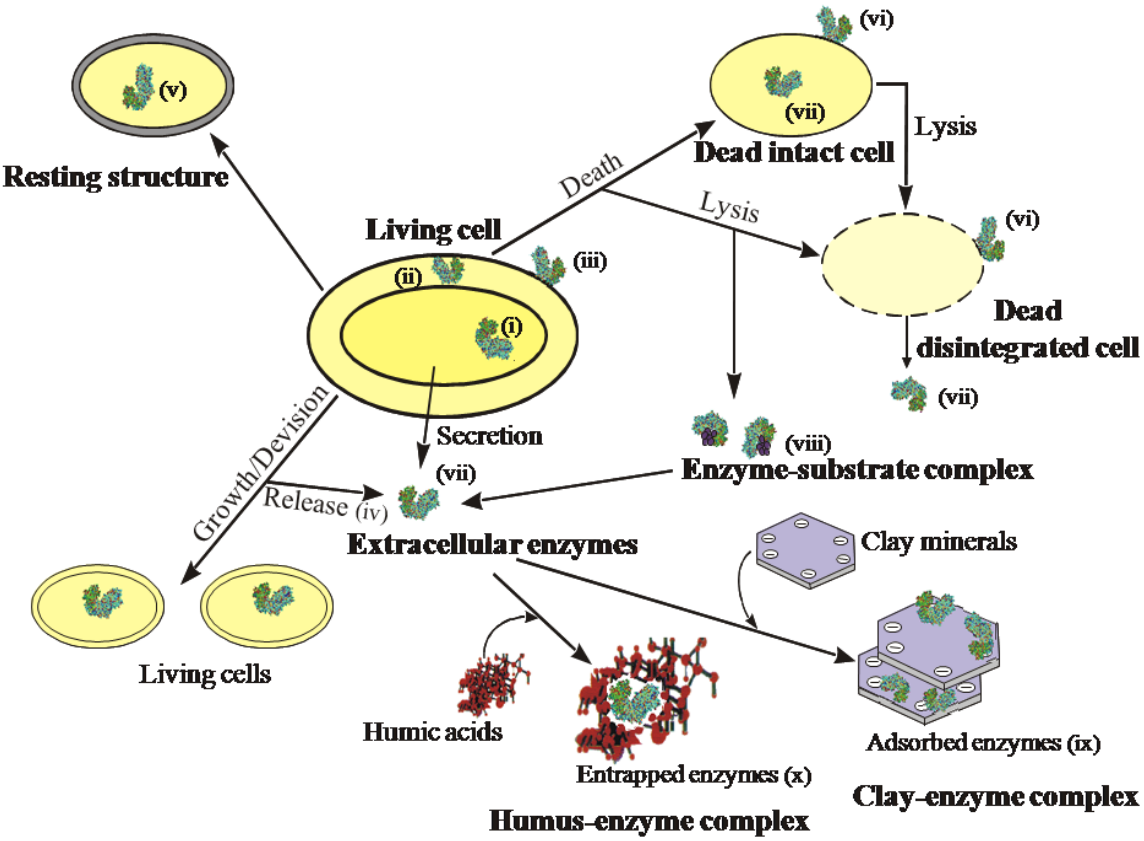

Figure 1.7 Soil enzymes location. (i) Intracellular enzymes, (ii) periplasmic enzymes, (iii) enzymes attached to outer surface of cell membranes, (iv) enzymes released during cell growth and division, (v) enzymes within non-proliferating cells (spores, cysts, seeds, endospores), (vi) enzymes attached to dead cells and cell debris, (vii) enzymes leaking from intact cells or released from lysed cells (viii), enzymes temporarily associated in enzyme-substrate complexes, (ix) enzymes adsorbed to surfaces of clay minerals, (x) enzymes complexed with humic colloids (according to Burns, 1982 and Nannipieri, 1994).

The activity and stability of enzymes in soil is regulated by pH (Frankenberger and Johanson, 1983; Trasar-Cepeda and Gil-Sotres, 1987; Dick et al., 1988), microbial biomass (Häussling and Marschner, 1989; Saffigna et al., 1989; Carter, 1991; Srivastava and Singh, 1991), vegetation (Juma and Tabatabai, 1978; Harrison, 1983; Perucci et al., 1984; Helal and Sauerbeck, 1987; Tarafdar and Jungk, 1987), soil and crop management practices (Perucci and Scarponi, 1985; Beck, 1990; Martens et al., 1992; Kandeler and Eder, 1993), soil OM (Juma and Tabatabai, 1978; Chhonkar and Tarafdar, 1984; Sparling et al., 1986), and soil moisture content (Harrison, 1983; West et al., 1988a,b).

Enzyme activities are important indicators of soil biological activity because they are involved in the dynamics of soil nutrient cycling and energy transfer. Indeed, they reflect the intensity and direction of biochemical processes in the soil matrix. Hence, their activity indicates the biological capacity of a soil to carry out the biochemical processes which are important to maintaining the soil fertility (Galstian, 1974; Dkhar and Mishra, 1983; Burns, 1986; Garcia et al., 1994), as soil fertility depends not only on the nutrient status and availability but also on the turnover of N, P and other nutrients (Lopez-Hernandez et al., 1989).

Actually, enzymatic processes are closely associated with soil fertility as they mediate the conversion of unavailable forms of nutrients to forms that are readily assimilable by plants and microbial biomass (Sarathchandra et al., 1984; Dick et al., 1988; Sarkar et al., 1989; Dick, 1992; Martens et al., 1992; Sinsabaugh, 1994). Soil enzymes also participate in the decomposition and synthesis of organic substances and are important for the formation of recalcitrant organic molecules (Galstian, 1974; Martens et al., 1992).

As enzymes do not react readily to environmental changes respect to soil microbial biomass, their activity is a more stable indicator of biological processes (Galstian, 1974).

1.6.1 Functions carried out by soil enzymes

Soil enzymes perform many functions which can be summarized as follows (Fig. 1.8):

- a key role in the process of decomposition of OM,
- catalysis of many reactions necessary for life processes of microorganisms,
- contribution to the stabilization of soil structure,
- make nutrients available for plants that appear to be immobilized in the soil,
- degradation of substrates too large or insoluble, making them available to the microorganisms that would otherwise not be able to use them.

In soil, most common enzyme activities belong essentially to four classes:

- oxidoreductases,
- lyases,
- hydrolases,
- transferases.

Among them, hydrolases and oxidoreductases are the most common. Hydrolytic enzymes, such as polysaccharidases and proteinases, responsible of the degradation of carbohydrates and proteins, respectively play an important role in ensuring the presence of monomeric products essential to the biological cycles of carbon and nitrogen.

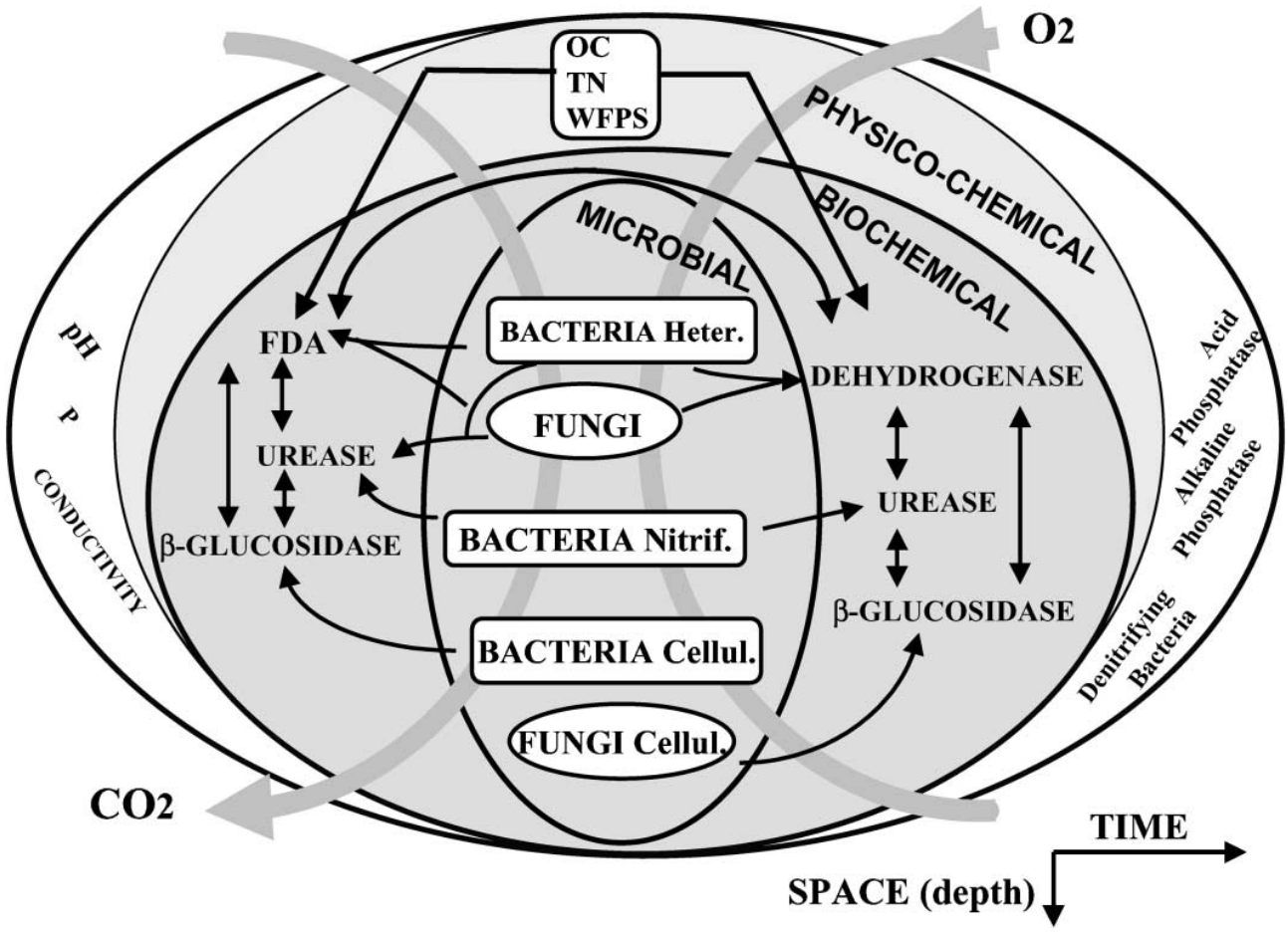

Figure 1.8 The soil as a bioreactor: a "hard-core" of interacting properties from microbial, biochemical and physico-chemical domains as a function of time and soil depth (Adapted from Aon and Colaneri, 2001).

Similarly, phosphatases and sulphatases, that hydrolyze organic phosphates and sulphur compounds, respectively, producing inorganic phosphorus and sulphur, carry

out essential biochemical functions, because they provide to plants and microorganisms both phosphorus and sulfur in available forms. Dehydrogenases, mainly intracellular enzymes, may give some information on the biological activity of soil microbial populations. Glycosidases are widely distributed in nature and their work reflects the effects of soil management. The enzyme β-glucosidase catalyzes the hydrolysis of cellobiose to glucose (last step of the catabolic way of cellulose) and is therefore important for the carbon cycle and the "quality" of soil in general. Invertase, another hydrolytic enzyme, is involved in the cleavage of sucrose into glucose and fructose and its presence in a soil is placed in relation to microbial activity, that's why activity was proposed as an index of fertility.

1.6.2 Factors affecting enzyme activities in soil

There are many causes that may alter the enzymatic activity of soil (Gianfreda and Bollag, 1996). Some of them are linked to natural variations in soil, while others are due to human activity and practices of soil management. Therefore, they can be grouped in *natural* and *anthropic* factors.

1.6.2.1 Natural factors

Seasonal changes

Several studies were carried out on the correlation between seasonal variations and the activity of enzymes in soil. These studies showed that during the warmer seasons (spring, summer) microbial populations are at their maximum development. Since the main source of soil enzymes is represented precisely by microorganisms, it follows that also the levels of enzyme activities are usually higher during the same period. An example is arylsulphatase that showed a peak of activity in summer (Niemi et al., 2005). Similarly, the activity of dehydrogenase is higher in spring due to increased bacterial and fungal proliferation, while urease showed two peaks of activity during

the months of May and September and a reduction in the month of June (Kumar et al., 1992).

Humidity and salinity

Another factor that influences the soil enzyme activities is humidity. Some authors have shown that the activity of dehydrogenase increases with the level of humidity (Gianfreda and Bollag, 1996), while enzymes such as urease and phosphatase express their maximum activity level in the optimal conditions of humidity values at 60% capacity field. The same phosphatase and arylsulphatase show an exponential reduction of their activity when the soil reaches moisture level of dry soil (Hyvönen and Persson, 1990).

Excess of salts in soil causes a high osmotic pressure of soil solution that alters the development of crops and microorganisms and enzyme activities. It is not, however, possible to generalize because enzymes show different behaviours with regard to salinity. For example, urease and phosphatase activities decrease with increasing salinity due to changes in the osmotic potential of soil (Iftikhar and Khan, 1988).

Distribution of the profile

Soil is divided into horizons with different physical, chemical and biological characteristics. These differences are reflected in different enzyme activity levels of soils. In fact, several studies have demonstrated that dehydrogenase, urease and phosphatase are, usually, higher in the organic horizon or in the first few centimeters of soil and decrease with depth (Taylor, 2001). This finding can be simply explained by the strong link between the organic component and the enzymatic activity, thus determining a gradual decrease of microbial population with depth.

Niemi et al. (2005) found, for example, that the activity of β-glucosidase and β-xylosidase subside strongly with increasing depth, as well as α-glucosidase activity, even if with less impact. Bergstrom and Monreal (1998) and Bergstrom et al. (1998, 2000) found that arylsulphatase activity decreased with increasing depth and water

content. In other studies it was observed that urease activity was higher in samples of the surface layers than deeper.

Soil physical and chemical properties

There are several soil physical and chemical properties that influence the enzyme activities, in particular pH, particle size, total organic C and N.
The enzymes most affected by soil physical and chemical conditions are involved in nutrient cycling (C, N, P).

The enzymes have maximum activity at a defined pH optimum (Gianfreda and Bollag, 1996), because at different pHs the structure of the active site of the enzyme changes and this decreases or inhibits the catalytic activity. For example, phosphatases are strongly correlated to soil pH so much that they can be distinguished in acidic and alkaline phosphatases. In acidic soils, phosphatase activity is expressed as acid phosphatase, while in alkaline soils alkaline phosphatases prevails. The liming increases the activity of many enzymes in the long-term indicating a positive correlation between enzyme activities (except acid phosphatase) and the soil pH (Ekenler and Tabatabai, 2003b).

Regard to texture, sandy soils have a less numerous and diversity of microbial biomass and enzyme activities than loam soils having a good structure, good porosity and good OM content.

In addition, microbial population and enzyme activities showed a strong sensitivity to the concentration of organic carbon (Powlson et al., 1987, Bergstrom et al., 1998). Aon and Colanieri (2001) found a quite strong correlation (r=0.68) between dehydrogenase activity and percentage of organic carbon. Urease activity increases with decreasing the C/N ratio, and is negatively correlated with the percentage of organic carbon, but positively with the amount of total nitrogen, while β-glucosidase strongly influenced by the content of total nitrogen, shows no correlation with organic carbon.

Clay minerals

When the enzymes, from any source, arrive to the soil solution, they are readily immobilized on mineral and/or organic colloids. Adsorption is achieved through various kinds of interactions such as electrostatic interactions, hydrogen bonds, Van der Waals forces. It usually involves changes in enzyme activity that is reduced or inhibited. This implies that the catalytic activity of the adsorbed enzyme is conditioned not only by the rate constants of the enzyme itself and the concentrations of the substrate, but also by the probability of contact between enzyme and substrate that it is minimized. In addition, the active site can be hidden due to the immobilization of protein molecules on colloidal particles, and this somehow reduces the efficiency of the enzyme. Another consequence of adsorption on clay minerals, or in general of immobilization on soil colloids, is generally a shift of optimal pH at which occurs the maximum catalytic activity of the enzyme (Demanèche et al., 2009).

Despite these problems, adsorbed enzymes have a range of benefits including a high resistance to microbial degradation and stability to heat denaturation.

Humic components

Soil extracellular enzymes are firmly bound to soil humic substances and they form complexes called "humo-enzymatic". The humo-enzymatic complexes are very important because they are an essential reserve of matter and energy of soil. In fact, being immobilized on the humic fraction, they are active even when conditions are prohibitive for the microorganisms. These humo-enzymatic complexes, called geo-enzyme or stable nucleus, have been extracted and purified from different soil types of the Mediterranean area (Nannipieri, 1994) and have been proposed as the latest biological defences of the soil exposed to a process of irreversible degradation (desertification).

The component of humic soil with biological activity can be considered an optimal indicator of the level of land degradation. It is related to soil resilience, and can

indicate the ability of soil to be recovered. The humo-enzymatic complexes can be considered a "recorder" in the history of the earth: they shall reflect the past, the presence of humic complexes, and the present, the catalytic activity for the role they play and their importance in nutrient cycling (Masciandaro et al., 1998). They represent a "transition point" between the chemical and microbiological soil (Ceccanti et al., 1997).

Biomass

Although plants and animals contribute significantly to the enzymatic activity of soil, enzymes of microbial origin are considered the most important so that microbial activity is considered an index of fertility.

Some authors have attempted to determine only the contribution of bacteria and fungi to the whole enzyme activity of soil. It was observed, for example, that urease and phosphatase activities are related to bacterial, but not to fungal biomass (Nannipieri et al., 1979). There is also a positive correlation between dehydrogenase and fungal populations and between urease activity and bacterial population, but this correlation is valid only at low altitudes (Kumar et al., 1992). These relationships are dependent on seasonal changes, type of vegetation cover and crop cultivation, the latter indirectly affecting enzyme activity because of their direct action on microbial population.

1.6.2.2 Anthropic factors

Human activities cause constant changes to the soil and thus to all enzymatic activities that are present in it. The human factors, interacting with the natural factors, can significantly influence the activity of enzymes.

Acid rains

Acid rains are called the "plague invisible" of the industrial era and are considered one of the most serious environmental problems of our time. This phenomenon is

caused mainly by the rising of atmospheric gaseous components such as sulfur dioxide (SO_2) and sulfur trioxide (SO_3), carbon monoxide (CO), nitrogen dioxide (NO_2) and carbon dioxide (CO_2) from industrial activities and combustion. These gases can be transformed in a few days in acid rains by various reactions such as acid combination with water (H_2O) or with the hydroxyl radical OH^-. These acids are then transported by rain on the earth's surface, thus increasing the concentration of hydrogen ions in soil.

Acid rains cause acidification of soil reducing the presence and availability of nutrients for plants. In addition, the lowering of soil pH mobilizes aluminum (usually enclosed in the silicate crystal lattice of soil), so it goes into the soil solution with severe poisoning (and consequent economic losses) to crops and to soil microorganisms. The deterioration caused by acid rains disturbs the soil enzymatic activity as it increases the solubility of some metals (Ni, Fe, Zn), and consequently reduces the stability of the protein or increases the solubility and ionization of substrates and enzyme cofactors. The degree of alteration of enzymatic activity by acid rains depends on the time of soil exposure to such precipitation; for example, brief exposure for dehydrogenase, urease and phosphatase does not appear lead to detectable changes, which are instead observed for longer exposures (Bitton and Boylan, 1985).

Fires

In literature, few and discordant are the data on the effect of fires on the activities of enzymes in soil. Skujins (1967) reports a total inactivation of enzymes in soils affected by fire and the request for a period of about 10 years to regain enzymatic activity values comparable to those present before the fire. Rutigliano et al. (1995) describe a reduced activity of dehydrogenase, phosphatase and sulphatase in soils covered by superficial and deep fires even after 8 years from the fire event.

Arianoutsou-Faraggitaki and Margaris (1982), by contrast, found no differences of dehydrogenase activity between burned and not burned soils. Boerner et al. (2000)

showed a reduced activity of acid phosphatase and chitinase in forest soils of southern Ohio by fire but not for phenoloxidase, that, on the contrary, increased its activity in the burned soil.

The passage of fire in an ecosystem influences the distribution and activity of populations of microorganisms in soil and consequently soil enzyme activity. The magnitude of fire effect depends on both the affected organisms and the fire intensity. In fact, very intense fires may temporarily sterilize the soil, while little intense fires may change only slightly its biological activity (Renbuss et al., 1973).

Soil management

Soil can be easily disturbed by inappropriate farming managements. The use of pesticides, monoculture, continuous tillage are all factors that lead to the loss of soil fertility and quality, which reflects negatively on the balance of vegetation and consequently on the microbial and enzymatic activities.

Despite the many information on enzymatic activities of the different soil managements, the results obtained are often disputed (Dick, 1992; Gil-Sotres et al., 2005). Many researchers have pointed out that tillage determs an increase of enzymatic activities in agricultural soils due to the exposure of new surfaces as soil aggregates are broken up (Dick, 1984; Khan, 1996; Latif et al., 1992; McGill et al., 1986), whereas other researchers have reported a decrease in enzymatic activities due to the decrease in OM content as a consequence of the tillage (Carter, 1986; Dick, 1994; Jensen et al., 1996).

The use of mineral fertilizers and organic amendments has also been reported to have contrary effects in these soils. In according to the literature use of organic amendments increases the biochemical activity of soil due to the addition of organic materials and microorganisms (Dick et al., 1988; Jenkinson, 1990; Kandeler et al., 1999), whereas others state that the activity decreases, particularly when poor quality manure is used (Garcìa et al., 1992; Perucci et al., 1984; Schipper and Sparling, 2000). Regarding inorganic fertilizers, although it is considered that the presence of

readily available inorganic nutrients inhibits enzymes synthesis in soil (Dick, 1992; Olander and Vitousek, 2000), some authors consider that the same presence may increase the biochemical activity of a soil by stimulating plant growth and the secretion of enzymes by roots (Lynch and Panting, 1980).

Thus, grass cutting determines an increase of soil biochemical activity due to the resulting increase of dead roots amount (Holland, 1995; Mawdsley and Bardgett, 1997), or may decrease this activity due to the decrease in root exudates (Northup et al., 1999), or may have no effect at all on soil biochemical properties (Kuzyakov et al., 2002; Wardle and Barker, 1997). Similar contradictory effects have been reported on the use of grazing animals. Thus, some authors have showed that grazing may determine an increase of the microbial activity due to the presence of the animals' excreta (Haynes and Williams, 1999; Zacheis et al., 2002), and others that it may cause a decrease in microbial activity, normally caused to the degradation of the soil structure by trampling (Conant et al., 2001; Cao et al., 2004).

The only management that doesn't produce contradictory effects is the use of OM. As reported above soil OM is one of the important soil properties that may considerably influence the activities of soil enzymes (Gianfreda and Bollag, 1996). Therefore, solid organic amendments could enhance soil enzyme activities by increasing soil OM and thus the microbial biomass (Crecchio et al., 2004; Acosta-Martinez and Harmel, 2006). The quality of soil OM can affect the composition and relative abundance of soil enzymes (Shi et al., 2006). For example, catalase activities of soils are based on the rates of release of oxygen from added hydrogen peroxide or on the recoveries of hydrogen peroxide (Wang and Lu, 2006). Zhao et al., (2009) showed that application of straw generated higher activity of invertase and catalase than manure treatment, which means higher redox-cycling in straw treatment.

Lagomarsino et al. (2009) demonstrated that not all studied enzymes (acid phosphatase, arylsulphatase, dehydrogenase and β-glucosidase) are valid indicators of changes under organic management. For instance, acid phosphatase and arylsulphatase activities did not respond to the different management, although other

studies reported significant changes of acid phosphatase (Pascual et al., 1999; Monokrousos et al., 2006). Arylsulphatase did not show significant variations also in the study of Mijangos et al. (2006). Dehydrogenase activity appeared to be a sensitive indicator of the impact of organic management in the studies of Mijangos et al. (2006) and Marinari et al. (2006). Conversely, β-glucosidase activity resulted to be suitable indicator of changes in C cycling.

Pesticides

Pesticides have a role in the current agriculture, being used to protect crops from pests (especially insects and mites) and pathogens (bacteria, fungi, viruses), to control the growth of weeds, and to ensure the achievement of high standards of quality of agricultural products.

Pesticides are, however, composed by toxic substances, and their improper use may determine risks and dangers to human and animal health. Their use has been largely confirmed to have an impact on physical and chemical properties and on microorganism of soils.

Pesticides arrive to the soil through direct application on the vegetation and indirect applications in the form of industrial wastes, municipal, or accidental release. Once introduced into the environment, pesticides are subjected to abiotic degradation processes (photolytic or chemical) and to biotic or biological processes. The photolytic degradation generally occurs when the pesticide molecule is irradiated by sunlight, chemical degradation occurs when the molecule is chemically unstable in the environment in which it is located, while the biotic degradation, defined by the term "biodegradation", is due to changes made by living organisms.

Typically, pesticides are used in optimal doses to not causing serious damage to microorganisms and to not alter the enzymatic activity (Schaffer, 1994), but if used in high doses they inhibit enzymatic activity. The negative effects are due to both a direct interaction pesticide-enzyme and also to an indirect action on edaphic microorganisms.

A study was carried out on phosphatase and dehydrogenase activity in a soil amended with compost or dissolved OM and then contaminated with different concentrations of pentachlorophenol (PCP, used in the past time in agriculture). Results showed that soil enzymatic activities responded differently to various concentrations of PCP (Scelza et al., 2008). The effects of pesticides on enzyme activities, in fact, depend not only on the type of enzyme and soil but also on the type and concentration of pesticide.

Fertilizers

In general, it is clear that biochemical and biological properties of soil may be affected by fertilization, whether organic or mineral, showing a close correlation between the kind of amendments and properties of soil (Marinari et al., 2001). The activities of β-glucosidase and protease were significantly influenced by repeated applications of inorganic nitrogen, and were stimulated by application of organic manure (Fauci and Dick, 1994). Dick et al. (1988) found that increasing the percentage of fertilizers based on ammonia a decrease of amidase and urease activities (both enzymes involved in nitrogen cycle) occurred. In contrast, the activity of other enzymes not directly involved in the nitrogen cycle, such as, for example, arylsulphatase and β-glucosidase, were not influenced. This mechanism was explained by assuming that these enzymes are inhibited by ammonia.

The supply of phosphorus-based fertilizers depressed phosphatase activity in both agricultural (Mathur and Rayment, 1977, Spiers and McGill, 1979) and forestry (Clarholm, 1993) systems. This negative action is probably linked to the type of soil as shown by Mathur and Rayment (1977). Indeed, in soils with low OM content, P fertilization increased phosphatase activity, while in soils with a high OM content fertilization did not cause any modification of that activity. Inhibition of phosphatase activity was correlated by Chunderova and Zubets (1969) to high levels of PO_4^{-2} in the soil solution. Phosphates may inhibit the synthesis of microbial phosphatase.

Moreover, orthophosphate behaves as competitive inhibitor of acid and alkaline phosphatase (Juma and Tabatabai, 1978).

The current problem of conventional agriculture is the continuous depletion of OM due to intensive farming and non-optimal use of mineral fertilizers, herbicides and pesticides. To overcome this problem, approaches using organic manure were tried.

The use of OM produced from municipal solid wastes may lead to both positive and negative effects, depending on its composition and used doses. (Marcote et al., 2001, Lakhdar et al., 2010). In general, an increase in all biological properties of soil is observed after the use of OM as fertilizer (Albiac et al., 2000). The use of compost also leads to a better development of the whole microflora, and beneficial fungi such as mycorrhizae, resulting in beneficial effects on crops (Perner et al., 2007).

Heavy metals

Heavy metals can reach the soil through the distribution of pesticides (copper, arsenic), mineral fertilizers (many phosphate fertilizers are rich in arsenic, cadmium and chromium), organic fertilizers (leather, roasted, etc, contain up to 3% of chromium, as well as by products of the tanning industry), slurry (for the presence of copper and zinc), but especially sewage sludge or compost. Once into the soil, heavy metals may have an impact on the biotic soil constituents and enzyme activities. The negative effects of heavy metals on soil enzymatic activities have been widely demonstrated (Deng and Tabatabai, 1995) and the pressing issue of the continuous enrichment of heavy metals in soils has led to exploit the potential of enzyme activities as biochemical indicators of the quality and pollution of the soil (Alef and Nannipieri, 1995).

1.6.3 Enzymatic activities as bioindicators

Biochemical properties of soil are an useful tool to monitor changes of microbial biomass in soil. As already said, biochemical properties can be used as "early indicators" of disturbances that degrade soil as they are more sensitive to changes

induced by anthropic and environmental activities than chemical and physical properties of soil as OM content (Dick, 1994). In general, biomonitoring is continuous, inexpensive and not complicated.

Soil enzymes can be considered as “good” biological indicators of the health status of a soil as they can perform excellent detections of conditions prevailing in soil (Dick et al., 1988; Ceccanti and Garcia, 1994; Masciandaro et al., 1998). Indeed, they respond quickly to physical, chemical and biological changes, are analytically simple inexpensive, measurable, easy to understand, reliable, reproducible and scientifically valid.

Enzymes have high specificity of recognition of their substrates, and may be able to detect the toxicity of a mixture of pollutants whose individual concentrations are below levels alarm. Therefore, they are important "soil sensors" and provide guidance on the metabolic state of microbial population and soil chemical and physical conditions. (Trasar-Cepeda et al., 2000). As a consequence, enzymatic activities represent a suitable tool to evaluate the influence of management techniques and land use on functional biodiversity.

Garcia et al. (1994) showed that dehydrogenase activity behaves as a good indicator of the microbial activity of soils in semi-arid areas. In areas subject to degradation processes, dehydrogenase activity is usually very low. In the last 20 years, biochemical properties (hydrolytic activity of enzymes and microbial indices) were used in many studies as indicators of soil quality in degraded environments or subject to pollution due to the presence of hydrocarbons or heavy metals. Being biomarkers they are also very sensitive to seasonal variables, they can be used in the comparison of study areas subject to the same climatic conditions or in the regular monitoring of changes induced by techniques of land management by considering a sufficiently long period.

The limitation of these biomarkers is the lack of universal threshold values that identify the condition of normality in a complex system like soil. This is the consequence of the absence of standardized, universally accepted methods for the

measurement of soil enzymatic activities. These limitations do not allow the immediate identification of a disturbance seen as deviation from "normal" and the identification of soil control is indispensable.

1.7 References

Acosta-Martinez V., Harmel R.D., 2006. Soil microbial communities and enzyme activities under various poultry litter application rates. *Journal of Environmental Quality* 35:1309-1318.

Adani F., Genevini P., Ricca G., Tambone F. and E. Montoneri., 2007. Modification of soil humic matter after 4 years of compost application. *Waste Management* 27:319-324.

Albiach R., Canet R., Pomares F., Ingelmo F., 2000. Microbial biomass content and enzymatic activities after the application of organic amendments to a horticultural soil. *Bioresource Technology* 75:43-48.

Alef K. and Nannipieri P., 1995. Methods in applied soil microbiology and biochemistry. 1995 Elsevier Ltd. ISBN: 978-0-12-513840-6.

Amlinger F., Gotz B., Dreher P., Geszti J., Weissteiner C., 2003. Nitrogen in biowaste and waste compost: dynamics of mobilization and availability a review. *European Journal of soil Biology* 39:107-116.

Anderson T.H. and Domsch K.H., 1993. The metabolic quotient CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. *Soil Biology and Biochemistry* 25: 393-395.

Andreoni V. and Gianfreda L., 2007. Bioremediation and monitoring of aromatic-polluted habitats. *Appl Microbiol Biotechnol* 76:287-308.

Andrews S.S., Carroll C.R., 2001. Designing a decision tool for sustainable agroecosystem management: soil quality assessment of a poultry litter management case study. *Ecological Applications* 11:1573-1585.

Andrews S.S., Mitchell J.P., Mancinelli R., Karlen D.L., Hartz T.K., Horwath W.R., Pettygrove G.S., Scow K.M., Munk D.S., 2002a. On-farm assessment of soil quality in California's central valley. *Agronomy Journal* 94:12-22.

Andrews S.S., Karlen D.L., Mitchell J.P., 2002b, A comparison of soil quality indexing methods for vegetable production systems in northern California. *Agriculture, Ecosystems and Environment* 90:25-45.

Annabi M., S. Houot F. Francou M. Poitrenaud and Le Bissonnais Y., 2007. Soil aggregate stability improvement with urban composts of different maturities. *Soil Science Society of America Journal* 71:413-423.

Aon M.A., Colanieri A.C., 2001. Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. *Applied Soil Ecology* 18: 255-270.

Arianoutsou-Faraggitaki M., Margaris N.S., 1982. Decomposer and the fire cycle in a phryganic (East Mediterranean) ecosystem. *Microbiology of Ecology* 8: 91-98.

ATTRA, National Sustainable Agriculture Information Service, Sustainable Soil Management (Soil Systems Guide) by Preston Sullivan 2004

Azaizeh H.A., Marschner H., Römheld V.,Wittenmayer L., 1995. Effects of a vesicular-arbuscular mycorrhizal fungus and other soil microorganisms on growth, mineral nutrient acquisition and root exudation of soil grown maize plants. *Mycorrhiza* 5:321-327.

Barron-Gafford G., Martens D., Grieve K., Biel K., Kudeyarov V., McLain J.E.T., Lipson D., Murthy R., 2005. Growth of eastern cottonwoods (*Populus deltoides*) in elevated [CO_2] stimulates stand-level respiration and rhizodeposition of carbohydrates, accelerates soil nutrient depletion, yet stimulates above- and belowground biomass production. *Global Change Biology* 11:1220-1233.

Bastian R.K., Ryan J.A., 1986. Design and management of successful land application system. Utilization, Treatment and Disposal of Waste on Land. *Soil Science Society of America* (Ed.), Inc, Madison, WI 217-234.

Beck T., 1990. Der Einfluß langjähriger Bewirtschaftungsweise auf bodenmikrobiologische Eigenschaften. *Kali-Briefe* 20:17-29.

Bergstrom D.W., Monreal C.M., King D.J., 1998. Sensivity of soil enzyme activities to conservation practices. *Soil Science Society of America Journal* 62:1286-1295.

Bergstrom D.W., Monreal C.M., Millette, J.A., King D.J., 1998. Spatial dependence of soil enzyme activities along a slope. *Soil Science Society of America Journal* 62:1302-1308.

Bergstrom D.W., Monreal C.M., Tomlin A.D., Miller J.J., 2000. Interpretation of soil enzyme activities in a comparison of tillage practices along a topographic and textural gradient. *Canadian Journal Soil Science* 80: 71-79.

Berthrong S.T., Jobbágy E.G., Jackson R.B., 2009. A global metaanalysis of soil exchangeable cations, pH, carbon, and nitrogen with afforestation. *Ecological Applications* 19:2228-2241.

Bitton G., Boylan R.A., 1985. Effect of acid precipitation on soil microbial activity. Soil core studies. *Journal of Environmental Quality* 14:66-69.

Boerner R.E.J., Decker, K.L.M., Sutherland E.K., 2000. Prescribed burning effects on soil enzyme activity in a southern Ohio hardwood: a landscape-scale analysis. *Soil Biology and Biochemistry* 32:899-908.

Bonanomi G., Antignani V., Capodilupo M., Cozzolino L., D'Ascoli R., Marzioli R., Puopolo G., Rao M.A., Rutigliano F.A., Scotti R., Zoina A., 2010. Assessing soil quality under intensive cultivation and tree orchards in Southern Italy. *Applied Soil Ecology* Accepted.

Boyd S.A. and Mortland M.M., 1990. Enzyme interactions with clays and clay-organic matter complexes. In: Bollag J.M. and Stotzky G. (Eds.), *Soil Biochemistry*. Marcel Dekker, Inc., New York, 6:1-28.

Brejda J.J., Karlen D.L., Smith J.L., Allan D.L., 2000a. Identification of regional soil quality factors and indicators: II. Northern Mississippi Loess Hills and Palouse Prairie. *Soil Science Society of America Journal* 64:2125-2135.

Brejda J.J., Moorman T.B., Karlen D.L., Dao T.H., 2000b. Identification of regional soil quality factors and indicators: I. Central and Southern High Plains. *Soil Science Society of America Journal* 64:2115-2124.

Brejda J.J., Moorman T.B., Smith J.L., Karlen D.L., Allan D.L., Dao, T.H., 2000c. Distribution and variability of surface soil properties at a regional scale. *Soil Science Society of America Journal* 64:974-982.

Brookes P.C., 1995. The use of microbial parameters in monitoring soil pollution by heavy metals. *Biology and Fertility of Soils* 19:269-279.

Burns R.G., 1982. Enzyme activity in soil: location and a possible role in microbial activity. *Soil Biology and Biochemistry* 14:423-427.

Burns R.G., 1986. Interaction of enzymes with soil mineral and organic colloids. In: Huang P.M. and Schnitzer M. (Eds.) Interactions of soil minerals and natural organics and microbes. SSSA Special Publication No. 17, Madison, Wisconsin, USA, 17: 429-451.

Busto M.D. and Perez-Mateos M., 1995. Extraction of humic-β-glucosidase fractions from soil. *Biology and Fertility of Soils* 20:77-82.

Cao G., Tang Y., Mo Y., Wang Y., Li Y., Zhao X., 2004. Grazing intensity alters soil respiration in an alpine meadow of the Tibetan plateau. *Soil Biology and Biochemistry* 36:237-243.

Caravaca F. and Roldán A., 2003. Assessing changes in physical and biological properties in a soil contaminated by oil sludges under semiarid Mediterranean conditions. *Geoderma* 117:53-61.

Carpenter S.R., Caraco N.F., Correll D.L., Howarth R.W., Sharpley A.N. and Smith V.H., 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications* 8:559-568.

Carter M.R., 1991. The influence of tillage on the proportion of organic carbon and nitrogen in the microbial biomass of medium-textured soils in a humid climate. *Biology and Fertility of Soils* 11:135-139.

Carter M.R., 1986. Microbial biomass as an index for tillage-induced changes in soil microbial properties. *Soil and Tillage Research* 7:29-40.

Cassman K.G. and Pingali P.L., 1995. Intensification of irrigated rice systems: learning from the past to meet future challenges. *GeoJournal* 35:299-305.

Cassman K.G., 1999. Ecological intensification of cereal production systems: yield potential, soil quality, and precision agriculture. *Proceedings of the National Academy of SciencesUSA* 96:5952-5959.

Ceccanti B. and Garcia C., 1994. Coupled chemical and biochemical methodologies to characterize a composting process and the humic substances. In: Humic Substances in the Global Environment and Implications on Human Health, ed. N. Senesi and T.M. Miano pp.1279-1285. Elsevier, New York.

Ceccanti B, Garcia C., Nogales R., Benitez E. and Masciandaro G., 1997. Attività e ruolo delle sostanze umiche nell' ambiente: aspetti chimico strutturali e biochimici. Dal suolo alla pianta dalla pianta al suolo. Le sostanze umiche come base della sostenibilità. Il convegno nazionale del capitolo italiano dell' IHSS.pp.1-9.

Centemero M. and Corti C., 2000. Caratteristiche tecniche del compost per un'agricoltura sostenibile. *L'Informatore Agrario* 6:23-39.

Chen X., Hutley L.B., Eamus D., 2005. Soil organic carbon content at a range of north Australian tropical savana with contrasting site histories. *Plant and Soil* 268:161-171.

Chhonkar P.K. and Tarafdar J.C., 1984. Accumulation of phosphatases in soils. *Journal of the Indian Society of Soil Science* 32:266-272.

Choi W.J. and Chang S.X., 2005. Nitrogen dynamic in co-composted drilling wastes: Effect of compost quality and N fertilization. *Soil Biology and Biochemistry* 37:2297-2305.

Chunderova A.I. and Zubets T., 1969. Phosphatase activity in dernopodzolic soils. *Pochvovedeniye* 11:47-53.

Ciccotti A.M., De Clauser R., Zorzi G., Cristoforetti A., Silvestri S., Jodice R., Gasperi F., Pinamonti F., 1988. Trasformazione in compost di residui di natura diversa. Aspetti biochimici e microbiologici nel corso del processo. Esperienze e ricerche XVIII. Anno 1988: pp. 75-120.

Clarholm M., 1993. Microbial biomass P, labile P, and acid phosphatase activity in the humus layer of a spruce forest, after repeated addition of fertilizers. *Biology and Fertility of Soils* 16:287-292.

Clark R.B. and Zeto S.K., 2000. Mineral acquisition by arbuscular mycorrhizal plants. *Journal of Plant Nutrition* 23:867-902.

Conant R.T., Paustian K., Elliot E.T., 2001. Grassland management and conversion into grasslands: effects on soil carbon. *Ecological Applications* 11:343-355.

Costantini E., 1995. Sostanza organica: conti e bilanci. *Agricoltura biologica*, suppl. al Notiziario ERSA n. 5, IX-X/95.

Crecchio C., Curci M., Pizzigallo M.D.R., Ricciuti P., 2004. Effects of municipal solid waste compost amendments on soil enzyme activities and bacterial genetic diversity. *Soil Biology and Biochemistry* 36:1595-1605.

Crippa L. and Zaccheo P. 1995. Effect of composting on short-term transformations in soil of 15N-labelled plant residues. *Soil Biology and Biochemistry* 27:247-250.

Demanèche S., Chapel J.P., Monrozier L.J., Quiquampoix H., 2009. Dissimilar pH-dependent adsorption features of bovine serum albumin and α-chymotrypsin on mica probed by AFM. *Colloids and Surfaces B: Biointerfaces* 70:226-231.

Deng S., Tabatabai M.A., 1995. "Cellulase activity of soils: effects of trace elements". *Soil Biology and Biochemistry* 27:977-979.

Dick W.A., 1984. Influence of long-term tillage and crop rotation combinations on soil enzyme activities. *Soil Science Society of America Journal* 48:569- 574.

Dick R.P., 1992. A review: Long-term effects of agricultural systems on soil biochemical and microbial parameters. *Agriculture Ecosystems and Environment* 40:25-36.

Dick R.P., 1994. Soil enzyme activities as indicators of soil quality. In: Doran J.W., Coleman D.C., Bezdicek D.F., Stewart B.A. (Eds.) Defining Soil Quality for a Sustainable Environment. Soil Science Society of America Special Publication No. 35, Madison, Winsconsin, pp. 107-124.

Dick R.P., Rasmussen P.E., Kerle E.A., 1988. Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat fallow system. *Biology and Fertility of Soils*, 6:159-164.

Dixon R.K., Brown S., Houghton R.A., Solomon A.M., Trexler M.C., Wisniewski J., 1994. Carbon pools and flux of global forest ecosystems. *Science* 263:185-190

Dkhar M.S. and Mishra R.R., 1983. Dehydrogenase and urease activities of maize (*Zea mays* L.) field soils. *Plant and Soil* 70:327-333.

Doran J.W. and Safley M., 1997. Defining and assessing soil health and sustainable productivity. In: Pankhurst C., Doube B.M., Gupta V. (Eds.) *Biological Indicators of Soil Health*. CAB International, Wallingford, pp. 1-28.

Doran J.W. and Parkin T.B., 1994. Defining and assessing soil quality. In: Defining Soil Quality or a Sustainable Environment. Doran J.W., Coleman D.C., Bezdicek D.F. and Stewart B.A. (Eds.), SSSA Inc Madison, USA.

Doran J.W., Sarrantonio M., Liebig M.A., 1996. Soil health and sustainability. In: Sparks D.L. (Ed.) Advances in Agronomy. Academic Press, New York, 56:1-54.

Edwards C.A., and Burrows I., 1988. The potential of earthworm composts as plant growth media. *Earthworms in Waste and Environmental Management* p. 211-219.

Ekenler M., Tabatabai M.A., 2003b. Responses of phosphatases and arylsulfatase in soil to liming and tillage systems. *Journalof Plant Nutrition and Soil Science* 166:281-290.

Elliot E. T., 1997. Rationale for developing bioindicators of soil heath. In Biological Indicators of Soil Health, Pankhurst C. E., Doube B. M., Gupta V. V. S. R. (Eds) CAB International, wellington, pp. 49-78.

Elsgaard L., Petersen S.O., Debosz K., 2001. Effect and risk assessment of linear alkylbenzenesulfonate (LAS) in agricultural soil. 2. Effect on soil microbiology as influenced by sewage sludge and incubation time. *Environmental Toxicology and Chemistry*, 20:1664-1672.

Faber B.A., Zasoski R.J., Burau R.G., Uriu K., 1990. Zinc uptake by corn as affected by vesicular-arbuscular mycorrhizae. *Plant and Soil* 129:121-130.

Fauci M.F. and Dick, R.P., 1994. Soil microbial dynamics: short- and long-term effects of organic and inorganic nitrogen. *Soil Science Society of America Journal* 58:801-806.

Felipò M.T., 1996. Compost as source of organic matter in Mediterranean soils. Pp 402-412. In: de Bertoldi, M; Sequi, P, Lemmes, B; Papi, T (eds.), The science of composting. Vol 2. Blackie Academic and Professional, UK.

Food and Agriculture Organization of the United Nations (FAO), 2001. FAO Statistical Databases <http://apps.fao.org/>.

Frankenberger W.T. and Johanson J.B., 1983. Influence of crude oil and refined petroleum products on soil dehydrogenase activity. *Journal of Environmental Quality* 11:602-607.

Gallardo-Lara F. and Nogales R., 1987. Effect of the application of town refuse compost on the soil-plant system: a review. *Biological Wastes*, 19:35-62.

Galstian, A.S. 1974. Enzymatic activity of soils. *Geoderma* 12:43-48.

Garcìa C., Hernàndez T., Costa F., Ceccanti B., Ciardi C., 1992. Changes in ATP content, enzyme activity and inorganic nitrogen species during composting of organic wastes. *Canadian Journal of Soil Science* 72:243-253.

Garcia C., Hernandez T., Costa F., 1994. Microbial activity in soils under Mediterranean environmental conditions. *Soil Biology and Biochemistry* 26:1185-1191.

George E., Marschner H., Jakobsen I., 1995. Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. *Critical Reviews in Biotechnology*15:257-270.

Gianfreda L., Bollag J-M., 1996. Influence of natural and anthropogenic factor on enzyme activity in soil. In: Stotzky G., Bollag J-M (Eds.). Soil Biochemistry, vol. 9. Marcel Dekker, New York, pp. 123-194.

Gil-Sotres F., Trasar-Cepeda C., Leirós M.C., Seoane S., 2005. Different approaches to evaluating soil quality using biochemical properties. *Soil Biology and Biochemistry* 37:877-887.

Gomez A.A., Swete Kelly D.E., Syers J.K., Coughlan K.J. 1996 Measuring sustainability of agricultural systems at the farm level. In Doran JW, Jones AJ, eds. Methods for Assessing Soil Quality. Spec. Publ. No. 49. Madison, WI: *Soil Science Society of America Journal,* pp. 401-409.

Griffith B.S., Bonkowski M., Roy J., Ritz K., 2001. Functional stability, substrate utilisation and biological indicators of soils following environmental impacts. *Applied Soil Ecology* 16:49-61.

Guo L.B., Gifford R.M., 2002. Soil carbon stocks and land use change: a meta analysis. *Global Change Biology* 8:345-360

Harrison A.F., 1983. Relationship between intensity of phosphatase activity and physico-chemical properties in woodland soils. *Soil Biology and Biochemistry* 15: 93-99.

Häussling M. and Marschner H., 1989. Organic and inorganic soil phosphates and acid phosphatase activity in the rhizosphere of 80-year-old Norway spruce (*Picea abies* (L.) Karst.) trees. *Biology and Fertility of Soils* 8:128-133.

Haynes R.J., Williams P.H., 1999. Influence of stock camping behaviour on the soil microbiological and biochemical properties of grazed pastoral soils. *Biology and Fertility of Soils* 28:253-258.

Hector A.B. et al., 1999. Plant diversity and productivity experiments in European grasslands. *Science* 286:1123-1127.

Helal H.M. and Sauerbeck D., 1987. Phosphatase-Aktivität von Pflanzenwurzeln und Böden in Abhängigkeit von der P-Versorgung. VDLUFA 23:195-201.

Hyvönen R. and Persson T., 1990. Effects of acidification and liming on feeding groups of nematodes in coniferous forest soils. *Biology and Fertility of Soils*9:205-210.

Hillel D., 1991. Out of the Earth: Civilization and the Life of the Soil. University of California Press, Berkeley, CA, pp. 321.

Hodge A., Campbell C.D., Fitter A.H., 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* 413:297-299.

Holland J.N., 1995. Effects of above-ground herbivory on soil microbial biomass in conventional and no-tillage agroecosystems. *Applied Soil Ecology* 2:275-279.

Hu Y.L., Zeng D.H., Fan Z.P., Chen G..S, Zhao Q., Pepper D., 2008. Changes in ecosystem carbon stocks following grassland afforestation of semiarid sandy soil in the southeastern Keerqin Sandy Lands, China. *Journal of Arid Environments*72:2193-2200.

Hussain I., Olson K.R., Wander M.M., Karlen D.L. 1999 Adaptation of soil quality indices and application to three tillage systems in southern Illinois. *Soil and Tillage Research* 50:237-249.

Iftikhar A., Khan, 1988. Studies on enzymes activity in normal and saline soils. Pak. *Journal of Agricultural Research* 9:505-508.

IPCC (2000) Land use, Land-use change, and forestry. Cambridge University Press, Cambridge

Jaenicke E.C., Lengnick L.L., 1999. A soil-quality index and its relationship to efficiency and productivity growth measures: two decompositions. *American Journal of Agricultural Economics* 81:881-893.

Jenkinson D.S., 1990. The turnover of organic carbon in the soil. Philosophical Transactions of the Royal Society of London B 329:361-368.

Jensen L.S., McQueen D.J., Sheperd T.G., 1996. Effects of soil compaction on N-mineralization and microbial-C and -N.I. Field measurements. *Soil and Tillage Research* 38:175-188.

Juma N.G. and Tabatabai M.A., 1978. Distribution of phosphomonoesterases in soils. *Soil Science* 126:101-108.

Kandeler E. and Eder G., 1993. Effect of cattle slurry in grassland on microbial biomass and on activities of various enzymes. *Biology and Fertility of Soils* 16:249-254.

Kandeler, E., Stemmer, M., Klimanek, E.M., 1999. Response of soil microbial biomass, urease and xylanase within particle size fraction to long-term soil management. *Soil Biology and Biochemistry* 31:261-273.

Karlen D.L., Andrews S.S., Doran J.W., 2001 “Soil quality : current concepts and applications" in Advances in Agronomy 74:1-39.

Karlen D.L., Gardner J.C., Rosek M.J., 1998. A soil quality framework for evaluating the impact of CRP. *Journal of Production Agriculture* 11:56-60.

Karlen D.L., Rosek M.J., Gardner J.C., Allan D.L., Alms M.J., Bezdicek D.F., Flock M., Huggins D.R., Miller B.S., Staben M.L., 1999. Conservation reserve program effects on soil quality indicators. *Journal of Soil and Water Conservation*54:439-444.

Kawasaki S., Maie N., Kitamura S., and Watanabe A., 2008. Effect of organic amendment on amount and chemical characteristics of humic acids in upland field soils. *European Journal of Soil Science* 59:1027-1037.

Khan A.R., 1996. Influence of tillage on soil aeration. *Journal of Agronomy and Crop Science* 177:253-259.

Knoepp J.D., Coleman D.C., Crossley D.A. Jr., Clark J.S., 2000. Biological indices of soil quality: an ecosystem case study of their use. *Forest Ecology and Management* 138:357-368.

Koide R., 1991. Nutrient supply, nutrient demand and plant-response to mycorrhizal infection. New Phytologist 117:365-386. Smith, S.E., Read, D.J., 1997. Mycorrhizal Symbiosis, second edn. Academic Press, London.

Kothari S.K., Marschner H., Römheld V., 1991a. Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in calcareous soil. *Plant and Soil* 131:177-185.

Kumar J.D., Sharma G.D., Mishra R.R., 1992. Soil microbial population number and enzyme activities in relation to altitude and forest degradation. *Soil Biology and Biochemystry* 24:761-767.

Kuzyakov Y., Biryukova O.V., Kuznetzova T.V., Mölter, K., Kandeler E., Stahr K., 2002. Carbon partitioning in plant and soil, carbon dioxide fluxes and enzyme activities as affected by cutting ryegrass. *Biology and Fertility of Soils* 35:348-358.

Laganière J., Angers D.A., Paré D., 2010. Carbon accumulation in agricultural soils after afforestation: a meta-analysis. *Global Change Biology* 16:439-453.

Lagomarsino A., Moscatelli M.C., Di Tizio A., Mancinelli R., Grego S., Marinari S. 2009. Soil biochemical indicators as a tool to assess the short-term impact of agricultural management on changes in organic C in a Mediterranean environment. *Ecological Indicators* 9:518-527.

Lähdesmäki P. and Piispanen R., 1992. Soil enzymology: role of protective colloid systems in the preservation of exoenzyme activities in soil. *Soil Biology and Biochemistry* 24:1173-1177.

Lakhdar A., Scelza R., Scotti R., Rao M.A., Jedidi N., Gianfreda L., Abdally C., 2010. The effect of compost and sewage sludge on soil biologic activities in salt affected soil. *Journal of Soil Science and Plant Nutrition* 10:40-47.

Lal R., 1999. Soil quality and food security: the global perspective. In: Lal R. (Ed.) Soil Quality and Soil Erosion. CRC Press, Boca Raton, FL, pp. 3-16.

Lal R., 2004. Soil carbon sequestration to mitigate climate change. *Geoderma* 123:1-22.

Lal R., 2005. Forest soils and carbon sequestration. *Forest Ecology and Management* 220:242-258.

Latif M.A., Mehuys G.R., Mackenzie A.F., Alli I., Faris M.A., 1992. Effects of legumes on soil physical quality in a maize crop. *Plant and Soil* 140:15-23.

Leroy B.L.M., H. Herath S., Sleutel S., De Neve D., Gabriel D., Reheul and M. Moens, 2008. The quality of exogenous organic matter: short-term effects on soil physical properties and soil organic matter fractions. *Soil Use and Management* 24:139-147.

Liebig M.A., Doran J.W., 1999. Impact of organic production practices on soil quality indicators. *Journal of Environmental Quality* 28:1601-1609.

Lopez-Hernandez D., Nino M., Nannipieri P., Fardeau J.C., 1989. Phosphatase activity in *Nasutitermes ephratae* termite nests. *Biology and Fertility of Soils* 7:134-137.

Loreau M. S. *et al.,* 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* 294:804-808.

Lynch J.M., Panting L.M., 1980. Cultivation and the soil biomass. *Soil Biology and Biochemistry* 12:29-33.

Marcote I., Hernandez T., Garcia C., Polo A., 2001. Influence of one or two successive annual application of organic fertilisers on the enzyme activity of a soil under barley cultivation. *Bioresource Technology* 79:147-154.

Marinari S., Masciandaro G., Ceccanti B., Greco S., 2001. Influence of organic and mineral fertilisers on soil biological physical properties. *Bioresource Technology* 72:9-17.

Marinari S., Mancinelli R., Campiglia E., Grego S., 2006. Chemical and biological indicators of soil quality in organic and conventional farming systems in Central Italy. *Ecological Indicators* 6:701-711.

Martens D.A., Johanson J.B., Frankeberger W.T. Jr, 1992. Production, and persistence of soil enzymes with repeated additions of organic residues. *Soil Science* 153:53-61.

Masciandaro G., Ceccanti B., Gallardo-Lancho J.F., 1998. Organic matter properties in cultivated versus set-aside arable soils. *Agriculture, Ecosystems and Environment* 67:267-274.

Mason J., 2003 Sustainable agriculture second edition National library of Australia Cataloguing ISBN 0 643 06876 7.

Mathur S.P., Rayment A.F., 1977. Influence of trace element fertilization on the decomposition rate and phosphatase activity of a mesic fibrisol. *Canadian Journal of Soil Science* 57:397-408.

Mawdsley, J.L., Bardgett, R.D., 1997. Continuous defoliation of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the microbial population of an upland grassland soil. *Biology and Fertility of Soils* 27:969-975.

McGill W.B., Cannon K.R., Robertson J.A., Cook F.D., 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. *Canadian Journal of Soil Science* 66:1-19.

Mijangos I., Pérez R., Albizu I., Garbisu C., 2006. Effects of fertilization and tillage on soil biological parameters. *Enzyme and Microbial Technology* 40:100-106.

Miller M. and Dick R.P., 1995. Thermal stability and activities of soil enzymes as influenced by crop rotations. *Soil Biology and Biochemistry* 27:1161-1166.

Monokrousos N., Papatheodorou E.M., Diamantopoulos J.D., Stamou G.P., 2006. Soil quality variables in organically and conventionally cultivated field sites. *Soil Biology and Biochemistry* 38:1282-1289.

Monreal C.M., Dinel H., Schnitzer M., Gamble D.S., Biederbeck V.O., 1998. Impact of carbon sequestration on functional indicators of soil quality as influenced by management in sustainable agriculture. In: Lal R., Kimble J.M., Follett R.F., Stewart B.A. (Eds.) Soil Processes and the Carbon Cycle. CRC Press, Boca Raton, FL, pp. 435-457.

Morris S.J., Bohm S., Haile-Mariam S., Paul E.A., 2007. Evaluation of carbon accrual in afforested agricultural soils. *Global Change Biology* 13:1145-1156.

Nannipieri P., 1994. The potential use of soil enzymes as indicators of soil productivity, sustainability, and pollution. In: Pankhurst C.E., Doube B.M., Gupta V.V.S.R., Grace P.R. (Eds.) Soil Biota: Management in Sustainable Farming Systems. CSIRO Information Services, Victoria, Australia, pp. 238-244.

Nannipieri P., Grego S., Ceccanti B., 1990. Ecological significance of the biological activity in soil. In: Bollag J.M. and Stotzky G. (Eds.) Soil Biochemistry. Dekker, New York, Vol. 6: 293-355.

Nannipieri P., Pedrazzini F., Arcara P.G., Piovanelli G., 1979. Changes in amino acids, enzyme activities and biomasses during soil microbial growth. *Soil Science* 127: 26-34.

National Research Council, 1993. Soil and Water Quality: an Agenda for Agriculture. National Academy Press, Washington, DC, pp. 516.

Niemi R.M., Vepsäläinen M, Wallenius K, Simpanen S, Alakukku L., Pietola L., 2005. Temporal and soil depth- related variation in soil enzyme activities and in root growth of red clover (*Trifolium pratense*) and timothy (*Phleum pratense*) in the field. *Applied Soil Ecology* 30:113-125.

Nissen T.M. and Wander M.M., 2003. Management and soil-quality effects on fertilizer-use efficiency and leaching. *Soil Science Society of America Journal* 67:1524-1532.

Northup B.K., Brown J.R., Holt J.A., 1999. Grazing impacts on the spatial distribution of soil microbial biomass around tussock grasses in a tropical grassland. *Applied Soil Ecology* 13:259-270.

Olander L.P. and Vitousek P.M., 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry* 49:175-190.

Ozores-Hampton M., Obreza T.A., Hochmuth G., 1998. Using composted wastes on Florida vegetable crops. *Horticulture Technology* 8:130-137.

Pascual J.A., Garcìa C., Hernandez T., 1999. Lasting microbiological and biochemical effects of the addition of municipal solid waste to an arid soil. *Biology and Fertility of Soils* 30:1-6.

Paul K.I., Polglase P.J., Nyakuengama J.G., Khama P.K., 2002. Change in soil carbon following afforestation. *Forest Ecology and Management* 168:241-257.

Peichl M. and Arain M.A., 2006. Above- and belowground ecosystem biomass and carbon pools an age-sequence of temperate pine plantation forests. *Agricultural and Forest Meteorology*140:51-63.

Perner H., Schwarz D., Bruns C., Mader P., George E., 2007. Effect arbuscular mycorrhizal colonization and two levels of compost supplì on nutrient uptake and flowering of pelargonium plants. *Mycorrhiza* 17:469-474.

Perucci P., Scarponi L., Businelli M., 1984. Enzyme activities in a clay-loam soil amended with various crop residues. *Plant and Soil* 81:345-351.

Perucci P. and Scarponi L., 1985. Effect of different treatments with crop residues on soil phosphatase activity. *Biology and Fertility of Soils* 1:111-115.

Pinstrup-Andersen, P. and Pandya-Lorch, R. Food for all in 2020—can the world be fed withoutdamaging the environment. *Environmental Conservation* 23:226-234.

Powers R.F., Tiarks A.E., Boyle J.R., 1998. Assessing soil quality: practicable standards for sustainable forest productivity in the United States. In: Adams M.B., Ramakrishna K., Davidson E.A. (Eds.) The Contribution of Soil Science to the Development and Implementation of Criterial and Indicators of Sustainable Forest Management. Soil Science Society of America, Special Publication No. 53, ASA, Madison, WI, pp. 53-80.

Powlson D.S., Brookes P.C., Christensen B.T., 1987. Measurement of soil microbial biomass provides an early indication of change in total soil organic matter due to straw incorporation. *Soil Biology and Biochemystry* 19:159-164.

Prather M. D. et al. in Climate Change 2001: The Scientific Basis (Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change) (eds Houghton, J. T. et al.) 239-287 (Cambridge University Press, Cambridge, 2001).

Preston G.S., 1998. Early Warning Monitoring Guide for Croplands. Center for Holistic Management, Albuquerque, NM. p.p. 22.

Puglisi E., Del Rea A.A.M., Rao M.A., Gianfreda L., 2006. Development and validation of numerical indexes integrating enzyme activities of soils. *Soil Biology and Biochemestry* 38:1673-1681.

Renbuss M., Chilvers G.A. and Pryer L.D. 1973 Microbiology of an ashbed. *Proceedings of Linnean Society* NS W 97:302-310.

Richter D.D., Markewitz D., Trumbore S.E., Wells C.G., 1999. Rapid accumulation and turnover of soil carbon in a reestablishing forest. *Nature* 400:56-58.

Ritter E., 2007. Carbon, nitrogen and phosphorus in volcanic soils following afforestation with native birch (*Betula pubescens*) and introduced larch (*Larix sibirica*) in Iceland. *Plant and Soil* 295:239-251.

Ros M., Pascual J.A., Garcia C., Hernandez M.T., Insam H., 2006. Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. *Soil Biology and Biochemistry* 38:3443-3452.

Rutigliano F.A., Virzo De Santo A., Alfani A., Esposito A., 1995. Attività metabolica di suoli campani in funzione degli incendi. S. It. E. Atti 16:535-538.

Saffigna P.G., Powlson D.S., Brookes P.C., Thomas G.A., 1989. Influence of sorghum residues and tillage on soil organic matter and soil microbial biomass in an Australian Vertisol. *Soil Biology and Biochemistry* 21:759-765.

Sanders F.E., Tinker P.B., 1971. Mechanism of absorption of phosphate from soil by Endogone mycorrhizas. *Nature* 233:278-279.

Sarathchandra S.U., Perrott K.W., Upsdell M.P., 1984. Microbiological and biochemical characteristics of a range of New Zealand soils under established pasture. *Soil Biology and Biochemistry* 16:177-183.

Sarkar J.M. and Burns R.G., 1984. Synthesis and properties of β-glucosidase-phenolic copolymers as analogues of soil humic-enzyme complexes. *Soil Biology and Biochemistry* 16:619-625.

Sarkar J.M., Leonowicz P., Bollag J.M., 1989. Immobilization of enzymes on clays and soils. *Soil Biology and Biochemistry* 21:223-230.

Scagliarini S., 1999. Le funzioni del compost nel terreno. Il Divulgatore Anno XXII. Centro Agricoltura Ambiente.

Scelza R., Rao M. A., Gianfreda L., 2008. Response of an agricultural soil to pentachlorophenol (PCP) and the addition of compost or dissolved organic matter. In *Soil Biology and Biochemistry* 40:2163-2169.

Schaffer A., 1994. Pesticide effects on enzyme activities in the soil ecosystem. Pp 273-340. In: Bollag J-M., Stotzky G. (Eds), Soil Biochemistry, Vol. 8. Marcel Dekker, New York.

Schipper, L.A. and Sparling, G.P., 2000. Performance of soil condition indicators across taxonomic groups and land uses. *Soil Science Society of America Journal* 64:300-311.

Schoenholtz S.H., Van Miegroet H., Burger J.A., 2000. A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. *Forest Ecology and Management* 138:335-356.

Schüssler A., Schwarzott D., Walker C., 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* 105:1413-1421.

Shi W., Dell E., Bowman D., Iyyemperumal, K., 2006. Soil enzyme activities and organic matter composition in a turfgrass chronosequence. *Plant and Soil* 288:285-296.

Sinsabaugh R.L., 1994. Enzymic analysis of microbial pattern and process. *Biology and Fertility of Soils* 17:69-74.

Six J., Conant R.T., Paul E.A., Paustian K., 2002. Stabilization mechanisms of soil organic matter: implications for C saturation of soils. *Plant and Soil 241*:155-176.

Skujinš J., 1967. Enzymes in soil. In: A.D. McLoren and G.H. Peterson (Eds.). Soil Biochemistry, Vol.1. Marcel Dekker, New York, pp. 371-414.

Skujinš J., 1976. Extracellular enzymes in soil. *CRC Critical Reviews in Microbiology* 4:383-421.

Smith S.E. and Read D.J., 1997. Mycorrhizal symbiosis. 2nd edn. Academic Press, London, p.p. 605.

Soumaré M. , Tack F.M.G., Verloo M.G., 2003. Characterization of Malian and Belgian solid waste composts with respect to fertility and suitability for land application. *Waste Management*, 23:517-522.

Sparling G.P., Speir T.W., Whale K.N., 1986. Changes in microbial biomass C, ATP content, soil phosphomonoesterase and phosphodiesterase activity following air-drying of soils. *Soil Biology and Biochemistry* 18:363-370.

Spiers G.A., McGill W.B., 1979. Effect of phosphorus addition and energy supply on acid phosphatase production and activity in soils. *Soil Biology and Biochemistry* 11:3-8.

Srivastava S.C. and Singh J.S., 1991. Microbial C, N, and P in dry tropical forest soils: effects of alternate land-uses and nutrient flux. *Soil Biology and Biochemistry* 23:117-124.

Tabaglio V., Gavazzi C., Menta C., 2008, Physico-chemical indicators and microarthropod communities as influenced by no-till, conventional tillage and nitrogen fertilisation after four years of continuous maize. *Soil and Tillage Research* 105:135-142.

Tabatabai M.A., 1994. Soil enzymes. In: R. W. Weaver, S. Angle, Bottomley P. (Eds.) Methods of Soil Analysis, Part 2: Microbiological and Biochemical Properties. Soil Science Society of America, Madison, pp. 775-883.

Tarafdar J.C. and Jungk A., 1987. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. *Biology and Fertility of Soils* 3:199-204.

Taylor J.P., Wilson M., Mills S., Burns R.G., 2002. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. *Soil Biology Biochemistry* 34:387-401.

Teklay T. and Chang S.X., 2006. Temporal changes in soil carbon and nitrogen storage in a hybrid poplar chronosequence in northern Alberta. *Geoderma* 144:613-619.

Tilman, D., Fargione J., Wolff B., D'Antonio C., Dobson A., Howarth R., Schindler D., Schlesinger W.H., Simberloff D., Swackhamer D., 2001. Forecasting agriculturally driven global environmental change. *Science* 292:281-284.

Tilman D., Cassman K.G., Matson P.A., Naylor R. and Polasky S., 2001. Agricultural sustainability and intensive production practices. *Nature* 418:671-677.

Trasar-Cepeda M.C. and Gil-Sotres F., 1987. Phosphatase activity in acid high organic matter soils in Galicia (NW Spain). *Soil Biology and Biochemistry* 19:281-287.

Trasar-Cepeda C., Leiros M.C., Seoane S., Gil Sotres F. 2000. Limitation of soil enzyme as indicators of soil pollution. *Soil Biology and Biochemistry* 32:1867-1875.

van Groenigen KJ, Six J, Hungate BA, de Graaff MA, van Breemen N, van Kessel C (2006) Element interactions limit soil carbon storage. *Proceedings of the National Academy of Sciences* U S A 103:6571-6574.

Vesterdal L., Ritter E., Gundersen P., 2002. Change in soil organic carbon following afforestation of former arable land. *Forest Ecology and Management* 169:137-147.

Visser S. and Parkinson D., 1992. Soil biological criteria as indicators of soil quality: soil microorganisms. *American Journal of Alternative Agriculture* 7:33-37.

Vitousek, P.M., Mooney, H.A., Lubchenco J., and Melillo J.M., 1997. Human domination of earth's ecosystems. *Science* 277:494-499.

Vizioli V., 1997. La fertilizzazione organica in agricoltura biologica. *Mediterraneo*, 4:70-77.

Waggoner P.E., 1995. How much land can ten billion people spare for nature? Does technology make a difference? *Technology in Society*17:17-34.

Wander M.M. and Bollero G.A., 1999. Soil quality assessment of tillage impacts in Illinois. *Soil Science Society of America Journal* 63:961-971.

Wang X.C., Lu Q., 2006. Beta-glucosidase activity in paddy soils of the Taihu lake region. *China Pedosphere* 16:118-124.

Wardle D.A., Barker G.M., 1997. Competition and herbivory in establishing grassland communities: Implications for plant biomass, species diversity and soil microbial activity. *Oikos* 80:470-480.

Weber J., Karczewska A., Drozd J., Licznar M., Licznar S., Jamroz E., Kocowicz, A., 2007. Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts. *Soil Biology and Biochemistry* 1294-1302.

West A.W., Sparling G.P., Speir T.W., Wood J.M., 1988a. Dynamics of microbial C, N-flush and ATP, and enzyme activities of gradually dried soils from a climosequence. *Australian Journal of Soil Research* 26:519-530.

West A.W., Sparling G.P., Speir T.W., Wood J.M., 1988b. Comparison of microbial C, N-flush and ATP, and certain enzyme activities of different textured soils subject to gradual drying. *Australian Journal of Soil Research* 26:217-229.

Whalen J.K., Hu Q.C., Liu A.G., 2003. Compost applications increase water-stable aggregates in conventional and no-tillage systems. *Soil Science Society of America Journal* 67:1842-1847.

Wienhold B.J., Andrews S.S. and Karlen D.L., 2004. Soil quality: a review of the science and experiences in the USA. *Environmental Geochemistry and Health* 26: 89-95.

World Health Organization (WHO). Public health impacts of pesticides used in agriculture (WHO in collaboration with the United Nations Environment Programme, Geneva, 1990).

Wortmann C.S., and Shapiro C.A., 2008. The effects of manure application on soil aggregation. *Nutrient Cycling in Agroecosystems* 80:173-180.

Zacheis A., Ruess R.W., Hupp J.W., 2002. Nitrogen dynamics in an Alaskan salt marsh following spring use by geese. *Oecologia* 130:600-608.

Zhao Y., Wang P., Li J., Chene Y., Yingf X., Liud S., 2009. The effects of two organic manures on soil properties and crop yields on a temperate calcareous soil under a wheat-maize cropping system. *European Journal of Agronomy* 31:36-42.

Chapter 2

Aim

The loss of organic matter in soils under intensive farming managements is one of the major concerns of the modern agriculture. Globalization and changes in technology, population growth and market demand have often intensified agricultural production. Based on projections for growth of the world's population, the current productivity of agriculture should be double by 2025 to meet the world's food needs.

Wide spread adoption of innovative technology is the key to continuing the increase in agricultural productivity. Profit-maximizing conventional agriculture, which has become increasingly intensified, not only has led to farm overproductions but has also dramatically increased soil degradation and environmental pollution by inducing erosion and poisoning water with agricultural chemicals.

The higher rates of plant uptake are compensated by increasing chemical fertilizers use, but matched by reductions in applications of organic amendments. Changes in soil physical and chemical properties, such as changing quality and quantity of organic matter also play a role in declining yields.

As currently practiced, however, intensive agriculture is degrading the soil resource base, posing a threat to its sustainability. Sustainable agriculture does not mean a return to either the low yields or poor farmers that characterized the 19th century. Rather, sustainability builds on current agricultural achievements, adopting a sophisticated approach that can maintain high yields and farm profits without undermining the resources on which agriculture depends.

The present study was based on the hypothesis that the long-term soil cultivation under intensive management, in particular under greenhouse, could affect negatively soil fertility, in physical, chemical and biological terms. Among all, a reduction in organic carbon content, and, consequently, a decrease in microbial activity were expected. On the other hand, sustainable managements can protect and enhance the productivity of soil by using cover crops, compost and/or manures, reducing tillage,

etc.. The lost soil fertility could be restore by ensuring a valid organic matter replenishment, but, as widely reported in literature, the recovery in soil fertility was shortly achieved and just as shortly lost, reaching the starting conditions. That could be ascribed to intensive farming as well as the prevailing labile fraction in compost generally used as amendments, and the environmental conditions favouring mineralization process.

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 besides compost, on the soil fertility of two farms selected among all previously monitored.
3. To characterize the organic matter of variously amended soils by conventional and innovative spectroscopic analyses.
4. To evaluate the effect of organic amendments on the development of indigenous micorrhiza (AMF) and on lettuce crops in a Chilean soil.

Chapter 3

Assessing soil quality of intensive farms in an important agriculture region of Southern Italy[1]

3.1. Introduction

As defined by Doran and Parkin (1994) (see Chapter 1) soil quality is a complex and multifaceted concept encompassing many properties and processes as the structural stability of aggregates (Abiven et al., 2009), the water retention capacity (Loveland and Webb, 2003), the capability of nutrient cycling (Tiessen et al., 1994), the ability to store organic carbon (Martens et al., 2003) and to naturally suppress soilborne plant pathogens (Janvier et al., 2007).

Soil quality is the outcome of interactions among physical, chemical and biological characteristics, and its proper assessment requires the determination of a large number of parameters (Bloem et al., 2006a; Marzaioli et al., 2010). In this context, soil enzymes and microbial-based processes are now considered particularly important because they usually respond more rapidly than chemical and physical parameters to environmental changes and stresses such as heavy metal pollution (Schloter et al., 2003; Gianfreda and Ruggiero, 2006). In fact, soil microbes are useful indicators of soil quality because they are involved in organic matter (OM) decomposition, nutrient cycling, maintenance of soil structure, and suppressiveness to plant pathogens.

In the last decades a significant decrease in primary productivity has been observed worldwide as a consequence of soil erosion, overgrazing, salinity and/or sodicity

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[1]A version of this Chapter has been published as:

Bonanomi G., D'Ascoli R., Antignani V., Capodilupo M., Cozzolino L., Marzioli R., Puopolo G., Rutigliano F.A., Scelza R., Scotti R., Rao M.A., Zoina A., 2010. Assessing soil quality under intensive cultivation and tree orchards in Southern Italy. *Applied Soil Ecology* 2010 Accepted

induced by irrigation (Sumner, 1995), pollution by heavy metals and xenobiotics (Moolenaar et al., 1997), reduction of soil organic carbon (Loveland and Webb, 2003) and loss of natural soil suppressiveness due to the heavy application of fumigants and fungicides (Weller et al., 2002).

Soil quality plays a key role in the development of a sustainable agriculture and several projects have been established in many countries (including US, Canada, France, Germany, Netherlands, UK, Switzerland, Czech Republic, Austria and New Zealand) with the aim of monitoring soil quality in relation to farming practices (Bloem et al., 2006b). Many studies investigated the impact of conventional vs. organic farming (Drinkwater et al., 1995; Birkhofer et al., 2008), grassland vs. arable soils (Haynes et al., 2000), and different fertilization approaches, including mineral and organic applications (Fließbach et al., 2007) on soil quality. In a leading study, Mäder et al. (2002) reported a higher soil quality and ecosystem biodiversity for organically managed plots compared to conventional cultivation.

Despite the large and growing body of available literature, no studies addressed the long-term impact of intensive cultivation under permanent plastic tunnels on soil quality. Cultivation under plastic tunnels is a steadily growing agricultural sector all over the world, and at present it covers more than 400,000 ha in the Mediterranean Basin alone (Enoch and Enoch, 1999). This type of cultivation is expected to profoundly affect soil quality because it drastically modifies water, carbon and nutrient cycles. In fact, the almost complete rainfall restriction and the consequent requirement of localized irrigation to support crop water demand is expected to increase soil salinity. Moreover, the widespread use of mineral fertilizer, the systematic removal of crop residues to limit plant diseases (Bonanomi et al., 2007), and the optimal temperature and water content that promote mineralization of OM are all factors expected to reduce soil organic C content, with a negative feedback on soil microbial populations. Therefore, the hypothesis advanced in the present work is that the long-term soil cultivation under permanent plastic cover reduce soil quality, both in chemical and biochemical terms. In detail, the expected results should be that

under intensive cultivation: i) a reduction in organic C content, and, consequently, a decrease in microbial activity, such as enzymatic activities; ii) an increase in soil salinity; iii) no effect on some basic chemical and physical parameters as pH, limestone content, and soil texture.

This study addressed this hypothesis with a multidisciplinary approach. To achieve this purpose two different soil management regimes, were compared: high-input management regime (HIMR), characterized by intensive cultivation under permanent plastic tunnels; and ii) low-input management regime (LIMR), constituted by tree orchards. Since soil quality cannot be summarized by a single property or process, its assessment necessarily requires measurement of a large number of parameters. Therefore, the main soil properties were measured (including physical, chemical and biochemical ones) to assess soil quality in five different sampling areas cultivated both under HIMR and LIMR.

The present work was carried out within the framework "Monitoraggio e recupero della Fertilità dei suoli in sistemi agricoli intensivi" a research project funded by CCIAA of Salerno (Italy) in collaboration with the research groups of Prof. Astolfo Zoina, Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, and Dr. Rosaria D'Ascoli, Dipartimento di Scienze Ambientali, Seconda Università di Napoli.

3.2 Materials and Methods

3.2.1 Study site description and selection of farms

All selected soils are located in agricultural farms of Salerno district (Southern Italy), a very productive area with ~3,500 ha cultivated under greenhouses. 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).

Two different soil management regimes were studied:

1) intensive cultivation under plastic cover for at least 6 years, classified as high-input management regime (HIMR);
2) tree orchards, classified as low-input management regime (LIMR).

In five sampling areas, named from A to E, characterized by different soil types along a gradient of soil texture ranging from heavy clay to sandy soil, 20 soils under HIMR were selected (four for each sampling area, named from 1 to 4), and in 15 of them (three for each sampling area, on the basis of field availability) adjacent fields cultivated with orchards were chosen as soils under LIMR (named Control). Tree orchards were selected as LIMR because such management regime is much more common compared with extensive arable cultivation or grassland in the study area. According to the USDA classification (1998), sandy Entisols, shallow Mollisols, Mollisols having a thick mollic epipedon, Mollisols with vertic properties, Alfisols and Vertisols having a mollic epipedon, Mollisols and Inceptisols having vitric properties, and Vitric Andosols with or without a mollic epipedon were included in the sampling (Table 3.1). This sampling strategy minimized the variability due to soil types and allowed a direct comparison of management regime effect on soil quality.

Table 3.1 Sampled soils classification according to Keys to Soil Taxonomy (USDA, 1998).

Sampling area	Soil	Type	Management regime
A	1	Pachic Haploxerolls	HIMR, LIMR
	2	Mollic Haploxeralf	HIMR
	3	Mollic Haploxeralf	HIMR, LIMR
	4	Pachic Haploxerolls	HIMR, LIMR
B	1	Typic Haploxererts	HIMR,
	2	Mollic Haploxererts	HIMR, LIMR
	3	Mollic Haploxererts	HIMR, LIMR
	4	Vertic Haploxerolls	HIMR, LIMR
C	1	Typic Xeropsamments	HIMR, LIMR
	2	Typic Xeropsamments	HIMR, LIMR
	3	Mollic Haploxererts	HIMR,
	4	Mollic Haploxererts	HIMR, LIMR
D	1	Lithic Haplustolls	HIMR,
	2	Lithic Haplustolls	HIMR, LIMR
	3	Lithic Haplustolls	HIMR, LIMR
	4	Fluvaquentic Haplustolls	HIMR, LIMR
E	1	Vitrandic Haplusteps	HIMR, LIMR
	2	Humic Ustivitrands	HIMR, LIMR
	3	Vitrandic Calciustolls	HIMR, LIMR
	4	Vitrandic Calciustolls	HIMR

3.2.2 Soil history assessment

The soil history of the six years preceding the sampling was assessed for all the selected farms. Information was gathered by interview and questionnaires to the farmers. The number of years of continuous intensive cultivation was recorded. In addition, the agronomic activities routinely carried out in the HIMR and LIMR farms were assessed as follows: i). crop rotation history; ii) soil tillage regime (types, depth and number per year); iii) application of mineral and organic amendments (type and application rate per year); iv) soil disinfestation treatments (application of chemical fumigants and soil solarization).

3.2.3 Soil sampling and analyses

In each HIMR farm three plastic tunnels were considered, and in each of them five soil sub-samples were collected following a W scheme (four sampling plots near to corners and one sampling plot in the centre of the tunnel) and pooling together, in order to have three composite soil sample for each farm. In each selected LIMR farm, a single composite soil sample was collected alone, because in the chosen farms only one orchard of small size (comparable with a single plastic tunnel) was available. In order to have a composite soil sample representing the variability of the field, the sample was obtained pooling together soil collected in five different plots, following a W scheme, under the trees row devoid of grass strips. Consequently, a total of 75 composite soil samples were collected for analyses (60 from HIMR and 15 from LIMR farms, respectively).

Soil samples (~2 kg) were collected from the topsoil (0-20 cm) in the Spring 2008 (April-May), considered the best time for soil collection (Bloem et al., 2006a). Samples were packed in polyethylene bags, transferred to laboratory quickly and sieved at 2 mm mesh. The biochemical analyses were carried out on fresh soils stored at +4 °C until time of measurements (within 10 days). Texture and chemical analyses were carried out on soil dried at room temperature until constant weight was reached.

3.2.4 Physical and chemical analyses

Physical and chemical properties of soils were determined by standard methods (Sparks, 1996). Particle size distribution analysis was carried out by the pipette method; pH and electrical conductivity were measured in 1:2.5 soil:water suspensions and 1:5 soil:water extracts, respectively; total carbonates (limestone) were determined by the Dietrich-Fruehling calcimeter method (Loeppert and Suarez, 1996); organic C content was assayed (on 1 g of pulverized soil) by 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.

Organic C content and total nitrogen analyses were performed by Dr. Rosaria D'Ascoli at the Department of Environmental Sciences, Second University of Naples.

3.2.5 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). 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*-nitrophenyl sulphate (*p*-NPS), *p*-nitrophenyl phosphate (*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. 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 AI 3 soil index (Puglisi et al. 2006) was useful to better discriminate between HIMR and LIMR soils. The AI 3 index, based on enzymatic activities, was able to discriminate between altered and unaltered soils, under a wide range of conditions, namely irrigation with brackish water, heavy metal contamination (Leirós et al., 1999; Hinojosa et al., 2004), intensive agricultural exploitation (Leirós et al., 1999; Caravaca et al., 2002). The index was developed from datasets of three enzyme

activities (β-glucosidase, phosphatase and urease) 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

The relationships between soil physical, chemical and biochemical properties were assessed by using Pearson correlation coefficients.

Analysis of variance (ANOVA-one way with replication) was used to evaluate the effect of different soil management regimes. The significance between means with $P < 0.01$ was determined using the Duncan test.

Principal component analysis (PCA) and all statistical analysis were performed by JMP 8 (SAS Institute, 2008).

3.3 Results and Discussion

3.3.1 Effect of management regimes on soil physical and chemical properties

In general, a different effect on soil physical and chemical properties of management regimes was observed in the different areas.

Texture soils, limestone and pH were no affected by management regime. The five sampling areas were characterized by different texture (Table 3.2), from clay to sandy loam soils. For each area, HIMR soils had similar texture to Control soil, LIMR.

Table 3.2 Texture of HIMR and LIMR soil samples (±sd), classified according to USDA classification.

			Texture			
	Area	**Soils**	**Sand %**	**Silt %**	**Clay %**	**USDA**
HIMR	A	1	39.63 ±1.9	26.84 ±1.4	33.53 ±1.7	Clay loam
		2	39.87 ±1.9	25.07 ±1.9	35.06 ±1.0	Clay loam
		3	37.32 ±2.7	25.60 ±2.0	37.08 ±2.0	Clay loam
		4	49.60 ±0.3	19.32 ±0.7	31.08 ±0.6	Sandy clay loam
	B	1	27.31 ±5.4	23.76 ±4.7	48.93 ±2.0	Clay
		2	27.46 ±1.9	21.46 ±0.8	51.09 ±1.0	Clay
		3	37.11 ±1.4	17.60 ±2.5	45.29 ±1.1	Clay
		4	41.01 ±0.9	14.41 ±0.5	44.59 ±1.4	Clay
	C	1	50.03 ±0.2	24.68 ±0.1	25.29 ±0.1	Sandy clay loam
		2	48.79 ±0.4	21.32 ±0.6	29.89 ±0.1	Sandy clay loam
		3	48.35 ±0.3	18.18 ±0.3	33.47 ±0.0	Sandy clay loam
		4	49.68 ±0.3	20.11 ±0.4	30.21 ±0.1	Sandy clay loam
	D	1	57.16 ±8.7	25.78 ±3.9	17.05 ±4.7	Sandy loam
		2	44.13 ±5.5	20.54 ±0.4	35.33 ±6.0	Sandy clay loam
		3	48.92 ±0.6	26.55 ±2.4	24.53 ±2.3	Loam
		4	55.86 ±1.2	27.43 ±0.9	16.70 ±2.1	Sandy loam
	E	1	74.37 ±2.8	16.65 ±1.3	8.98 ±2.3	Sandy loam
		2	63.89 ±4.1	22.87 ±1.9	13.24 ±2.3	Sandy loam
		3	72.10 ±5.1	15.43 ±2.2	12.47 ±2.5	Sandy loam
		4	75.29 ±3.1	14.63 ±1.1	10.07 ±2.1	Sandy loam
LIMR	Control	A	41.82 ±14.0	22.35 ±5.7	35.83 ±9.3	Clay
		B	41.08 ±14.0	17.15 ±1.3	41.77 ±1.4	Clay
		C	49.23 ±2.3	22.54 ±2.8	28.23 ±8.6	Sandy clay loam
		D	48.69 ±7.9	32.41 ±3.5	18.90 ±1.6	Loam
		E	73.84 ±4.0	15.51 ±5.5	10.64 ±8.6	Sandy loam

All soils sampled in different areas showed sub-alkaline pH values ranging from 7.59 to 8.34, except soils 4E, Control E, and 3C having pH 6.50, 7.20, 7.21, respectively (Fig. 3.1). No differences between HIMR and LIMR were observed.

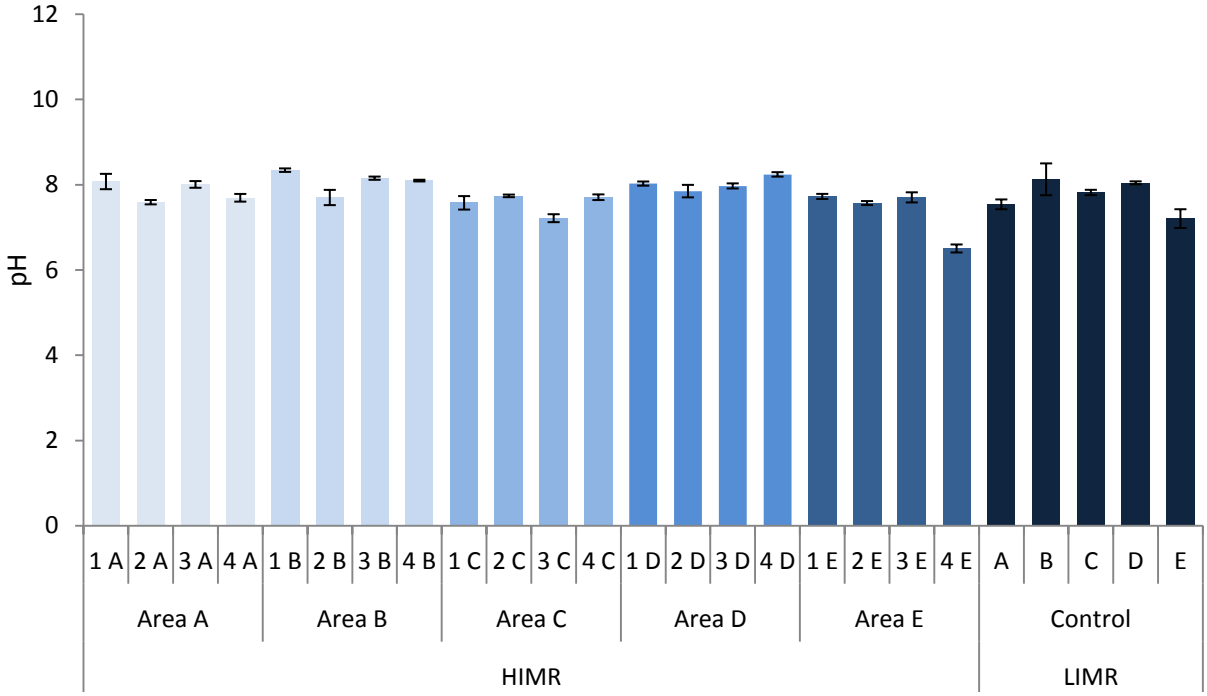


Figure 3.1 pH values determined of all soil samples.

Limestone content which generally depends on the geopedologic characteristics of soils was not affected by management regime (Fig. 3.2). The values were very different among soil samples and areas. Soils from area A can be considered uncalcaric, while only same soils from areas C and D were over 200 g kg^{-1} of lime, the limit to define a very calcaric soil. In soils 4D, the most calcaric soil, more than 500 g kg^{-1} of limestone was measured. All others fell into category weakly calcaric soils (Fig. 3.2).

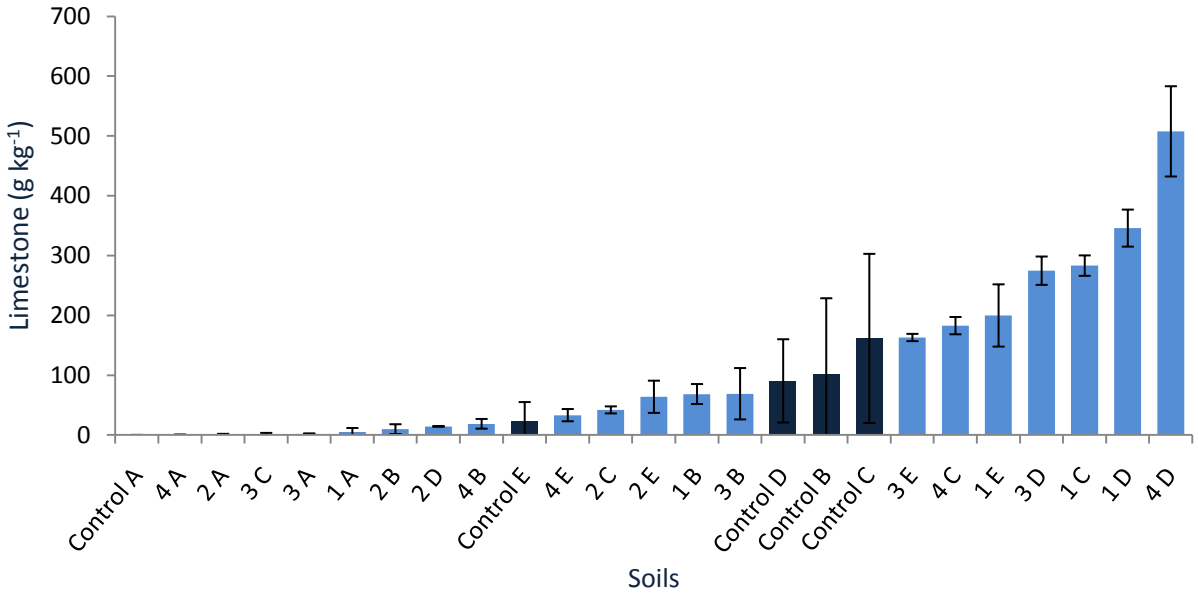


Figure 3.2 Limestone determined of all soil samples.

Soil organic C content was affected either by soil management regime and/or sampling area (Fig. 3.3). Only areas B and E showed a higher organic C content in soils under LIMR than HIMR, whereas areas A, C and D didn't show significant differences in organic C values of HIMR soils compared to LIMR soil. In general a decrease of organic C content in HIMR soils respect to LIMR soils was observed, although discordance trends in some areas occurred, indicating that the carbon balance of HIMR soils were altered.

Total soil organic C content is considered a stable parameter compared to labile C in terms of light C fractions or microbial biomass (Haynes, 2000). Usually, several years of different land use are required to detect significant changes of the total soil organic C pool (Gregorich et al., 1994). Under HIMR, because of the systematic elimination of crop residues to limit plant diseases, the amount of organic input returned to the soil is much less than under LIMR, where an input of leaves, twigs and root of orchard trees as well as cutting of inter-row grassland occurred.

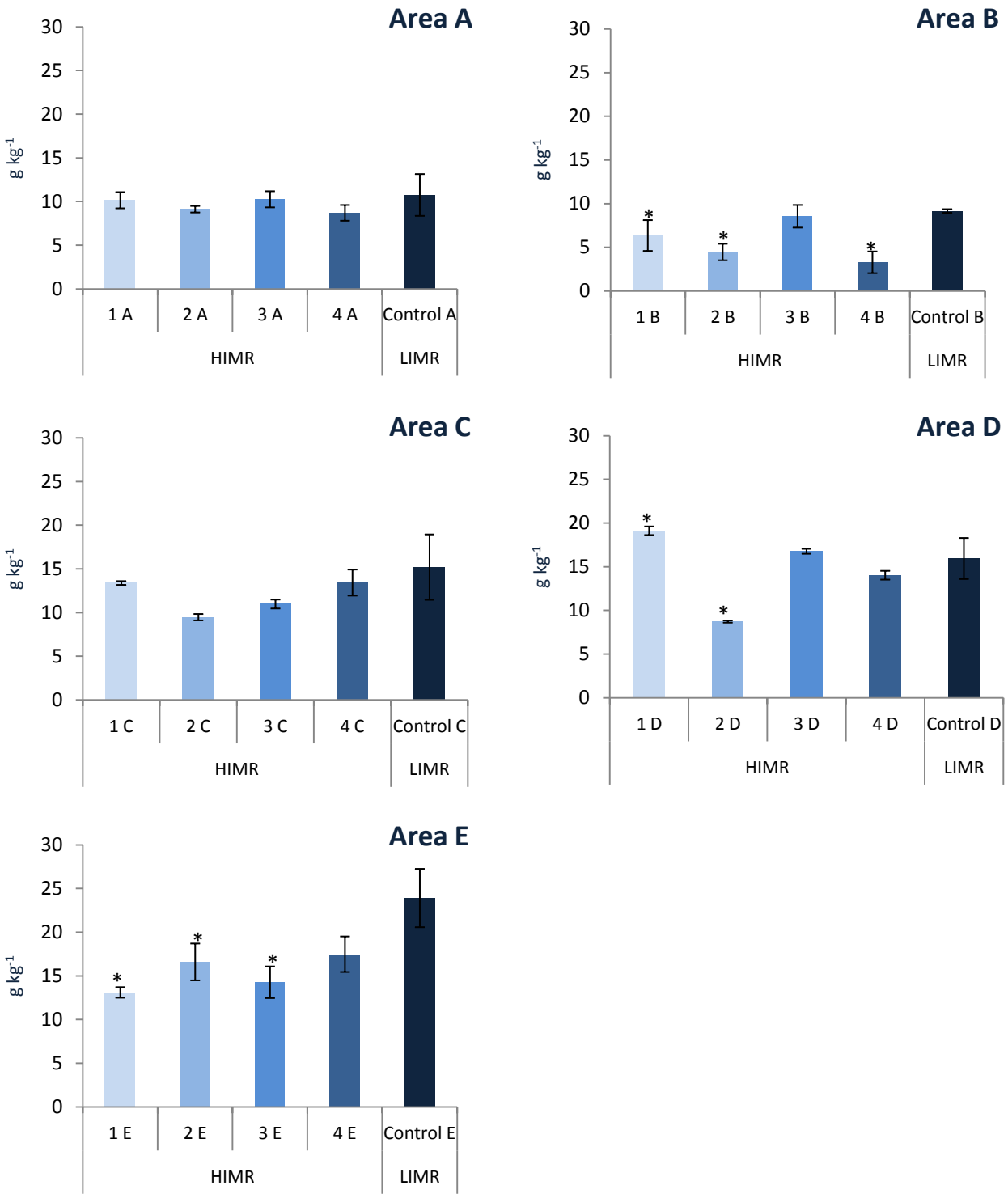


Figure 3.3 Effect of management regime on organic C content in soils of different areas. Asterisks indicate significant statistical differences (Duncan test: *=P<0.01).

In addition, the intensive tillage regime and the more favourable environmental conditions (higher temperature in winter and optimal soil water content also in dry summer months) under the plastic cover of HIMR farms stimulate a faster decomposition of native and exogenous OM.

All sampling areas, in general, showed a total N content ranging from 1 and 4 g kg^{-1}, and no effect due to management regimes was observed only in areas C and D (Fig. 3.4), In area A and area B some HIMR soils (2A, 1B, and 2B) showed higher values than Control soil; in area E, instead, soils 3E and 4E contained very lower amount of total N (1.23 and 1.17 g kg^{-1}, respectively) than Control soil (4.13 g kg^{-1}). Nevertheless, in soils under different management regimes, with similar total N, the organic C content determined higher C/N ratio in LIMR than in HIMR soils (Table 3.3). This is the case of the Control soil under LIMR of area C characterized by a C/N ratio of 9 against the lower values of respective HIMR soils. The higher C/N values were calculated in soil sited in areas C, D and E, which showed higher organic C content. Only in few cases, however, these values were enough high to favor optimal conditions to microbial activity.

Table 3.3 C/N ratio of all different sampling area.

	Area				
Sample	**A**	**B**	**C**	**D**	**E**
1	3	2	7	10	4
2	2	1	5	5	5
3	3	5	6	9	12
4	2	2	7	7	15
Control	3	4	9	8	6

Among micronutrients phosphorus could be strongly affected by management regime. Figure 3.5 shows the P_2O_5 content of studied soils. The reported results confirmed the influence of farming management and also of sampling area. Soils belonging to area B showed the lowest P_2O_5 content, while soil in area E the higher.

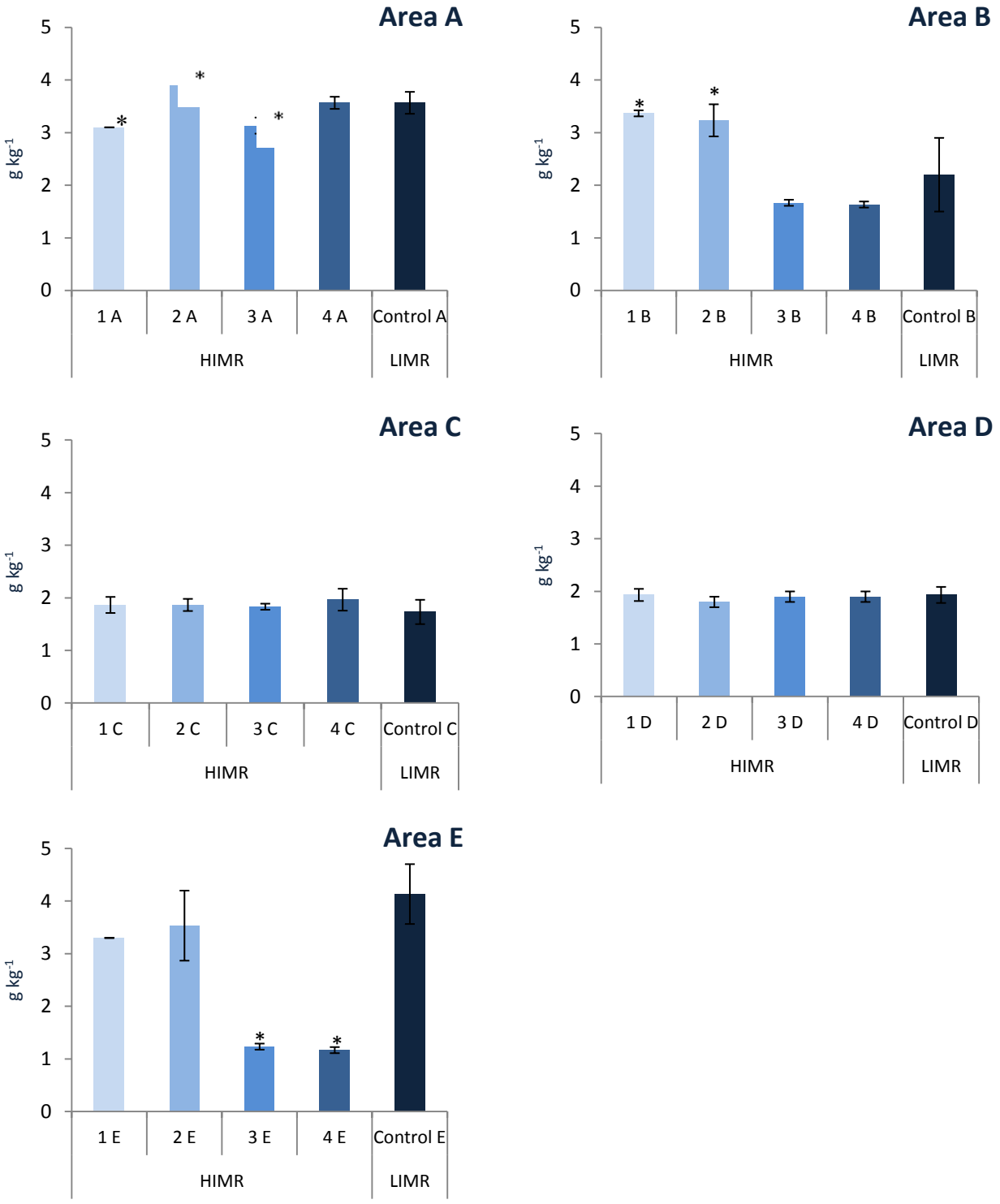


Figure 3.4 Effect of management regime on total N content in soils of different areas. Asterisks indicate significant statistical differences (Duncan test: *=P<0.01).

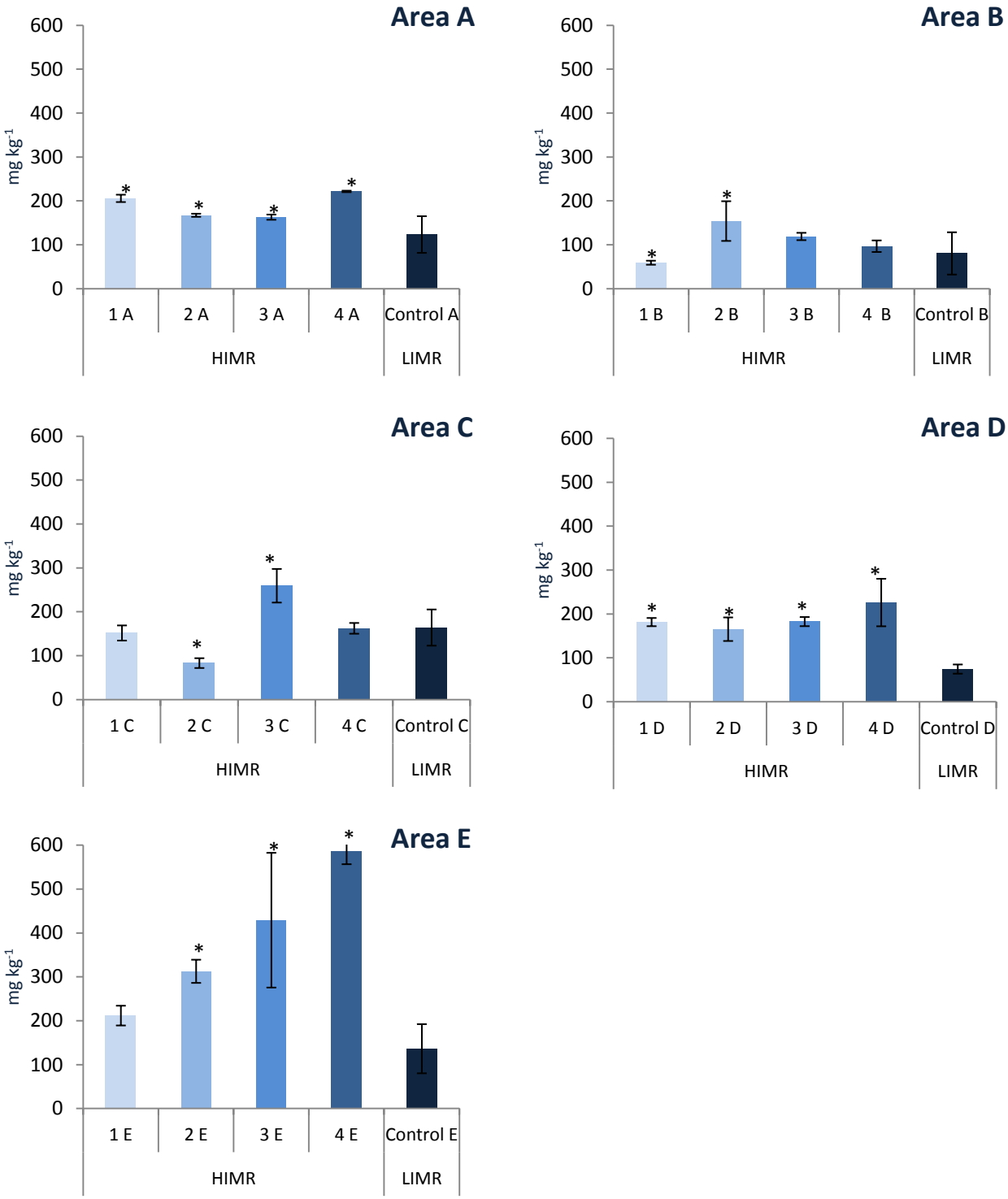


Figure 3.5 Effect of management regime on available phosphorus (P_2O_5) content in soils of different areas. Asterisks indicate significant statistical differences (Duncan test: *=P<0.01).

These differences, related to belonging areas, could be due to different cropping uptake and different mineral fertilizations in past years. An effect of management regime on P_2O_5 content was also considered. In all sampling areas, LIMR soils showed lower available phosphorus than HIMR soils. The more reduced doses of mineral phosphate given to tree orchards than those used in soils under intensive farming can be responsible for those results.

Cation exchange capacity (CEC) and exchangeable Ca^{2+}, Mg^{2+} and K^{+} were not affected by soil management, but some differences of soil texture and organic carbon content were observed among sampling areas (Tables 3.2 and 3.4; Fig. 3.3). Soil richer in clay fraction showed higher CEC and exchangeable base values than soils characterized by sandy or sandy loam characteristics.

Finally, exchangeable Na^{+} (Table 3.4), and electrical conductivity (EC) were influenced by management regime (Fig. 3.6). HIMR determined a drastic increase of electrical conductivity and exchangeable Na^{+} content compared to LIMR. EC values increased up to nearly 3000 $\mu S\ cm^{-1}$ in soil 4E, ten-fold more than the respective Control (Fig. 3.6) and exchangeable Na^{+} was two/three fold compared to LIMR soils (Table 3.4).

It is well-know that only management regime affects EC and Na^{+} content, indicating that some ecological factors linked to HIMR cause an increase of such parameters. Salinity and/or sodicity are widespread problems in irrigated areas characterized by low rainfall level and high evapo-transpiration demand that determines salt accumulation at the soil surface (Sumner et al., 1995).

The increase of soil electrical conductivity could be due to the almost total rainfall restriction under the plastic film, especially when HIMR is prolonged for many years, and/or to the use of poor quality irrigation water (Rietz and Haynes, 2003). The last factor, however, could be ruled out because of the high quality of the water used for irrigation in our study area.

Salinity and sodicity are well known for their detrimental effect on crop yields (Maas and Hoffman, 1977), microbial activities (Rietz and Haynes, 2003) and soil structure (Sumner, 1995). Commonly, soils are classified as saline when they have an electrical conductivity of 400 μS cm^{-1} or higher (Rietz and Haynes, 2003). Electrical conductivity values were high (>400 μS cm^{-1}, maximal values of 2983 μS cm^{-1}) in nine soils analyzed (3D, 1C, 4C, 2C, 2E, 1D, 3C, 1E and 4E; Fig. 3.6). This trend of electrical conductivity in HIMR soils suggests that these and above all further increases could be detrimental to crop productivity (Fig. 3.6).

Table 3.4 Cation exchange capacity and exchangeable bases of all soil samples (±sd).

	Sample	CEC	Ca	Mg	Na	K
				$cmol_{(+)}\ kg^{-1}$		
HIMR	1 A	25.21 ± 2.8	18.19 ± 0.4	4.80 ± 0.2	0.61 ± 0.1	1.54 ± 0.1
	2 A	22.18 ± 3.0	16.14 ± 1.0	3.90 ± 0.4	0.37 ± 0.0	1.51 ± 0.1
	3 A	22.89 ± 1.2	15.68 ± 1.4	4.20 ± 0.4	0.15 ± 0.0	1.43 ± 0.0
	4 A	23.33 ± 3.2	16.51 ± 0.6	4.38 ± 0.1	0.30 ± 0.1	1.34 ± 0.0
	1 B	32.94 ± 0.8	26.66 ± 1.5	4.46 ± 0.1	0.95 ± 0.1	1.51 ± 0.1
	2 B	30.47 ± 1.7	26.63 ± 1.1	6.31 ± 0.2	0.52 ± 0.0	1.55 ± 0.1
	3 B	30.28 ± 1.8	27.10 ± 1.6	4.56 ± 0.3	0.63 ± 0.1	1.21 ± 0.0
	4 B	31.65 ± 1.7	26.70 ± 0.9	5.82 ± 0.5	0.75 ± 0.1	0.57 ± 0.1
	1 C	27.09 ± 0.4	21.29 ± 1.7	4.29 ± 0.5	0.33 ± 0.1	1.37 ± 0.2
	2 C	25.03 ± 2.1	20.15 ± 2.1	3.70 ± 0.1	1.02 ± 0.1	0.74 ± 0.1
	3 C	30.32 ± 3.5	20.11 ± 2.1	6.39 ± 0.8	0.65 ± 0.1	2.02 ± 0.0
	4 C	28.73 ± 0.7	20.89 ± 6.0	4.16 ± 1.0	0.66 ± 0.1	1.72 ± 0.0
	1 D	22.75 ± 1.0	29.50 ± 1.2	3.48 ± 0.1	1.16 ± 0.1	1.51 ± 0.1
	2 D	22.21 ± 1.0	16.92 ± 8.0	2.31 ± 1.2	0.55 ± 0.1	1.43 ± 0.0
	3 D	26.19 ± 0.1	20.12 ± 3.3	2.56 ± 0.5	0.77 ± 0.1	2.34 ± 0.0
	4 D	21.42 ± 0.8	16.48 ± 1.7	1.74 ± 0.1	0.48 ± 0.1	0.68 ± 0.1
	1 E	27.33 ± 3.8	20.55 ± 1.0	3.37 ± 0.2	1.12 ± 0.1	1.90 ± 0.1
	2 E	33.25 ± 4.6	25.97 ± 5.4	4.16 ± 0.9	0.72 ± 0.1	1.95 ± 0.1
	3 E	29.31 ± 0.4	22.92 ± 3.1	3.80 ± 0.5	0.43 ± 0.0	1.34 ± 0.1
	4 E	30.35 ± 2.2	23.94 ± 4.4	2.85 ± 0.6	0.61 ± 0.1	2.98 ± 0.3
LIMR	Control A	25.18 ± 7.7	16.55 ± 2.7	3.74 ± 1.6	0.27 ± 0.1	1.45 ± 0.4
	Control B	28.37 ± 4.8	23.40 ± 3.9	4.15 ± 2.5	0.72 ± 0.9	1.29 ± 0.2
	Control C	24.97 ± 4.6	19.49 ± 3.8	2.70 ± 0.4	0.11 ± 0.0	1.52 ± 0.3
	Control D	29.92 ± 2.8	24.03 ± 2.7	2.21 ± 1.3	0.35 ± 0.2	1.54 ± 0.4
	Control E	22.56 ± 0.7	18.16 ± 3.9	1.76 ± 0.4	0.29 ± 0.1	1.54 ± 0.1

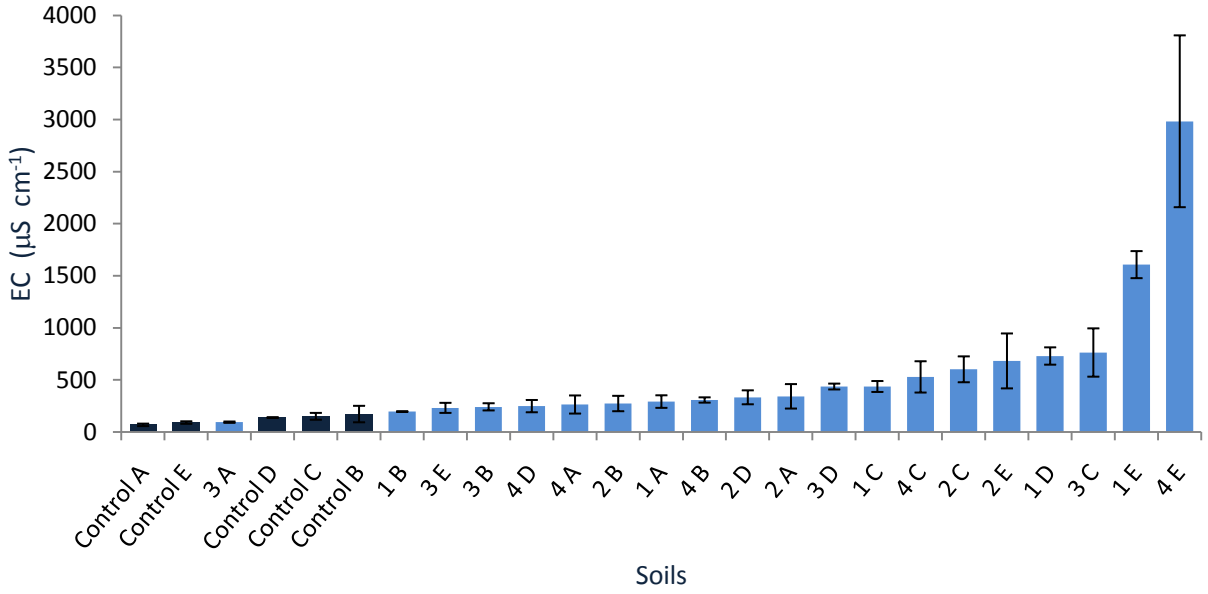


Figure 3.6 Electrical conductivity determined of all soil samples.

3.3.2 Effect of management regime on enzymatic activities

All studied enzymatic activities were affected by management regime and the effect can be related to sampling areas and, therefore, to soil physical and chemical properties. Numerous papers have demonstrated that intensive soil management results in decreased enzyme activity (Dick, 1992; Bandick and Dick, 1999; Haynes and Tregurtha, 1999; Islam and Weil, 2000; Dilly et al., 2003).

The DHY activity was different among soils samples (Fig. 3.7). It varied from 0.07 µg TPF g^{-1} h^{-1}, in 1A sample, to 10.88 µg TPF g^{-1} h^{-1} in Control D sample. However, HIMR soils of the same area generally had the same average activity level, significantly lower compared to those LIMR soil. These results indicated that the DHY activity of studied soil was strongly affected by intensive management regime in according to Lupwayi et al. (2007) and Udawatta et al. (2008), who report that the dehydrogenase activity is strongly influenced by intensive farming and use of pesticide and herbicide (Reinecke et al., 2002). These latter usually enter in intensive farming, as useful tools in pest control, causing serious troubles to the biotic soil components.

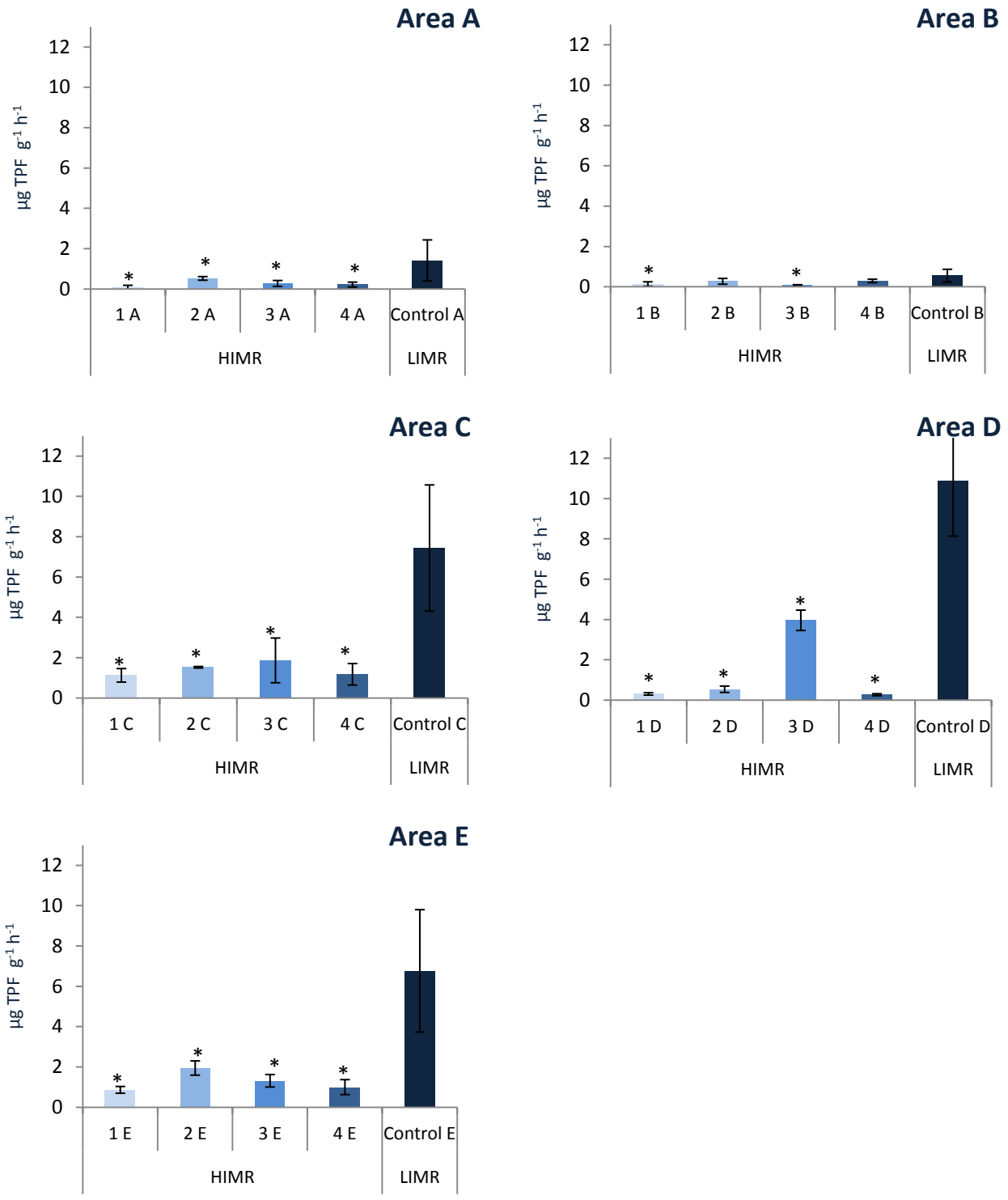


Figure 3.7 Effect of management regime on dehydrogenase activity of soils in different areas. Asterisks indicate significant statistical differences (Duncan test: *=P<0.01).

Dehydrogenase activity provides information on soil chemical processes, nutrient mineralization rates, and organic C accumulation. This enzyme is related to OM oxidation since it catalyzes the aerobic respiration process occurring in the electron transport chain (Ross, 1971). DHY activity depends on the metabolic state of soil microorganism, although its use as indicator of soil microbial activity has been criticized because these enzymes are affected by numerous factors (soil type, pH, etc.) (Nannipieri et al., 1990; Beyer et al., 1992).

Also GLU activity was strongly affected by management regime (Fig. 3.8). In all sampling areas, LIMR soils showed significantly higher GLU activity levels than HIMR soils, with values ranging from 0.44 to 1.22 μmol *p*-NP g^{-1} h^{-1}. In all HIMR samples values varied from 0.13 μmol *p*-NP g^{-1} h^{-1} (1D) to 0.33 μmol *p*-NP g^{-1} h^{-1} (3D), except 2B sample having a higher activity (0.73 μmol *p*-NP g^{-1} h^{-1}). β-Glucosidase catalyses the hydrolysis of β-glucosides in soils and is one of enzyme involved the decomposition of the plant residues. GLU activity reflects the state of OM and the processes occurring therein. Moreover it has been used as sensitive indicator of soil farming managements (Ndiaye et al., 2000; De la Horra et al., 2003; Lagomarsino et al., 2009) as well as for the development of complex indices (Puglisi et al., 2006).

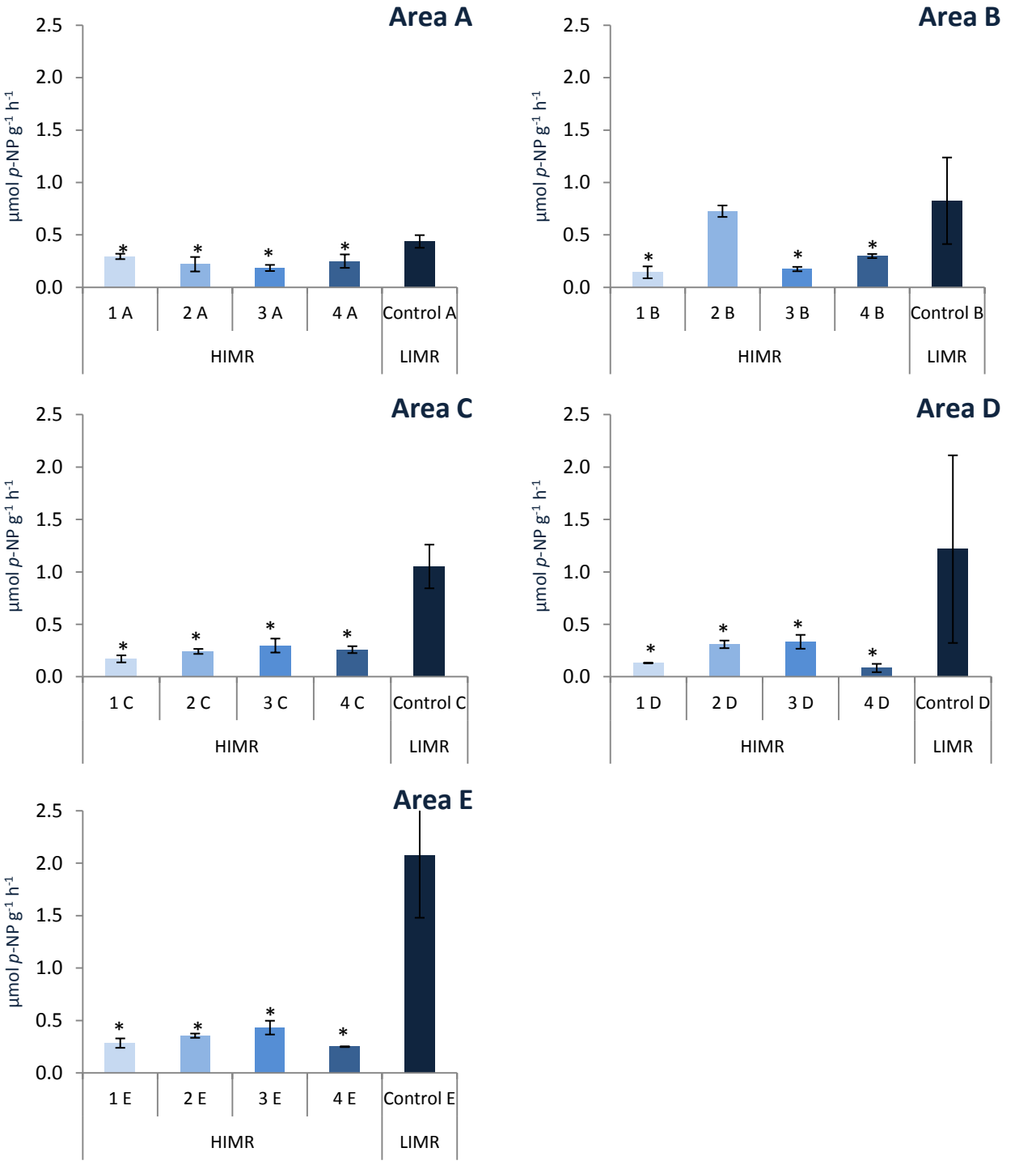


Figure 3.8 Effect of management regime on β-glucosidase activity of soils in different areas. Asterisks indicate significant statistical differences (Duncan test: *=P<0.01).

Phosphatase activity is strongly correlated with phosphorus cycle, an important plant nutrient. In the studied soils, it was affected by management regime in all areas (Fig. 3.9). In the sampling area A, PHO showed lower activity (from 0.31 to 0.56 μmol *p*-NP g^{-1} h^{-1}) than in other areas, and no differences between the two management regimes were observed. In the other sampling areas, HIMR soils showed high variability of PHO. In general, LIMR soils always showed higher PHO (from 0.49 to 2.83 μmol *p*-NP g^{-1} h^{-1}), than HIMR soils (from 0.32 to 1.64 μmol *p*-NP g^{-1} h^{-1}). These results are according to those of Trasar-Cepeda et al. (2008) who reported a negative effect of intensive farming on PHO, due to extensive use of mineral fertilizations, such as phosphate fertilizers.

Acid phosphomonoesterase catalyses the hydrolysis of organic phosphorus to release free orthophosphate, its activity is regulated by a negative feedback mechanism where products of its reaction (phosphates) may inhibit the activity of enzyme (Juma and Tabatabai, 1978).

Urease activity showed a greater variability among LIMR soils than HIMR soils (Fig. 3.10). In some areas UR activity was higher in LIMR than HIMR soils (Area A, D and E), in other areas it had similar values in both soils. This behavior could be explained by the intensive use of mineral N fertilization, as occurred in HIMR, that makes the synthesis of new urease unnecessary (Dick et al., 1988a; Marcote et al., 2001).

Arylsulphatase activity behaved according to the trend shown by the other studied enzymes. ARYL always showed lower activity in HIMR soils than LIMR soils (Fig. 3.11). As for the behavior of PHO, the intensive use of copper sulphate in HIMR, used as fungicide, could led to an excess of sulphate anion, the hydrolysis product of the ARYL-catalyzed reaction, thus determining a feedback inhibition (Trasar-Cepeda et al., 2008).

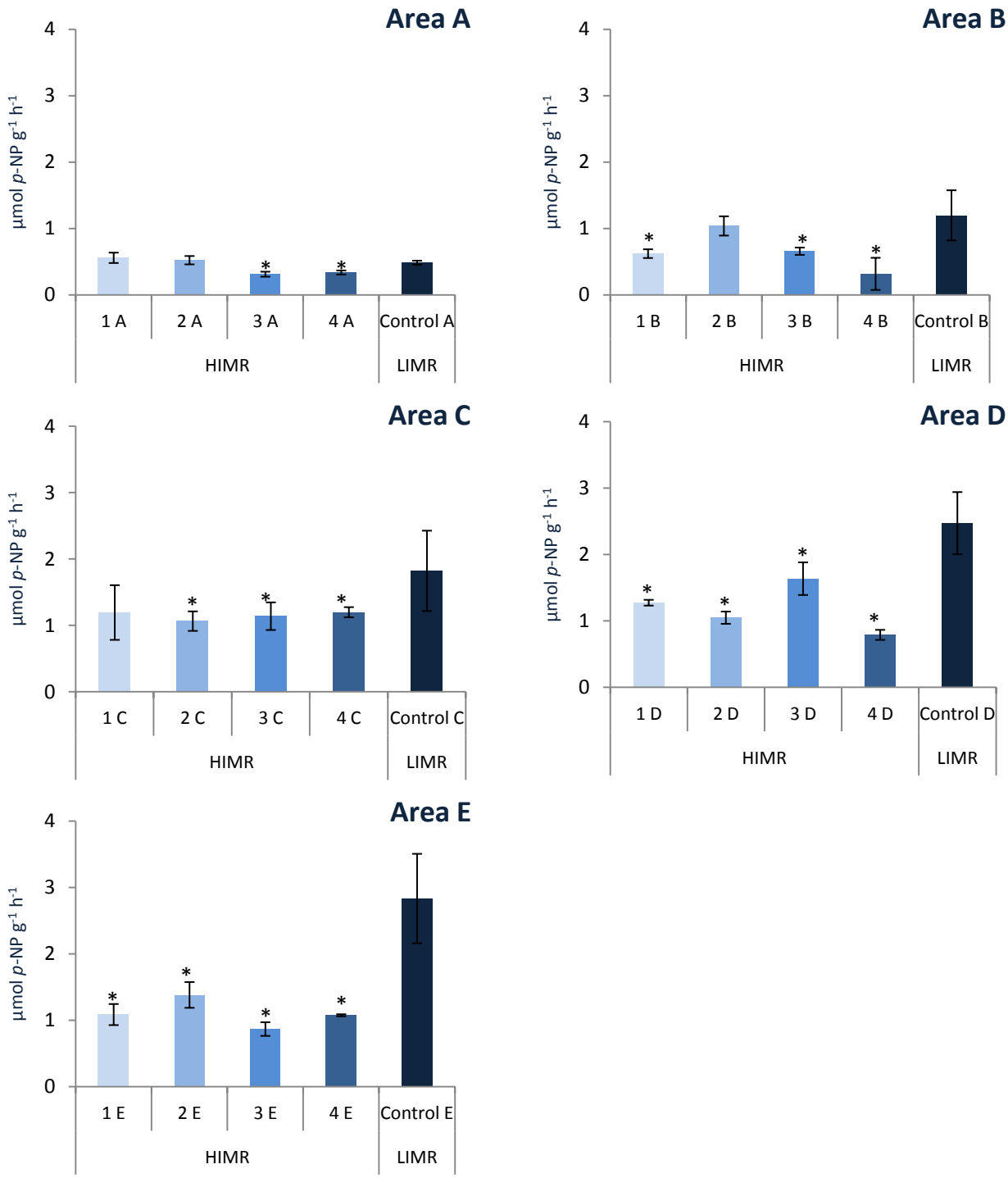


Figure 3.9 Effect of management regime on phosphatase activity of soils in different areas. Asterisks indicate significant statistical differences (Duncan test: *=P<0.01).

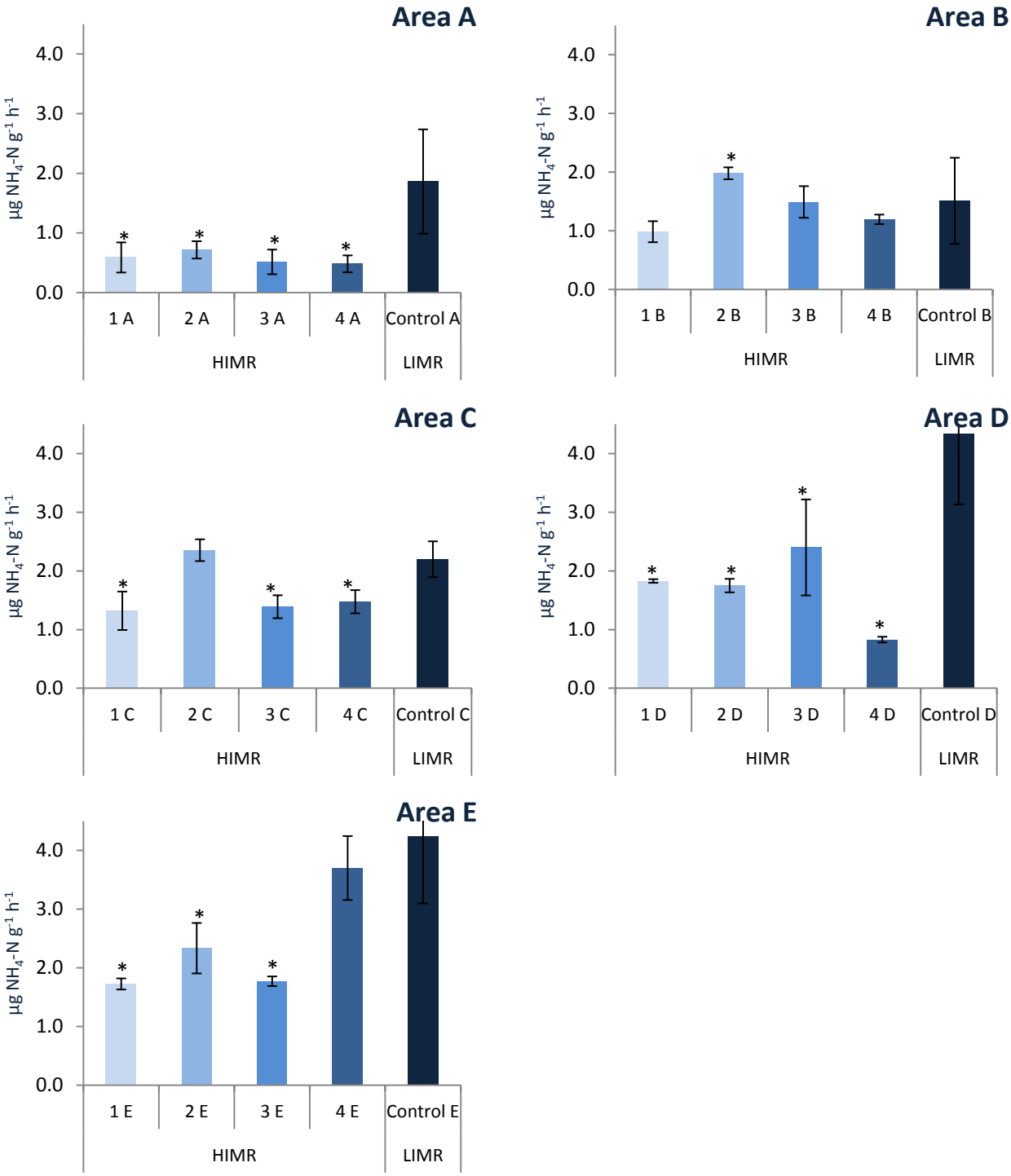


Figure 3.10 Effect of management regime on urease activity of soils in different areas. Asterisks indicate significant statistical differences (Duncan test: *=P<0.01).

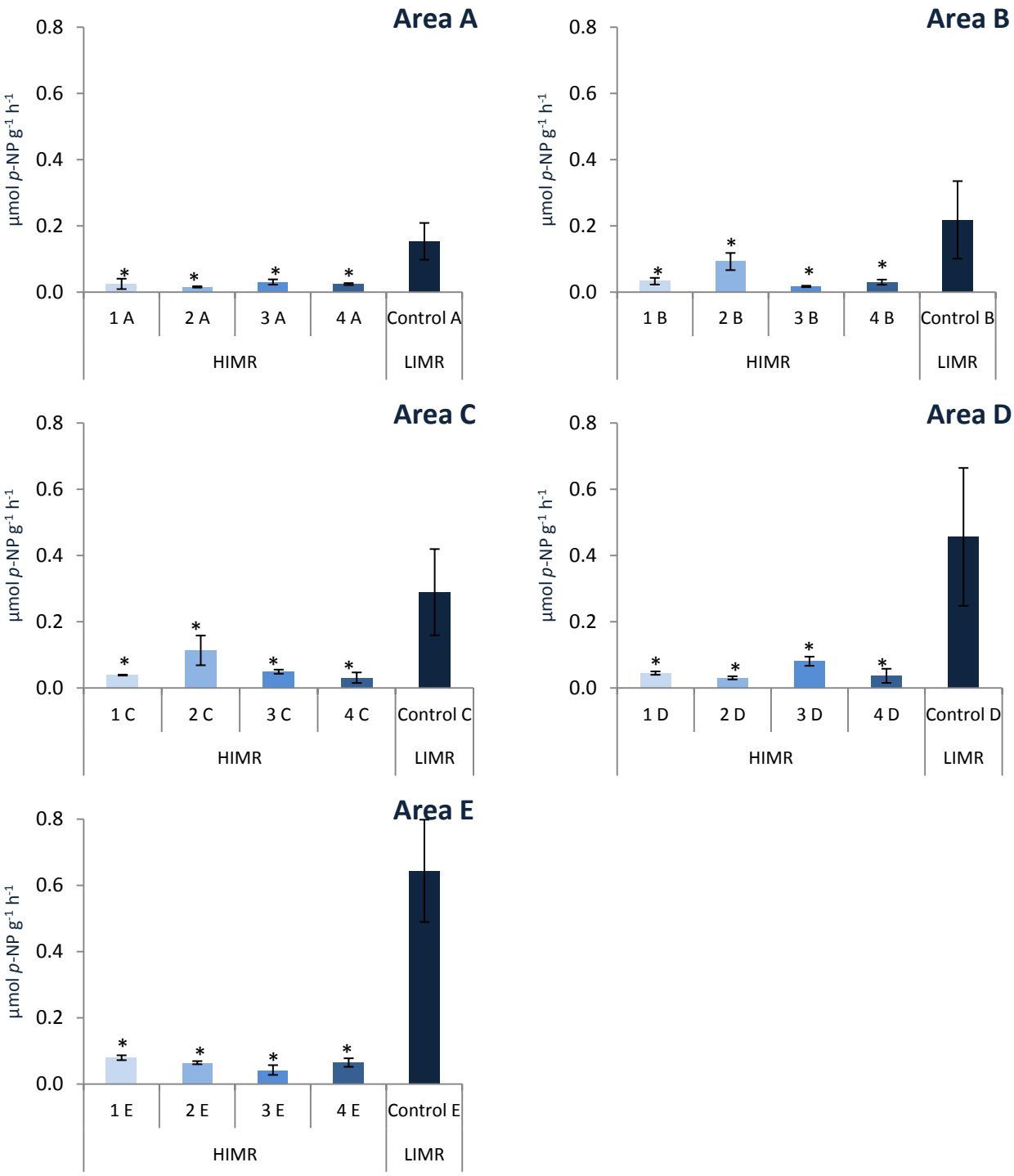


Figure 3.11 Effect of management regime on arylsulphatase activity of soils in different areas. Asterisks indicate significant statistical differences (Duncan test: *=P<0.01).

To better understand how enzymatic activities could be valid indicators of soil quality, an index developed by Puglisi et al. (2006) was used. Authors developed three indices (AI 1, AI 2, and AI 3) able to discriminate altered and not altered soils. Several enzymatic activities were considered and each of them was taken in account with different weight. A wide research in literature allowed to Puglisi et al. (2006) to validate the three indices, initially calculated from own experimental data.

The AI 3 index 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; higher values indicate more altered soils. In the present work, this index allowed to discriminate between LIMR soils and soils under intensive agricultural management (HIMR). AI 3 index was -6 for HIMR soils and -7 for LIMR soils. (Fig. 3.12) thus highlighting an alteration of HIMR soils compared to LIMR soils.

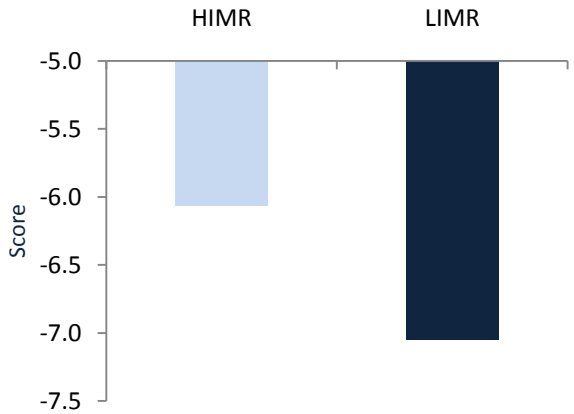


Figure 3.12 Scores of soil index AI3, applied to mean values of enzymatic activities of all HIMR and LIMR soils.

The effect of different management regimes may appear more evident by an overall view of all studied physical, chemical and biochemical parameters. Figure 3.13 summarizes the effect of different management regimes on soil characteristics. Values are expressed as deviation to respective Control (LIMR) in percentage of mean values in all HIMR soils. It is clear that management regimes had a significant effect on organic C content: it decreased by 24% in the HIMR compared to LIMR farms (11.4 vs. 14.9 g kg^{-1}, respectively) (Fig. 3.13). In contrast, electrical

conductivity, exchangeable Na^+ and P_2O_5 increased by 370% (579 vs. 123 µS cm^{-1}), 86% (147 vs. 79 $cmol_{(+)}$ kg^{-1}) and 78% (206 vs. 115 mg kg^{-1}), respectively in HIMR compared to LIMR soils (Fig. 3.13). Exchangeable Mg^{2+} was slightly higher in HIMR than in LIMR soils (Fig. 3.13). Also all enzymatic activities were significantly affected by management regime. Dehydrogenase, arylsulphatase, β-glucosidase, phosphatase and urease were reduced by 84% (0.89 vs. 5.41 µg TPF g^{-1} h^{-1}), 87% (0.04 vs. 0.35 µg *p*-NF g^{-1} h^{-1}), 76% (0.27 vs. 1.12 µg *p*-NF g^{-1} h^{-1}), 49% (0.90 vs. 1.76 µg *p*-NF g^{-1} h^{-1}) and 46% (1.54 vs. 2.83 µg NH_4-N g^{-1} h^{-1}) in the HIMR compared to the LIMR, respectively (Fig. 3.13).

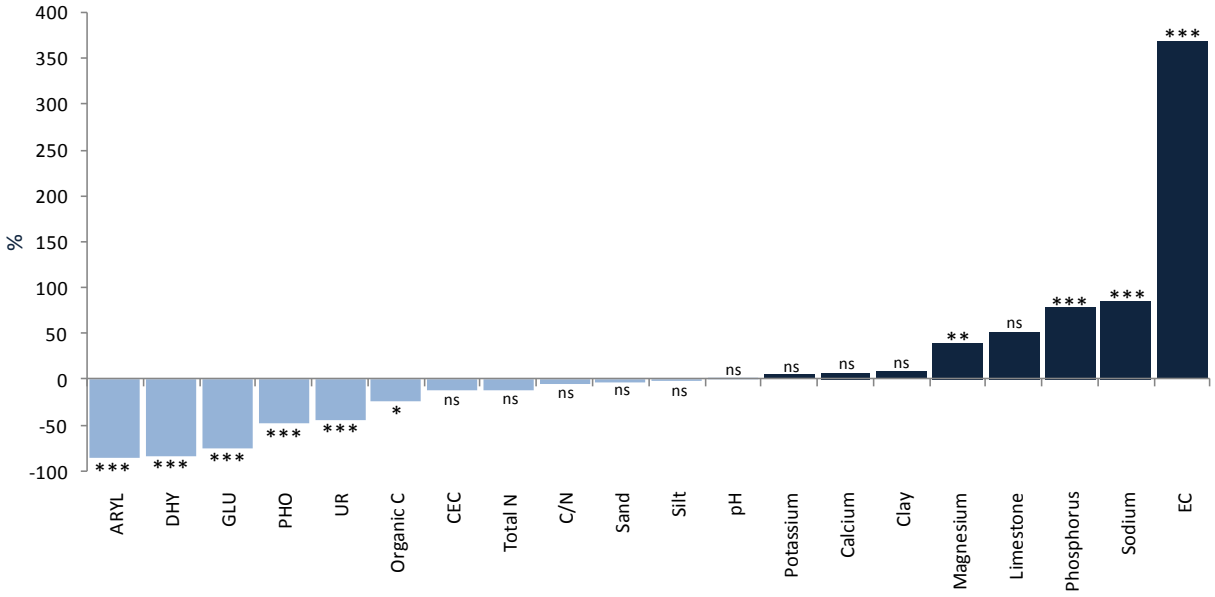


Figure 3.13 Effect of soil management on physical, chemical and biochemical properties. Values are expressed as percentage decrease or increase in HIMR compared to LIMR (=0) soils, used as Control. Asterisks indicate significant statistical differences (Duncan test: ***=*P*<0.001; **=*P*<0.01; *=*P*<0.05; ns=not significant).

All reported results showed a great variability in biochemical properties among farms and areas, due to differences in physical-chemical soil properties and to different farm history. Clear evidence was that LIMR, not subjected to intensive farming, generally showed higher enzymatic activities compared to HIMR, indicating a negative effect of intensive agriculture practices on biochemical properties of studied soils. This result is consistent with the findings of many studies that reported

a decline of enzymatic activities in cultivated soils when compared to the corresponding uncultivated or less-disturbed soils (Drinkwater et al., 1995; Gianfreda et al., 2005; Acosta-Martínez et al., 2008).

3.3.3 Relationship between soil physical, chemical and biochemical parameters

The whole cross-correlation matrix, being composed of 20 parameters and indices, produced 190 Pearson correlation coefficients (Table 3.5). The most relevant results are described below.

Soil organic C content strongly correlated negatively with clay and positively with sand content. Moreover, it showed a strong negative correlation with exchangeable Mg^{2+}. When all HIMR farms were considered, a weak positive correlation was found between their organic C and limestone contents. Soil electrical conductivity reported significant correlations with potassium and phosphorus content, but not with the biological soil functions, as enzymatic activities. In our study all enzymatic activities showed strongly positive correlations with soil organic C content ($P<0.01$), except for β-glucosidase ($P<0.05$). Their values higher in LIMR than HIMR soils indicate that even a slight increase of organic carbon can promote enzymes protection and induce significant changes of biochemical activity and demonstrating the efficacy of this enzyme to value different agricultural managements. However, other soil factors also significantly affect enzymatic activities.

Rietz and Haynes (2003) found strong negative correlations between soil electrical conductivity and enzymatic activities (hydrolysis of fluoresceine diacetate, β-glucosidase, alkaline phosphatase and arylsulphatase). In our study the weak, but not significant, negative correlation between enzymatic activities and both electrical conductivity and Na^{+} content could be explained by the narrower range of soil salinity values measured in our farms compared to those reported by Rietz and Haynes (2003).

The consistent negative correlations between enzymatic activities and clay content and exchangeable Mg^{2+} could be accounted for by the high affinity of protein

molecules for clay surfaces. This could strongly reduce their catalytic activity because of induced conformational changes, inter-layering phenomena, and inhibition effects (Gianfreda and Ruggiero, 2006). In general the lower enzymatic activities in the HIMR soils indicate a reduced microbial activity and, probably, a lower capability to cleave organic P, S and to degrade OM compared to LIMR soils. Moreover, the consistent decline of enzymatic activities across the soil texture gradient indicates that such parameters provide sensitive indicators of the impact of intensive cultivation on soil quality.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)
Limestone**(1)**																			
EC**(2)**	0.025																		
N total**(3)**	-0.375	-0.258																	
Organi C**(4)**	0.405(*)	0.263	-0.050																
C/N**(5)**	0.449(*)	0.519(**)	-0.674(**)	0.683(**)															
CEC**(6)**	-0.309	-0.141	-0.030	-0.100	-0.147														
Ca^{2+}**(7)**	0.092	0.190	-0.307	-0.052	0.171	0.150													
Mg^{2+}**(8)**	-0.424(*)	-0.083	0.084	-0.675(**)	-0.486(*)	0.235	0.328												
Na^{+}**(9)**	0.220	0.372	-0.156	-0.075	0.004	0.025	0.540(**)	0.160											
K^{+}**(10)**	-0.117	0.663(**)	0.013	0.443(*)	0.458(*)	0.086	0.068	-0.101	0.060										
P_2O_5**(11)**	0.050	0.706(**)	-0.269	0.363	0.649(**)	-0.112	0.014	-0.092	-0.046	0.594(**)									
Sand**(12)**	0.293	0.556(**)	-0.141	0.759(**)	0.643(**)	-0.128	-0.040	-0.500(*)	0.093	0.366	0.648(**)								
Silt**(13)**	0.320	-0.354	0.123	0.103	-0.054	-0.158	-0.156	-0.248	-0.143	-0.075	-0.335	-0.357							
Clay**(14)**	-0.431(*)	-0.464(*)	0.106	-0.851(**)	-0.668(**)	0.195	0.101	0.626(**)	-0.047	-0.364	-0.570(**)	-0.939(**)	0.013						
ARYL**(15)**	-0.106	-0.199	0.198	0.533(**)	0.126	0.283	-0.069	-0.480(*)	-0.320	0.023	-0.248	0.275	0.016	-0.301					
PHO**(16)**	0.174	0.003	-0.058	0.729(**)	0.413(*)	0.284	0.107	-0.532(**)	-0.085	0.278	-0.068	0.429(*)	0.114	-0.501(*)	0.836(**)				
GLU**(17)**	-0.200	-0.247	0.236	0.447(*)	0.060	0.340	-0.050	-0.349	-0.382	0.038	-0.198	0.231	-0.103	-0.210	0.960(**)	0.794(**)			
UR**(18)**	-0.057	0.302	-0.144	0.634(**)	0.513(**)	0.332	0.234	-0.497(*)	-0.033	0.393	0.168	0.499(*)	-0.073	-0.507(**)	0.753(**)	0.832(**)	0.686(**)		
DHY**(19)**	0.017	-0.160	-0.059	0.542(**)	0.317	0.284	-0.044	-0.499(*)	-0.355	0.157	-0.155	0.239	0.285	-0.361	0.828(**)	0.841(**)	0.757(**)	0.740(**)	
pH**(20)**	0.302	-0.656(**)	-0.018	-0.395	-0.408(*)	-0.088	0.144	0.039	0.188	-0.627(**)	-0.659(**)	-0.560(**)	0.445(*)	0.435(*)	-0.165	-0.226	-0.191	-0.458(*)	-0.098

Table 3.5 Cross-correlation matrix among soil physical, chemical and biochemical parameters of HIMR and LIMR soils. Significant correlations from regression analysis: *=P<0.05; **= P<0.01.

3.3.4 Analysis of principal components

Analysis of the principal components of the variables affected by management regimes (EC, total N, organic C, CEC, P_2O_5, dehydrogenase, β-glucosidase, phosphatase, urease and arylsulphatase) showed that the first and second components explained 71.79 % of the total variance. The score plot indicates that the samples can be divided into two groups, HIMR and LIMR, confirming that the two different management regimes had a strong effect on soil properties (Fig. 3.14). The parameters studied were more variable (i.e. sparse in the score plot) within the HIMR soils than within LIMR soils, probably due to different management history that occurred in studied farms.

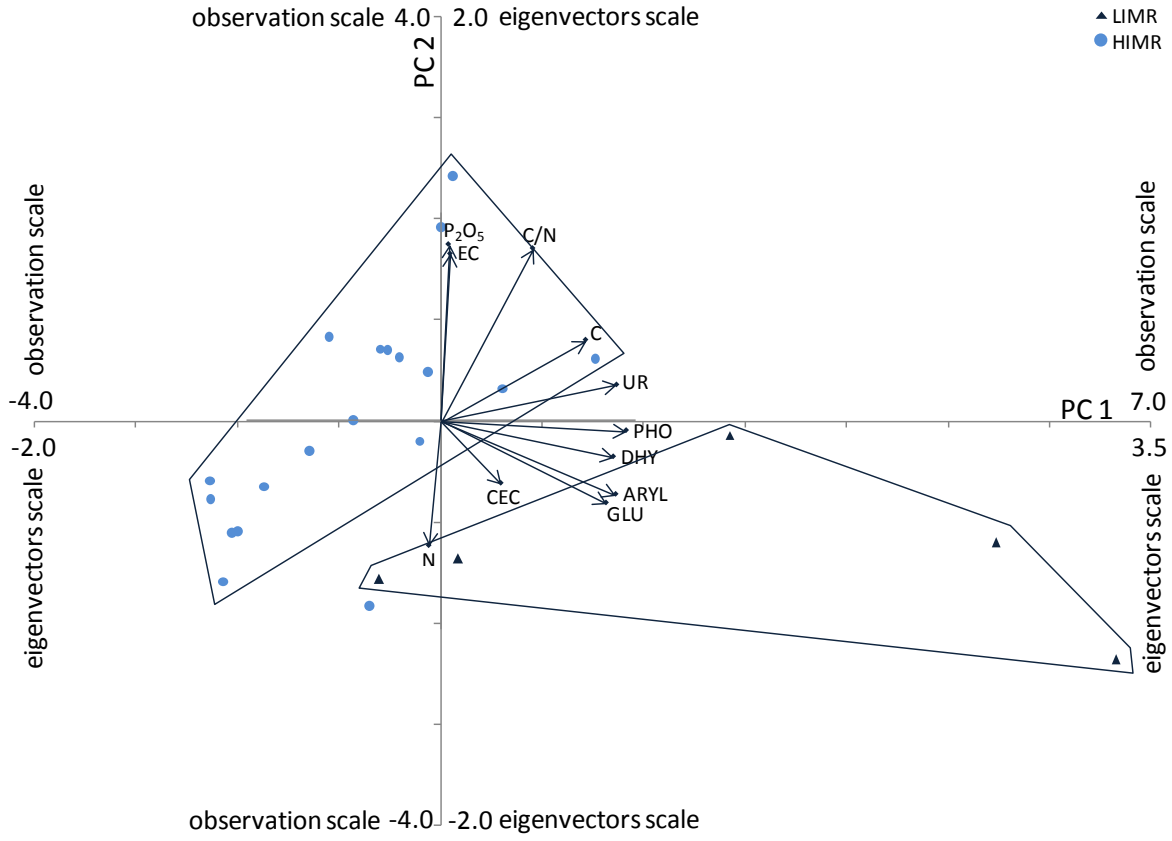


Figure 3.14 Biplot of soil properties affected by management regime. EC, electrical conductivity; C, total organic carbon; UR, urease activity; PHO, phosphatase activity; DHY, dehydrogenase activity; ARYL, arylsulphatase activity; GLU, β-glucosidase activity; CEC, cation exchangeable capacity; N, total nitrogen.

3.4 Conclusions

This study has demonstrated that long-lasting soil cultivation under plastic cover negatively affects chemical and biochemical soil quality. The reduction in soil C content and soil enzymatic activities, determining a decrease of functional and species diversity, and the drastic increase in soil salinity are the result of such land use. The full consequences of such changes on crop productivity are still unknown, but they probably determine a multiple stress to plants with potential negative consequences on crop yield. Phytopathological disorders with complex, often unidentified ethyology are growing under the HIMR, and farmers continuously increase the cultivation inputs (mainly soil fumigations and mineral fertilizers) to overcome such problems and maintain crop productivity. Further studies are needed to identify agronomic strategies that will mitigate the negative effects of HIMR cultivation on soil quality.

3.5 References

Abiven S., Menassero S., Chenu C., 2009. The effect of organic inputs over time on soil aggregate stability, A literature analysis. *Soil Biology and Biochemistry* 41:1-12.

Acosta-Martínez V., Acosta Mercado D., Sotomajor-Ramírez D., Cruz-Rodríguez, L., 2008. Microbial community and enzymatic activities under different management in semiarid soils. *Applied Soil Ecology* 38:249-260.

Bandick A.K. and Dick R., 1999. Field management effects on soil enzyme activities. *Soil Biology and Biochemistry* 31:1471-1479.

Beyer L., Wackendorf C., Balzen F.M., Balzer-Graf,V.R., 1992. The effect of soil texture and soil management on microbial biomass and soil enzyme activities in arable soils of Northwest Germany. *Agrobiology Research* 45:276-283.

Birkhofer K., Bezemer T.M., Bloem J., Bonkowski M., Christensen S., Dubois D., Ekelund F., Fließbach A., Gunst L., Hedlund K., Mäder P., Mikola J., Robin C., Setälä H., Tatin-Froux F., Van der Putten W.H., Scheu S., 2008. Long-term organic farming fosters below and aboveground biota: Implications for soil quality, biological control and productivity. *Soil Biology and Biochemistry* 40:2297-2308.

Bloem J., Hopkins D.W., Benedetti A., 2006a. Microbiological methods for assessing soil quality. CABI Publishing, Oxfordshire, UK..

Bloem J., Schouten A.J., Sørensen S.J., Rutger M., van der Werf A., Breure A.M., 2006b. Monitoring and evaluating soil quality. In: Bloem, J. Hopkins, D.W., Benedetti, A., (Eds.), Microbiological methods for assessing soil quality. CABI Publishing, Oxfordshire, UK..

Bonanomi G., Antignani V., Pane C., Scala F., 2007. Suppression of soilborne fungal diseases with organic amendments. *Journal of Plant Pathology* 89:311-340.

Caravaca F., Masciandaro G., Ceccanti B., 2002. Land use in relation to soil chemical and biochemical properties in a semiarid Mediterranean environment. *Soil Tillage Research 68*:23-30.

De la Horra A.M., Conti M.E., Palma R.M., 2003. β-Glucosidase and protease activities as affected by long-term management practices in a Typic Argiudoll soil. Commun. *Soil Science & Plant Nutrition* 34:2395-2404.

Dick R.P., Myrold D.D., Kerle E.A., 1988. Microbial biomass and soil enzyme activities in compacted and rehabilitated skid trail soils. *Soil Science Society of America Journal* 52:512-516.

Dick R.P., 1992. A review: Long term effects of agricultural systems on soil biochemical and microbial parameters. *Agriculture, Ecosystems and Environment* 40:25-36.

Dilly O., Blume H.P., Munch J.C., 2003. Soil microbial activities in Luvisols and Anthrosols during 9 years of region-typical tillage and fertilization practices in northern Germany. *Biogeochemistry* 65:319-339.

Doran J.W. and Parkin T.B., 1994. Defining and assessing soil quality. In: Defining Soil Quality or a Sustainable Environment. Doran J.W., Coleman D.C., Bezdicek D.F. and Stewart B.A. (Eds.), SSSA Inc Madison, USA..

Drinkwater L.E., Letourneau D.K., Workneh F., van Bruggen A.H.C., Shennan C., 1995. Fundamental differences between conventional and organic tomato agroecosystems in California. *Ecological Applications* 5, 1098-1112.

Enoch H.Z., Enoch Y., 1999. The history and geography of the greenhouse. In: Stanhil G., Enoch H.Z., (Eds.), Greenhouse Ecosystems. *Ecosystems of the World* 20. Elsevier, Amsterdam, pp. 1-15.

Fließbach A., Oberholzer H.-R., Gunst L., Mäder P., 2007. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. *Agriculture, Ecosystems and Environment* 18:273-284.

Gianfreda L., Rao M.A., Piotrowska A., Palombo G., Colombo C., 2005. Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. *Science of the Total Environment* 341:265-279.

Gianfreda L., Ruggiero P., 2006. Enzyme Activities in Soil. In: Soil Biology, Volume 8: Nucleic Acids and Proteins in Soil. (P. Nannipieri and K. Smalla, Eds.), Springer-Verlag Berlin Heidelberg, pp.257-311.

Gregorich E.G., Carter M.R., Angers D.A., Monreal C.M., Ellert B.H., 1994. Towards a minimum data set to assess soil organic quality in agricultural soils. *Canadian Journal of Soil Science* 74:367-385.

Haynes R.J., 2000. Labile organic matter as an indicator of organic matter quality in arable and pastoral soils in New Zealand. *Soil Biology and Biochemistry* 32:211-219.

Haynes R.J., Tregurtha R., 1999. Effects of increasing periods under intensive arable vegetable production on biological, chemical and physical indices of soil quality. *Biology and Fertility of Soils* 28:259-266.

Hinojosa M.B., Carreira J.A., García- Ruiz R., Dick R.P., 2004. Soil moisture pre-treatment effects on enzyme activities as indicators of heavy metal-contaminated and reclaimed soils. *Soil Biology and Biochemistry 36*:1559-1568.

Islam K.R., Weil R.R., 2000. Land use effects on soil quality in a tropical forest ecosystem of Bangladesh. *Agriculture, Ecosystems and Environment* 79:9-16.

Janvier C., Villeneuve F., Alabouvette C., Edel-Hermann V., Mateille T., Steinberg C., 2007. Soil health through soil disease suppression: Which strategy from descriptors to indicators? *Soil Biology and Biochemistry* 39:1-23.

Juma N.G. and Tabatabai M.A., 1978. Distribution of phosphomonoesterases in soils. *Soil Sci* 126:101-108.

Kandeler E. and Gerber H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biology and Fertility of Soils* 6:68-72.

Lagomarsino A., Di Tizio A., Marinari S., Moscatelli M.C., Mancinelli R., Grego S., in press. Soil organic matter pools under different system management and tillage level in a three-year crop rotation. *Agrochimica*.

Leirós M.C., Trasar-Cepeda C., García-Fernández F., Gil- Sotres F., 1999. Defining the validity of a biochemical index of soil quality. *Biology and Fertility of Soils* 30:140-146.

Loeppert R.H., Suarez D.L., 1996. Carbonate and gypsum. In: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3 e Chemical Methods. SSSA Book Series no. 5. SSSA-ASA, Madison, WI, USA, pp. 437-474.

Loveland P., Webb J., 2003. Is there a critical level of organic matter in the agricultural soils of temperate regions: a review. *Soil Tillage Research* 70:1-18.

Lupwayi N.Z., Hanson K.G., Harker K.N., Clayton G.W., Blackshaw R.E., O'Donovan J.T., Johnson E.N., Gan Y., Irvine R.B., Monreal M.A., 2007. Soil microbial biomass functional diversity and enzyme activity in glyphosate-resistant wheat-canola rotations under low-disturbance direct seeding and conventional tillage. *Soil Biology and Biochemistry* 39:1418-1427.

Maas E.V., Hoffman G.J., 1977. Crop salt tolerance - current assessment. *Journal of Irrigation and Drainage Engineering* 103:115-134.

Mäder P., Flieβbach A., Dubois D., Gunst L., Fried P., Niggli U., 2002. Soil fertility and biodiversity in organic farming. *Science* 296, 1694-1697.

Marcote I., Hernandez T., Garcia C., Polo A., 2001. Influence of one or two successive annual applications of organic fertilisers on the enzyme activity of a soil under barley cultivation. *Bioresource Technology* 79, 147-154.

Martens D.A., Reedy T.E., Lewis D.T., 2003. Soil carbon content and composition of 130-year crop, pasture and forest land-use managements. *Global Change Biology* 10:65-78.

Marzaioli R., D'Ascoli R., De Pascale R.A., Rutigliano F.A., 2010. Soil quality in a Mediterranean area of Southern Italy as related to different land use types. *Applied Soil Ecology* 44:205-212.

Moolenaar S.W., Van Der Zee S.E.A.T.M., Lexmond T.M., 1997. Indicators of the sustainability of heavy-metal management in agro-ecosystems. *Science of the Total Environment* 201:155-169.

Nannipieri P., Greco S., Ceccanti B., 1990. Ecological significance of the biological activity in soil. In: Bollag, J.M., Stotzky, G. (Eds.), *Soil Biochemistry*, 6:293-355.

Ndiaye E.L., Sandeno J.M., McGrath D., Dick R.P., 2000. Integrative biological indicators for detecting change in soil quality. *American Journal of Alternative Agriculture* 15:26-36.

Puglisi E., Del Rea A.A.M., Rao M.A., Gianfreda L., 2006. Development and validation of numerical indexes integrating enzyme activities of soils. *Soil Biology and Biochemestry* 38:1673-1681.

Reinecke A.J., Helling B., Louw K., Fourie J., Reinecke S.A., 2002. The impact of different herbicides and cover crops on soil biological activity in vineyards in the Western Cape, South Africa. *Pedobiologia* 46:475-484.

Rietz D.N., Haynes R.J., 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. *Soil Biology and Biochemistry* 35:845-854.

Ross D.J., 1971. Some factors influencing the estimation of dehydrogenase activities of some soil under pasture. *Soil Biology and Biochemistry* 3:97-110.

SAS Institute, 2008. SAS/STAT Users' Guide. Release 6.08. Ed. SAS, Cary, NC.

Schloter M., Dilly O., Munch J.C., 2003. Indicators for evaluating soil quality. *Agriculture, Ecosystems and Environment* 98:255-262.

Sparks D.L., 1996. Methods of Soil Analysis. Part 3. Chemical Methods. SSSA Book Series 5 SSSA and ASA, Madison, WI.

Sumner M.E., 1995. Sodic soils: new perspectives. In: Naidu, R., Sumner, M.E., Rengasamy, P., (Eds), Australian Sodic Soils: Distribution, Properties, and Management. CSIRO, Melbourne, pp. 1-34.

Tiessen H., Cuevas E., Chacon, P., 1994. The role of soil organic matter in sustaining soil fertility. *Nature* 371:783-785.

Trasar-Cepeda C., Leiròs M.C, Gil-Sotres F., 2008. Hydrolytic enzyme activities in agricultural and forest soils. Some implications for their use as indicators of soil quality. *Soil Biology and Biochemistry* 40:2146-2155.

Trevors J.T., 1984. Dehydrogenase activity in soil. A comparison between the INT and TTC assay. *Soil Biology and Biochemistry* 16:673-674.

Udawatta R.P., Kremer R.J., Adamson B.W., Anderson S.H., 2008. Variations in soil aggregate stability and enzyme activities in a temperate agroforestry practice. *Applied Soil Ecology* 39:153-160.

United States Department of Agriculture Natural Resources Conservation Service (USDA) Soil Survey Staff, 1998. Keys to Soil Taxonomy. Washington.

Weller D.M., Raaijmakers J.M., Gardener B.B.M., Thomashow L.S., 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology* 40:309-348.

Chapter 4
Organic amendments to improve soil quality under intensive farming

4.1 Introduction

Demand for organically produced food has increased by 24% yearly in the USA in the 1990s, as many consumers have expressed concern over pesticide residues on foods (Govindasamy and Italia, 1998; Thompson, 1998). Food and environmental safety are often-cited reasons for the use of alternative soil amendments, but increasingly economic considerations are becoming important with a rise in popularity of organically produced foods (Govindasamy and Italia, 1998; Klonsky and Tourte, 1998; Thompson, 1998).

On the other hand, the growing demand of food and the necessity to produce agricultural products throughout the year have prompted farmers to adopt more intensive cultivation techniques.

The 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 can provide key data for understanding many relevant effects of intensive agriculture managements on soil properties (Bonanomi et al., 2010).

The intensive agriculture determines a decrease of soil fertility, due to crop systems based on monoculture, continuous tillage, and excessive use of pesticides and mineral fertilizations (Marcote et al., 2001). In particular, the intensive agriculture under greenhouse, due to high temperature under cover and the rains lack, determines a strong decrease of organic carbon in the soil, favoring mineralization processes.

The decline in organic matter (OM) content of many soils is becoming a major process of soil degradation, particularly in European semi-arid Mediterranean

regions. Degraded soils are not fertile and thus cannot maintain sustainable production.

Use of organic amendments is generally seen as a key tool for soil health and sustainability in intensive agriculture systems, both in terms of maintaining the amount and quality of soil OM and in terms of supplying important micronutrients (Gill and Meelu, 1982; Ponnamperuma, 1984; Mahapatra et al., 1991; Bronson et al., 1997; Nambiar, 1997; Singh et al., 1997; Reichardt et al., 2000; Yadav et al., 1998; Timsina and Connor, 2001).

The use of organic amendments has been associated with desirable soil properties including higher plant available water holding capacity, lower bulk density and CEC, and can foster beneficial microorganisms (Doran, 1995; Drinkwater et al., 1995). Benefits of compost amendments to soil also include pH stabilization and faster water infiltration rate due to enhanced soil aggregation (Stamatiadis et al., 1999). If from one hand, soil chemical properties are positively affected by organic amendment, from the other hand, yields of crops grown, under organic amendment, can be equivalent to those under conventional fertilizers. Vegetable fields under organic amendments in California produced yields equal to those under conventional production (Drinkwater et al., 1995; Stamatiadis et al., 1999).

The application of organic amendments, such as compost, has been proposed, in successfully many cases, to improve the structural and chemical fertility of soils (Zucconi, 1996; Magid et al., 2001; Conklin et al. 2002; Cavigelli and Thien, 2003), the biological (Borken et al. 2002; Ros et al 2003), as well as for monitoring telluric pathogens (Hoitink and Boehm, 1999; Bonanomi et al., 2007).

The same studies have demonstrated that the effectiveness of organic amendments to recovery of soil fertility depends on the kind of used OM, doses and agronomic practices. If these variables are not handled properly, the amendment may result in insignificant or even harmful effects on the productivity of the crop (phytotoxicity, increase in the incidence of pathogens) and environmental (release of nitrates) due to rapid mineralization and low efficiency of humification (Zucconi, 1996). Most of the

studies carried out on different Italian soil also in the context of the Piana del Sele (Morra et al., 2006; Zaccardelli et al., 2006) indicated that the application of organic amendments characterized by easily decomposable material (low C/N) could lead to a not persistent increase of OM. For example, Morra et al. (2006) found no positive change in the balance of C three years after the yearly compost supply of 30 t ha^{-1}.

In this context it is clear that it weeds to set up experimental designs aimed to identify conditions which can maximize the humification efficiency.

The amendment should be not limited to guarantee the labile OM, that ameliorates physical properties, enhances nutrient availability and improve soil biological activity in short term. The treatment should produce long-term advantages and therefore, besides having beneficial effect on physical, chemical and biological properties, described above, it should favour humification processes and retard OM mineralization rate.

Aim of this work was to study the fertility recover in agricultural soils, under intensive farming for long time, by supplying compost enriched with wood scraps, in order 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 has been conducted in two farms selected among those studied in the previous research step (Chapter 3). 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, were used.

After the amendments at different ratio and doses, main soil chemical properties (pH, electrical conductibility, CEC, organic carbon content, total nitrogen, available phosphorus) and main enzymatic activities (dehydrogenase, β-glucosidase,

phosphatase, invertase and arylsulphatase) were determined to assess the effects of organic amendments on soil fertility during one year.

The present work was carried out within the framework “Monitoraggio e recupero della Fertilità dei suoli in sistemi agricoli intensivi” a research project funded by CCIAA of Salerno (Italy) in collaboration with the research groups of Prof. Astolfo Zoina, Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, and Dr. Rosaria D’Ascoli, Dipartimento di Scienze Ambientali, Seconda Università di Napoli.

4.2 Material and Methods

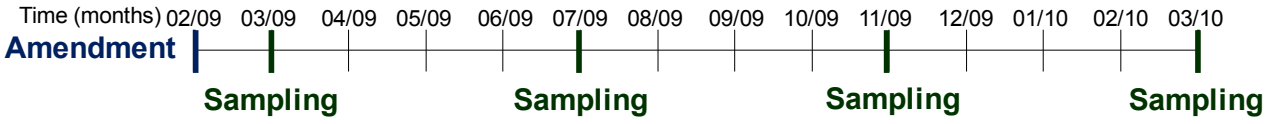
4.2.1 Description of the study site and experimental design

Two intensive farms, named F1 and F2, were selected amonf the farms of the previous study on assessing soil quality (see Chapter 3) in the Plain of Sele River (Salerno, Southern Italy). The two selected farms, characterized by different geopedologic characteristics, have provided a field, under greenhouse, of 160 m^2 that was divided in thirty plots, to allow the execution of the experimental design.

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:

- melon in both farms in the springtime;
- two crop cycles of lettuces and kohlrabi in F1 and F2, respectively, during the autumn and the winter.

Soil samples were collected after one month from the amendments (March 2009), and then every four month until to one year, in according to the following scheme:



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:

- compost from municipal solid waste (GeSeNu Srl, Perugia, Italy), whose properties are reported in Table 4.1;

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

Table 4.1 Chemical properties (on dry matter) of compost from municipal solid waste used for the experimental study.

Umidity %	pH	TOC %	HAs + FAs %	Total N %	Organic N %	P_2O_5 %	K_2O %	C/N	Cu ppm	Zn ppm	Salinity $cmol_{(+)}\ kg^{-1}$
25	7.9	28	14.2	2.1	2	0.8	1.8	13.3	67	146	53.2

Two amendments were obteined 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. In some plots 200 kg ha^{-1} of mineral fertilizer with N-P-K (14-7-17) and microelements (m) were also added, at the start of every crop cycle.

Furthermore, in the experimental field Control plots (Control and Control m) were also designed without organic amendments and mineral fertilizer to compare the effect of the treatments.

In each farm, the experimental design under greenhouse was set up in triplicate (three plots) in according to the scheme in Figure 4.1.

A1L	A1Lm	A1L	Control	Control m	Control
A2L	A2Lm	A2L	A1H	A1Hm	A1H
A1H	A1Hm	A1H	A2H	A2Hm	A2H
A2H	A2Hm	A2H	A1L	A1Lm	A1L
Control	Control m	Control	A2L	A2Lm	A2L

Figure 4.1 Amendment scheme. The grey plots ▢ indicate with mineral background fertilization, while the white plots ▢ indicate without mineral background fertilization.

4.2.3 Soil chemical and biochemical properties

Samples collected, in each sampling, have been characterized for the main chemical properties (pH, EC, CEC, exchangeable basis, organic C content, total N and available phosphorus) following the methodologies already described in Chapter 3, Par. 3.2.4. Organic C content and total nitrogen analyses were performed by the research group of Dr. Rosaria D'Ascoli at Dipartimento di Scienze Ambientali, Seconda Università di Napoli.

The activities of arylsulphatase, β-glucosidase, phosphatase and dehydrogenase were detected as described in detail in Chapter 3, Par. 3.2.5. The invertase activity 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). 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 sugar content analysis of the melons was performed using undiluted juice from the fruits just harvested. Approximately 20 μL of juice was placed in the well of a refractometer (Spear Scientifc Digital refractometer model 300 016; Scottsdale, AZ, USA) to measure the Brix value (%). The average weights of lettuces and kohlrabies were assessed by recording the weights of 15 plants collected per plot.

4.2.5 Statistical analysis

The relationships among soil physical, 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-one way with replication) was used to evaluate the effect of different organic amendments on crop yields. The significance between means with $P < 0.01$ was determined using the Tukey test.

Principal component analysis (PCA), and all statistical analysis were performed by JMP 8 (SAS Institute, 2008).

4.3 Results and Discussion

4.3.1 Soil chemical properties of studied farm soils

The starting step of the study was the soil characterization of two farms where the experiment have been carried out, in order to better assess the effects of organic amendments on soil properties. Physical and chemical properties of the F1 and F2 soils are showed in Table 4.2.

Table 4.2 Soil physical and chemical properties (±sd) of the two farms soils (F1 and F2) at the starting time.

Properties	F1 soil	F2 soil
Texture	Clay loam	Sandy loam
Sand, %	37 ±3	56 ±1
Silt, %	26 ±2	27 ±1
Clay, %	37 ±2	17 ±2
pH	7.74 ±0.12	7.65 ±0.12
EC, dS m^{-1}	0.08 ±0.02	0.17 ±0.03
Limestone, g kg^{-1}	1.55 ±1.03	639 ±75
Organic C, g kg^{-1}	10.47 ±0.56	16.19 ±0.39
Total N, g kg^{-1}	4.13 ±0.31	3.90 ±0.03
C/N	2.5 ±0.2	4.11 ±0.15
P_2O_5, mg kg^{-1}	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

The F1 soil was a clay loam soil, a *Mollic Haploxeralf* according to Soil Taxonomy (USDA, 1998; Regione Campania, 2004), with sub-alkaline pH, low electrical conductivity and limestone and high cation exchange capacity.

The farm F2, by contrast, was characterized by sandy loam soil, a *Lithic Haplustolls* according to Soil Taxonomy (USDA, 1998; Regione Campania, 2004), with sub-alkaline pH, high electrical conductivity, 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.

4.3.2 Effect of organic amendments on soil chemical properties

In Tables 4.3 and 4.4 pH values of F1 and F2 soils during the experiment time are shown.

Table 4.3 Effect of organic amendments on pH (± sd) of F1 soils.

	pH			
Plot	**I Sampling**	**II Sampling**	**III Sampling**	**IV Sampling**
Control	7.7 ±0.1	8.1 ±0.1	8.3 ±0.1	8.1 ±0.3
A1L	8.0 ±0.0	8.2 ±0.1	8.2 ±0.0	7.8 ±0.1
A1H	7.9 ±0.0	8.3 ±0.1	8.4 ±0.1	7.8 ±0.1
A2L	7.8 ±0.1	8.2 ±0.1	8.5 ±0.0	8.1 ±0.2
A2H	7.9 ±0.1	8.3 ±0.1	8.3 ±0.0	7.8 ±0.1
Control m	7.5 ±0.2	8.2 ±0.1	8.2 ±0.0	7.9 ±0.1
A1Lm	8.0 ±0.1	8.3 ±0.1	8.2 ±0.0	7.7 ±0.1
A1Hm	7.9 ±0.0	8.4 ±0.1	8.4 ±0.1	8.1 ±0.1
A2Lm	7.9 ±0.1	8.2 ±0.0	8.4 ±0.0	7.9 ±0.1
A2Hm	7.9 ±0.1	8.3 ±0.1	8.3 ±0.0	7.8 ±0.0

Table 4.4 Effect of organic amendments on pH (±sd) of F2 soils.

	pH			
Plot	**I Sampling**	**II Sampling**	**III Sampling**	**IV Sampling**
Control	7.6 ±0.1	7.8 ±0.0	8.1 ±0.1	8.1 ±0.1
A1L	7.5 ±0.3	7.5 ±0.1	8.0 ±0.0	7.9 ±0.1
A1H	7.9 ±0.1	7.5 ±0.0	8.1 ±0.1	8.1 ±0.1
A2L	7.9 ±0.1	7.7 ±0.1	8.0 ±0.0	8.1 ±0.1
A2H	8.0 ±0.1	7.8 ±0.1	8.1 ±0.0	8.1 ±0.1
Control m	7.8 ±0.2	7.7 ±0.1	7.9 ±0.0	7.9 ±0.1
A1Lm	7.7 ±0.1	7.4 ±0.2	8.1 ±0.0	8.1 ±0.1
A1Hm	7.5 ±0.2	7.6 ±0.1	8.2 ±0.1	8.2 ±0.2
A2Lm	7.8 ±0.1	7.5 ±0.1	7.9 ±0.1	8.1 ±0.1
A2Hm	7.8 ±0.2	7.8 ±0.1	8.0 ±0.0	8.2 ±0.2

The addition of whichever organic amendment, as well as mineral fertilization (m), caused in F1 soil a little increase of pH after one month, but after one year (IV sampling) the pH values of amended soils did not differ from that of Control soil (Table 4.3). The F2 soils showed not varied pH values neither upon treatment or over time. (Table 4.4). In Figures 4.2 and 4.3 the EC of F1 and F2 soils are shown. The addition of organic amendments determined an immediate increase of EC in F1 soils (around 0.2 dS m^{-1} against 0.07 dS m^{-1} of Control at I sampling) and a further increase at second sampling in the plots A1H and A2H (0.32 and 0.29 dS m^{-1}, respectively). The only mineral fertilization determined already an increase of EC, also in Control m plot compared to Control (+ 50%). The EC of mineral fertilized soils followed a similar trend to that of only organic amended soils over time (Figs. 4.2 and 4.3).

It is interesting to highlight the role of compost in the EC behavior, as it represents a precious source of electrolytes going into soil solution. The higher increase of EC values measured in A1H compared to those of other plots could be explained, if the absolute amount of compost added to different plots is considered. It is possible to observe that A1H and A1Hm plots received the higher compost amount (54 t ha^{-1}), whereas A2H and A2Hm received 40 t ha^{-1}, A1L and A2L (as well as A1Lm and A2Lm) received the lower amounts (27 and 20 t ha^{-1}).

Afterwards, at the third sampling an increase of EC in the Control plots and a considerable decrease in the plots under amendments was observed. The seasonal effect could be the cause of these results, because the third sampling occurred after the summer, when the high temperatures may have caused a solute concentration in soil and therefore higher EC value in Control soil. Organic amendments contrasted this phenomenon as organic colloids are able to better retain water and limit the solute concentration effect. At last sampling (IV), then, the EC further decreased, even in Control plots, returning to nearly the initial values (0.08 dS m^{-1}).

The F2 soils were characterized by higher initial values of EC (0.17 dS m^{-1}) and also in this case the addition of organic amendments determined a significant increases of

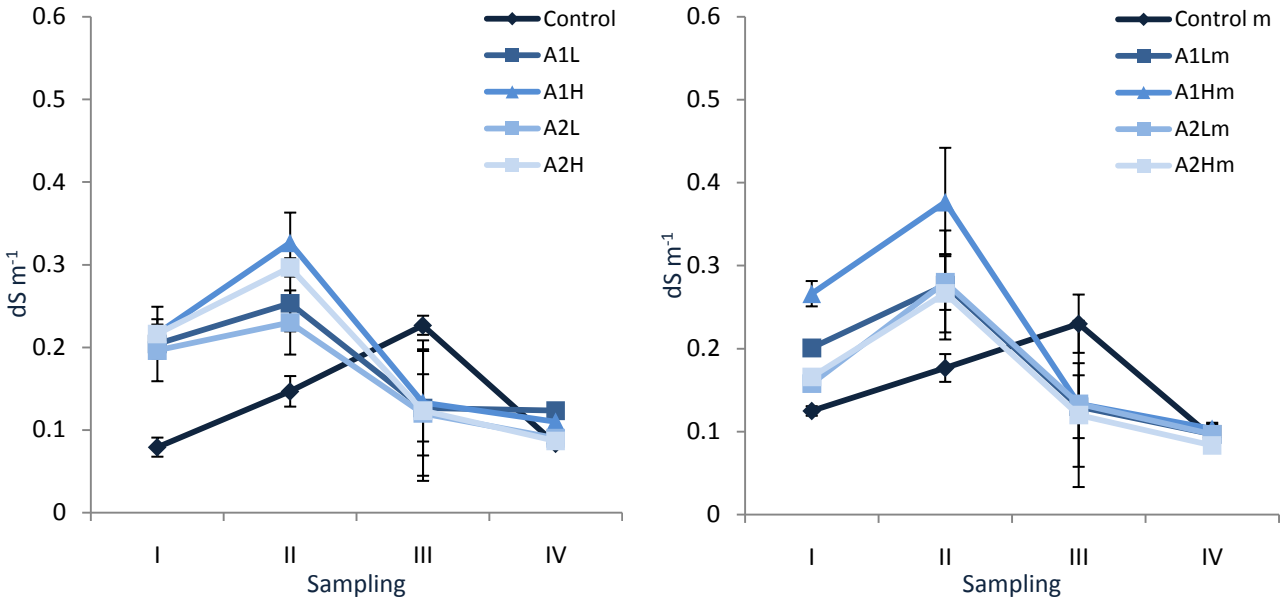


Figure 4.2 Effects of organic amendments on electrical conductivity (EC) in F1 soils during the four samplings.

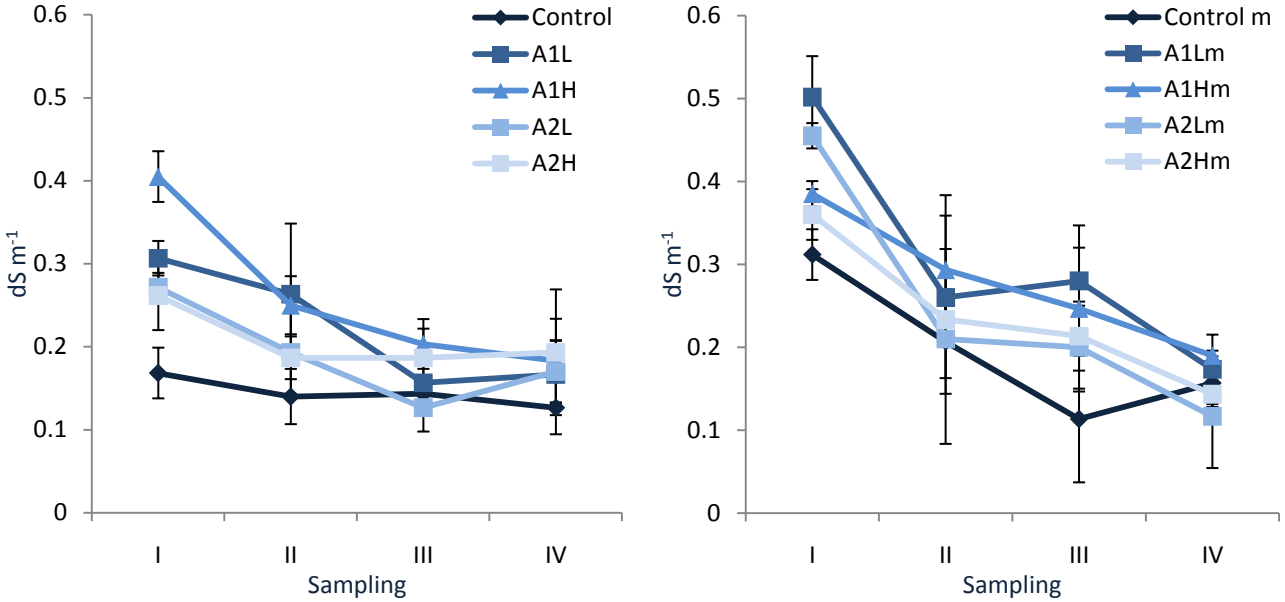


Figure 4.3 Effects of organic amendments on electrical conductivity (EC) in F2 soils during the four samplings.

these values especially in the plots A1Lm and A2Lm (0.50 and 0.45 dS m^{-1}, respectively), also due to mineral fertilization. During the annual experiment a continuous decrease of EC values in all plots, also in those mineral fertilized, was observed (Fig. 4.3). At fourth sampling, after one year, the EC further decreased, even in Control plots, returning to nearly the initial values.

The sandy loam texture could have favored the electrolyte leaching over time; because of the low specific area and CEC, and the high porosity sandy fractions were not able to retain soil solution and dissolved ions. Thus EC values decreased also in summer when evaporation processes would have concentrated soil solution. Conversely, clay texture of F1 soils made soils enable to better retain liquid phase that became more concentrated in electrolytes because of the summer temperature and therefore of evapotranspiration.

However, the crop uptake could be not excluded to explain the EC reduction in all plots of both soils.

In Tables 4.5 and 4.6 the CEC values of F1 and F2 soils are shown. The cation exchange capacity is a very important chemical property of soil, as it gives information on the amount of exchangeable cations that soil can absorb on its surface. The F1 soil was clay loam and therefore rich in fine materials, such as clays, having high specific surface, that contributed to the well-exchange process. At first sampling, CEC of all plots were similar to Control soil (21.09 $cmol_{(+)}$ kg^{-1}) and did not changed during the study, except in A1H, A1Hm and A2Hm plots, where an increase by around 10-11% against Control plot was observed.

The F2 soil, instead, was sandy loam, characterized by lower CEC values (13.6 $cmol_{(+)}$ kg^{-1}) at the first sampling. After one year, a general increase of CEC values in all plots, including Control plot, was observed. 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 (Sequi, 2005).

Table 4.5 Effect of organic amendments on cation exchangeable capacity (±sd) of F1 soils over time.

	CEC			
Plot	**I Sampling**	**II Sampling**	**III Sampling**	**IV Sampling**
Control	21.09 ±0.3	20.63 ±1.7	19.69 ±1.1	17.98 ±0.5
A1L	21.89 ±0.5	18.08 ±1.2	20.16 ±0.5	19.12 ±0.3
A1H	23.28 ±0.3	16.59 ±3.5	20.20 ±0.9	19.58 ±0.8
A2L	21.14 ±1.8	16.65 ±4.6	19.96 ±0.3	20.45 ±0.0
A2H	20.83 ±1.3	16.42 ±3.3	19.09 ±0.4	20.38 ±0.1
Control m	21.89 ±1.4	15.86 ±2.0	20.61 ±0.5	18.42 ±0.4
A1Lm	22.28 ±0.7	15.53 ±2.5	19.13 ±0.5	19.06 ±0.3
A1Hm	23.39 ±0.2	16.40 ±2.2	19.52 ±0.5	19.78 ±0.8
A2Lm	21.39 ±0.4	15.46 ±4.7	18.39 ±0.6	20.54 ±0.0
A2Hm	23.65 ±0.9	19.30 ±1.1	18.25 ±0.6	20.13 ±0.3

Table 4.6 Effect of organic amendments on cation exchangeable capacity (± sd) of F2 soils over time.

	CEC ($cmol_{(+)}\ kg^{-1}$)			
Plot	**I Sampling**	**II Sampling**	**III Sampling**	**IV Sampling**
Control	13.55 ±0.9	10.95 ±0.8	16.30 ±0.6	19.82 ±0.7
A1L	14.24 ±0.7	12.35 ±0.2	16.26 ±0.3	18.05 ±1.0
A1H	15.93 ±0.5	13.79 ±1.2	16.71 ±1.7	18.10 ±1.3
A2L	15.88 ±0.4	14.89 ±2.1	16.11 ±0.6	17.43 ±1.2
A2H	14.79 ±1.8	12.31 ±1.2	17.01 ±0.8	17.69 ±0.2
Control m	15.26 ±0.2	13.60 ±0.8	15.61 ±0.1	18.83 ±1.0
A1Lm	15.25 ±0.5	13.59 ±3.4	16.79 ±0.2	17.70 ±0.6
A1Hm	15.98 ±1.7	10.04 ±2.0	15.99 ±0.7	16.71 ±2.2
A2Lm	16.43 ±1.1	12.57 ±0.7	16.31 ±0.4	18.67 ±1.0
A2Hm	17.77 ±1.2	12.76 ±0.8	15.93 ±0.6	17.56 ±0.1

The exchangeable bases of F1 and F2 soils are shown in Tables 4.7 and 4.8. In the F1 soils, an increase of exchangeable Na^+ in all plots under organic amendments respect to Control plot (0.03 $cmol_{(+)}\ kg^{-1}$) was observed, especially in A2H plot (0.26 $cmol_{(+)}\ kg^{-1}$) at the first sampling. After one year, the supplied organic amendments determined a further increase of exchangeable Na^+ compared to not amended soils. The addition of OM did not affected exchangeable K^+, Ca^{2+} and Mg^{2+} after one

month, but after one year from the amendment K^+ and Ca^{2+} remained unchanged during the experimental study, whereas the exchangeable Mg^{2+} decreased.

The F2 soils showed an increase of exchangeable K^+ and Na^+ in the amended plots, at the first sampling, but these values decreased until the starting content after one year. The alkaline earth metals, Ca^{2+} and Mg^{2+}, were not affected by used organic amendments.

The supplied OM determined only few changes in the exchangeable bases content of the studied soils, in particular, a increase of extractable ions, as K^+ and Na^+, was observed, in according to others authors (Bougnom et al., 2009; Smith, 2009)

Table 4.7 Effect of organic amendments on exchangeable bases (±sd) of F1 soils, over time.

	Exchangeable bases ($cmol_{(+)}\ kg^{-1}$)									
	Control	**A1L**	**A1H**	**A2L**	**A2H**	**Control m**	**A1Lm**	**A1Hm**	**A2Lm**	**A2Hm**
I sampling										
K^+	1.47±0.12	1.70±0.12	1.80±0.13	1.73±0.12	1.85±0.51	1.50±0.12	1.75±0.22	2.02±0.53	1.61±0.24	1.81±0.15
Na^+	0.03±0.07	0.13±0.02	0.20±0.03	0.14±0.04	0.26±0.07	0.06±0.02	0.16±0.05	0.21±0.03	0.16±0.05	0.16±0.08
Ca^{2+}	12.06±0.3	12.70±0.7	14.15±0.4	12.38±0.8	12.33±0.9	13.29±1.0	13.24±0.5	13.95±0.1	13.08±0.3	13.92±0.6
Mg^{2+}	3.94±0.41	3.41±0.96	4.30±0.51	3.27±0.11	4.14±0.46	3.63±0.53	3.72±0.36	4.29±0.23	4.03±0.24	4.37±0.32
II sampling										
K^+	1.52±0.23	1.79±0.34	2.43±0.71	2.05±0.22	2.27±0.45	1.45±0.12	1.91±0.16	2.32±0.51	1.85±0.16	2.13±0.12
Na^+	0.14±0.06	0.85±0.02	0.84±0.06	0.43±0.01	0.57±0.03	0.11±0.02	0.86±0.02	0.55±0.06	0.38±0.04	0.47±0.06
Ca^{2+}	14.22±3.7	14.35±0.6	14.97±0.5	14.50±0.4	14.09±0.3	14.35±0.4	14.02±0.6	14.10±0.4	16.11±6.3	13.14±0.3
Mg^{2+}	5.22±0.93	5.18±0.32	5.30±0.41	5.31±0.35	5.12±0.23	5.21±0.34	4.52±0.12	5.50±0.24	4.23±0.42	4.50±0.51
III sampling										
K^+	1.38±0.22	1.28±0.13	1.36±0.14	1.28±0.1	1.22±0.0	1.21±0.0	1.20±0.1	1.30±0.1	1.26±0.0	1.33±0.1
Na^+	0.05±0.05	0.04±0.06	0.04±0.07	0.02±0.08	0.03±0.09	0.03±0.00	0.01±0.01	0.02±0.02	0.02±0.03	0.05±0.04
Ca^{2+}	16.22±0.7	14.85±0.6	15.07±0.5	14.80±0.4	13.89±0.3	15.55±0.4	14.22±0.6	14.40±0.4	17.41±0.3	13.34±0.3
Mg^{2+}	5.42±0.91	5.28±0.31	5.02±0.42	5.13±0.33	5.16±0.25	5.14±0.31	4.92±0.16	5.04±0.21	4.53±0.48	4.70±0.51
IV sampling										
K^+	1.10±0.01	1.18±0.03	1.22±0.01	1.21±0.01	1.31±0.02	1.04±0.00	1.17±0.01	1.27±0.03	1.30±0.01	1.22±0.04
Na^+	0.05±0.01	0.08±0.02	0.17±0.02	0.33±0.01	0.34±0.01	0.05±0.03	0.07±0.01	0.35±0.03	0.35±0.02	0.36±0.01
Ca^{2+}	13.35±0.3	14.54±0.2	14.92±0.6	15.59±0.2	15.52±0.1	13.68±0.2	14.51±0.2	15.13±0.6	15.73±0.1	15.29±0.2
Mg^{2+}	4.63±0.31	4.54±0.21	4.53±0.42	4.62±0.13	4.52±0.02	4.82±0.31	4.52±0.11	4.28±0.23	4.47±0.15	4.55±0.21

Table 4.8 Effect of organic amendments on exchangeable bases (±sd) of F2 soils, over time.

	Exchangeable bases ($cmol_{(+)}\ kg^{-1}$)									
	Control	**A1L**	**A1H**	**A2L**	**A2H**	**Control m**	**A1Lm**	**A1Hm**	**A2Lm**	**A2Hm**
I sampling										
K^+	0.75±0.21	1.68±0.52	1.86±0.41	1.37±0.63	1.86±0.41	0.72±0.13	2.01±0.24	1.89±0.21	1.91±0.32	2.10±0.11
Na^+	0.41±0.11	0.72±0.21	1.00±0.01	0.56±0.11	0.88±0.21	0.33±0.02	0.87±0.21	0.84±0.23	0.77±0.22	0.92±0.12
Ca^{2+}	10.68±0.7	9.65±0.5	11.52±0.4	11.53±0.4	10.23±0.4	12.21±0.1	10.24±0.6	11.71±0.9	11.17±0.6	12.86±1.8
Mg^{2+}	1.71±0.11	2.19±0.14	2.17±0.13	2.42±0.12	2.44±0.61	2.00±0.22	2.13±0.11	2.16±0.41	2.58±0.31	2.60±0.41
II sampling										
K^+	0.30±0.11	1.51±0.12	1.22±0.23	1.00±0.41	0.81±0.33	0.48±0.32	1.32±0.14	1.07±0.11	0.86±0.25	1.38±0.51
Na^+	0.14±0.12	0.44±0.21	0.51±0.16	0.27±0.12	0.35±0.15	0.07±0.14	0.47±0.14	0.56±0.44	0.31±0.12	0.53±0.21
Ca^{2+}	14.61±0.6	13.73±0.5	14.46±0.8	14.79±0.5	15.26±0.7	14.50±0.2	14.44±0.3	14.59±0.7	14.42±0.1	14.63±0.6
Mg^{2+}	1.99±0.11	2.13±0.32	2.09±0.21	1.99±0.12	1.91±0.21	1.73±0.16	2.10±0.14	2.13±0.14	2.16±0.21	1.99±0.12
III sampling										
K^+	0.55±0.12	0.89±0.11	0.32±0.41	1.00±0.31	1.42±0.22	0.57±0.16	0.90±0.41	0.41±0.25	1.08±0.41	0.76±0.11
Na^+	0.16±0.17	0.33±0.15	0.43±0.11	0.21±0.13	0.26±0.11	0.11±0.16	0.31±0.11	0.32±0.22	0.26±0.24	0.32±0.21
Ca^{2+}	15.61±0.6	14.73±0.5	15.63±0.8	14.79±0.5	15.26±0.7	14.90±0.2	15.44±0.3	14.99±0.7	14.72±0.1	14.73±0.6
Mg^{2+}	1.79±0.34	2.11±0.31	2.19±0.24	1.89±0.13	1.95±0.21	1.77±0.11	2.00±0.12	2.03±0.13	2.06±0.22	1.89±0.12
IV sampling										
K^+	0.61±0.21	0.74±0.12	0.95±0.11	0.68±0.12	0.66±0.13	0.60±0.15	0.72±0.21	0.68±0.33	0.76±0.15	0.61±0.15
Na^+	0.48±0.11	0.44±0.12	0.49±0.17	0.46±0.14	0.44±0.16	0.38±0.14	0.47±0.12	0.50±0.12	0.48±0.23	0.42±0.25
Ca^{2+}	18.12±0.3	16.64±0.9	16.23±0.9	16.18±0.9	16.43±0.3	17.79±1.0	16.43±0.7	15.31±0.9	17.23±1.1	16.43±0.1
Mg^{2+}	2.81±0.55	2.24±0.22	2.45±0.51	2.05±0.31	2.120.25	2.15±0.23	2.05±0.21	2.08±0.25	2.27±0.22	2.05±0.22

Figures 4.4 and 4.6 report the organic C content of differently F1 and F2 amended soils, respectively, over time.

First it should be considered that F1 and F2 soils were characterized by different initial organic carbon content (10.47 and 16.19 g kg^{-1}, respectively).

Then in F1 soils a slight increase in the treated plots was observed, after one month from amendments. The ready response could be missed as, on the one hand, the compost was still mostly in pellet form after one month and, on the other hand, wood scraps were not yet degraded, because of their recalcitrant nature to the mineralization.

In the following samplings (II and III samplings) the used organic amendments were better incorporated determining a significant increase of organic carbon in the amended plots. At the third sampling, all plots upon amendments showed organic C content two folds increased compared to Control plot (~15 and 8.32 g kg^{-1},

respectively). After one year, higher organic C content in amended plots than Control plot was still observed. No effects of mineral fertilization were observed.

The positive effect of organic amendment on organic C content could be better observed if the data were analyzed in percentage deviation from initial value of the Control soil (Fig. 4.5). After the third sampling these increases reached 40 until to 80% compared to Control plot and remained unchanged over time, also one year from amendments, except in A2H plot (Fig. 4.5).

These results testify the positive effect in terms of OM recovery, especially if these results are considered in long-term (after 12 months from the treatment) and after three crop cycles. The clay nature of F1 soils had an important role in this recovery. In fact, clay particles are involved in biophysical and chemical processes of C 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; Anderson and Paul, 1984; Schulten and Leinweber, 2000).

In F2 soils, as occurred in the F1, only a slight increase of organic carbon in the amended plots was observed after one month (Fig. 4.6). Only at the third sampling the supplied organic amendments determined a strong increase of organic C (~22 g kg^{-1} in the amended plots against 16.19 g kg^{-1} of Control plot). The increase, in general, was by around 40% compared to Control plot, except in A1Lm and A1Hm, where deviation percentage from Control plot were 64 and 48%, respectively (Fig. 4.7). In these plots, the addition of mineral fertilizer determined a further increase of organic carbon content, in according to Kiem and Kandeler (1997) that found, after mineral fertilization, an increase of microbial activity and consequently, an increase of OM degradation. After one year, the added OM was almost completely degraded, and the organic carbon content of treated plots were similar to Control plot, also in the mineral fertilized plots, as shown in Figure 4.6.

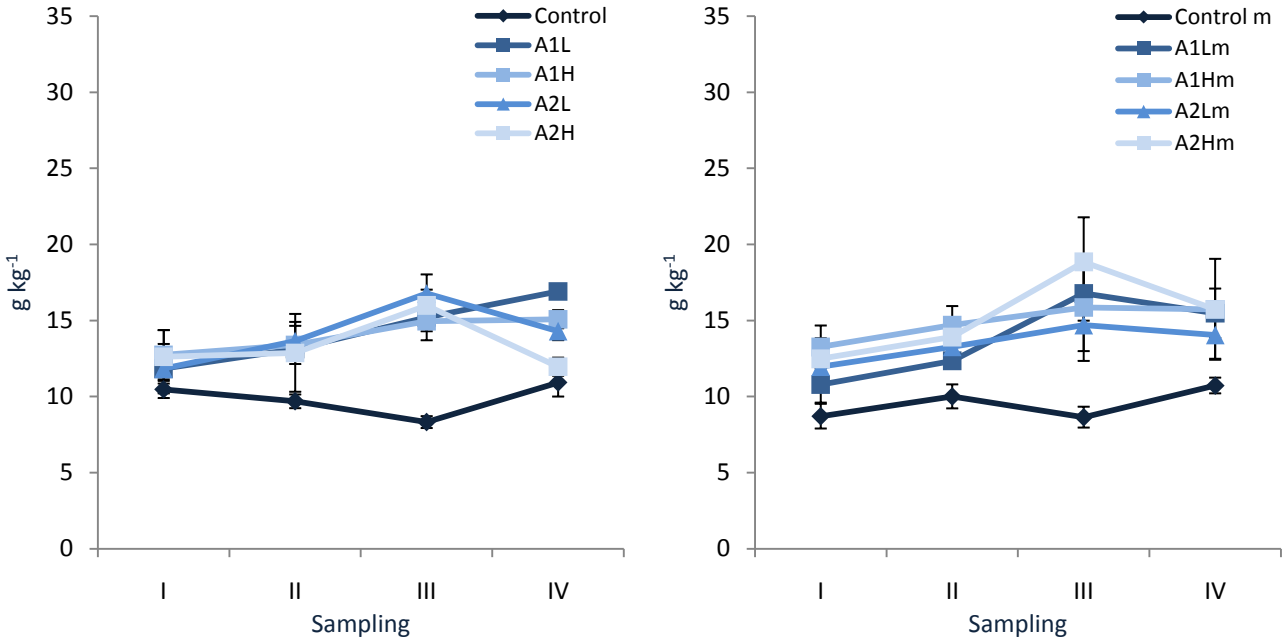


Figure 4.4 Effects of organic amendments on organic carbon content in the F1 soils during the four samplings.

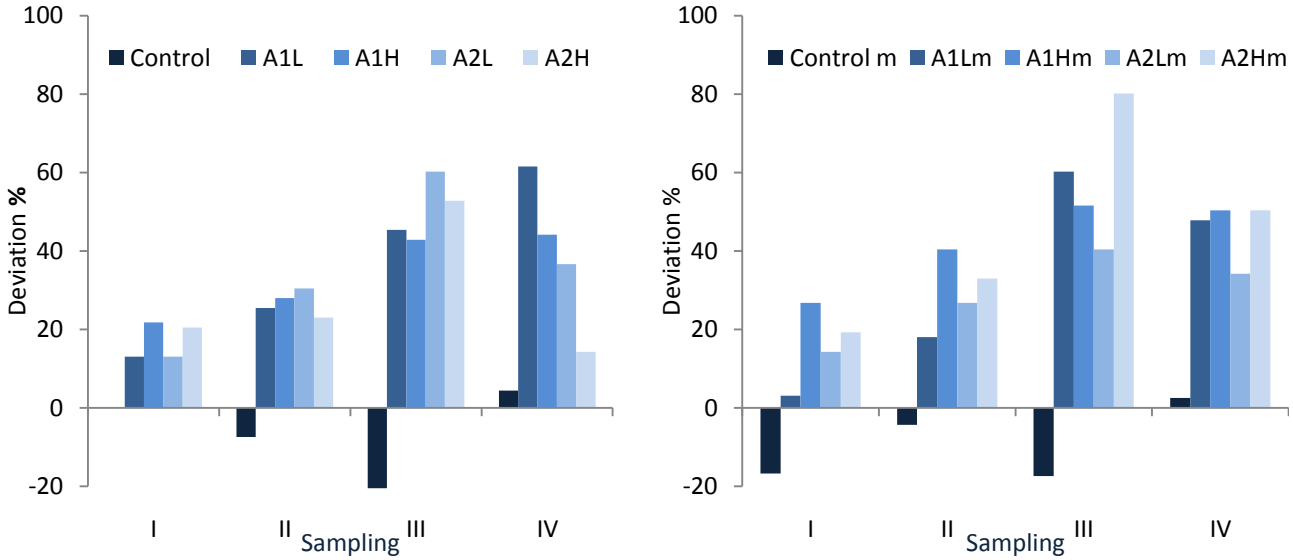


Figure 4.5 Effect of organic amendment on organic carbon content in F1 soils during the four samplings. Values are expressed as percentage decrease or increase in treated plots compared to Control at first sampling.

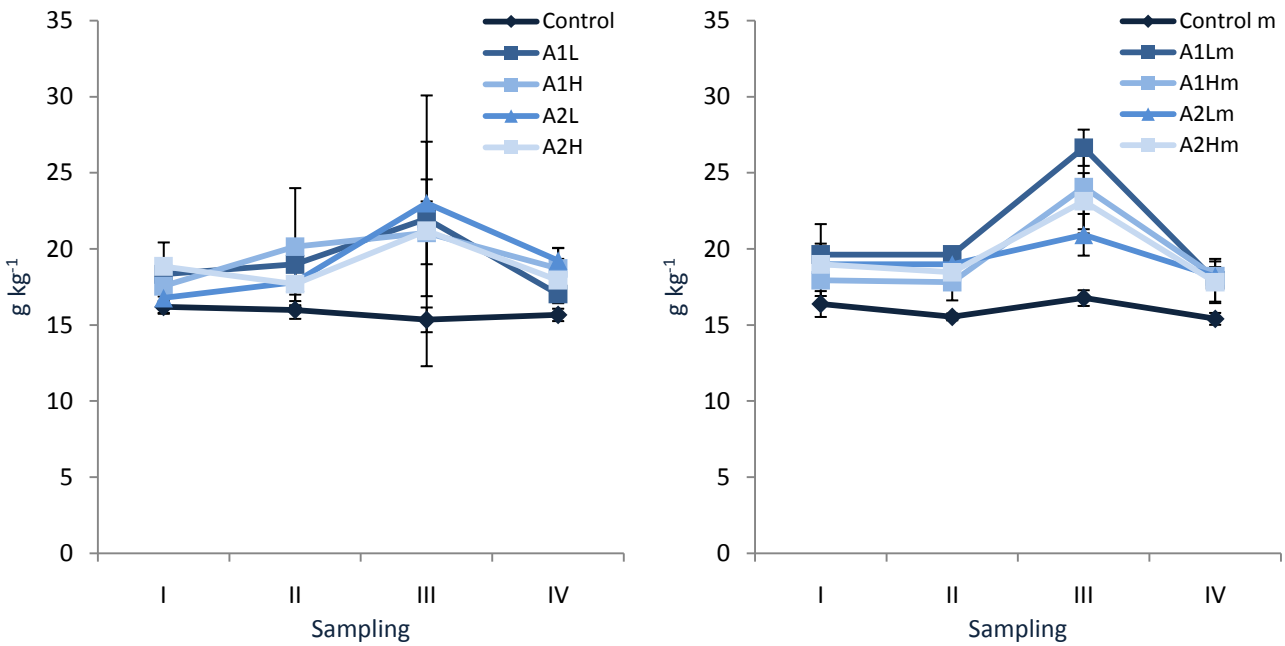


Figure 4.6 Effects of organic amendments on organic carbon content in the F2 soils during the four samplings.

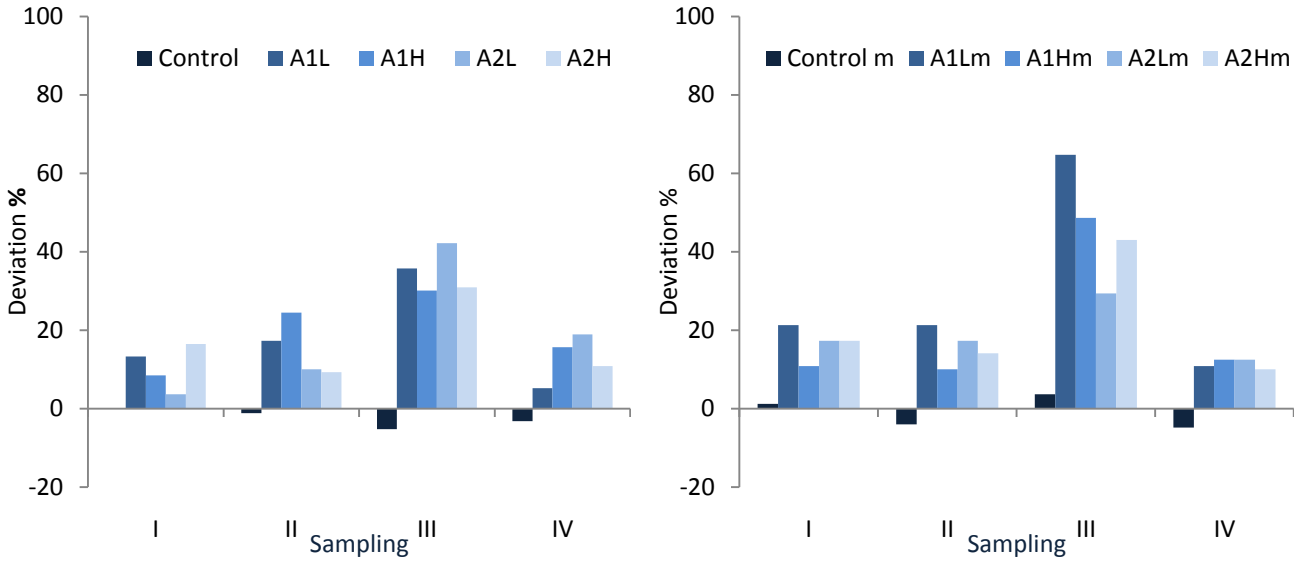


Figure 4.7 Effect of organic amendment on organic carbon content in F2 soils, during the four samplings. Values are expressed as percentage decrease or increase in treated plots compared to Control at first sampling.

The sandy loam nature, and consequently the low clay content, determined aired conditions that favoured the biomass activity, oxidative processes and leaching of OM, that contributed to lead to faster degradation and removal of fresh OM added to F2 soils in according to Christensen (1996) end Hassink et al. (1997).

The total nitrogen of amended F1 and F2 soils was reported in Figures 4.8 and 4.9, respectively. Both soils were characterized by a high total N content (about 4 g kg^{-1}), higher than the normal content of an agricultural soil (0.7 to 2 g kg^{-1}) (Sequi, 2005).

In F1 soils, only after one year a slightly increase of total N in the amended plots was observed, whereas in F2 soils, the total N content was not significantly affected by organic amendments over time. In both soils no further effects due to mineral fertilization were observed.

Differently from chemical fertilizations, that determine a rapid N release, organic amendments determine a slow N release, but extended over time (Claassen and Carey, 2006). In our case, used organic amendments showed a slow release of N, but sufficient to maintain a high total N content, considering the uptake from three crop cycles and mineralization processes occurred during the experimental time. Besides the plant uptake, the microbial biomass use of nitrogen could not be excluded (Borken et al., 2002).

By the values of organic carbon and total nitrogen it was possible to calculate the C/N ratios that providing information about the amount of carbon relative to the amount on nitrogen present. The ratio generally falls within well defined limit, usually from about 10 to 12. 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 forms of N to NH_3 and NO_3^-, and soil microorganisms utilize part of this N for synthesis of new cells. The gradual transformation of organic material into stable OM (humus) leads to the establishment of consistent relationship between C and N. Factors which may be involved in narrowing of the C/N ratio include chemical fixation of NH_3 as amines by lignin like substances.

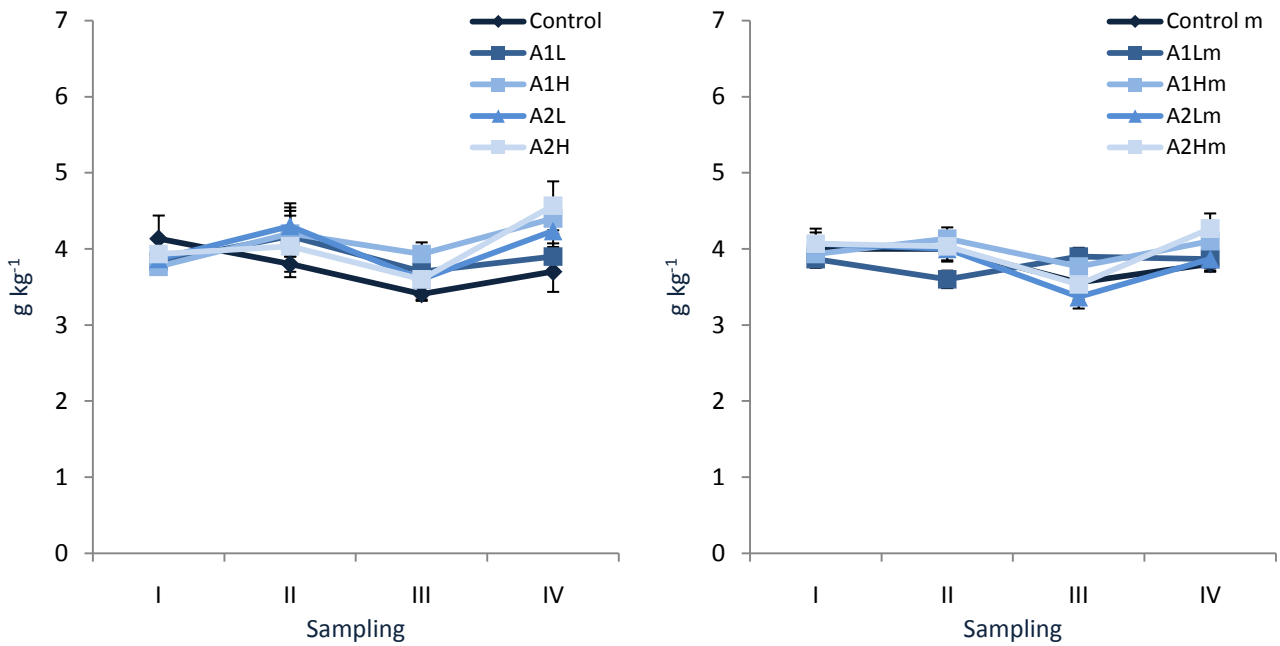


Figure 4.8 Effects of organic amendments on total nitrogen content, in the F1 soils, during the four samplings.

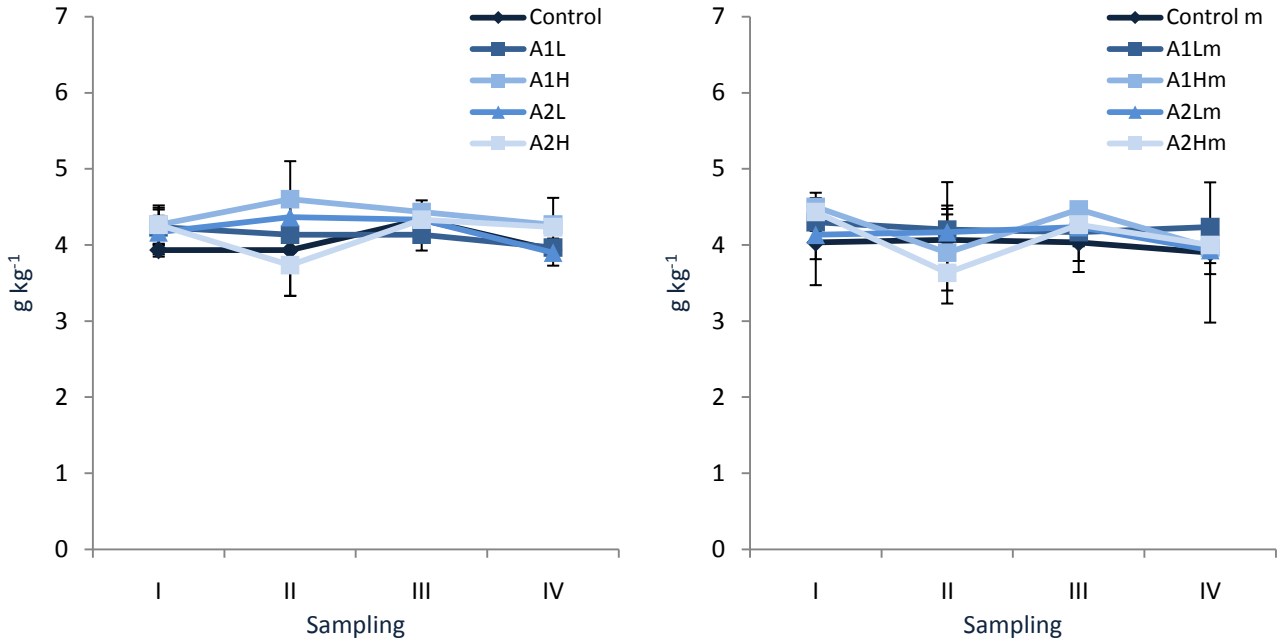


Figure 4.9 Effects of organic amendments on total nitrogen content, in the F2 soils, during the four samplings.

In Tables 4.7 and 4.8 are shown C/N ratios of F1 and F2 soils, respectively, over the experimental time. 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.

Table 4.7 C/N ratios of treated plots, in F1 soils, during the four sampling.

	C/N ratio			
Plot	**I Sampling**	**II Sampling**	**III Sampling**	**IV Sampling**
Control	2.5	2.5	2.4	3.0
A1L	3.1	3.2	4.1	4.3
A1H	3.4	3.2	3.8	3.4
A2L	3.1	3.2	4.7	3.4
A2H	3.2	3.2	4.4	2.6
Control m	2.2	2.5	2.4	2.8
A1Lm	2.8	3.4	4.3	4.0
A1Hm	3.4	3.6	4.2	3.8
A2Lm	2.9	3.3	4.4	3.6
A2Hm	3.1	3.4	5.3	3.7

Table 4.8 C/N ratios of treated plots, in F2 soils, during the four sampling.

	C/N ratio			
Plot	**I Sampling**	**II Sampling**	**III Sampling**	**IV Sampling**
Control	4.1	4.1	3.5	4.0
A1L	4.3	4.6	5.3	4.3
A1H	4.1	4.4	4.8	4.4
A2L	4.0	4.1	5.3	4.9
A2H	4.4	4.7	4.9	4.2
Control m	4.1	3.8	4.2	4.0
A1Lm	4.6	4.7	6.4	4.2
A1Hm	4.0	4.6	5.4	4.6
A2Lm	4.6	4.6	4.9	4.6
A2Hm	4.3	5.1	5.4	4.5

At the first sampling in F1 soils, plots under organic amendments, showed C/N ratios higher than the Control plot value, especially those without mineral fertilization. At the third sampling C/N ratio further increased until to double the Control value as in A2Hm plot. After one year the values of amended plot slightly decreased but remained however higher than Control plot.

In F2 soils hiving higher organic carbon content and therefore with C/N ratios higher (4.1), a slight increase was observed, except in A1Lm (6.4). However, after one year, C/N ratio returned to initial levels, in according to the reduction of organic carbon occurred in these soils.

When OM was incorporated into soils microorganisms start to decompose it through enzymatic hydrolysis. Nutrients released in this process could be used by bacteria and fungi, and, if they were in excess, also by other soil organisms, as plants (Borken et al., 2002). In this soils the C/N ratio was enough low to avoid a nitrogen deficiency to microorganisms as well as the plants deficiency. Incorporating OM that had a high C/N ratio, like wood, may have been cause same nitrogen deficiency in crops at least in the short time.

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. Also the missed increase of nitrogen leads to believe that soil microorganism, in their metabolic activity utilized nitrogen liberated from decomposed OM.

However, the high N content showed in both soils, promoting microbial activity, favored a fast degradation of supplied OM, in particular in F2 soil due to its geo-geopedologic characteristics, and to C/N ratios more favorable to microbial activity. (Sequi, 2005)

In Figures 4.10 and 4.11 the available phosphorus in F1 and F2 soils after the amendments over time, is shown.

As reported in Table 4.2, the initial values of P_2O_5, were almost similar (~160 mg kg^{-1}) in both soils and however far beyond the normal content of an agricultural soil, due to the continuous mineral fertilization that occurred under past intensive farming. At

the first sampling, in both soils, a slight decrease of available phosphorus in the treated plots was observed.

In F1 soils, during the experimental time, treated plots showed a slight increase of P_2O_5 compared to Control plot, until after one year from the amendment. At the last sampling, available phosphorus content of amended plots were higher than Control plot (230 mg kg^{-1} in A2H against 209 mg kg^{-1} of Control) (Fig. 4.10).

In F2 soils, on the contrary, a decrease of available phosphorus in plots under organic amendments, compared to Control, was observed during all the experimental time. However after one year the phosphorus content of the amended plots (~180 mg kg^{-1}) was very similar to that of Control plot , indicating a slow release of phosphorus by supplied OM.

The ready reduction of phosphorus after OM addition could be ascribed to the strong impulse to microbial activity. Firstly microorganisms used the already available phosphorus and then they contributed by enzymatic hydrolysis of OM to replenish the nutrient reserves. As regards phosphorus, phosphatase enzymes have an important role in the transformation of organic P in inorganic P (phosphate), the only form available to plant as well as microorganisms (Toor and Bahl, 1997; Cavigelli and Thien, 2003; Sequi, 2005).

The reduction of available phosphorus, in F2 soils compared to F1 soils, was determined by its calcareous nature, because the presence of insoluble precipitates of Ca phosphates, that occurred in this soil, are one of the major cause of the loss of available phosphorus (Sample et al., 1980; Ruiz et al., 1997; Delgado and Torrent, 2000).

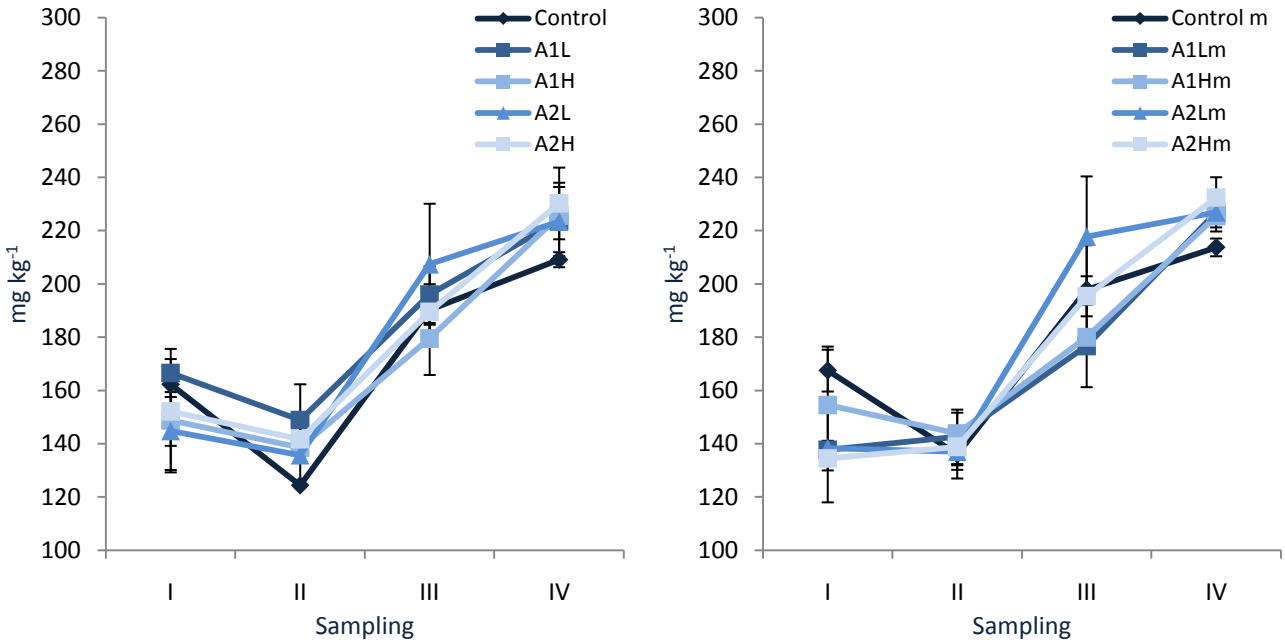


Figure 4.10 Effects of organic amendments available phosphorus (P_2O_5) content in the F1 soils, during the four samplings.

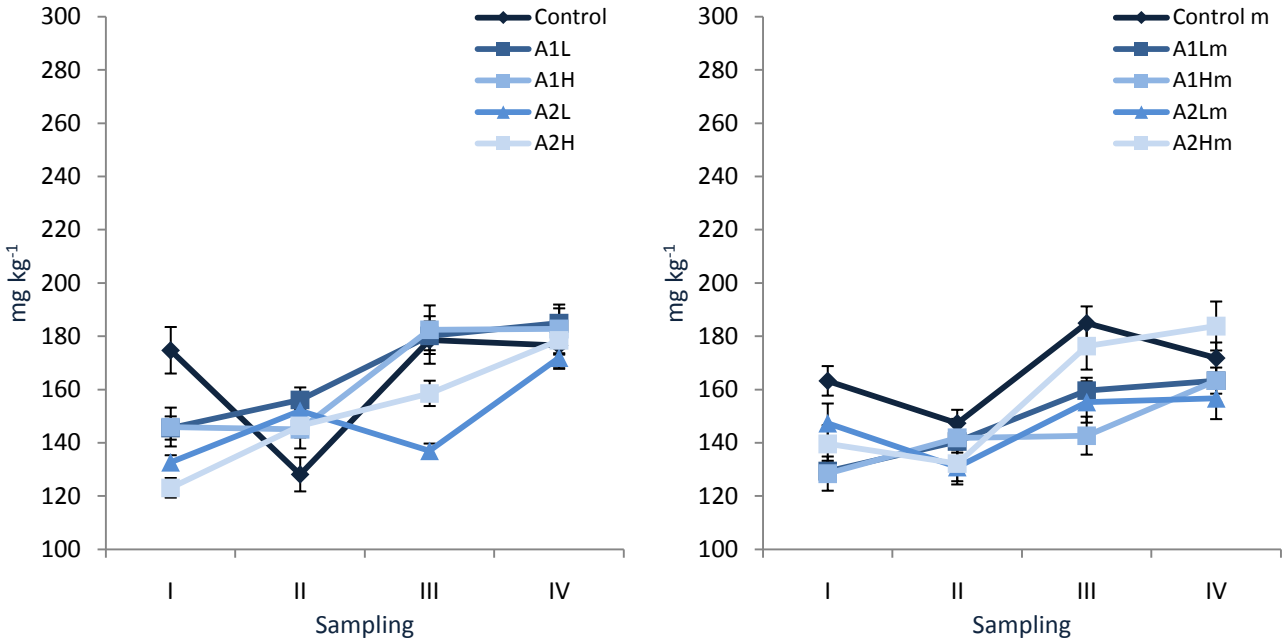


Figure 4.11 Effects of organic amendments available phosphorus (P_2O_5) content in the F2 soils, during the four samplings.

4.3.3 Effect of organic amendments on enzymatic activities

The incorporation of OM in soil determined changes in soil physical, chemical and biochemical properties. Among these, soil enzymes are considered good indicators of soil quality and are used to evaluate all those positive or negative changes, going to alter the dynamic equilibrium reached in soil at a given time (Dick, 1994). For this reason, during this study, some enzymatic activities directly involved in biogeochemical cycles of main elements (C. N, P, S), in particular, dehydrogenase, β-glucosidase, invertase, phosphatase, arylsulfatase, are determined during the experiment.

In the figures 4.12 and 4.13 the dehydrogenase activities (DHY) of F1 and F2 soils, respectively, over time are shown. At the first sampling, it is clear that the treatment strongly enhanced DHY activity in both soils.

In all treated plots of F1 soil activity were higher than in Control plot. In particular, in soils treated with the highest amendment dose (A1H and A2H), 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 second sampling the activity values strongly felt down until to initial value of Control soil. A similar trend was also observed in mineral fertilized plots.

In F2 soils the addition of OM led the immediately DHY activity to higher absolute values than in F1 soils, but the percentage increase was similar. The mineral fertilization contributed to the very strong impulse to DHY in amended plots. At the second sampling DHY started to decrease and continued to fall down even below the Control plot values.

The amendments used in this study were a mixture of compost and wood which have different behavior and fate. Compost contributed to ameliorate soil chemical properties immediately, as already detailed in previous paragraph especially in terms of organic carbon content and nutrients.

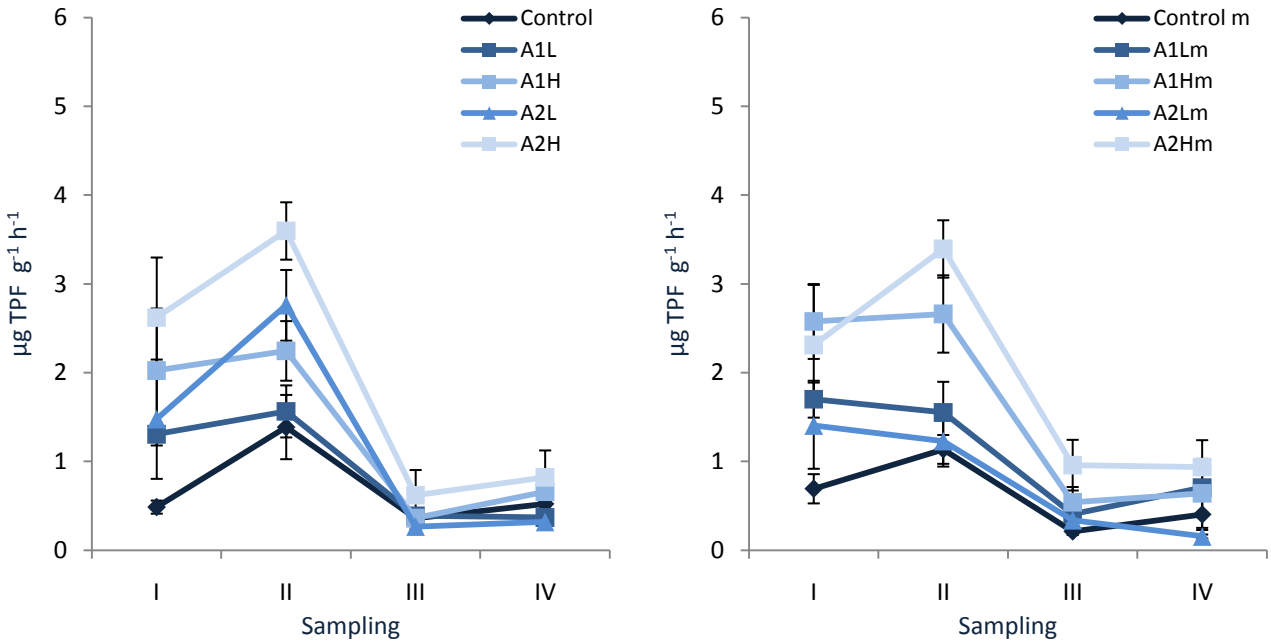


Figure 4.12 Effect of organic amendments on dehydrogenase activity of F1 soils, at different samplings.

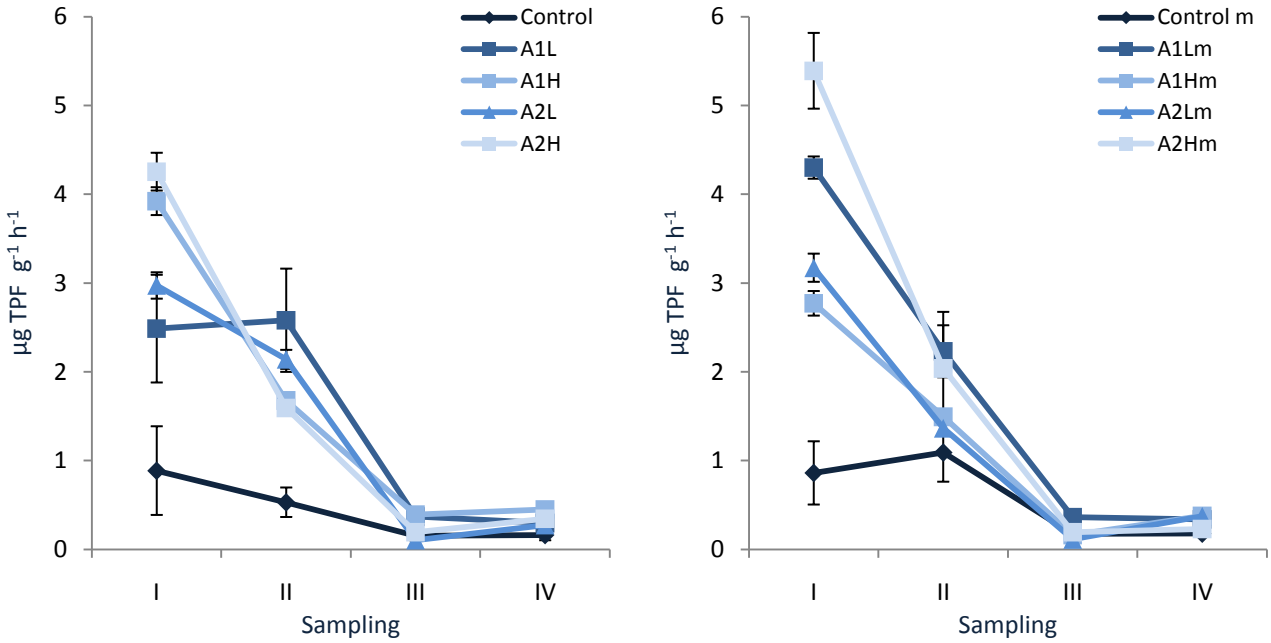


Figure 4.13 Effect of organic amendments on dehydrogenase activity of F2 soils, at different samplings.

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, at first sampling in both soils. DHY, as expected, was in according to the other parameters closely related to this enzyme, in particular to organic carbon (Aon and Colanieri, 2001). In fact, DHY was higher in F2 than F1 soils, according to their organic carbon content (respectively 10.47 and 16.19 g kg^{-1}). In according to Kiem and Kandler (1997) the positive effect of mineral fertilization could be due to the further stimulation of soil microbial biomass. The dehydrogenase is a soil enzyme involved in all degradation reactions of OM (Dick, 1994). Its activity is closely related to the activity of soil microbial biomass and directly reflects the conditions of the biological activity in the soil. Therefore, DHY performance is strongly affected by those factors that modify the entity and the activity of the soil biomass (Dick, 1994).

To explain the behavior of the studied soils, seasonal effects should be also taken in account. In fact samplings occurred in different seasons. The third and fourth sampling were carried out in autumn and winter, when soil biological activity is reduced compared to the spring and summer (first and second samplings) (Ros et al., 2003). However also the reduced amount of labile organic C fraction over time should be not excluded since microbial biomass is able to consume for metabolic needs above all more available compounds, like polysaccharides, lipids etc (Kandeler et al., 1999).

The β-glucosidase activity (GLU) of F1 and F2 soil, is shown in Figures 4.14 and 4.15, respectively.

In F1 soils, the addition of organic amendments determined no significant increase of GLU activities at the first as well as second sampling, except in the plots with mineral fertilization (m), indicating a positive effect of mineral fertilization on the microbial activities. At the third and fourth sampling the activities decreased reaching similar level (~0.1 μmol *p*-NP $g^{-1}h^{-1}$) in all plots, included mineral fertilized ones.

In the F2 soils, on the contrary, the use of OM determined a significant increase of the GLU activity compared to Control plot, but already from the second sampling a

significant decrease of β-glucosidase activity in all plots, even in the Control, was observed. Nevertheless after one year the activity level remained high in amended plots than in Control soil. The GLU activity trend was in according to DHY activity and consequently to microbial activity. In F2 soils amendments induced already in the first months a faster OM decomposition, that furnished more rapidly suitable substrate to GLU. In fact, β-glucosidase is an enzyme that catalyzes the hydrolysis of cellobiose into glucose, the last step of the cellulose breakdown, and its performance is closely related to the contribution of new organic material which gives a boost to this enzyme activity. However, unlike the dehydrogenase, GLU is not so dependent on the nature of OM (fresh or well-stabilized) (Dick, 1994).

Figures 4.16 and 4.17 report the invertase (INV) activity of F1 and F2 soils, under amendments, over time. The INV activity of the F1 plots showed different behavior during the experiment time (Figures 4.16). At the first sampling a significant increase only in A2L and A2H plots (0.47 and 0.44 μmol glucose g^{-1} h^{-1}, respectively) compared to Control plot was observed. At the second and the third sampling, the INV activity decreased or remained unchanged, whereas at the last sampling, after one year, the activity values increased, especially in the plots under organic amendments (~0.8 μmol g^{-1} h^{-1}).

In the F2 soils the INV activity increased in all treated plots at the first sampling, but then, it decreased at the second sampling and increased again after one year (Fig. 4.17). A similar trend was also observed in mineral fertilized plots (Fig. 4.17).

As for invertase, it is important to highlight that this enzyme is involved when OM degradation regards simpler compounds, in particular in disaccharide hydrolysis. Therefore, it was expected to observe an increase of the INV activity in the final phase of the study, when the added OM had already undergone degradation processes. The different behavior in the two soils could be due, also in this case, to the different nature and properties of soils.

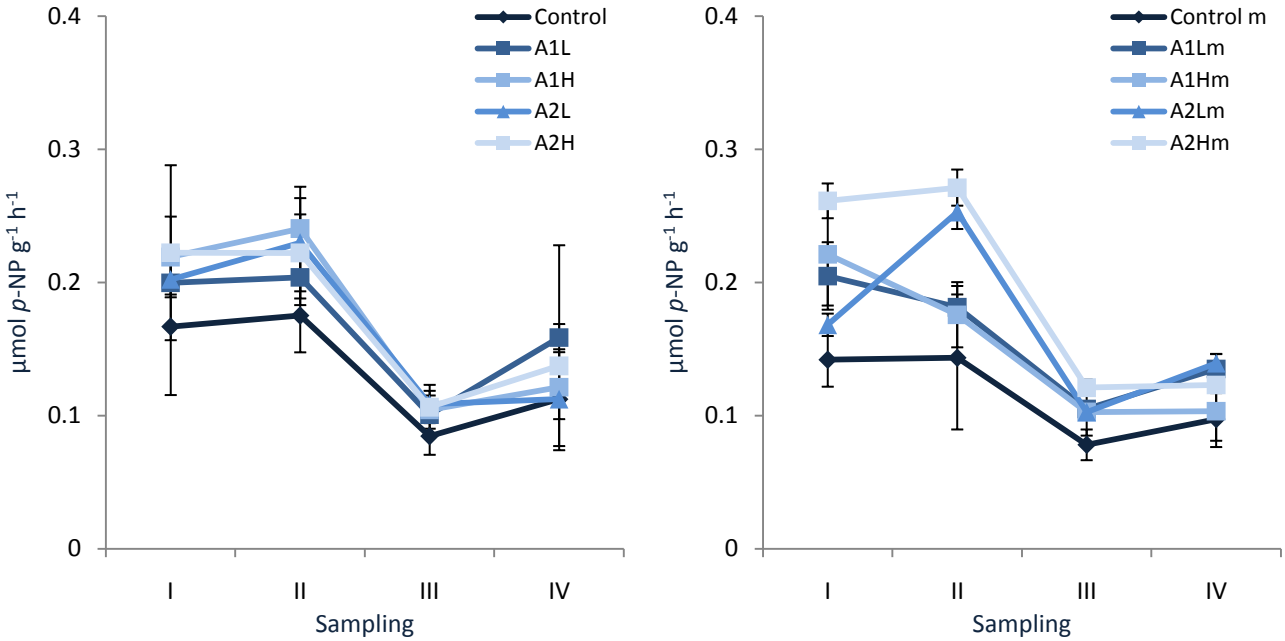


Figure 4.14 Effect of organic amendments on β-glucosidase activity of F1 soils, at different samplings.

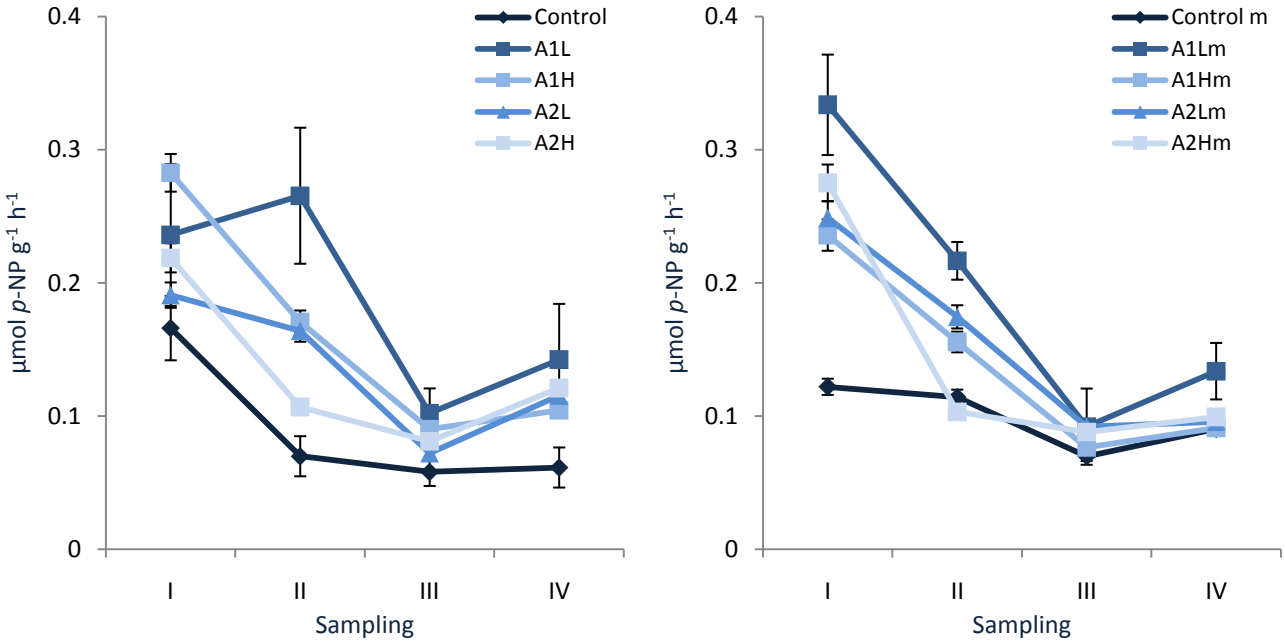


Figure 4.15 Effect of organic amendments on β-glucosidase activity of F1 soils, at different samplings.

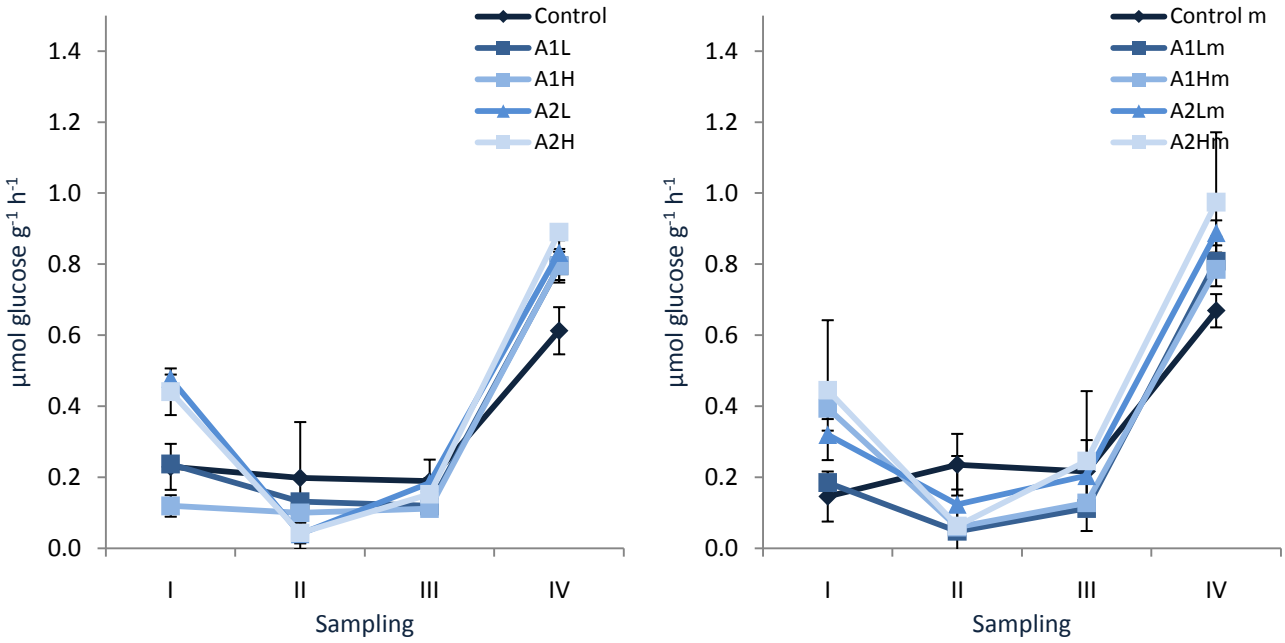


Figure 4.16 Effect of organic amendments on invertase activity of F1 soils, at different samplings.

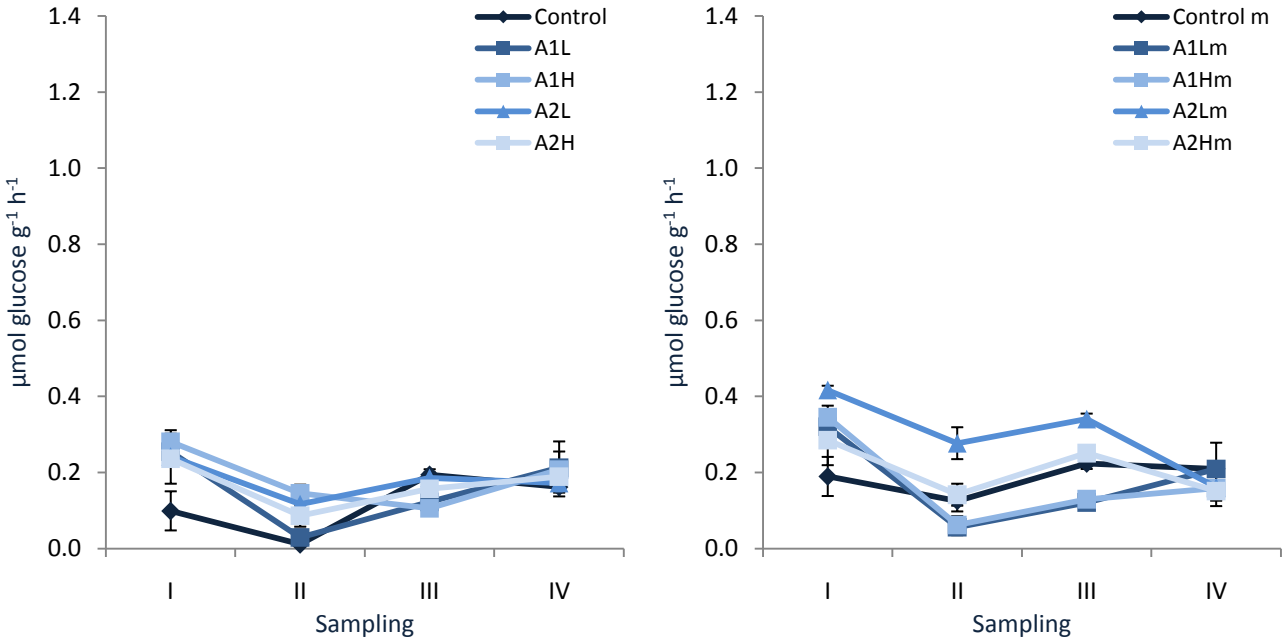


Figure 4.17 Effect of organic amendments on invertase activity of F2 soils, at different samplings.

The activity of phosphomonoesterase (PHO), commonly known as phosphatase, of F1 and F2 soils is shown in Figures 4.18 and 4.19, respectively.

In F1 soils an increase of PHO in all plots under organic amendments compared to the Control plots (Control and Control m) was observed at the first sampling, . At the third sampling a significant activity decrease affected all plots including Controls. At the last sampling, finally, PHO activity increased again in the treated plots, but not to the levels recorded at the first sampling.

In F2 soils PHO activity showed a strong significant increase in all amended plots, especially in A1H (1.67 μmol *p*-NP g^{-1} h^{-1}), and then it decreased in all plots over time, but remaining higher than Control plots.

To better understand the PHO behavior in both soils, it is important to consider that PHO activity markedly feels inhibitor effects of phosphate, its hydrolysis product (Burns, 1978). Therefore when phosphorus content increased in studied soils (Figures 4.10 and 4.11), consequently PHO activity decreased (Figures 4.18 and 4.19). This happened especially in the last period, when microbial activity had abundantly decomposed OM and released inorganic P.

Figures 4.20 and 4.21 show the arylsulphatase activity (ARYL) of both farm soils.

In F1 soils, the use of organic amendments determined no significant changes in ARYL activity. At the third sampling, the activity fell down nearly zero in all plots except in A2H and A2Hm (0.022 and 0.026 *p*-NP g^{-1} h^{-1}, respectively). In the fourth sampling it strongly went up in all plots.

In F2 soils, ARYL activity was higher in organic amended plots at the first sampling, then, at the second sampling, the activity levels increased only in A1L plots, whereas remained unchanged in the other plots. While at the third sampling ARYL activity generally decreased, at the last sampling a different behavior between A1 and A2 amendments could be observed. In the plots amended with A2, as well as the mineral fertilizers (A2Lm and A2Hm), the ARYL activity increased again, indicating that A2 amendment, which was richer in wood scraps and therefore more slowly degradable,

furnished suitable substrate to ARYL, also in long-term. A similar trend was observed also in A1Hm.

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 (Al-Khafaji and Tabatabai, 1979; Bardgett et al., 1994) and the intensive use of fungicide, based on sulphaets, in both farms (Trasar-Cepeda et al., 2008).

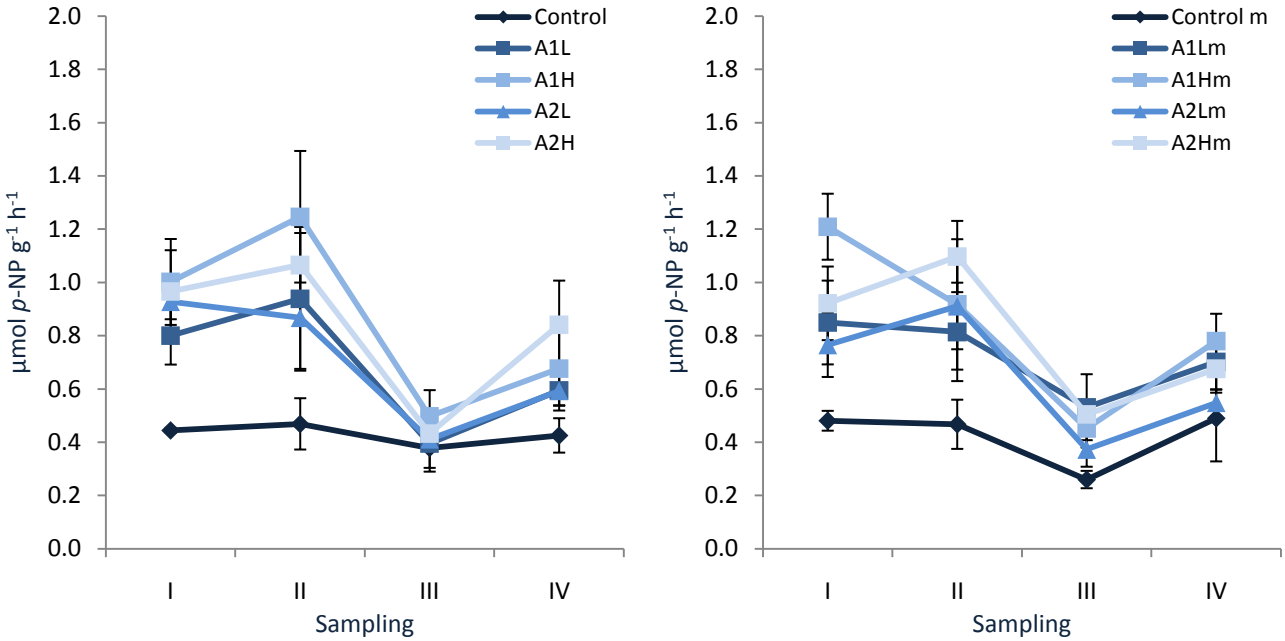


Figure 4.18 Effect of organic amendments on phosphatase activity of F1 soils, at different samplings.

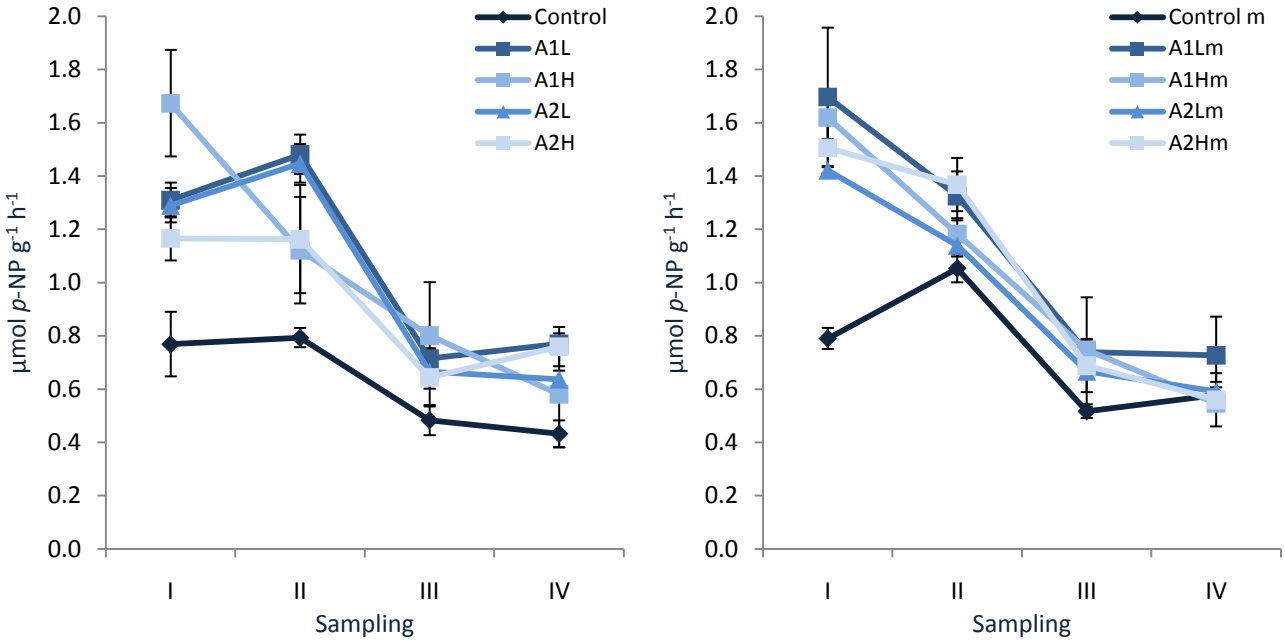


Figure 4.19 Effect of organic amendments on phosphatase activity of F2 soils, at different samplings.

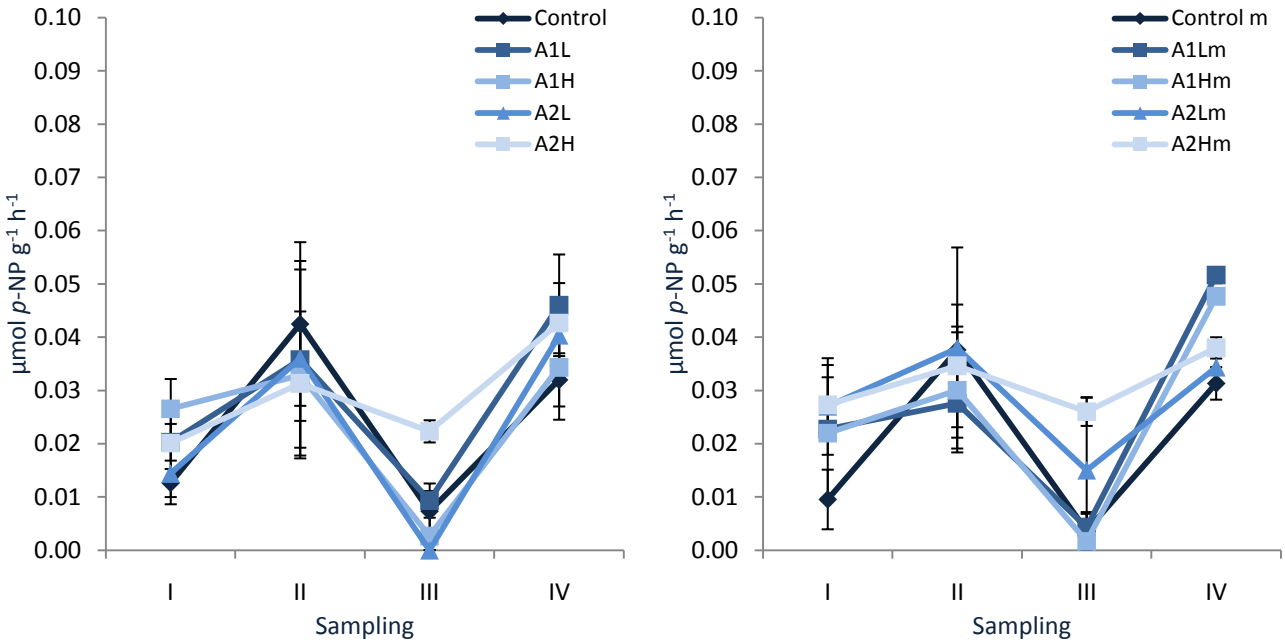


Figure 4.20 Effect of organic amendments on arylsulphatase activity of F1 soils, at different samplings.

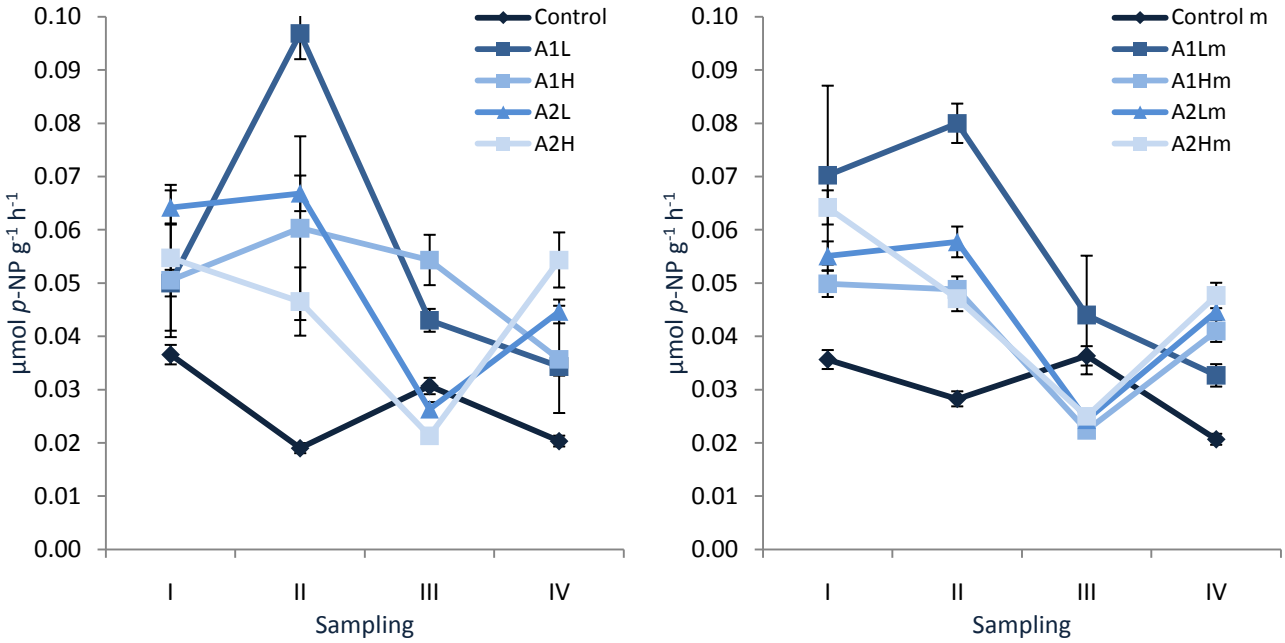


Figure 4.21 Effect of organic amendments on arylsulphatase activity of F2 soils, at different samplings.

4.3.4 Effect of organic amendments on crop production

In Figure 4.22 crop productions obtained in F1 plots are shown. During the first crop cycle a significantly difference among soils under organic amendment was observed. The treated plots produced lower melon yields (from 59.3 to 79.7 kg per plot, in A2H and A2Lm, respectively) than Control and Control m (89.7 and 101 kg per plot, respectively), whereas the sugar content of melon was no affected by organic amendments (data not showed).

During the second and the third crop cycle, the average weight of lettuces (AWL) in F1 soils was calculated (Fig. 4.23a) and this was significantly and positively affected by organic amendments. In fact, in the second crop cycle Control and Control m showed lower AWL (193.56 and 211.60 g plant^{-1}, respectively) than treated plots. In particular, the amendment A1determined the higher AWL (A1L and A1H, 291.38 and 309.09 g, respectively). Similar trend was observed in the third cycle: 301.27 and 317.00 g in A1L and A1H plots, respectively (Fig. 4.23b).

In F2, during the first crop cycle, few differences among plots were observed (Fig. 4.22b). Also in these soils Control plot produced the highest melon yield (63.7 kg), while the amendment A2 determined the lowest (38 and 39 kg per plot in A2L and A2H, respectively). As occured in F1, also in these soils the sugar content of melon was no affected by organic amendments (data not showed).

As in F1 soils, in F2 soils crop production was significantly enhanced by organic amendments, in the second and third crop cycles (Fig. 4.24). While in the second cycle only A1L and A1Lm (372.27 and 369.82 g plant^{-1}, respectively) determined a significant improvement to kohlrabi weight compared to Control plot (256.93 g plant^{-1}), in the third crop cycle, all amendment led to significant differences against Control plot. Also in this case, A1H determined the higher average weight of kohlrabi (479.53 g plant^{-1}).

Therefore, by summarize crop yield results, the positive effects of all used organic amendment in this study was highlighted only from the second crop cycle, 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).

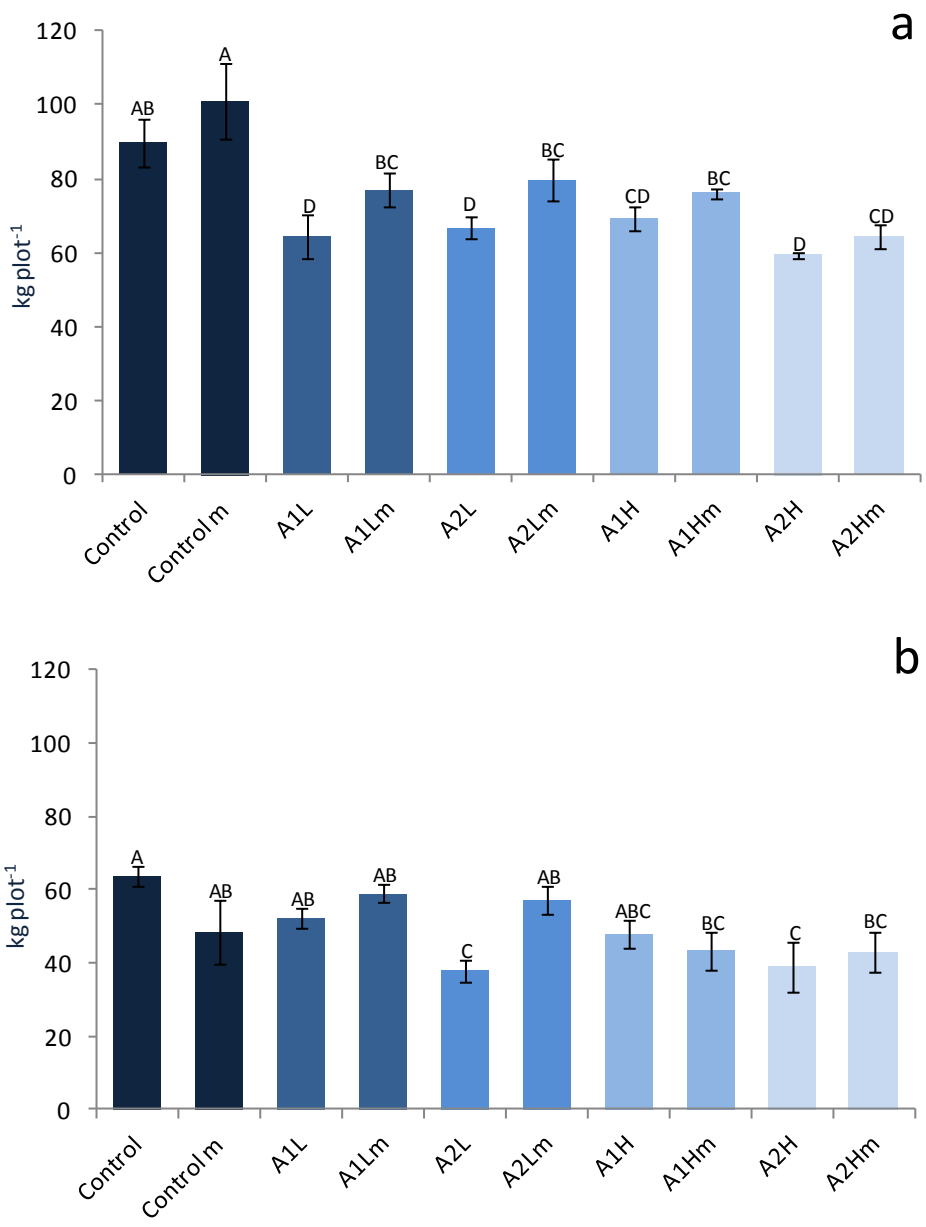


Figure 4.22 Yield of melon in the plots under different organic amendments, in F1 and F2 (a and b, respectively), during the first crop cycle. Samples not connected by same letter are significantly different (P<0.05).

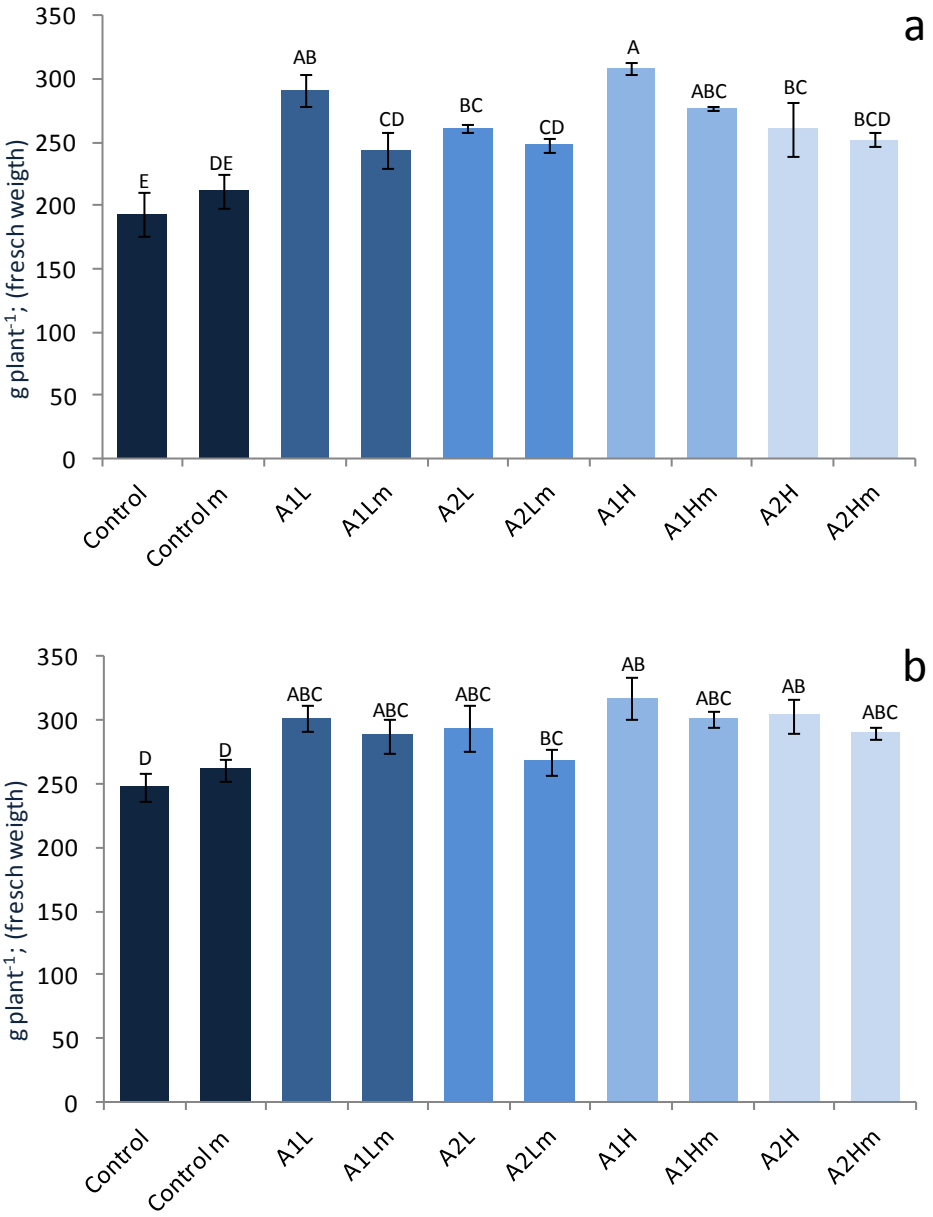


Figure 4.23 Average fresh weight of lettuces product in the plots under different organic amendments, in F1 during (a) the second and (b) the third crop cycle. Samples not connected by same letter are significantly different (P<0.05).

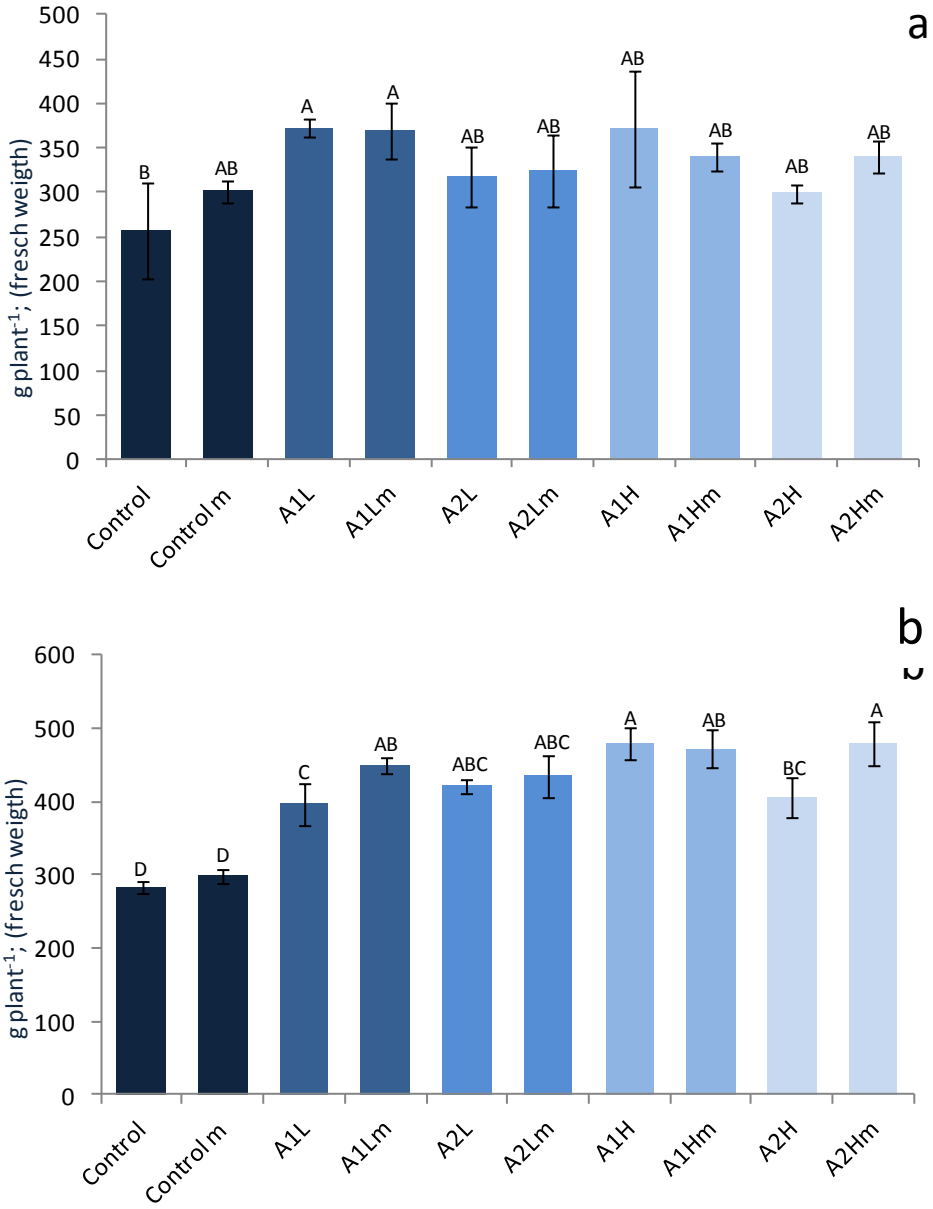


Figure 4.24 Average fresh weight of kohlrabi product in the plots under different organic amendments, in F2 during (a) the second and (b) the third crop cycle. Samples not connected by same letter are significantly different (P<0.05).

4.3.5 Principal component analysis of soil properties affected by organic amendments

Factorial map of the principal component analysis of F1 soil properties, accounting for 59.77% of the variation in the data, showed two distinct clusters of variables (Fig. 4.25). The first (PC 1, 42.73%) was positively correlated with PHO, GLU and DHY activities, EC, exchangeable Na^+ and K^+, and was opposed to pH and available phosphorus. The second (PC 2, 17.03%) was correlated with ARYL and INV activities and total nitrogen. The score plot indicated that samples could be divided in three clusters. In the first, all samples of the first two samplings were clustered, whereas, the other two clusters represented the third and the fourth sampling, respectively. Within each cluster, Control samples were always distinct from treated samples, indicating that important changes in the analyzed soil properties upon amendments occurred.

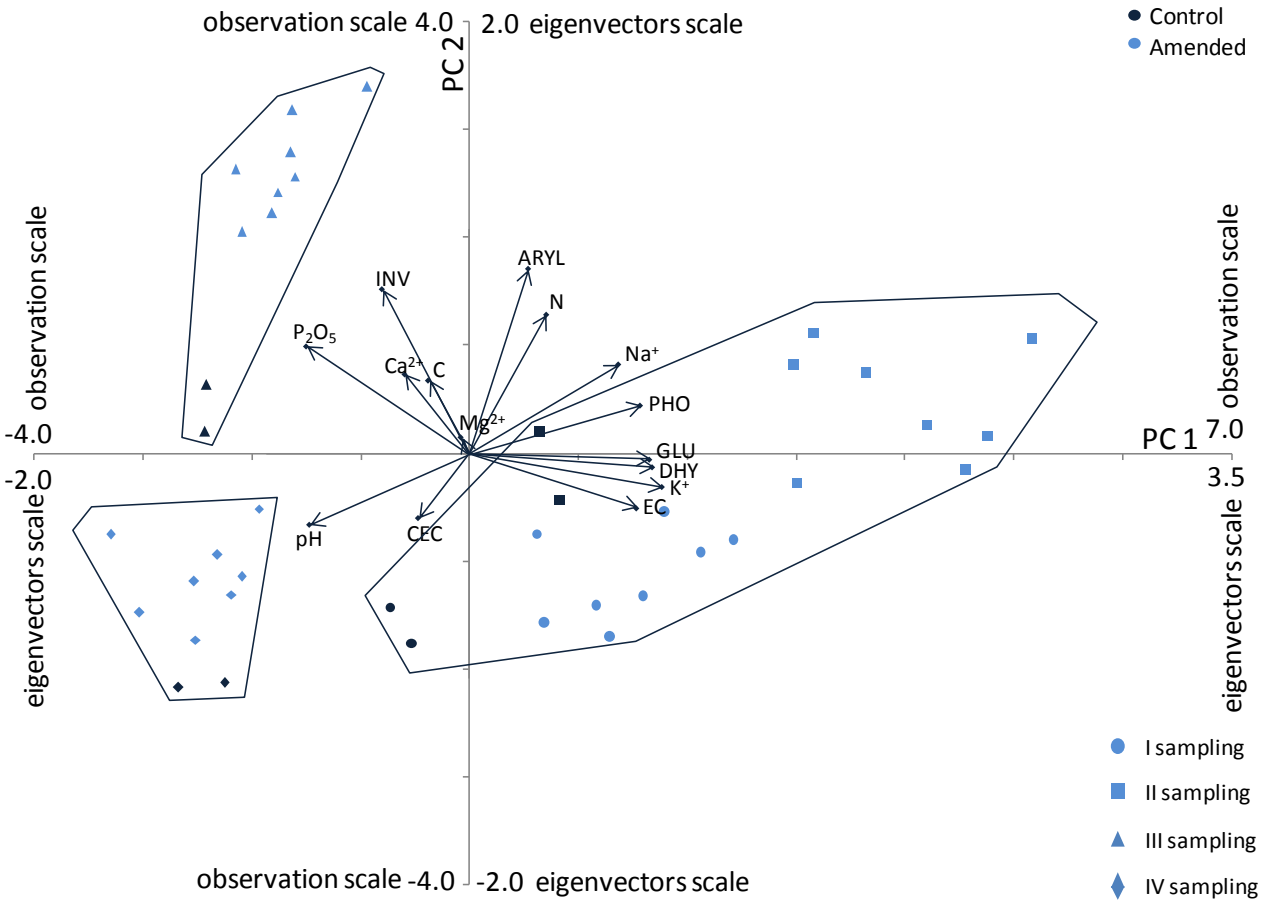


Figure 4.25 Biplot of analyzed soil properties of F1 soils under organic amendment.

The first cluster had positive values of PC 1, in particular in treated samples. This result suggested and confirmed that the addition of organic amendments have determined an increase of microbial activity and exchangeable Na^+ and K^+, but a decrease of phosphorus content, probably because microorganisms, stimulated by added OM, used available phosphorus for their metabolic needs.

The second cluster, formed by all samples of the third sampling, had negative values of PC 1 and PC 2, in particular untreated samples. The seasonal effect on this sampling and the reduction of labile OM could be responsible for the microbial activity reduction. Negative values of PC 1 indicate an increase of available phosphorus likely caused by the OM degradation, and the consequently increase of available phosphate. Reduction of total nitrogen observed from negative values of PC 2, instead, could be due to intensive microbial activity that metabolized available nitrogen for its metabolic needs in the first phase of study.

The last cluster represented in the score plot, collected soils samples of the last sampling. These samples were characterized by negative values of PC 1 and by positive values of PC 2.Treated samples had higher values of PC 2 than Control samples belonging to the same cluster, highlighting higher values of total nitrogen, ARYL and INV activities in the treated samples, after one year from the OM addition, compared to Control plot.

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

The analysis of principal components of F2 soils showed that the first and second clusters of variables explained 66.75% of the total variance (Fig. 4.26). The first one was correlated with PHO, GLU, ARYL and DHY activities, EC, exchangeable Na^+ and K^+, and negatively correlated with available phosphorus, whereas the second with INV activity, CEC and exchangeable Mg^{2+}.

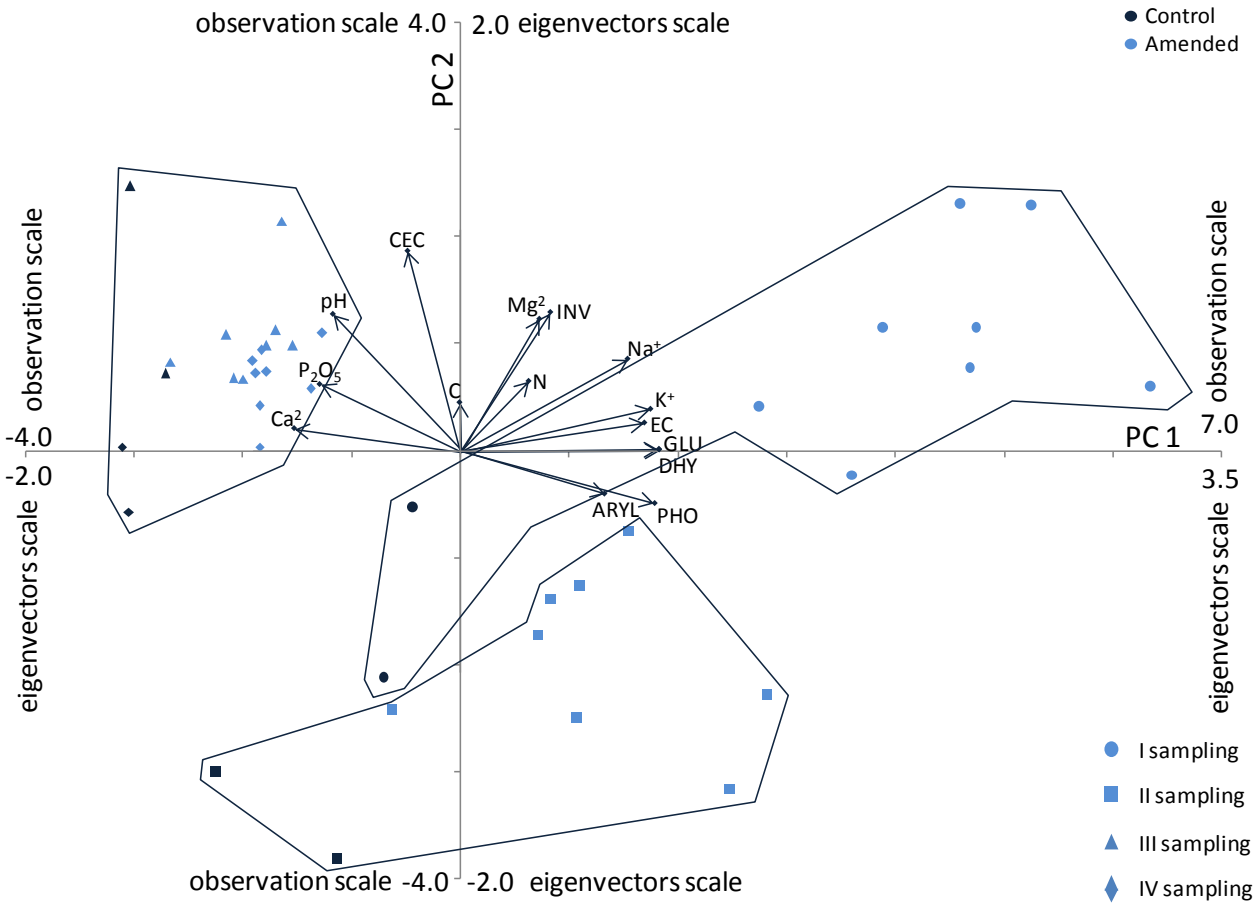


Figure 4.26 Biplot of analyzed soil properties of F2 soils under organic amendment.

As occured in F1 soil samples, also F2 score plot showed three clusters, representing samples of the first, second, and the third and fourth samplings, respectively. It is clear that the studied parameters were more variable (*i.e.* sparse in the score plot) within the first two clusters than within the third. Also in these soils, an increase of values correlated to PC 1, such as GLU, DHY and PHO activities, EC, exchangeable Na^+ and K^+, occurred in the treated samples compared to Control in the first two cluster of the score plot. The third cluster showed Control samples separated from the treated samples. This cluster had negative values of PC 1 and positive values of PC 2, and showed a decrease of enzymatic activities that were correlated to PC 1, and an increase of soil properties correlated to PC 2, such as available phosphorus and INV activity, as occurred in the F1 soils.

Differences between F1 and F2 soils observed in the principal component analysiswere 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.

4.4 Conclusions

This study confirmed the positive effects of organic amendments on soil fertility. The amended soils of both farms, after one year, showed an improvement of soil chemical properties, in particular in terms of organic carbon content. In disaccording to previous studies that showed no positive change in the C balance after three years of 30^{-1} t ha compost yearly supply (Morra et al., 2006), in the present study the use of compost mixed with wood scraps, as low mineralization rate material, determined a stable increase of organic C content 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, so demonstrating to be a good alternative to conventional fertilizers.

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

At the end of the study, no 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 of A1 mixture led to significant advantages, in particular in terms of OM recovery.

In this study, crop yields were higher in fields under organic amendments than in the Control plots under conventional farming, after the second crop cycle. The effects of amendments were correlated to the time analysis: negative correlation in the short time (first crop cycle) due to an initial phytotoxicity of supplied compost, but strongly positive correlation over time for the beneficial effects on soil nutrients and OM.

Our results demonstrated that the use of compost enriched with low mineralization materials, such as wood scraps, can enhance soil biochemical and chemical properties

compared with synthetic fertilizers, and consequently improve crop yield, but above all to guarantee an OM recovery that kept stable over time.

4.5 References

Al-Khafaji A.A. and Tabatabai M.A., 1979 Effects of trace elements on arylsulfatase activity in soils. *Soil Science* 127:129-133.

Amato M. and Ladd, J.N., 1980. Studies of nitrogen immobilization and mineralization in calcareous soils. V. Formation and distribution of isotope-labelled biomass during decomposition of ^{14}C- and ^{15}N carbon and nitrogen isotope-labelled plant material. *Soil Biology and Biochemistry* 12:405-411.

Anderson D.W. and Paul E.A., 1984. Organo-mineral complexes and their study by radiocarbon dating. *Soil Science Society of America Journal* 48:298-301.

Aon M.A., Colanieri A.C., 2001. Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. *Applied Soil Ecology* 18:255-270.

Bardgett R.D., Speir T.W., Ross D.J., Yeates G.W. and Kettles H.A., Impact of pasture contamination by copper, chromium and arsenic timber preservative on soil microbial properties and nematodes. *Biology and Fertility of Soils* 18:71-79.

Bonanomi G., Antignani V., Capodilupo M., Cozzolino L., D'Ascoli R., Marzioli R., Puopolo G., Rao M.A., Rutigliano F.A., Scotti R., Zoina A., 2010. Assessing soil quality under intensive cultivation and tree orchards in Southern Italy. *Applied Soil Ecology Accepted*.

Bonanomi G., Antignani V., Pane C., Scala F. 2007. Suppression of soilborne fungal diseases with organic amendments. *Journal of Plant Pathology* 89:311-340.

Borken W., Muhs A., Beese F. 2002. Changes in microbial and soil properties following compost treatment of degraded temperate forest soils. *Soil Biology and Biochemistry* 34: 403-412.

Bougnom B.P., Mair J., Etoa F.X., Insam H. 2009 Composts withwood ash addition: A risk or a chance for ameliorating acid tropical soils? *Geoderma* 153:402-407.

Bronson K.F., Cassman K.G., Wassman R., Olk D.C., van Noordwijk M., Garrity D.P., 1997. Soil carbon dynamics in different cropping systems in principal ecoregions of Asia. In: Lal, R., et al. (Eds.), Management of Carbon Sequestration in Soil. CRC Press, Boca Raton, FL, pp. 35-57.

Bulluck III L.R., Brosius M., Evanylo G.K., Ristaino J.B. 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. *Applied Soil Ecology* 19:147-160.

Burns R.G. 1978. Soil Enzymes. Academic Press, New York.

Cavigelli M.A., Thien S.J., 2003. Phosphorus bioavailability following incorporation of green manure crops. *Soil Science Society American Journal* 67:1186-1194.

Christensen B.T., 1996. Carbon in primary and secondary organomineral complexes. In: Carter, M.R., Stewart, B.A. (Eds.), Structure and Organic Matter Storage in Agricultural Soils. Lewis Publ., Boca Raton, FL, pp. 97-165.

Claassen V.P. and Carey J. L. 2006 Comparison of slow-release nitrogen yield from organic soil amendments and chemical fertilizers and implications for regeneration of disturbed sites. *Land Degradation and Development* 18:119-132.

Conklin A.E., Erich M.S., Liebman M., Lambert D., Gallandt E.R., Halteman W.A. 2002. Effects of red clover (*Trifolium pratense*) green manure and compost soil amendments on wild mustard (*Brassica kaber*) growth and incidence of disease. *Plant and Soil* 238: 245-256.

Delgado A. and Torrent J., 2000. Phosphorus forms and desorption patterns in heavily fertilized calcareous and limed soils. *Soil Science Society of America Journal* 64:2031-2037.

Dick R.P., 1994. Soil enzyme activities as indicators of soil quality. In: Doran J.W., Coleman D.C., Bezdicek D.F., Stewart B.A. (Eds.) Defining Soil Quality for a Sustainable Environment. Soil Science Society of America Special Publication No. 35, Madison, Winsconsin, pp. 107-124.

Doran, J., 1995. Building soil quality. In: Proceedings of the 1995 Conservation Workshop on Opportunities and Challenges in Sustainable Agriculture. Red Deer, Alta., Canada, Alberta Conservation Tillage Society and Alberta Agriculture Conservation, Development Branch, pp. 151-158.

Drinkwater L.E., Letourneau D.K., Workneh F., van Bruggen A.H.C., Shennan C., 1995. Fundamental differences between conventional and organic tomato agroecosystems in California. *Ecological Applications* 5:1098-1112.

Gill H.S. and Meelu O.P., 1982. Studies on the substitution of inorganic fertilizers with organic manure and their effect on soil fertility in rice-wheat rotation. *Fertilezer Research* 3:303-314.

Govindasamy R. and Italia J., 1998. A willingness-to-purchase comparison of integrated pest management and conventional produce. *Agribusiness* 14:403-414.

Hassink J., Whitmore A.P., Kubat J., 1997. Size and density fractionation of soil organic matter and the physical capacity of soil to protect organic matter. *European Journal of Agronomy* 7:189-199.

Hoitink H.A.J., Boehm M.J., 1999. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. *Annual Review of Phytopathology* 37:427-446.

Kandeler E., Stemmer M., Klimanek E.M., 1999. Response of soil microbial biomass, urease and xylanase within particle size fraction to long-term soil management. *Soil Biology and Biochemistry* 31:261-273.

Kiem R. and Kandeler E., 1997. Stabilization of aggregates by the microbial biomass as affected by soil texture and type. *Applied Soil Ecology* 5:221-230.

Klonsky K. and Tourte L., 1998. Organic agricultural production in the United States: debates and directions. *American Journal of Agriculture Economy* 80:1119-1124.

Magid J., Henriksen O., Thorup-Kristensen K., Mueller T. 2001. Disproportionately high N-mineralisation rates from green manures at low temperatures - implications for modelling and management in cool temperate agro-ecosystems. *Plant and Soil* 228:73-82.

Mahapatra B.S., Sharma G.L., Singh N., 1991. Integrated management of straw and urea nitrogen in lowland rice under a rice-wheat rotation. *Journal of Agriculture Science Cambridge* 116:217-222.

Marcote I., Hernandez T., Garcia C., Polo A., 2001. Influence of one or two successive annual application of organic fertilisers on the enzyme activity of a soil under barley cultivation. *Bioresource technology* 79:147-154.

Morra L., Bilotto M., Pagano L., Calì., Iovieno P., Tonini A., 2006. Studio degli effetti dell'ammendamento con compost in un agrosistema orticolo sotto serra attraverso l'analisi della produzione colturale, della fertilità chimica del terreno e della micorrizzazione radical. Atti del convegno: *Utilizzo del compost da frazione organica dei rifiuti solidi urbani : attualità e prospettive*. Napoli, Citta della Scienza.

Nambiar K.K.M., 1997. Soil health and organic matter—changing scenario. *Proceedings of the Indian National Science Academy* 67B:141-160.

Nelson N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry* 153:375-380.

Ponnamperuma F.N., 1984. Straw as a source of nutrients for wetland rice. In: Organic Matter and Rice. International Rice Research Institute, Manila, Philippines, pp. 117-136.

Regione Campania - Assessorato Agricoltura - Settore SIRCA, 2004. *Carta dei Suoli della Piana in sinistra Sele.*

Reichardt W., Inubushi K., Tiedje J., 2000. Microbial processes in C and N dynamics. In: Kirk, G.J.D., Olk, D.C. (Eds.), Carbon and Nitrogen Dynamics in Flooded Soils. International Rice Research Institute, Makati City, Philippines, pp. 101-146.

Ros M., Hernandez M.T., Garcìa C., 2003. Soil microbial activity after restoration of a semiarid soil by organic amendments. *Soil Biology and Biochemistry* 35:463-469.

Ruiz J. M., Delgado A., Torrent J., 1997. Iron-related phosphorus in over fertilized European soils. *Journal of Environmental Quality* 26:1548-1554.

Sample E. C., Soper R. J., Racz G. J., 1980. Reactions of phosphate fertilizers in soils. In: The role of phosphorus in agriculture. Eds. Khassawneh F. E., Sample E. C., Kamprath. pp. 263-310. ASA, CSSA, SSSA, Madison, WI.

SAS Institute, 2008.SAS/STAT Users' Guide. Release 6.08. Ed. SAS, Cary, NC.

Schulten H.-R., Leinweber P., 2000. New insights into organic-mineral particles: composition, properties and models of molecular structure. *Biology and Fertility of Soils* 30:1-34.

Sequi P., 2005. Fondamenti di Chimica del Suolo. Patron Editore.

Singh R.P. and Agrawal M., 2007. Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of Beta vulgaris plants. *Chemosphere* 67:2229-2240.

Singh V., Singh Y., Maskina M.S., Meelu O.P., 1997. The value of poultry manure for wetland rice grown in rotation with wheat. *Nutrient Cycling in Agroecosystems* 47:243-250.

Smith S.R., 2009. A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. *Environmental International* 35:142-156.

Stamatiadis S., Werner M., Buchanan M., 1999. Field assessment of soil quality as affected by compost and fertilizer application in a broccoli field (San Benito County, California). *Applied Soil Ecology* 12:217-225.

Thompson G.T., 1998. Consumer demand for organic foods: what we know and what we need to know. *American Journal of Agriculture Economy* 80:1113-1118.

Timsina J. and Connor D.J., 2001. Productivity and management of rice-wheat cropping systems: issues and challenges. *Field Crops Research* 69:93-132.

Toor G. S. and Bahl G. S., 1997. Effect of solitary and integrated use mineral of poultry manure and fertilizer phosphorus on the dynamics of P availability in different soils. *Bioresource Technology* 62:25-28.

Trasar-Cepeda C., Leiròs M.C, Gil-Sotres F. 2008. Hydrolytic enzyme activities in agricultural and forest soils. Some implications for their use as indicators of soil quality. *Soil Biology and Biochemistry* 40:2146-2155.

United States Department of Agriculture Natural Resources Conservation Service (USDA) Soil Survey Staff, 1998. Keys to Soil Taxonomy. Washington.

Yadav R.L., Yadav D.S., Singh R.M., Kumar A., 1998. Long-term effects of inorganic fertilizer inputs on crop productivity in a rice-wheat cropping system. *Nutrient Cycling in Agroecosystems* 51:193-200.

Zaccardelli M., Mori M., Teshale A.B., Di Mola I., Perrone D., Giordano I. 2006. Dinamica dei nitrati e bilancio della fertilità chimica in un terreno agrario della Piana del Sele ammendato con compost da F.O.R.S.U. Atti del convegno: *Utilizzo del compost da frazione organica dei rifiuti solidi urbani : attualità e prospettive*. Napoli, Citta della Scienza.

Zucconi F. 1996. Declino del suolo e stanchezza del terreno. Edizioni Spazio Verde Padova.

Chapter 5
Long-term effects
of organic amendments on soil organic matter

5.1 Introduction

One of the most worrying aspects of intensive agriculture is the gradual loss of soil fertility due to organic matter (OM) decrease. The delicate balance between OM accumulation and consumption must be retained in agricultural systems in order to prevent soil fertility reduction.

The maintenance or even an improvement of soil fertility as well as of OM content, can be achieved through the use of different types of composts such as those produced by municipal organic wastes, sewage sludges, agricultural wastes, animal manures and some industrial wastes (e.g. char and bio-char by-products produced by energy-developing companies) (Masciandaro et al., 2000; Soumaré et al., 2003).

Compost represents an available and low cost organic amendment. It is obtained by the degradation, at different extents, of plant biomasses deriving either from mowing and yard trimmings or from lignocellulose-containing plant residues. These latter can also be used as bulking and carbon sources for the stabilization of selected municipal solid wastes and sewage sludges.

Soil amendments with composts positively affect physical, chemical and biochemical soil quality, thereby imporving soil capacity for plant nutrition (Kowaljow and Mazzarino, 2007;Weber et al., 2007; Hargreaves et al., 2008). In fact, as an example, compost-amended soils reveal larger cation exchange capacity (CEC), as well as larger nitrogen and organic carbon contents as compared to Control soils which did not receive any treatment (Adani et al., 2006). In addition, compost amendment increase the content of the colloidal humified OM which, in turn, affects the quantitative and qualitative long-term status of soil OM (Adani et al., 2006, 2007; Gonzales-Vila et al., 1999; Quedraogo et al., 2001; Leifeld et al., 2002). In fact,

incorporation of compost in soils strongly modifies quality of endogenous humic substances (Rivero et al., 2004; Adani et al., 2007) and reduces mineralization of biolabile compounds, thereby enhancing the role of soil OM as a sink of organic carbon (Spaccini et al., 2002; Fortuna et al., 2003; Piccolo et al., 2004).

As carbon is the most important element for living organisms, soil OM represents the main source for CO_2 in nature due to soil respiration. However, the drastic increase in atmospheric CO_2 concentration, mainly due to land use changes since industrial revolution, has leaded to the identification of sustainable strategies for threat offsetting of the global climate change (Lal, 2004). By considering that the carbon accumulated in soils accounts for over two-thirds of the total carbon in forestal ecosystems (Dixon et al., 1994) and that it is more stable than the one stored in living plant biomasses (Vesterdal et al., 2002; Chen et al., 2005; Laganière et al., 2010), a deeper understanding of the dynamics of soil organic carbon stocks in agricultural fields is of paramount importance to address the modern agriculture towards sustainable practices.

Study of dynamics and fate of soil OM necessarily passes through the comprehension of its structure and conformation. In fact, it has been already clarified that nature of soil OM strongly affects, for example, soil aggregate stability, fate of organic and inorganic contaminants, and nutrient availability for plant nutrition (Stevenson, 1994). In general, the importance of examining threshold values at which organic carbon becomes effective and asserts a positive influence on soil properties should not be underestimated, as detrimental effects can occur if too much carbon is added to the soil. Therefore, although carbon increase is usually helpful to improve soil functions, more is not always better. For example, too much carbon can result in surface crusting, increased detachment by raindrops and decreased hydraulic conductivity (Haynes and Naidu, 1998). One reason for structural breakdown is a high content of monovalent cations, which can occur if too much animal waste is added.

Among the different techniques that can be used for structural and conformational analyses of soil OM, the main ones are spectroscopic techniques such as UV, fluorescence, FT-IR, and NMR spectroscopy.

FT-IR stands for Fourier Transform InfraRed, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiations are absorbed by the sample and others pass through (i.e. they are transmitted). The resulting spectrum represents the molecular absorption and transmission which creates a molecular fingerprint of the sample. Like a fingerprint, two unique molecular structures cannot produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis. In fact, FT-IR spectroscopy can be used to identify unknown materials, to determine quality or consistency of a sample and quantify the amount of components in a complex mixture (Grube et al., 1999). In the case of environmental analysis, FT-IR spectroscopy has been applied to recognize quality of soil OM (Olk et al., 1999; Mermut and Eswaran, 2001), nature of humic and fulvic acids from different sources (Naidja et al., 2002; Filip and Bielek, 2002; Chien et al., 2003; Senesi et al., 2003), extent of degradation of organic wastes (Reeves and Van Kessel, 2002; Reveille et al., 2003), compost quality and compost maturity degree (Tseng et al., 1996; Provenzano et al., 2001; Smidt et al., 2002; Grube et al., 2006).

Deeper structural and conformational information on soil OM characteristics can be obtained by high field (HF) nuclear magnetic resonance (NMR) spectroscopy either in the liquid or in the solid state (Martin et al., 2001; Lguirati et al., 2005; Conte, et al., 2004). The technique is based on the interaction between a radio-frequency and the magnetic moments of selected nuclei as far as they are subjected to a Zeeman magnetic field. In general, liquid state NMR spectroscopy is applied on soluble OM (dissolved OM also referred to as DOM), whereas solid state NMR spectroscopy is mainly used when water solubilisation is impossible as in the case of highly hydrophobic natural OM (Conte et al., 1997). The results obtained from NMR spectrocopy either in the liquid or in the solid state are spectra from which structure

and amount of soil OM constituents can be retrieved (Conte et al., 2004; Albers and Hansen, 2010). In addition, NMR spectroscopy can be applied to recognize interactions among pollutants and soil components (Russo et al., 2010).

Very recently, development of a new low field NMR technique has been achieved. Namely, this technique investigates the effect of variable Zeeman magnetic fields over the spectral densities (J(ω)) and the strength (C) of the dipolar interactions being modulated. The technique known as Fast Field Cycling (FFC) NMR relaxometry allows a fast investigation of the conformational and dynamic properties of whole complex molecular systems through measurement of longitudinal or spin-lattice relaxation rates ($R_1 = 1/T_1$) (Kimmich and Anoardo, 2004). The latter is the lifetime of the first order process that returns the magnetization to the Boltzman equilibrium (Bakhtumov, 2004). The R_1 magnitude depends on the nature of the nuclei, the physical state of the system, its viscosity and temperature (Bakhtumov, 2004). Spin-lattice relaxation occurs when the lattice creates magnetic fields fluctuating at frequencies resembling those of the observed nuclei (e.g. protons). Fluctuating fields are created by molecular motions which strongly affect dipolar interactions (Bakhtumov, 2004). In particular, the faster the motions are, the less efficient is the dipolar interaction, thereby favouring lower R_1 values (Bakhtumov, 2004). Conversely, slower molecular dynamics can be associated with faster spin-lattice relaxation rates due to a higher intra and inter nuclear dipolar interaction efficiency (Bakhtumov, 2004).

FFC relaxometry is based on the cycling of the Zeeman magnetic field (B_0) through three different values traditionally indicated as polarization (B_{pol}), relaxation (B_{relax}), and acquisition (B_{acq}) fields (Kimmich and Anoardo, 2004; Ferrante and Sykora, 2005). B_{pol} is applied for a limited and fixed period of time in order to obtain magnetization saturation and sensitivity enhancement (Kimmich and Anoardo, 2004). Then, the magnetic field is switched to a new one, B_{relax}, applied for a period (τ) during which the intensity of the magnetization changes (or relaxes) to reach a new equilibrium condition. Finally, the application of the acquisition magnetic field

together with a ^{1}H 90° pulse makes the magnetization observable and the free induction decay (FID) acquirable. The proton longitudinal relaxation times are obtained at the fixed B_{relax} intensity through a progressive variation of the τ values.

The objective of this work was to study the effect of different organic amendments on an agricultural soil and, in particular, on its OM over time. Innovative and conventional spectroscopic techniques were used such as low field NMR relaxometry for the direct analysis of soil, and FT-IR and solid phase NMR for humic fraction extracted from the same soil samples.

The present work was carried out in collaboration with Prof. Pellegrino Conte, Dipartimento di Ingegneria e Tecnologie Agro-Forestali, Università degli Studi di Palermo, and with Dr. Anne E. Berns, Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences, IBG-3: Agrosphere, 52425 Jülich, Germany.

5.2 Materials and Methods

5.2.1 Description of the study site and sampling

Two commercial farms (F1 and F2) were selected in the Plane of Sele river (Salerno, Southern Italy), one of the most productive farming area (See Chapter 4, Par. 4.2.1). The two selected farms, characterized by different geopedologic characteristics (Table 4.2, Par. 4.3.1), provided a field under greenhouse of 160 m^2 that was divided in thirty plots, as described in Par. 4.2.2, Figure 4.1.

On February 2009, the soils of two farms were treated with organic amendments. Soil samples were collected after one (March 2009) and twelve months (February 2010) from treatment. In each plot, five sub-samples, were taken, following a W scheme from the topsoil (0-20 cm), to form a sample. Samples were packed in polyethylene bags, dried at room temperature and sieved (mesh size 2 mm).

5.2.2 Organic amendments

In this study, two different organic amendments were used. They were a compost from municipal solid waste (GeSeNu Srl, Perugia, Italy) (Chapter 4, Par. 4.2.2., Table 4.1) and wood from scraps of poplars pruning (Experimental Regional Farm Improsta), as descibed in Chapter 4, Paragraph 4.2.2, Figure 4.1.

Compost and wood were mixed in different two ratios: A1 amendment with compost:wood 10:1 and a C/N ratio of 15, and A2 amendment with compost:wood 2:1 and a C/N ratio of 25. The two amendments (A1 and A2) were supplied in two doses: 30 and 60 tons ha^{-1}, named L and H, respectively. Also 200 kg ha^{-1} of mineral fertilizer with N-P-K (14-7-17) and microelements (m), were added. Minelar fertilizers were applied at the start of every crop cycles. Each treatment was studied in triplicate (three plots), as shown in Figure 4.1, Paragraph 4.2.2.

Furthermore, in the experimental field Control plots (Control and Control m) were designed without organic amendments and mineral fertilizer to compare the effect of the treatments.

5.2.3 Extraction of organic matter

OM was extracted from all samples collected after one month and after one year from amendment (March 2009 and February 2010). Soil (150 g) was shaked for 24 h with 750 ml of 1 M 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 resuspend in deionized water, dialyzed against deionized water, frezeed and lyophilized.

The supernatans, formed by FA + NH, were 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) were discarded. The fractions retained on the column (fulvic acids, FAs) were eluted with 0.5 M NaOH and collected in a beaker, and then acidified to pH 1 with concentrated HCl. The fulvic acids were dialyzed against deionized water, frezeed and lyophilized.

5.2.4 Elemental analyses

The elemental composition (C, H, N, O) of HAs and FAs, of the samples 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, H and N were obtained directly from analysis, whereas O was

calculated by difference, i.e. O % = 100 - C % - H % - N %, and therefore it can include trace fractions of S or P.

5.2.5 FT-IR analyses

Diffuse reflectance infrared Fourier transform spectroscopy (DRIFT) spectra of HA, of the samples collected in the second sampling (February 2010), were recorded with a Perkin Elmer Spectrum-One spectrometer, equipped with a diffuse reflectance accessory, and by accumulating up to 100 scans with a resolution of 4 cm^{-1}. Before DRIFT analysis, dry samples were finely ground in an agate mortar while diluting with oven-dried KBr powder (5/100, w/w).

5.2.6 Fast Field Cycling (FFC) NMR relaxometry

A Stelar Spinmaster-FFC-2000 field-cycling relaxometer (Stelar s.r.l., Mede, PV - Italy) was used to run relaxometry experiments directly on soil samples collected in the first sampling (March 2009). The solid state HA samples were studied at the constant probe temperature of 298 K. The experimental relaxometry setting consisted of a polarization field with a flux density of 580 mT corresponding to a proton Larmor frequency (ω_L) of 25 MHz applied for a period of time (T_{pol}) of 0.1 s; a relaxation field with a flux density of 500 mT (ω_L = 20 MHz) chosen inside the highest sensitivity instrumental region and applied for a period τ arrayed with 64 values varying from 1 to 800 ms. τ array was chosen in a geometrical progression in order to cover the entire relaxation curve of interest. Finally, a 380 mT (ω_L = 16.2 MHz) acquisition field with a 1H 90° pulse of 7 μs in order to obtain observable magnetization and reveal free induction decay (FID) with a time domain of 100 μs sampled with 512 points. 64 scans were accumulated. Acquisition of relaxation curves was achieved with the AcquNMR V95® software provided by Stelar. The experimental data were processed with UPEN algorithm (Alma Mater Studorium, Università di Bologna), which gives the distributions of T_1 values (Borgia et al., 1998, 2000). The T_1 distribution curves (also indicated as relaxograms or T_1-D

curves) were exported to OriginPro 7.5 SR6 (Version 7.5885, OriginLab Corporation, Northampton, MA, USA) in order to perform deconvolution with Gaussian functions and to recover the different components giving rise to the longitudinal relaxation time distributions. The number of Gaussian functions that were used for the deconvolution without unreasonably increasing the number of parameters were determined by means of the Merit function analysis (Halle et al., 1998).

5.2.7 CPMAS ^{13}C NMR spectroscopy

Cross polarization magic angle spinning (CPMAS) ^{13}C NMR experiments were done on HA of two sampling by a 14.1 T Varian NMR System (Varian Inc., Palo Alto CA, USA) with a 6 mm T3 NB HX probe head. The samples were spun at 7500 ± 1 Hz. All free induction decays (FID) were recorded with a recycle delay of 6 s, an acquisition time of 20 ms and a sweep width of 50 kHz. Cross-polarization was performed with a contact time of 1 ms and a ^{13}C r.f. field set at 39 kHz, while the ^{1}H r.f. field was ramped from 40 to 45.5 kHz. Decoupling was done using a SPINAL sequence with a ^{1}H r.f. field of 35.5 kHz, a pulse width of 9 µs and a phase of 9.98. All the experiments were done with the classical TOSS (Total Suppression of Spinning Side Bands) sequence. The FIDs were acquired with VNMRJ version 2.2C software (Varian Inc., Palo Alto CA, USA) and elaborated with MestReNova 6.1.1 software (Mestrelab Research, Santiago de Compostela, Spain).

5.3 Results and Discussion

5.3.1 Yields and elemental content of organic fractions in amended soils

Organic fractions were extracted from the soils sampled at the end of the experimental session, one year after the organic amendment. HA and FA yields of extraction from soils F1 and F2 soils are reported in Table 5.1.

An increase of both organic fractions was observed as a consequence of the amendment. In particular, the increase extent after one year from the starting of the experiment appeared to be farm-specific, very likely because of the geopedologic differences between the two soils.

The amount of HAs extracted from F1 soils, under organic amendments, increased also until two fold, compared to Control soil (Table 5.1), suggesting a progressive incorporation of organic compounds in HA with increasing stabilization of compost over time. The elemental analysis of HAs extracted by differently treated soils showed higher C contents than those from Control soils (Table 5.2).

The amount of extracted FAs was not affected by organic amendments, as the values remained unchanged or only slightly reduced (Table 5.1) similarly to the trend of the C content determined by elemental analysis (Table 5.3). 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).

In the F2 soils, the amount of extracted HAs was larger in the amended soils than in the F1 ones (Table 5.1). Moreover, C content in F2 soils also resulted larger than in F1 soils (Table 5.2). Also FAs content increased upon organic amendments, although their C content showed an opposite trend (Table 5.3) . 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 Chapter 4, Figure 4.7). In these environmental conditions the higher FAs content

indicated that more intensive degradation processes occurred. In fact, FAs can be considered intermediate products during OM oxidation towards the complete mineralization in CO_2. Conversely, the calcaric nature of F2 soils due to the high amount of lime (~600 g kg^{-1}) seemed not to affect OM behaviour. As matter of fact, it is well known that $CaCO_3$ stabilizes HAs and FAs, thereby preserving them from degradation. However, F2 soils, thought being calcaric, revealed low exchangeable Ca^{2+} concentration which did not cause this process. Conversely, in F1 soil, the clay nature favoured the formation of organo-mineral complexes in which OM is stabilized and preserved from degradation and mineralization processes. Moreover, the lower porosity and, therefore, the less aired environment could slow down soil microbial activity and, consequently, OM degradation processes (Schulten and Leinweber, 2000; Amato and Ladd, 1980; Anderson and Paul, 1984).

Table 5.1 Extraction yields (g kg^{-1} of soil), and % respect to Control, of HAs and FAs from soils of F1 and F2 farms, collected after 12 months from organic amendments.

	Soil F1				Soil F2			
Sample	**HA**	**%**	**FA**	**%**	**HA**	**%**	**FA**	**%**
Control	1.83	0	1.89	0	1.45	0	0.98	0
Control m	2.08	13	1.24	-34	1.19	-18	1.49	53
A1L	3.05	66	1.68	-11	1.83	26	1.36	39
A1Lm	4.49	145	1.80	-4	2.57	77	1.86	90
A1H	3.46	89	2.61	38	2.37	63	2.05	110
A1Hm	3.93	115	1.23	-34	2.64	82	1.69	73
A2L	2.98	63	2.15	13	2.69	85	1.97	101
A2Lm	3.80	108	2.17	15	1.67	15	0.82	-15
A2H	4.71	157	2.14	13	2.01	38	1.76	80
A2Hm	4.71	157	1.99	5	2.13	47	1.73	77

The O/C, H/C and C/N atomic ratios are often used to identify humic substances from different sources, to monitor structural changes of humic substances in different environments and to elucidate structural formulae for humic substances (Steelink, 1985). For instance, the O/C ratio is believed to be an indicator of the carbohydrate and carboxylic acid contents of humic substances and can be used to compare humic substances from different depositional environments. The value of a H/C ratio shows the degree of maturity in humic substances (Gonzalez-Vila et al., 1992) and can also

be considered as a source indicator of OM (Bourbonniere and Meyers, 1978). The C/N ratio of humic substances reflects the original proportions of algae and land-derived material and can also be used as an indicator of different sources (Ishiwatari, 1985).

According to Rice and MacCarthy (1991), the studied fulvic fractions showed H/C ratios higher than humic fractions (Tables 5.2 and 5.3), thereby indicating presence of larger content of carbohydrates. In both farms, the soil treatment with organic amendments determined an increase of H/C ratio in the FAs and a decrease in the HAs (Tables 5.2 and 5.3), due to the increase of the maturity degree of the humic substances after the use of organic amendments (Belize et al., 1997).

Table 5.2 Elemental analyses of HAs fraction of soils treated with different amendments after one year from the amendment .

	Soil F1							Soil F2						
	Mass % of humic acids				Atomic ratio			Mass % of humic acids				Atomic ratio		
Sample	N	C	H	O	C/N	H/C	O/C	N	C	H	O	C/N	H/C	O/C
Control	4.67	28.83	5.07	61.43	7.20	2.10	1.60	4.49	33.99	5.51	56.01	8.83	1.93	1.24
Control m	3.99	31.44	5.28	59.29	9.19	2.00	1.42	5.86	52.03	5.63	36.48	10.36	1.29	0.53
A1L	4.37	32.92	4.95	57.76	8.79	1.79	1.32	5.65	35.12	5.73	53.50	7.25	1.94	1.14
A1Lm	4.27	32.18	5.33	58.22	8.80	1.98	1.36	6.01	56.18	5.55	32.26	10.91	1.18	0.43
A1H	3.86	33.24	4.95	57.95	10.05	1.77	1.31	4.42	34.22	5.52	55.84	9.03	1.92	1.23
A1Hm	3.91	34.41	4.52	57.16	10.25	1.56	1.25	4.64	36.55	5.93	52.88	9.19	1.93	1.09
A2L	3.48	29.34	4.45	62.74	9.83	1.81	1.61	4.35	37.02	5.44	53.19	9.92	1.75	1.08
A2Lm	4.33	32.10	5.37	58.20	8.65	1.99	1.36	4.56	44.78	5.76	44.90	11.46	1.53	0.75
A2H	4.69	34.52	5.53	55.26	8.59	1.91	1.20	5.03	51.66	4.37	38.94	11.97	1.01	0.57
A2Hm	4.63	32.85	5.34	57.19	8.28	1.94	1.31	5.05	51.29	5.33	38.33	11.84	1.24	0.56

The O/C ratios decreased from 1.60 to 1.20 in HAs, and from 7.13 to 3.00 in the fulvic fractions of F1 soils, and from 1.24 to 0.43 and from 2.45 to, 0.94, respectively, in F2 soils (Tables 5.2 and 5.3). Belzile et al. (1997) made a comparison of elemental ratios of humic substances from different sources and revealed that organic material with a relatively high O-alkyl and carboxylic acid composition would result in a high O/C ratio. They also concluded that fulvic acids isolated from various sources appeared to contain a higher proportion of O-alkyl and carboxylic acid functional groups than the corresponding humic acids. In this work, the trend of

O/C ratios indicates a decrease in the amounts of oxygen-containing functional groups such as methoxy carbon, carboxylic acid and carbonyl carbon in the humic substances and an increase in the fulvic substances from soils under organic amendments, in both farm soils, in according to H/C ratios.

The N content in the organic fractions varied in soils at the two farms. In general, the F2 soils showed N content higher than F1 ones, and an inverse C/N ratio trend (Tables 5.2 and 5.3). In both farms C/N ratios of fulvic fraction did not change upon organic amendments, while the C/N ration of humic fraction increased (Tables 5.2 and 5.3), probably because this fraction is a better C sink than fulvic acids. The C/N ratio showed a different trend in the organic fractions of soils of two farms: F1 soils had C/N ratio higher in the fulvic than in the humic acids, while F2 soils showed an opposite behaviour. This results are due to the presence of larger amounts of protein or peptide fragments in the organic fractions of the two soils (Stevenson, 1994).

Table 5.3 Elemental analyses of FAs fractions of soils treated with different amendments after one year from the amendment.

	Soil F1							Soil F2						
	Mass % of fulvic acids				Atomic ratio			Mass % of fulvic acids				Atomic ratio		
Sample	**N**	**C**	**H**	**O**	**C/N**	**H/C**	**O/C**	**N**	**C**	**H**	**O**	**C/N**	**H/C**	**O/C**
Control	2.18	19.02	2.74	76.06	10.16	1.72	3.00	5.48	40.00	4.55	49.97	8.51	1.35	0.94
Control m	2.41	21.71	2.80	73.08	10.51	1.53	2.53	3.78	28.38	3.72	64.13	8.77	1.56	1.70
A1L	2.30	19.79	3.32	74.59	10.05	2.00	2.83	4.15	29.56	3.97	62.32	8.31	1.60	1.58
A1Lm	3.25	27.65	3.84	65.26	9.93	1.66	1.77	3.22	21.87	3.59	71.32	7.91	1.96	2.45
A1H	2.23	19.28	3.77	74.73	10.09	2.33	2.91	3.52	25.61	3.29	67.58	8.47	1.53	1.98
A1Hm	3.18	27.82	3.81	65.18	10.19	1.63	1.76	3.17	23.15	3.50	70.18	8.52	1.80	2.28
A2L	2.20	18.32	2.64	76.83	9.69	1.72	3.15	3.86	28.47	4.13	63.54	8.59	1.73	1.68
A2Lm	1.08	9.30	1.24	88.38	10.07	1.59	7.13	4.86	35.78	4.64	54.73	8.58	1.55	1.15
A2H	1.79	15.82	2.60	79.80	10.31	1.96	3.79	4.23	18.94	3.69	73.13	5.22	2.32	2.90
A2Hm	1.60	13.56	1.83	83.00	9.87	1.61	4.59	3.95	29.81	3.95	62.28	8.79	1.58	1.57

5.3.2 FT-IR Spectroscopy

The FT-IR spectra of humic acids extracted from soils sampled after one year from the amendment are shown in Figures 5.1 and 5.2.

The main absorption bands and corresponding assignments are summarized in Table 5.4.

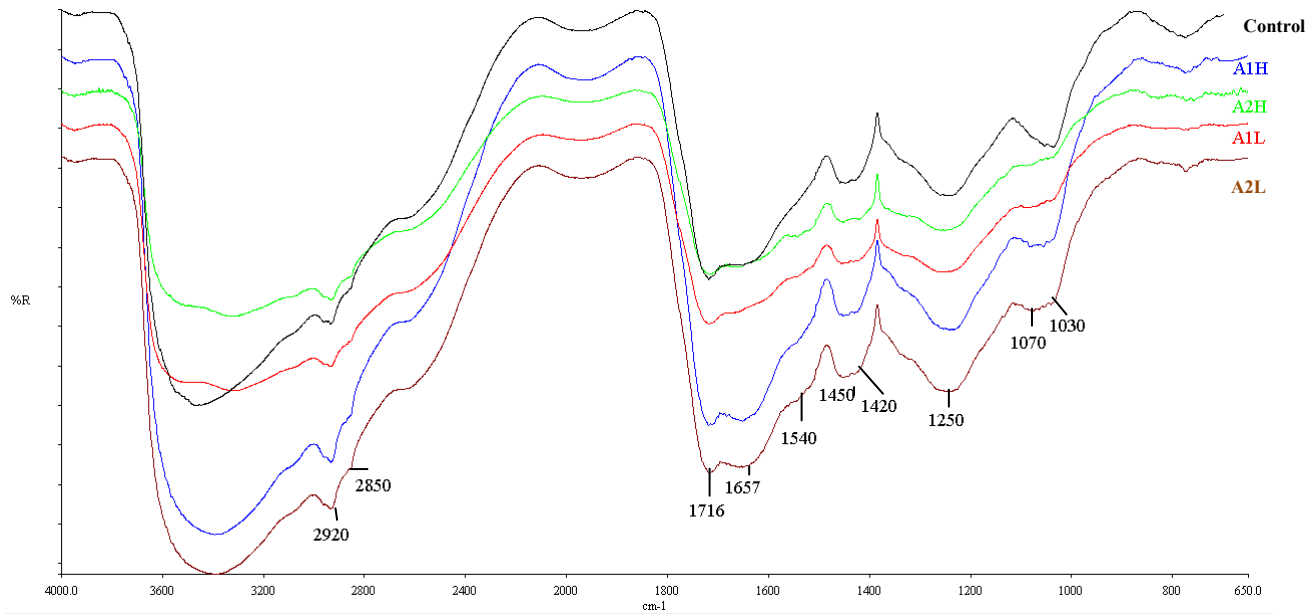


Figure 5.1 FT-IR/Drift spectra of HAs extracted from F1 soils after one year from the addition of organic amendments.

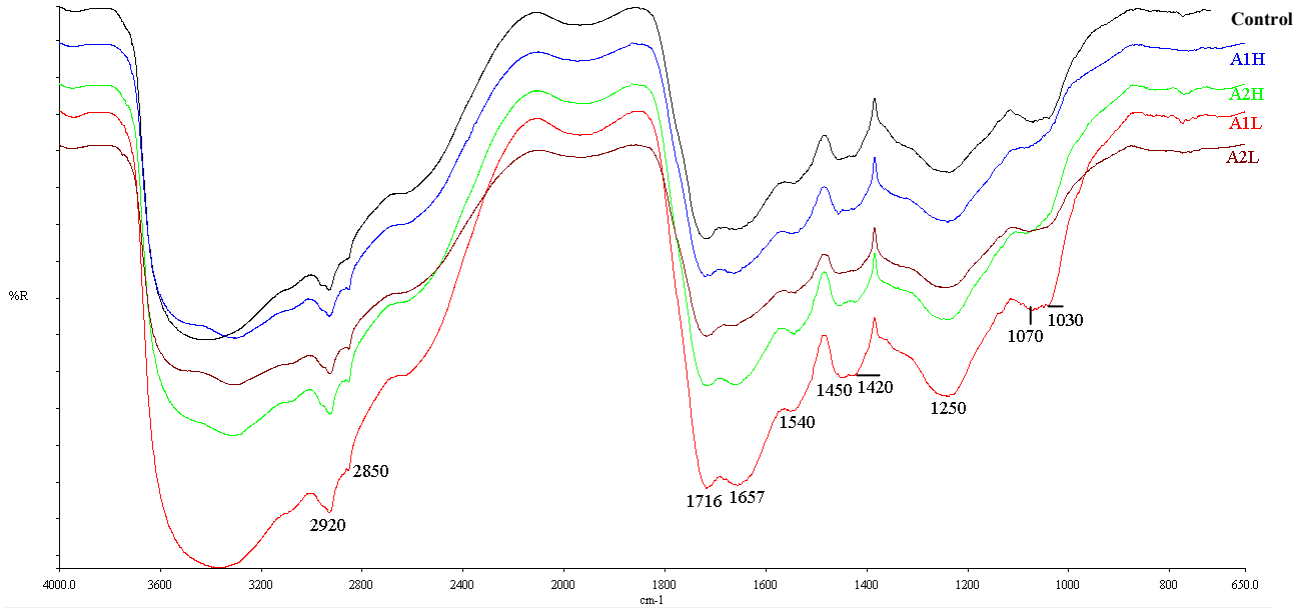


Figure 5.2 FT-IR/Drift spectra of HAs extracted from F2 soils after one year from the addition of organic amendments.

Table 5.4 Major FT-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

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). The signal at 1716 cm^{-1} and 1250 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), 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). 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).

The effects of the organic amendments on the soils F1 and F2 are clearly revealed by the FT-IR spectra. In fact, all the spectra of the humic acids extracted from the aforementioned soils, showed a decrease of the bands between 1030 and 1070 cm^{-1} as

compared to the untreated soils. These bands are typical for carbohydrates, and the intensity diminution is in line with the H/C ratio decrease (Table 5.2). Clearly, supply of organic amendment favored microbial activity, thereby leading to an increase of the consumption of the polysaccharidic OM fraction. In addition, the spectra of the humic acids extracted from the untreated F1 farm soils (Figure 5.1) revealed lower signal intensity at 1540-47 cm^{-1} and 1507 cm^{-1}, typical regions for stretching of C-N amide II groups and C=C in aromatic systems. These signals were more intense in A2Lm and A2Hm than in the other soils since the addition of wood-rich amendment mixture (A2) supplied greater amount of lignin-derived compounds rich in aromatic groups. Presence of wooden residues together with mineral fertilizer produced a stronger degradative attack by microorganism, because of the nutrient provided by the mineral fertilizers (Juan et al., 2008).

As compared to the HAs from F1 soils, the FT-IR spectra of the humic acids extracted from F2 soils (Figure 5.2) showed stronger signals around 1540-47 cm^{-1} and 1507 cm^{-1} to indicate higher amounts of nitrogen organic compounds, such as proteins and peptide fragments. These results are also confirmed by higher N content of HAs of F2 soils (Tables 5.2 and 5.3). The use of organic amendments stimulated biomass growth, but when substrates of their metabolic activity (more labile OM) decreased and microenvironment conditions are no more suitable, microorganisms came at an end of their life cycle, leaving in soil cells debris and their molecular components represented a reservoir/source of organic C as well as N (Bengtsson et al., 2003; Iyyemperumal et al., 2007).

5.3.3 ^{13}C CPMAS-NMR Spectroscopy

Figure 5.3 reports the solid state ^{13}C CPMAS-NMR spectra of some HAs extracted from F1 soils, after one month from amendment.

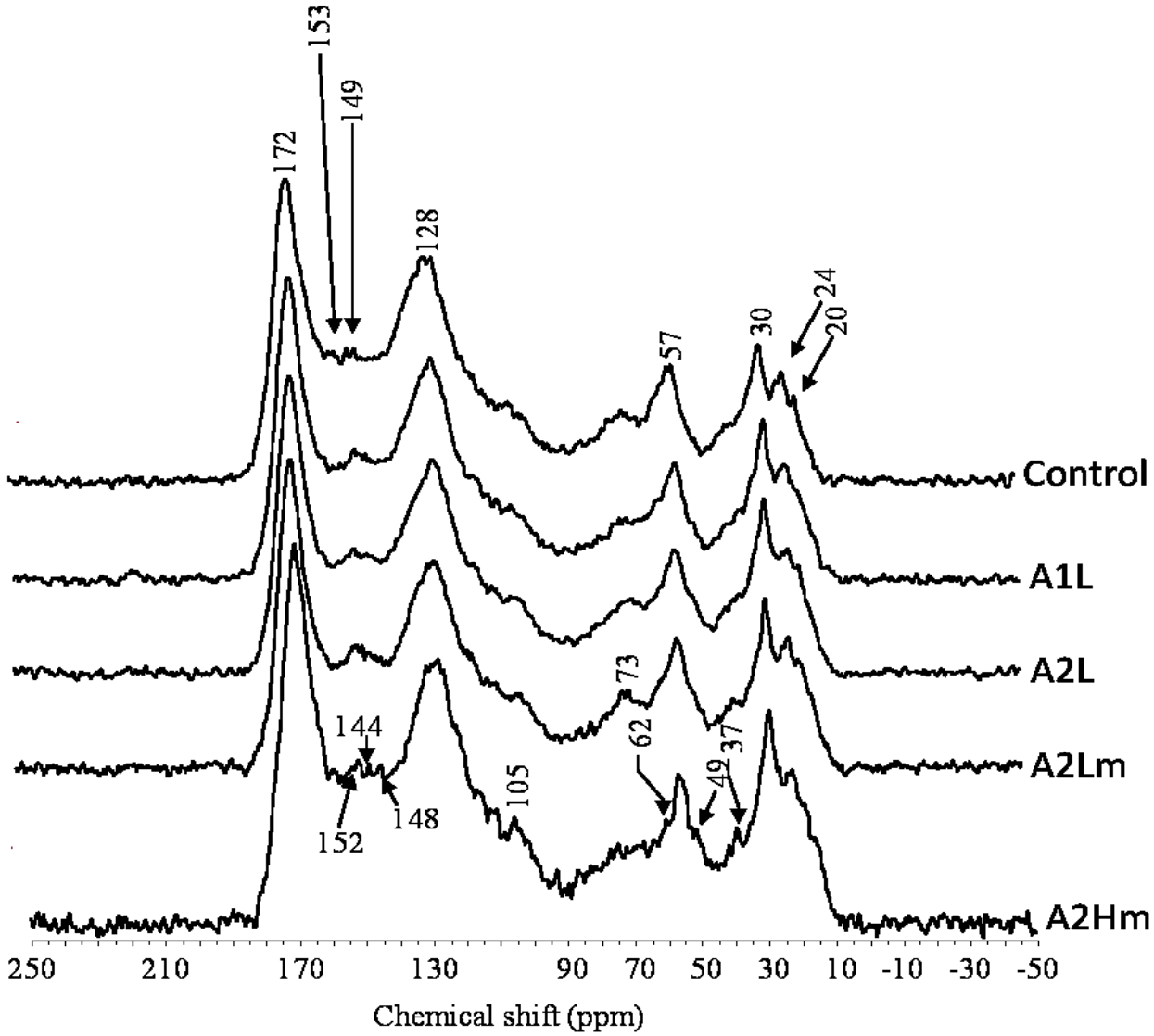


Figure 5.3 Solid state ^{13}C CPMAS-NMR spectra of some HAs extracted from F1 soils.

According to literature (Knicker and Lüdemann, 1995; Dignac et al., 2002; Lima et al., 2009; Conte et al. 2010) seven different regions were assigned to the spectra (Table 5.5). The first region, in the interval 0-45 ppm, can be assigned to aliphatic compounds. In this region, the most important signals of humic substances extracted from studied soils were at 20 ppm, assigned to methyl groups which terminate alkyl chains, 24 and 30 ppm that could be attributed to linear methylene chains belonging to lipids and cutin-like structures (Conte et al., 2010). The second spectral region,

between 45 and 60 ppm, was attributed to methoxyl groups in lignin and polysaccharides (Knicker and Lüdemann, 1995), such as hemicelluloses, and to N-alkyl carbons in amino acids (Conte et al., 2010).

In the analyzed spectra, two signals at 49, and 57 ppm were the most common. The shoulder at 49 ppm can be assigned to N-alkyl carbons in amino acids (Quideau et al., 2001; Lemma et al., 2007). The signal at 57 ppm is attributed to O-alkyl groups such as methoxyls in lignin-like structures and -CH_2O- systems into branched molecules.

Table 5.5 Tentative assignment of signals in the 13C NMR spectra (Knicker and Lüdemann, 1995; Conte et al. 2010).

Chemical shift range (ppm)	Assignment
0-45	Terminal methyl groups, methylene groups in long chains
45-60	Methoxyl groups in lignin and polysaccharides, N-alkyl C
60-90	O-alkyl C in higher alcohols, C-2 to C-5 of hexoses
90-120	di-O-Alkyl C (anomeric C-1 in polysaccharides) and C-2 and C-6 in syringyl units (lignin)
120-160	Aromatic C-C, C-O and C-N groups
160-180	Carboxyl, amide and ester-C (lipids, proteins)
180-250	Carbonyl groups belonging to aldehides and ketones

The third region comprised between 60 and 90 ppm is characteristic of O-alkyl carbon in higher alcohols and C-2 to C-5 of hexoses, indicating a presence of cellulose and hemicelluloses (Knicker and Lüdemann, 1995; Conte et al. 2010).The signal at 72 ppm, in the studied spectra, is due to carbons in positions 2, 3 and 5 in cellulose (either crystalline or not) (Taylor et al., 2008).

The spectral region included in the chemical interval 90-120 ppm contains the typical signal for the carbon C1 in carbohydrates (Conte et al., 2010).

The region between 120 and 160 ppm is traditionally assigned to aromatic systems, such as those in lignin and lignin-derived compounds. Defined peaks at about 144, 148, and 152 ppm were relieved and they were attributed to O-aryl carbons from guaiacyl- and syringyl-units that may give resonances at 144 ppm, and to high lignin-like products that give characteristic peaks at 148 and 152 ppm. In soils fertilized

with farmyard manure a higher content of lignin and carbons bound to phenolic -OH generally occurs and seems to confirm the positive effect of organic amendments already stated by FT-IR spectroscopy (Sierra et al., 2005; Ussiri and Johnson, 2003).
The spectral region between 160 and 180 ppm, showed the peak at 172 ppm that is due to -COOH and amide groups as already reported in Conte et al., (2010). Finally, the last chemical shift region 180-250 ppm is assigned to carbonyls in aldehides and ketones.
The spectra of humic acids extracted from F1 soils (Fig. 5.3) revealed that the peaks at 148 ppm and 113-101 ppm in A1L and A2L had a relative intensity larger than in the Control soil, to indicate, in this latter, a low content of O-substituted carbons in aromatic rings and a low content of oligo- and polysaccharides.
The A2Lm spectrum showed a signal at 73 ppm more intense than in the Control soil, thereby suggesting a higher content in polysaccharides due to the addition of organic amendment. In the A2Hm spectrum, peaks at 105, 112 and 115 ppm, assigned to aromatic carbons in polysaccharides derived by degradation of lignin materials, more abundant in A2H treatment, were observed (Malcom, 1989).
One year after the amendment, HAs extracted from amended soils showed spectra with relative more intense signal in the chemical shift region between 140 and 160 ppm as reported, for example, in Figure 5.4 for A1L treatment. This behavior indicates a higher content of lignin and lignin-like products in soils after one year from the amendment, due to slow degradation of wood scraps present in the used amendment. Furthermore, an intensification of the signal centered at 216 ppm, typical of carbonyl carbons, was observed, thereby indicating that the possible mechanism for OM degradation passes through oxidative processes.
When A2 amendment was added, in the HAs spectra relative intense peaks at around 76 and 100 ppm were observed, indicating that upon this treatment carbohydrates were not completely degraded after one year, because derived, in this case, from wood scraps degradation (data not shown).

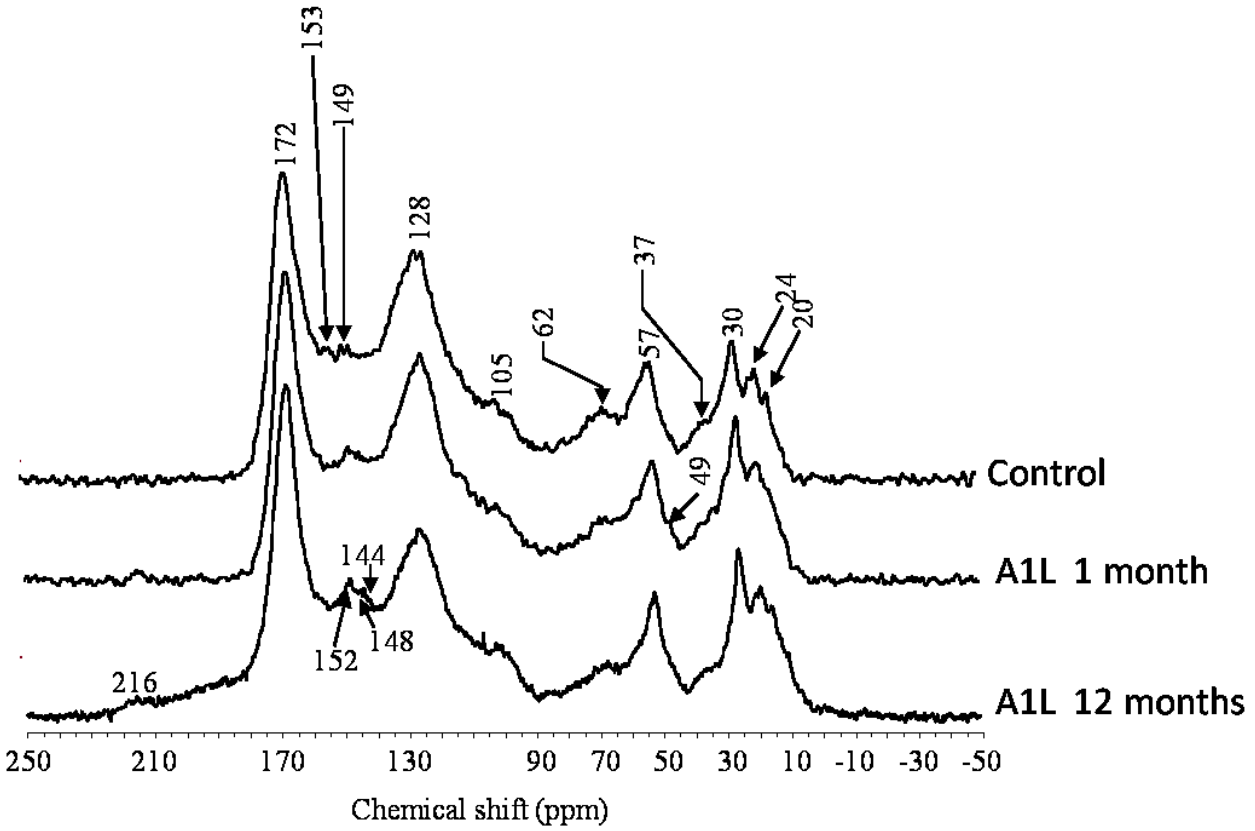


Figure 5.4 Solid state ^{13}C CPMAS-NMR spectra of HAs extracted from A1L plots of F1 soils, after one and twelve months from amendment.

By comparing results obtained in two samplings, changes in the aliphatic region, between 0-45 ppm attributed to -CH_2 of aliphatic lipid chains, cutin and cutin-like structures, were observed (Conte et al., 2010). After one month, two peaks at 24 and 30 ppm appeared, whereas after twelve months spectrum showed three peaks at 20, 24, and 30 ppm, as Control soil, indicating a initial change just after the amendment and a return to starting condition after one year, due to degradation of these compounds.

The spectra of HAs extracted from F2 soils (Fig. 5.5) showed, in general, the same trend of HAs extracted from F1 soils, except few differences in the spectral 60-90 ppm region, where a more relative intense peak at 72 ppm, and a new peak at 84 ppm, due to amorphous cellulose, hemicelluloses and cellulose oligomers (Wickholm et al., 1998), were observed. These changes could be explained by the high microbial activity occurred in this soil (as demonstrate in detail in Chapter 4) that strongly contributed to degrade the OM.

After one year, the spectra of HAs extracted from soils of the last sampling (Fig. 5.5) were similar to Control, showing that the return to initial characteristic of humic compound occurred, thought, after one month the 60-90 ppm region was modified.

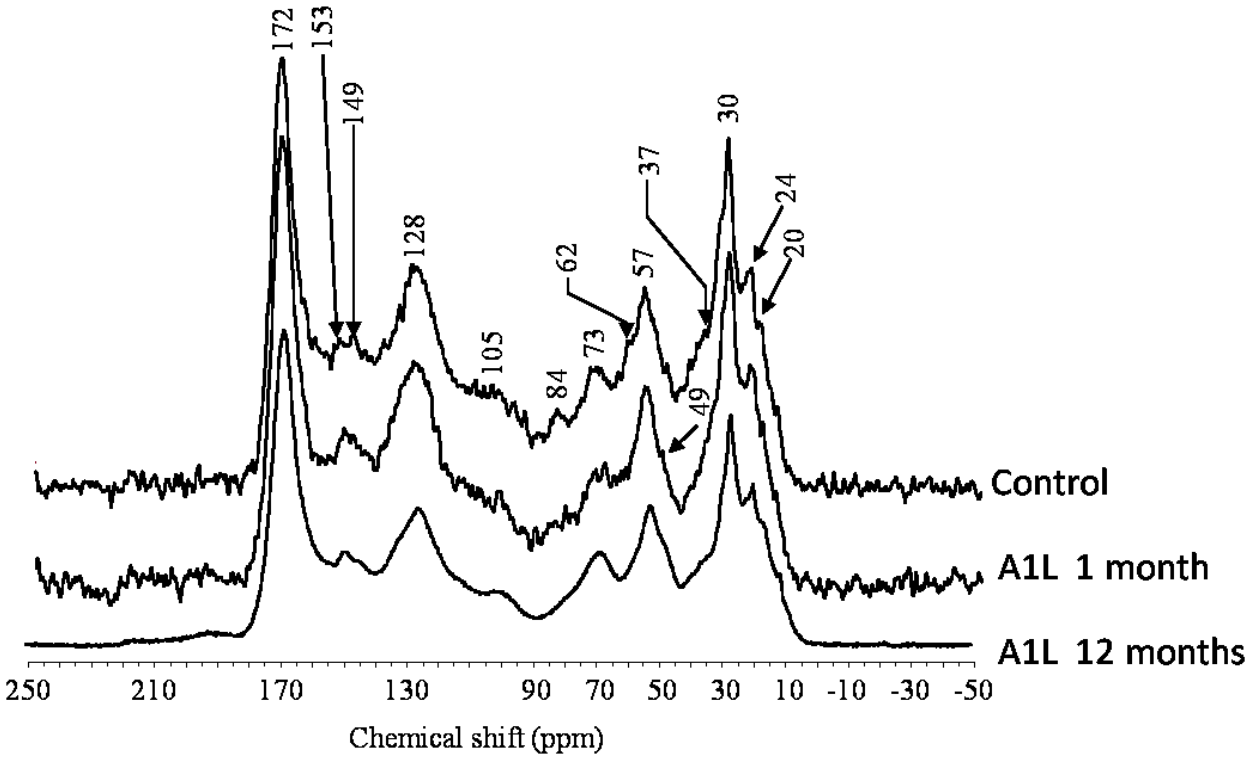


Figure 5.5 Solid state ^{13}C CPMAS-NMR spectra of HAs extracted from A1L plots of F2 soils, after one and twelve months from amendment.

5.3.4 Longitudinal relaxation time distribution by Fast Field Cycling (FFC) NMR relaxometry setup

Figure 5.6 reports the longitudinal relaxation time (T_1) distributions of the different soil samples used in the present study. According to Kimmich and Anoardo (2004), the longer the T_1 values, the faster are the molecular motions. Conversely, when motions are slow, more efficient are the proton-proton dipolar interactions, thereby resulting in shorter longitudinal relaxation rates. The efficiency of the dipolar interactions depends upon the size of the molecular systems. In particular, large-sized systems are characterized by short T_1 values, whereas small-sized systems have long T_1s.

As soils are analysed by nuclear magnetic resonance relaxometry with fast field cycling setup, dynamics of water molecules retained in soils is monitored (Kimmich

and Anoardo, 2004). Based on the aforementioned remarks, it is possible to state that the less mobile water molecules are trapped in the smallest soil pores, while those which are present in the largest soil pores are the more mobile (Pohlmeier et al., 2009).

The Control soil, which was not subjected to any treatment, revealed a T_1 distribution which was deconvoluted into four components centred at 8, 19, 77 and 502 ms. On the other hand, either the soils amended with A1L and A1H or those amended with A2L and A2H showed similar T_1 distribution between each other, which appeared different from those of the Control systems (Figure 5.6). In particular, while the first two components at the lowest T_1 values remained unaltered, the others changed to shorter values. According to the model described above, the effect of the amendments was an increase of the soil pore size, which, in turn, can have positive effects on soil structure and soil aeration (Spaccini et al., 2004).

Although at a different extent, also the soil samples that, in addition to organic amendments, received mineral fertilizations (m) revealed an increase of pore size as previously evidenced for the organically amended soils.

Several workers have noted that the addition of readily decomposable substrate causes a rapid stimulation of the soil microflora and this is accompanied by a significant increase of stability (Kiem and Kandeler, 1997). Among the different mechanisms by which microorganism interact with the soil structure the entanglement of particles by fungal hyphae and polysaccharide-mediated aggregation or stabilization by bacteria are well understood (Kiem and Kandeler, 1997).

After one month from amendment, the readily available OM leads to a rapid increase of the microbial biomass pool and to an increase in aggregate stability. In according to Kiem and Kandeler (1997) polysaccharides are active glues and aggregating agents in soil, whose presence was confirmed by NMR spectra of HAs from amended soils (Figure 5.3).

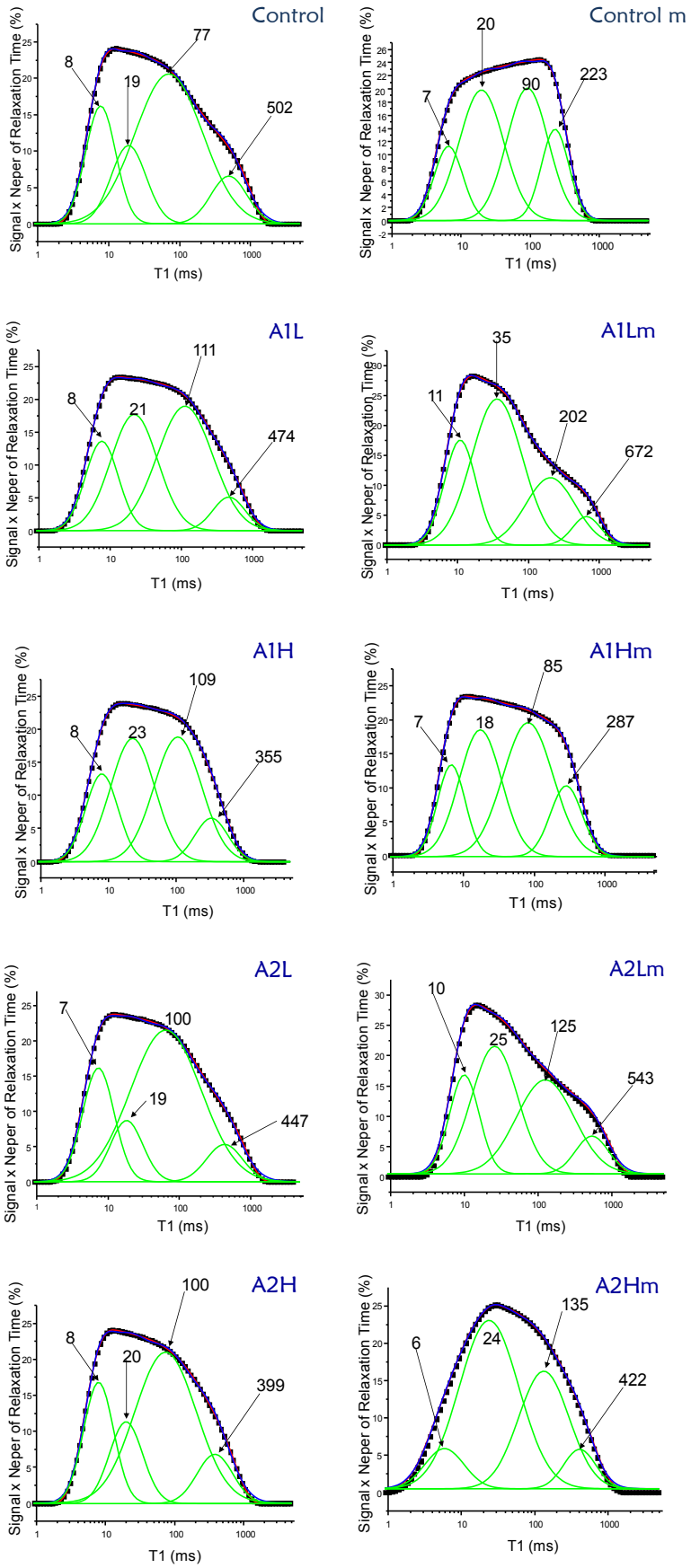


Figure 5.6 FFC NMR relaxometry spectra of F1 soils at first sampling (March 2009).

5.4 Conclusions

The results obtained by elemental analysis, FT-IR and ^{13}C CPMAS NMR spectroscopy gave important information on soil OM evolution. Changes in elemental composition of both HAs and FAs, depending on soil geopedologic characteristics (F1 and F2) and on experimental time (one and twelve months) were observed after the addition of different organic amendments.

In both amended soils OM fractions showed an increase of HAs fractions, characterized by a lower content of more labile fraction, such as polysaccharides and lipid components, and a higher content of aromatic compounds, indicating a selective preservation of recalcitrant hydrophobic molecules, such as lignin and lignin-like compounds, and confirming the positive effects of wood materials add in mix with compost.

The nature of soils affected the behaviour and fate of OM. In fact, on the one hand the clay nature of F1 soil favoured processes leading to more stable OM, rich in carbonyl groups belonging to aldehydes and ketones, on the other hand the sandy nature of F2 soil induced faster degradative processes, due to oxidative conditions and more intensive microbial activity, in according to results obtained from soil chemical and biochemical characterization.

The ^{13}C CPMAS NMR spectroscopy showed important changes in their functional composition that allow to better understand also the physical, chemical and biochemical results previously described. Also FFC NMR relaxometry as innovative application of spectroscopic technique to soil, allowed to obtained interesting information on the effects of organic amendments on soil properties. In particular results suggest that the amendments induced an increase of the soil pore size, by organo-mineral aggregates formation, which, in turn, can have positive effects on soil structure and soil aeration.

5.5 References

Adani A., Genevini P., Tambone F., Montoneri E., 2006. Compost effect on soil humic acid: A NMR study. *Chemosphere* 65:1414-1418.

Adani F., Genevini P., Ricca F. Tambone and Montoneri E., 2007. Modification of soil humic matter after 4 years of compost application. *Waste Management* 27:319-324.

Albers C. N. and Hansen P. E., 2010. ^{13}C-NMR Chemical Shift Databases as a Quick Tool to Evaluate Structural Models of Humic Substances. *The Open Magnetic Resonance Journal* 3:96-105.

Amato M. and Ladd J.N., 1980. Studies of nitrogen immobilization and mineralization in calcareous soils. V. Formation and distribution of isotope-labelled biomass during decomposition of ^{14}C- and ^{15}N carbon and nitrogen isotope-labelled plant material. *Soil Biology and Biochemistry* 12:405-411.

Anderson D.W. and Paul E.A., 1984. Organo-mineral complexes and their study by radiocarbon dating. *Soil Science Society of America Journal* 48:298-301.

Bakhmutov V. I., 2004. *Pratical NMR Relaxation for Chemists*, John Wiley and Sons Ltd: Chichester, West Sussex, UK..

Belzile N., Joly H.A., Li H., 1997. Characterization of humic substances extracted from Canadian lake sediments. *Canadian Journal of Chemistry* 75:14-27.

Bengtsson G., Bengtson P., Mansson, K.F., 2003. Gross nitrose mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. *Soil Biology and Biochemistry* 35:143-154.

Borgia G.C., Brown R.J.S., Fantazzini P., 1998. Uniform-penalty inversion of multiexponential decay data. *Journal of Magnetic Resonance* 132:65-77.

Borgia G.C., Brown R.J.S., Fantazzini P., 2000. Uniform-penalty inversion of multiexponential decay data II: data spacing, T2 data, systematic data errors, and diagnostics. *Journal of Magnetic Resonance* 147:273-285.

Bourbonniere R.A. and Meyers P.A., 1978. Characterization of sedimentary humic matter by elemental and spectroscopic methods. *Canadian Journal of Spectroscopy* 23:35-41.

Chen X., Hutley L.B., Eamus D., 2005. Soil organic carbon content at a range of north Australian tropical savana with contrasting site histories. *Plant and Soil* 268:161-171.

Chien S.-W., Huang C.-C., Wang M.-C., 2003. Analytical and spectroscopic characteristics of refuse compost-derived humic substances. *International Journal of Applied Science and Engineering* 1:62- 71.

Conte P., De Pasquale C., Novotny E.H., Caponetto G., Laudicina V. A., Ciofalo M., Panno M., Palazzolo E., Badalucco L. and Alonzo G. 2010 CPMAS ^{13}C NMR Characterization of Leaves and Litters from the Reafforestated Area of Mustigarufi in Sicily (Italy). *The Open Magnetic Resonance Journal* 3:89-95.

Conte P., Piccolo A., van Lagen B., Buurman P., de Jager P. A., 1997. Quantitaitve differences in evaluating soil humic substances by liquid- and solid-state ^{13}C-NMR spectroscopy *Geoderma* 80:339-352.

Conte P., Spaccini, R., Piccolo, A., 2004. State of the art of CPMAS ^{13}C-NMR spectroscopy applied to natural organic matter. *Progress in Nuclear Magnetic Resonance Spectroscopy* 44:215-223.

Dignac M.-F., Kögel-Knabner I., Michel K., Matzner E., Knicker H., 2002. Chemistry of soil organic matter as related to C/N in Norway spruce forest (*Picea abies* (L.) Karst.) floors and mineral soils. *Zeitschrift für Pflanzenernährung und Bodenkunde* 165, 281-289.

Dixon R.K., Brown S., Houghton R.A., Solomon A.M., Trexler M.C., Wisniewski J., 1994. Carbon pools and flux of global forest ecosystems. *Science* 263:185-190.

Droussi Z., D'Orazio V., Hafidi M., Ouatmane A., 2009. Elemental and spectroscopic characterization of humic-acid-like compounds during composting of olive mill by-products. *Journal of Hazardous Materials* 163:1289-1297.

Ferrante G., Sykora S., 2005. Technical aspects of fast field cycling. *Advances in Inorganic Chemistry* 57:405-470.

Filip Z., Bielek P., 2002. Susceptibility of humic acids from soils with various contents of metals to microbial utilisation and transformation. *Biology and Fertility of Soils* 36:426- 433.

Fortuna A., Harwood R., Kizilkaya K., Paul E.A., 2003. Optimizing nutrient availability and potential carbon sequestration in an agroecosystem. *Soil Biology and Biochemistry* 35:1005-1013.

Gonzàles-Vila F.J., Almendros G., Madrid F., 1999. Molecular alterations of organic fractions from urban waste in the course of composting and their further transformation in amended soil. *Science of the Total Environment* 236:215-229.

Gonzalez-Vila F.J., Martin F., del Rio J.C., Fruünd, R., 1992. Structure characteristics and geochemical signifcance of humic acids isolated from three Spanish lignite deposits. *Science of the Total Environment* 117/118:335-343.

Grube M., Lin J.G., Lee P.H., Kokorevicha S., 2006. Evaluation of sewage sludgebased compost by FT-IR spectroscopy. *Geoderma* 130:324-333.

Grube M., Zagreba J., Gromozova E., Fomina M., 1999. Comparative investigation of the macromolecular composition of mycelia forms *Thielavia terrestris* by infrared spectroscopy. *Vibration Spectroscopy* 19:301-306.

Halle B., Johannesson H., Venu K., 1998. Model-Free Analysis of stretched relaxation dispersions. *Journal of Magnetic Resonance* 135:1-13.

Hargreaves J.C., Adl M.S., Warman P.R., 2008. A review of the use of composted municipal solid waste in agricolture. *Agriculture, Ecosystems and Environment* 123:1-14.

Haynes R.J., Naidu R., 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. *Nutrient Cycling in Agroecosystems* 51:123-137.

Ishiwatari R., 1985. Geochemistry of humic substances in lake sediments. In: Aiken G.R., McKnight D.M., Wershow R.L., MacCarthy P. (Eds.), Humic Substances in Soil, Sediment, and Water. John Wiley and Sons, New York, pp. 147-180.

Iyyemperumal K., Israel D.W., Shi W., 2007. Soil microbial biomass, activity and potential nitrogen mineralization in a pasture: Impact of stock camping activity. *Soil Biology and Biochemistry* 39:149-157.

Juan Li., Bing-qiangl Z., Xiu-yingl L., Rui-bol J., Hwat Bing S., 2008. Effects of long-term combined application of organic and mineral fertilizers on microbial biomass, soil enzyme activities and soil fertility. *Agricultural Sciences in China* 7:336-343.

Kiem R. and Kandeler E., 1997. Stabilization of aggregates by the microbial biomass as affected by soil texture and type. *Applied Soil Ecology* 5:221-230.

Kimmich R. and Anoardo E., 2004. Field-cycling NMR relaxometry. *Progress in Nuclear Magnetic Resonance Spectroscopy* 44:257-320.

Knicker H. and Lüdemann H.-D., 1995. N-15 and C-13 CPMAS and solution NMR studies of N-15 enriched plant material during 600 days of microbial degradation. *Organic Geochemistry* 23:329-341.

Komaljow E. and Mazzarino M.J., 2007. Soil restoration in semiarid Patagonia: Chemical and biological response to different compost quality. *Soil Biology and Biochemistry* 39:1580-1588.

Laganière J., Angers D.A., Paré D., 2010. Carbon accumulation in agricultural soils after afforestation: a meta-analysis. *Global Change Biology* 16:439-453.

Lal R., 2004. Soil carbon sequestration to mitigate climate change. *Geoderma* 123:1-22.

Leifeld J., Siebert S., Kogel-Knaber I., 2002. Changes in the chimica composition of soil organic matter after application of compost. *European Journal of Soil Science* 53:299-309.

Lemma B., Nilsson I., Kleja D.B., Olsson M., Knicker H., 2007. Decomposition and substrate quality of leaf litters and fine roots from three exotic plantations and a native forest in the southwestern highlands of Ethiopia. *Soil Biology and Biochemestry* 39:2317-28.

Lguirati A., Ait Baddi G., El Mousadik A., Gilard V., Revel J.C., Hafidi M., 2005. Analysis of humic acids from aerated and non-aerated urban landfill composts. *International Biodeterioration and Biodegradation* 56:8-16.

Lima D.L.D., Santos S.M., Scherer H.W., Schneider R.J., Duarte A.C., Santos E.B.H., Esteves V.I., 2009. Effects of organic and inorganic amendments on soil organic matter properties. *Geoderma* 150:38-45.

Liu N., He H., Xie H., Bai Z., Zhang X., Peng C., Zhu P., Ren J., Wang L., 2010. Impacts of long-term inorganic and organic fertilization on lignin in a Mollisol. *Journal of Soils Sediments* 10:1466-1474.

Malcolm R.L., 1989. Application of solid state ^{13}C NMR spectroscopy to Geochemical structure of humic sub- stances. Hayes MHB, MacCarthy P., Malcolm R.L., Swift R.S.. In: Humic Substances II. In Search of Structure. John Wiley and Sons, New York, pp. 339±372.

Martin F., Almendros G., Gonzàlez-Vila F.-J., Verdejo T., 2001. Experimental reappraisal of flash pyrolysis and low-temperature thermally assisted hydrolysis and and methylation using tetramethylammonium hydroxide for the molecular characterization of humic acids. *Journal of Analytical and Applied Pyrolysis* 61:133-145.

Masciandaro G., Ceccanti B., Gracía C., 2000. In situ vermicomposting of biological sludges and impact on soil quality. *Soil Biology and Biochemistry* 32:1015-1024.

Mermut A.R., Eswaran H., 2001. Some major developments in soil science since the mid-1960s. *Geoderma* 100:403- 426.

Naidja A., Huang P.M., Anderson D.W., Van Kessel J.S., 2002. FT-IR, UV-visible, and X-ray diffraction analyses of organic matter in humin, humic acid, and fulvic acid fractions in soil exposed to elevated CO_2 and N fertilisation. *Applied Spectroscopy* 56:318-324.

Olk D.C., Brunetti G., Senesi N., 1999. Organic matter in doublecropped lowland rice soils: chemical and spectroscopic properties. *Soil Science* 164:633- 649.

Piccolo A., Spaccini R., Nieder R., Richter J., 2004. Sequestration of a biologically labile organic carbon in soils by humified organic matter. *Climatic Change* 67:329-343.

Pohlmeier A., Haber-Pohlmeier S., Stapf S., 2009. A Fast Field Cycling Nuclear Magnetic Resonance Relaxometry Study of Natural Soils, *Vadose Zone Journal*, 8, 735-742.

Provenzano M.R., de Oliveira S.C., Silva M.R.S., Senesi N., 2001. Assessment of maturity degree of composts from domestic solid wastes by fluorescence and Fourier transform infrared spectroscopy. *Journal of Agricultural and Food Chemistry* 49:5874-5879.

Quèdraogo E., Mando A., Zombre` N.P., 2001. Use of compost to improve soil properties and crop productivity under low input agricultural system in West Africa. *Agriculture, Ecosystems and Environment* 84:259-266.

Quideau S.A., Chadwick O.A., Benesi A., Graham R.C., Anderson M.A., 2001. A direct link between forest vegetation type and soil organic matter composition. *Geoderma* 104: 41-60.

Reeves III J.B., Van Kessel J.S., 2002. Spectroscopic analysis of dried manure: near- versus mid-infrared spectroscopy for the analysis of dried dairy manure. *Journal of Near Infrared Spectroscopy* 10:93-101.

Reveille V., Mansuy L., Jarde E., Garnier-Sillam E., 2003. Characterization of sewage sludge-derived organic matter: lipids and humic acids. *Organic Geochemistry* 34:615-627.

Rice J.A., MacCarthy P., 1991. Statistical evaluation of the elemental composition of humic substances. *Organic Geochemestry* 17:635-648.

Rivero C., Chirenje T., Ma L.Q., Martinez G., 2004. Influence of compost on soil organic matter quality under tropical conditions. *Geoderma* 123, 355-361.

Russo F., Gianfreda L., Conte P. and Rao M. A., 2010, Interaction of a Recombinant Prion Protein with Organo-Mineral Complexes as Assessed by FT-IR and CPMAS 13C NMR Analysis, *The Open Magnetic Resonance Journal* 3:84-89.

Schulten H.-R., Leinweber P., 2000. New insights into organic-mineral particles: composition, properties and models of molecular structure. *Biology and Fertility of Soils* 30:1-34.

Senesi N., Orazio V.D., Ricca G., 2003. Humic acids in the first generation of EUROSILS. *Geoderma* 116:325-344.

Sierra M.M.D., Giovanela M., Parlanti E., Esteves V.I., Duarte A.C., Fransozo A., Soriano-Sierra E.J., 2005. Structural description of humic substances from subtropical coastal environments using elemental analysis, FT-IR and ^{13}C-solid state NMR data. *Journal of Coastal Research* 42:370-382.

Silverstein R. M., Webster F. X., Kiemle D., 2005. The Spectrometric Identification of Organic Compounds, 7th ed. J. Wiley and Sons, NJ, USA.

Smidt E., Lechner P., Schwanninger M., Haberhauer G., Gerzabek M.H., 2002. Characterization of waste organic matter by FT-IR spectroscopy: application in waste science. *Applied Spectroscopy* 56:1170-1175.

Soumaré M., Tack F.M.G., Verloo M.G., 2003. Effects of a municipal solid waste compost and mineral fertilization on plant growth in two tropical agriculture soils of Mali. *Bioresource Technology* 86:15-20.

Spaccini R., Mbagwu J.S.C., Igwe C.A., Conte P., Piccolo A., 2004. Carbohydrates and aggregation in lowland soils of Nigeria as influenced by organic inputs, *Soil and Tillage Research* 75:161-172.

Spaccini R., Piccolo A., Conte P., Haberhauer G., Gerzabek M.H., 2002. Increased soil organic carbon sequestration through hydrophobic protection by humic substances. *Soil Biology and Biochemistry* 34:1839-1851.

Steelink C. 1985. Implications of elemental characteristics of humic substances. In: Aiken G.R., McKnight D.M., Wershow R.L., MacCarthy P. (Eds.), Humic Substances in Soil, Sediment, and Water. John Wiley and Sons, New York, pp. 457±476.

Stevenson F.J., 1994. Humus chemistry: genesis, composition, reactions. *Journal of Chemical Education* 72:A93.

Tatzber M., Stemmer M., Spiegel H., Katzlberger C., Haberhauer G., Mentler A., and Gerzabek M.H., 2007. FTIR-spectroscopic characterization of humic acids and humin fractions obtained by advanced NaOH, $Na_4P_2O_7$, and Na_2CO_3 extraction procedures *Journal of Plant Nutrition and Soil Science* 170:522-529.

Taylor R.E., French A.D., Gamble G.R., *et al*. ^{1}H and ^{13}C solid-state NMR of *Gossypium barbadense* (Pima) cotton. *Journal of Molecular Structure* 878:177-84.

Tseng D.Y., Vir R., Traina S.J., Chalmers J.J., 1996. A Fourier transform infrared spectroscopic analysis of organic matter degradation in a bench-scale solid substrate fermentation (composting) system. *Biotechnology and Bioengineering* 52:661-671.

Ussiri D.A.N. and Johnson C.E., 2003. Characterization of organic matter in a northern hardwood forest soil by ^{13}C NMR spectroscopy and chemical methods. *Geoderma* 111:123-149.

Vesterdal L., Ritter E., Gundersen P., 2002. Change in soil organic carbon following afforestation of former arable land. *Forest Ecology and Management* 169:137-147.

Weber J., Karczewska A., Drozd J., Licznar M., Licznar S., Jamroz E., Kocowicz A., 2007. Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts. *Soil Biology and Biochemistry* 39:1294-1302.

Wickholm K., Hult E.-L., Larsson P.T., Iversen T., Lennholm H.,1998. Assignment of non-crystalline forms in cellulose I by CP/MAS ^{13}C NMR spectroscopy. *Carbohydrates research* 312:123-129.

Zaccheo P., Cabassi G., Ricca G., Crippa L., 2002. Decomposition of organic residues in soil: experimental technique and spectroscopic approach. *Organic Geochemistry* 33:327-345.

Chapter 6

Effects of organic fertilizers on development of arbuscular mycorrhizal fungi indigenous in lettuce

6.1 Introduction

In the natural ecosystem, the symbiotic association between the roots of plants and fungi is very common. Among the different types of fungi forming these associations the most important are the arbuscular mycorrhizal fungi (AMF) of the Phylum Glomeromycota (Schussler et al., 2001). AMF form a symbiotic association with more than 80% of cultivated plants, though crops in the Brassicaceae and Chenopodiaceae generally do not form mycorrhizal associations (Newman and Reddell, 1987). The AMF consists of an internal part inside the plant root and an external part, called extra radical mycelium, which can form an extensive network within the soil.

A widely accepted definition, that includes all aspects of the association between the host plant and the AMF, does not appear in literature. Fitter and Moyersoen (1996) defined AMF association as ‘‘a sustainable non-pathogenic biotrophic interaction between a fungus and a root’’, but although having merit, it does not emphasize the importance of the extra radical phase (Hodge, 2000; Gosling et al., 2005).

The AMF facilitate their host principally by increasing the uptake of relatively immobile phosphate ions, due to the ability of the fungal extra radical mycelium to grow beyond the phosphate depletion zone that quickly develops around the root (Sanders and Tinker, 1971; Koide, 1991; George et al., 1995; Smith and Read, 1997). In return, the fungi receive carbon from the host plant. Other benefits to the host include: increased resistance to foliar-feeding insects (Gange and West, 1994), improved drought resistance (Augé et al., 1994), increased resistance to soil pathogens (Newsham et al., 1995; Lingua et al., 2002; Pozo et al., 2002) and increased tolerance to salinity and heavy metals (Shetty et al., 1995; Diaz et al., 1996;

Al-Karaki et al., 2001; Feng et al., 2002; Mohammad et al., 2003). Increased uptake of macronutrients besides phosphorus (P), including nitrogen (N) potassium (K) and magnesium (Mg) has also been reported (Smith and Read, 1997; Hodge et al., 2001) as well as increased uptake of some micronutrients (Faber et al., 1990; Kothari et al., 1991a; Li et al., 1991; Azaizeh et al., 1995). In addition, mycorrhizas have been shown to play an important role in maintaining soil aggregate stability (Tisdall, 1991; Degens et al., 1996).

Crop management can affect AMF association both directly, by damaging or killing AMF and indirectly, by creating conditions either favorable or unfavorable to AMF. In general, intensive agricultural managements negatively affected the AMF association, determining an impoverished AMF in the agro-ecosystems, particularly in terms of biodiversity (Helgason et al., 1998; Menéndez et al., 2001).

The intensive use of mineral fertilizers, such occurs in intensive farming, depleted the AMF development (Gosling et al., 2005).

Use of phosphate fertilizers has a significant impact on the relationship between the plant and the fungus. The intensive use of phosphate fertilizers, used in excess respect to crop requirements, determines a build-up of total and, in some cases, of easily available phosphorus in the soil (Boehm and Anderson, 1997; Withers et al., 2001; De Clerck et al., 2003; Kogelmann et al., 2004). This has led, in turn, to less reliance of crops on the AMF association and lower AMF colonization and propagule density.

Use of nitrogen fertilizers it has also been reported to have a negative effect on AMF colonization and/or diversity in some cases (Miller and Jackson, 1998; Liu et al., 2000; Burrows and Pfleger, 2002; Treseder and Allen, 2002). On the other hand, organic amendments, such as farmyard manure, compost and crop residues, and slow release mineral fertilizers, such as rock phosphate, do not seem to suppress AMF and could even stimulate them (Douds et al., 1997; Kabir et al., 1998; Miller and Jackson, 1998; Joner, 2000; Alloush and Clark, 2001).

Low input practices, such as occur in organic farming, are generally more favourable to AMF and this fungi have the potential to replace synthetic fertilizers and biocides,

which are forbidden in organic systems. However, the available evidence suggests that, despite the use of generally favourable management practices, organic agro-ecosystems do not always have large, diverse or efficient AMF communities. Many authors report higher levels of AMF colonization, higher propagule numbers or higher diversity in organic farming (Douds et al., 1993; Kahiluoto and Vestberg, 1998; Eason et al., 1999; Ryan and Ash, 1999; Ryan et al., 2000, 2004; Galvez et al., 2001; Oehl et al., 2003, 2004; Bending et al., 2004).

To better understand how organic fertilizers can affect AMF, the aim of this work was to evaluate the effect of nitrogen and phosphorus, by organic fertilizations, on the development of indigenous AMF and on the growth of lettuce plants, an important crop widely cultivated under organic farming and with high percentage of root colonization by AMF.

This study was performed, during the period of Ph doctorate foreign, at the Departamento de Ciencias Químicas, Universidad de la Frontera, Temuco, Chile, under the supervision of Prof. Fernando Borie.

6.2 Materials and Methods

6.2.1 Description of study

Four different commercial cultivars of *Lactuca sativa* (Romana, Milanesa, Grande Lagos e Escarola) were used to study mycorrhization under organic agricultural system, using compost from agricultural wastes (1 kg m^{-2}) (Table 6.1) as background fertilization for all plots, red guano as phosphorus source (75 U and 150 U ha^{-1} of P_2O_5), lupine flour as nitrogen source (75 and 150 U ha^{-1} of N) and a combination of both, according to the experimental design in Figure 6.1.

Table 6.1 Chemical composition of soil and compost used in the experimentation.

	N	P	pH	SOM	K	Na	Ca	Mg	Al	CEC	Al Sat
	mg kg^{-1}			%	$cmol_{(+)}$ kg^{-1}						%
Soil	41	11	5.78	12	1.46	0.27	6.7	1.39	0.01	9.87	0.10
Compost	34	142	6.34	27	3.81	0.40	22.3	7.67	0.01	34.24	0.03

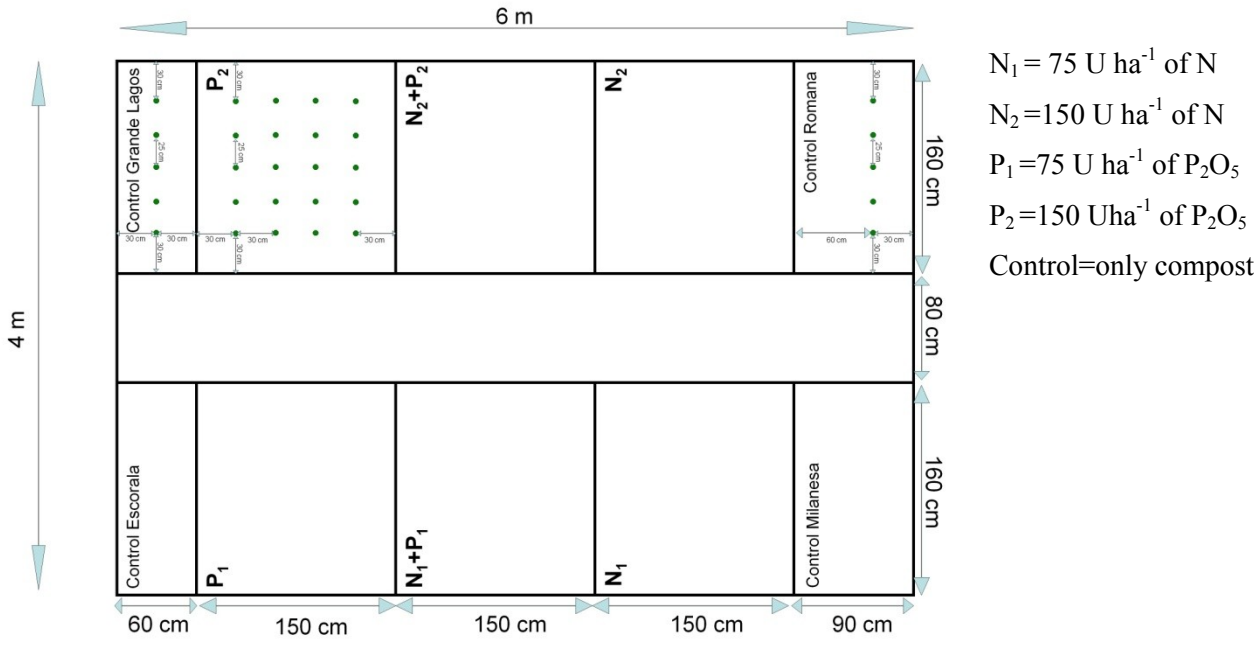


Figure 6.1 Experimental design of pots under greenhouse.

Four different cultivars of *L. sativa* were seeded in seedbeds with a mixture of soil and compost (1 kg m^{-2}) (Table 6.1). After 40 days of growth, lettuces were transplanted in a field under greenhouse, with organic fertilizations; for each cultivar

5 plants in each amendment condition were transplanted. After 60 days of growth under greenhouse, when lettuces were ready to commerce, the plants were harvested.

6.2.2 Plant analyses

Plants were harvested by recording of shoot and root parts and rhizosphere soils were collected. The roots were washed under a stream of cold tap water to remove soil residues and fresh and dry (65 °C, 24 h) weight of both shoot and roots was recorded. Before drying, roots were subsampled in three 2-cm cross-sections of the upper, middle, and lower root system to determine mycorrhizal colonization. To assess colonization, roots were cleared with 10% KOH at room temperature, rinsed with water, neutralized with 1 N HCl, and stained with 0.05% trypan blue for 24 h (Phillips and Hayman, 1970). The percentage of root colonized by AMF was calculated by the gridline intersect method (Giovanetti and Mosse, 1980). Positive counts for AM colonization included the presence of vesicles or arbuscules or typical mycelium within the roots.

6.2.3 Rhizosphere soil analysis

Mycorrhizal spores were collected from soil by wet sieving and decanting as described by Sieverding (1991). Glomalin extraction from whole-soil subsamples (1 g) was conducted as described by Wright and Upadhyaya (1998) with three replicates. Total glomalin (TG) was extracted with 50 mM sodium citrate, at pH 8.0 at 121 °C in rounds of 60 min cycles until supernatant showed none of the red-brown colour typical of glomalin. Extracts from each replicate were centrifuged to remove soil particles, and protein in the supernatant was analyzed using a Bradford assay with bovine serum albumin as standard (Wright and Upadhyaya, 1998).

6.2.4 Statistical analysis

Analysis of variance (ANOVA-one way with replication) was used to evaluate the effect of different organic fertilizers on crop yields. The significance between means

with $P < 0.01$ was determined using the Duncan test and all statistical analysis were performed for all samples values by using JMP 8 (SAS Institute, 2008).

6.3 Results and Discussion

6.3.1 Effect of organic fertilization on lettuces growth

The use of red guano, as sole phosphorus (P) fertilizer determined a significant increase of weight in all cultivars (Fig. 6.2). The high dose of phosphorus fertilizer (P_2) determined a significant increase of weight in cultivar Romana (5.0 g) compared to Control (2.0 g), whereas in cultivars Grande Lagos and Milanesa a strongly increase of weight (8.1 g and 8.3 g, respectively) was detected. In this latter cultivar also the low dose of phosphorus (P_1) positively affected the growth of lettuces, determining the same dry weight of P_2. In cultivar Escarola, P fertilization determined an increase of dry weight, but not as in the other cultivars.

Lupine flour used as nitrogen (N) fertilizer did not determined the same effect of red guano (Fig. 6.2a). In this case the weight of all plants was lower than those grown under only P fertilization. In general, no significant differences of growth were observed in plants under N fertilization, compared to not fertilized plants. Only in cultivar Milanesa the higher dose of N (N_2) determined an increase of dry weight (3.1 g) compared to the Control (1.3 g).

Combined use of nitrogen and phosphorus fertilization (N+P) determined higher growth in cultivars Milanesa, Grande Lagos and Escarola, whereas in cultivar Romana no positive effects on plant growth were observed (Fig. 6.3). The higher dose (N_2+P_2) produced higher dry weight values in cultivars Escarola and Grande Lagos (3.9 g and 3.6 g, respectively) compared to the others three cultivars.

Dry weight roots were affected by organic fertilizers in different ways. In general, a decrease of root weight in all cultivars under the only nitrogen fertilization compared to plants cultivated with P fertilization was observed; at low N availability, plant increases the proportion of biomass allocated into roots, to promote the uptake of limited nutrients (Chapin, 1991).

The differences of lettuces growth depending on the two organic fertilizers could be explained by the different amendment nature. Red guano is a phosphorus source easily available, so it determined a strong increase of plant weight, whereas lupine

flour is a slow release N source which takes long time to make N available, so it determined low N concentration in soil solution with a consequently reduction of lettuce growth compared to plants growth under phosphorus fertilization.

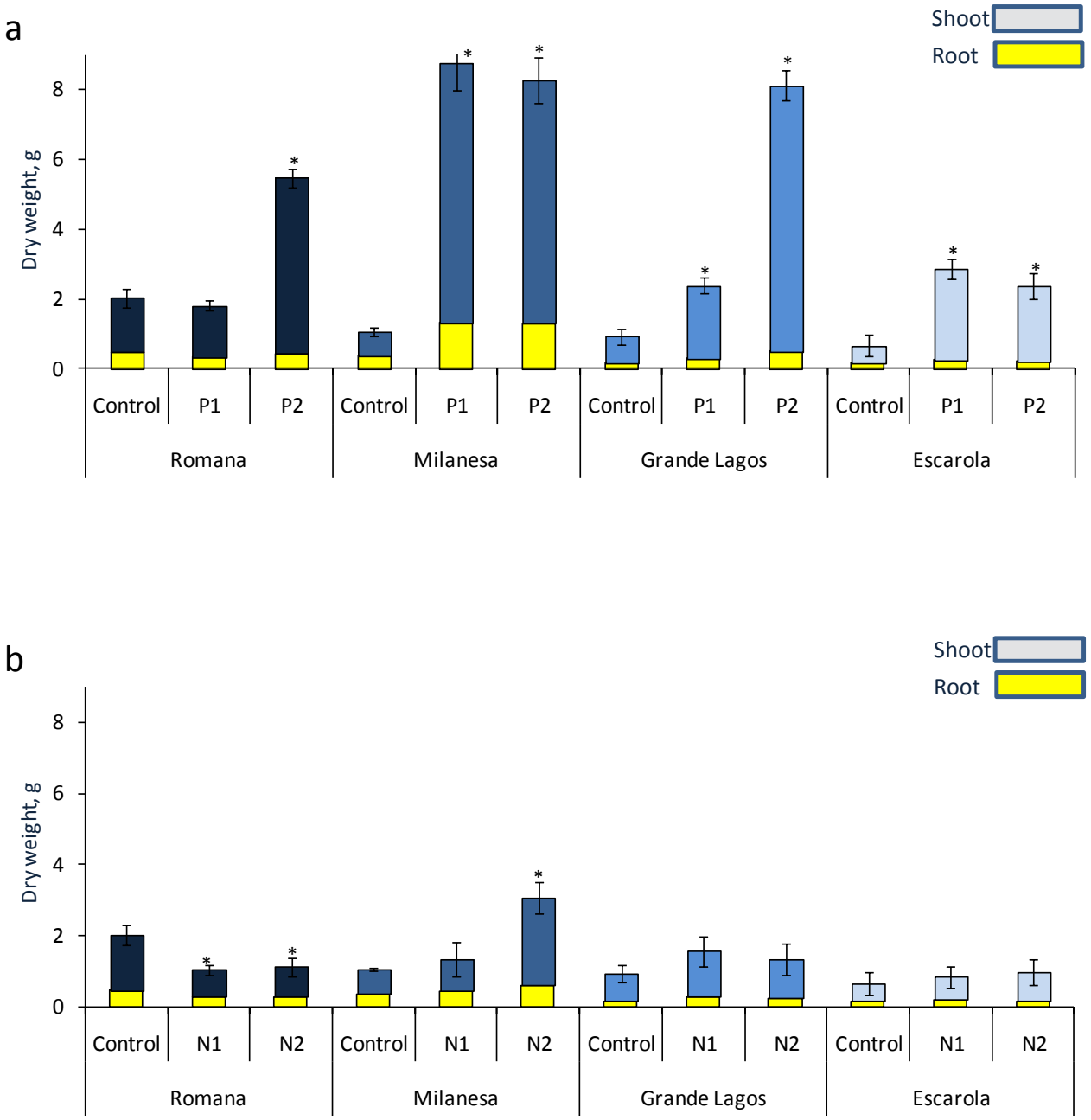


Figure 6.2 Effect of a) phosphorus and b) nitrogen organic fertilization on lettuces growth (*=P<0.01).

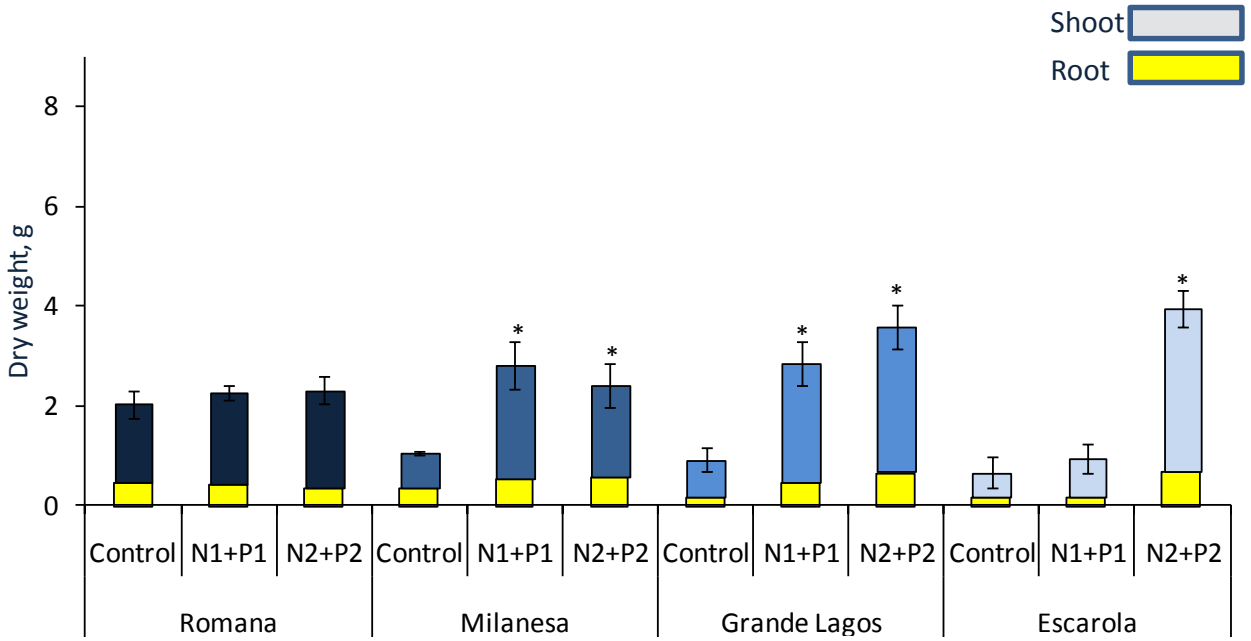


Figure 6.3 Effect of combined phosphorus and nitrogen fertilization on lettuces growth. (*=P<0.01).

6.3.2 Effect of organic fertilization on indigenous mycorrhizas

Figure 6.4 reports the percentage of root colonization in the 4 cultivars of lettuces, under organic fertilization. Higher dose of P fertilizers (P_2) decreased root colonization in all the samples, except in cultivar Milanesa (36%), whereas P_1 did not negatively affect this mycorrhizal process. The use of lupine flour, as nitrogen fertilizer, determined negative effect on cultivars Escarola and Romana, in both doses, whereas, the higher dose (N_2) provided an increase of root colonization (65% and 77%, respectively) in cultivars Grande Lagos and Milanesa compared to Control (47% and 25%, respectively). In these latter cultivars, the combined use of nitrogen and phosphorus fertilization, in the higher dose (N_2+P_2), determined the same root colonization levels of the only nitrogen fertilization (N_2) (73.5 and 74.3 %, respectively), indicating a positive effect of combined fertilization on root colonization.

In literature it is reported that fertilizers in organic form are less inhibitor of AMF root colonization than synthetic mineral fertilizers (Harinikumar and Bagyaraj, 1989).

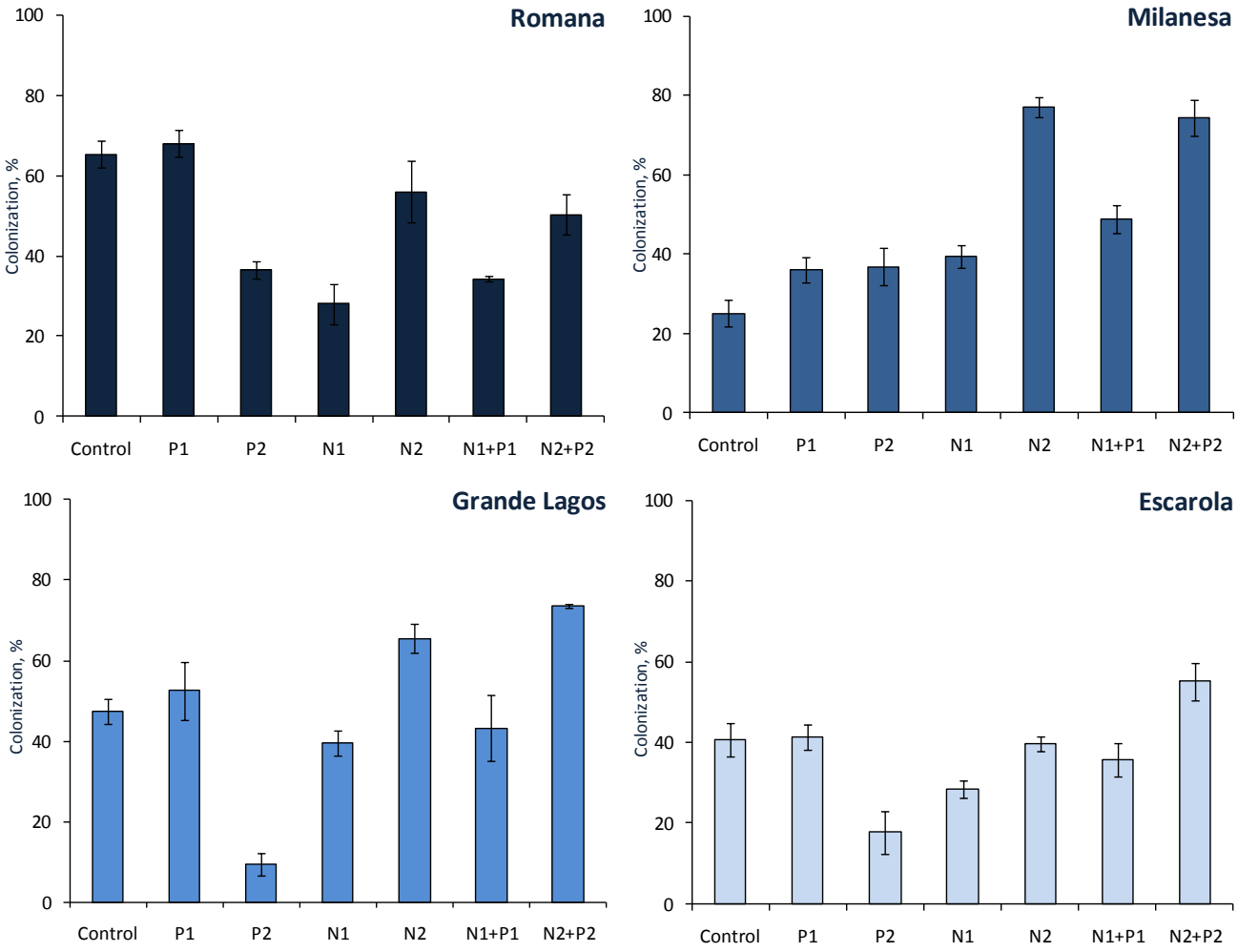


Figure 6.4 Effect of organic fertilizations on root colonization in lettuces cultivars by AMF indigenous.

The different root colonization by indigenous AMF reflected partly the intensity of fertilizer input and the soluble-nutrients content, especially of P, in the soils. This was in accordance with Hayman (1982) and Gosling (2006) that demonstrated as P has the most pronounced effect on the development of AMF symbiosis. In general, the percentage of AMF root colonization decreases by increasing P status of the host plant (Smith and Read, 1997). In our case, a decrease of root colonization in lettuces under the only phosphorus fertilization was observed, due to easily available P of red guano, which had strongly increased P in soil solution, inhibiting root colonization by indigenous AMF.

As occur for phosphorus, high solubility of N fertilizers generates high soil N concentrations detrimental to many soil microorganisms and mycorrhizal fungi

(O'Donnell et al., 2001; Scagel, 2005). By contrast, organic fertilizers often promote activity and diversity of these microorganisms because of low-solubility and slow release of N (such as lupine flour) and other nutrients released by microbial degradation of the organic compounds (Goulart et al., 1995; van Bruggen and Semenov, 2000). For this reason, the use of lupine flour as N source did not affect the root colonization in the studied lettuces, and in some cases, such as in cultivars Milanesa and Grande Lagos, positively affected the colonization by AMF.

In general the indigenous AMF spore density was positively affected by organic fertilizers, as shown in Figure 6.5. The use of only P fertilization slightly raised spores, in all studied cultivars; whereas, when lupine flour was used as N fertilization, a strong increase, particularly in cultivars Romana and Escarola, was observed. The combined use of N and P fertilization favoured, in both doses, in cultivars Milanesa a strongly increase in spores number, compared to Control; while in cultivars Escarola and Grande Lagos, the low dose determined similar spore numbers, compared to N_2.

The increase of AMF spores number observed after the addition of organic fertilizers, in particular with N fertilizers, was in according to the generally observed positive effect of organic fertilizer amendments on AMF (Oehl et al., 2004; Gosling et al., 2006). This result could relates to the characteristics of lupine flour, an organic fertilizer with a low-solubility and a slow release of N that determined low N concentration in the soil solution. Consequently higher soil colonization occurred in order to keep plants supply the supplied with more soluble N (Koide, 1991; Smith and Read, 1997).

These soils showed an active soil microbial community due to the exclusion of soluble mineral fertilizers and the limited use of biocides, as it occurs in organic farming (Gosling et al., 2006).

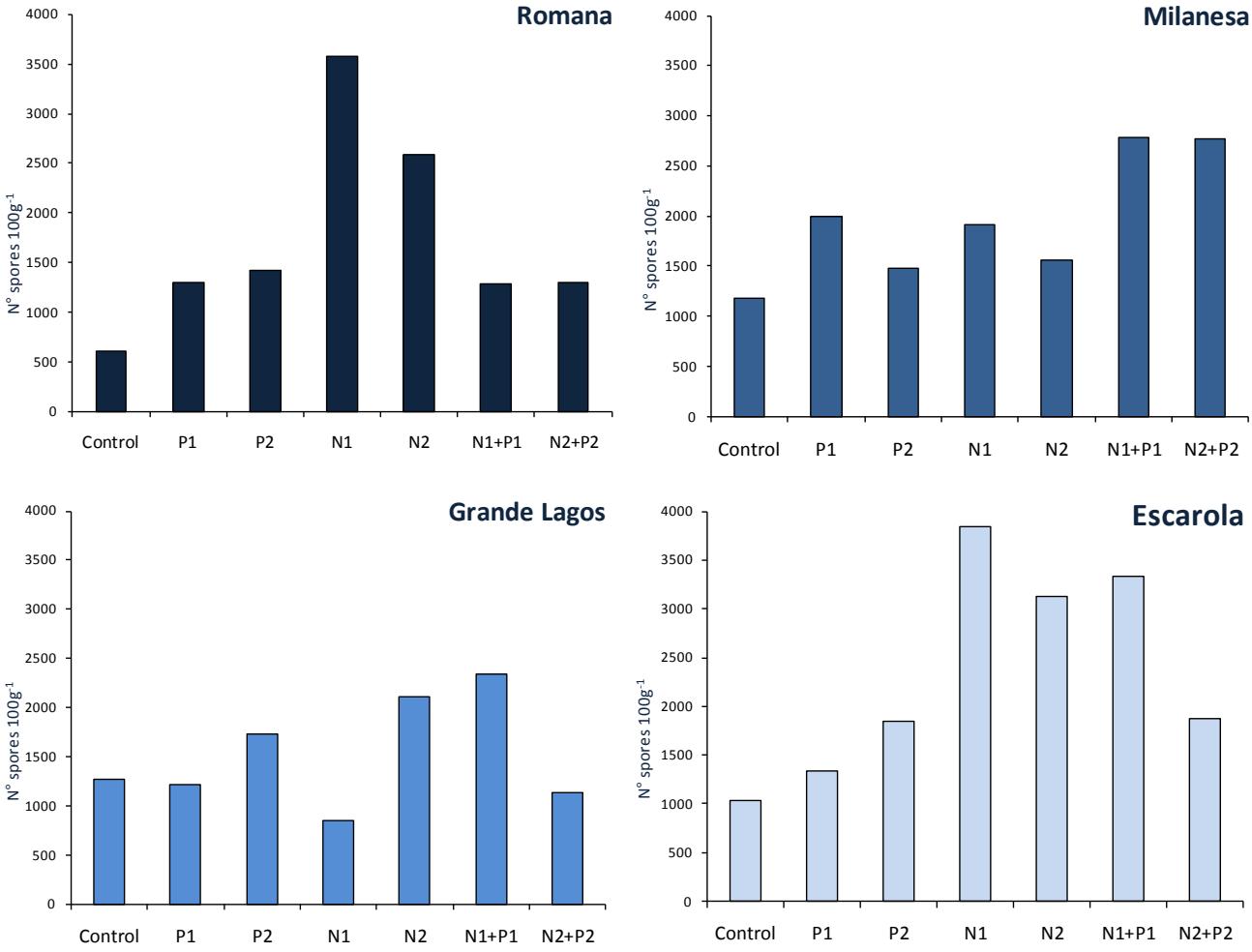


Figure 6.5 Effect of organic fertilizations on AMF indigenous spores numbers.

In the rhizospheric soils of all studied cultivars, under organic fertilizer, 10-11 different spore ecotypes were identified. This result confirmed that under low-input fertilizers, as it occurs under organic fertilizations, a high AMF biodiversity can be established (Oehl et al., 2009). The same ecological AMF groups in soils under organic fertilizer, compared with no treated soils, could indicate no loss of soil function and soil quality.

Total glomalin content ranged from 5 to 6 mg g^{-1} in all studied soils (Table 6.2), and remained unchanged by organic fertilizers, according to the average values of agriculture soil (Borie et al., 2006).

Table 6.2 Effect of organic fertilizations on total glomalin content (±sd).

	Glomalin mg g^{-1}			
Plot	**Romana**	**Milanesa**	**Grande Lagos**	**Escarola**
Control	5.39 ±0.07	5.46 ±0.39	5.04 ±0.31	5.58 ±0.16
P_1	5.10 ±0.17	5.32 ±0.10	5.05 ±0.15	5.11 ±0.01
P_2	5.78 ±0.17	5.58 ±0.15	5.76 ±0.10	6.18 ±0.24
N_1	5.43 ±0.12	5.56 ±0.16	5.43 ±0.17	4.43 ±0.16
N_2	6.60 ±0.16	5.38 ±0.15	5.65 ±0.11	5.94 ±0.16
N_1+P_1	5.34 ±0.18	5.69 ±0.25	4.77 ±0.15	6.40 ±0.05
N_2+P_2	5.89 ±0.23	5.82 ±0.10	6.00 ±0.16	6.32 ±0.15

This protein is produced by arbuscular mycorrhizal fungi and released abundantly in the soil (Wright et al., 1996; Rillig and Mummey, 2006). Glomalin concentration in soil was related to the abundance of water-stable aggregates (Borie et al., 2006; Wright et al., 2007), and so it may influence tilth.

Glomalin is also known as a proxy measure of AMF growth (Krivtsov et al., 2004; Lovelock et al., 2004b) because of the labor and subjectivity involved in quantifying soil hyphae (Millner and Wright, 2002; Rosier et al., 2006).

The results obtained in this study showed the same total glomalin content in soils treated with organic fertilizers as in Control soil, indicating the absence of negative effects of organic amendments on its content, and a consequently possible positive effect on soil fertility.

6.4 Conclusions

The nature of the used organic fertilizers has had a crucial role in the experimentation.

Red guano, due to its nature of easily available P source, determined a strongly increase of biomass in all lettuce cultivars. In particular, cultivar Milanesa showed the higher growth than all studied cultivars, also with the lower level of phosphorus fertilization (P_1). On the other hand, the increase of soil P concentration negatively affected root colonization by AMF, according to literature (Gosling et al., 2006).

Conversely, the use of lupine flour, as N source, determined a lower growth of lettuce plant, because of its low solubility. For this reason therefore, it cannot be considered a good organic fertilizer for plants as lettuce, having a very short crop cycle. Although lupine flour was recalcitrant to degradation it enhanced root colonization, in particular in cultivars Grande Lagos and Milanesa. In this latter cultivars the combined use of red guano and lupine flour led to a high percentage of root colonization as well as a good green biomass development, and affected positively AMF biodiversity, as it occurred also with the others organic treatments.

In conclusion, agriculture management practices like the use of different organic fertilizers, soil tillage, etc., always interact with crop biological factors, as AMF (Ngosong et al., 2010), highlighting the need for farmers to always consider the crops related to their implication on soil microbial communities. This, in a sustainable agriculture, is essential to preserve crop associated microorganisms involved in plant growth and soil fertility.

6.5 References

Al-Karaki G.N., Hammad R., Rusan M., 2001. Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. *Mycorrhiza* 11:43-47.

Alloush G.A., Clark R.B., 2001. Maize response to phosphate rock and arbuscular mycorrhizal fungi in acidic soil. *Communications in Soil Science and Plant Analysis* 32:231-254.

Augé R.M., Duan X., Ebel R.C., Stodola A.J.W., 1994. Non hydraulic signalling of soil drying in mycorrhizal maize. *Planta* 193:74-82.

Azaizeh H.A., Marschner H., Römheld V., Wittenmayer L., 1995. Effects of a vesicular-arbuscular mycorrhizal fungus and other soil microorganisms on growth, mineral nutrient acquisition and root exudation of soil grown maize plants. *Mycorrhiza* 5:321-327.

Bending G.D., Turner M.K., Rayns F., Marx M.C., Wood M., 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. *Soil Biology and Biochemistry* 36:1785-1792.

Boehm M.M., Anderson D.W., 1997. A landscape-scale study of soil quality in three prairie farming systems. *Soil Science Society of America Journal* 61:1147-1159.

Borie F., Rubio R., Rouanet J.L., Morales A., Borie G., Rojas C., 2006. Effects of tillage systems on soil characteristics, glomalin and mycorrhizal propagules in a Chilean Ultisol. *Soil and Tillage Research* 88:253-261.

Burrows R.L., Pfleger F.L., 2002. Arbuscular mycorrhizal fungi respond to increasing plant diversity. *Canadian Journal of Botany* 80:120-130.

Chapin F.S., 1991. Effects of multiple environmental stresses on nutrient availability and use Accademy press , San Diego, CA pp 67-88.

De Clerck F., Singer M.J., Lindert P., 2003. A 60-year history of California soil quality using paired samples. *Geoderma* 114:215-230.

Degens B.P., Sparling G.P., Abbott L.K., 1996. Increasing the length of hyphae in a sandy soil increases the amount of water-stable aggregates. *Applied Soil Ecology* 3:149-159.

Diaz G., Azcon Aguilar, C., Honrubia M., 1996. Influence of arbuscular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of *Lygeum spartum* and *Anthyllis cytisoides*. *Plant and Soil* 180:241-249.

Douds D.D., Galvez L., Franke-Snyder M., Reider C., Drinkwater L.E., 1997. Effect of compost addition and crop rotation point upon VAM fungi. *Agriculture, Ecosystems and Environment* 65:257-266.

Douds D.D., Janke R.R., Peters S.E., 1993. VAM fungus spore populations and colonization of roots of maize and soybean under conventional and low-input sustainable agriculture. *Agriculture, Ecosystems and Environment* 43:325-335.

Eason W.R., Scullion J., Scott E.P., 1999. Soil parameters and plant responses associated with arbuscular mycorrhizas from contrastino grassland management regimes. *Agriculture, Ecosystems and Environment* 73:245-255.

Faber B.A., Zasoski R.J., Burau R.G., Uriu K., 1990. Zinc uptake by corn as affected by vesicular-arbuscular mycorrhizae. *Plant and Soil* 129:121-130.

Feng G., Zhang F.S., Li X.L., Tian C.Y., Tang C., Rengel Z., 2002. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza* 12:185-190.

Fitter A.H., Moyersoen B., 1996. Evolutionary trends in root-microbe symbioses. *Philosophical Transactions: Biological Sciences* 351:1367-1375.

Galvez L., Douds Jr. D.D., Drinkwater L.E., Wagoner P., 2001. Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. *Plant and Soil* 118:299-308.

Gange A.C., West H.M., 1994. Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata* L. *New Phytologist* 128:79-87.

George E., Marschner H., Jakobsen I., 1995. Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. *Critical Reviews in Biotechnology* 15:257-270.

Giovanetti M. and Mosse B., 1980. An evolution of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* 84:489-499.

Gosling P., Hodge A., Goodlass G., Bending G.D., 2006. Arbuscular mycorrhizal fungi and organic farming. *Agriculture, Ecosystem and Environment* 113:17-35.

Goulart B.L., Demchak K., Yang W.Q., 1995. Organic matter and nitrogen level effects on mycorrhizal infection in 'Bluecrop' highbush blueberry plants. *Journal of Small Fruit & Viticulture* 3:151-164.

Harinikumar K.M. and Bagyaraj D.J., 1989. Effect of cropping sequence, fertilizers and farmyard manure on vesicular-arbuscular mycorrhizal fungi in different crops over three consecutive seasons. *Biology and Fertility of Soils* 7:173-175.

Hayman D.S., 1982. Influence of soil and fertility on activity and survival of vesicular-arbuscular mycorrhizal fungi. *Phytopathology* 72:1119-1125.

Helgason T., Daniell T.J., Husband R., Fitter A.H., Young J.P.W., 1998. Ploughing up the wood-wide web? *Nature* 394:431.

Hodge A., 2000. Microbial ecology of the arbuscular mycorrhiza. *FEMS Microbiology Ecology* 32:91-96.

Hodge A., Campbell C.D., Fitter A.H., 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* 413:297-299.

Joner E.J., 2000. The effect of long-term fertilization with organic or inorganic fertilizers on mycorrhiza-mediated phosphorus uptake in subterranean clover. *Biology and Fertility Soils* 32:435-440.

Kabir Z., O'Halloran I.P., Fyles J.W., Hamel C., 1998. Dynamics of the mycorrhizal symbiosis of corn (*Zea mays* L.), effects of host physiology, tillage practice and fertilization on spatial distribution of extra-radical mycorrhizal hyphae in the field. *Agriculture, Ecosystems and Environment* 68:151-163.

Kahiluoto H. and Vestberg M., 1998. The effect of arbuscular mycorrhiza on biomass production and phosphorus uptake from sparingly soluble sources by leek (*Allium porrum* L.) in Finnish field soils. *Biology, Agriculture and Horticulture*16, 65-85.

Kogelmann W.J., Lin H.S., Bryant R.B., Beege D.B., Wolf A.M., Petersen G.W., 2004. A state wide assessment of the impacts of phosphorus-index implementation in Pennsylvania. *Journal of Soil and Water Conservation* 59:9-18.

Koide R., 1991. Nutrient supply, nutrient demand and plant-response to mycorrhizal infection. *New Phytologist* 117:365-386.

Kothari S.K., Marschner H., Römheld V., 1991a. Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in calcareous soil. *Plant and Soil* 131:177-185.

Krivtsov V., Griffiths B.S., Salmond R., Liddell K., Garside A., Bezginova T., Thompson J.A., Staines H.J., Watling R., Palfreyman J.W., 2004. Some aspects of interrelations between fungi and other biota in forest soil. *Mycological Research* 108:933-946.

Li X.L., Marschner H., George E., 1991. Acquisition of phosphorus and copper by VA-mycorrhizal hyphae and root-to-shoot transport in white clover. *Plant and Soil* 136:49-57.

Lingua G., D'Agostino G., Massa N., Antosiano M., Berta G., 2002. Mycorrhiza-induced differential response to a yellows disease in tomato. *Mycorrhiza* 12:191-198.

Liu A., Hamel C., Hamilton R.I., Ma B.L., Smith D.L., 2000. Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. *Mycorrhiza* 9:331-336.

Lovelock C.E., Wright S.F., Nichols K.A., 2004b. Using glomalin as an indicator for arbuscular mycorrhizal hyphal growth: an example from a tropical rain forest soil. *Soil Biology and Biochemistry* 36:1009-1012.

Menéndez A.B., Scervino J.M., Godeas A.M., 2001. Arbuscular mycorrhizal populations associated with natural and cultivated vegetation on a site of Buenos Aires province, Argentina. *Biology and Fertility of Soils* 33:373-381.

Miller R.L. and Jackson L.E., 1998. Survey of vesicular-arbuscular mycorrhizae in lettuce production in relation to management and soil factors. *Journal of Soil and Water Conservation* 130:173-182.

Millner P.D. and Wright S.F., 2002. Tools for support of ecological research on arbuscular mycorrhizal fungi. *Symbiosi*s 33:101-123.

Mohammad M.J., Malkawi H.I., Shibli R., 2003. Effects of mycorrhizal fungi and phosphorus fertilization on growth and nutrient uptake of barley grown on soils with different levels of salts. *Journal of Plant Nutrition* 26:125-137.

Newman E.I., Reddell P., 1987. The distribution of mycorrhizas among the families of vascular plants. *New Phytologist* 106:745-751.

Newsham K.K., Fitter A.H., Watkinson A.R., 1995. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends Ecology and Evolution* 10:407-411.

Ngosong C., Jarosch M., Raupp J., Neumann E, Ruess L., 2010. The impact of farming practice on soil microorganisms and arbuscular mycorrhizal fungi: Crop type versus long-term mineral and organic fertilization. *Applied Soil Ecology* 46:134-142

O'Donnell A.G., Seasman M., Macrae A., Waite I., Davies J.T., 2001. Plants and fertilisers as drivers of change in microbial community structure and function in soils. *Plant and Soil* 232:135-145.

Oehl F., Sieverding E., Ineichen K., Mader P., Wiemken A., Boller T., 2009. Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long-term microcosms. *Agriculture, Ecosystems and Environment* 134:257-268.

Oehl F., Sieverding E., Ineichen K., Mader P., Boller T., Wiemken A., 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of central Europe. *Applied Environmental Microbiology* 69:2816-2824.

Oehl F., Sieverding E., Mader P., Dubois D., Ineichen K., Boller T., Wiemken A., 2004. Impact of long-term conventional and organic farming on the diversity or arbuscular mycorrhizal fungi. *Oecologia* 138:574-583.

Phillips J.M., Hayman D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55:158-161.

Pozo M.J., Cordier C., Dumas-Gaudot E., Gianinazzi S., Barea J.M., Azcòn-Aguilar C., 2002. Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to Phytophthora infection in tomato plants. *Journal of Experimental Botany* 53:525-534.

Rillig M.C., Mummey D.L., 2006. Mycorrhizas and soil structure. *New Phytologist* 171:41-53.

Rosier C.L., Hoye A.T., Rillig M.C., 2006. Glomalin-related soil protein: assessment of current detection and quantification tools. *Soil Biology and Biochemistry* 38:2205-2211.

Ryan M.H. and Ash J., 1999. Effects of phosphorus and nitrogen on growth of pasture plants and VAM fungi in SE Australian soils with contrastino fertiliser histories (conventional and biodynamic). *Agriculture, Ecosystems and Environment* 73:51-62.

Ryan M.H., Derrick J.W., Dann P.R., 2004. Grain mineral concentrations and yield of wheat grown under organic and conventional management. *Journal of the Science of Food and Agriculture* 84:207-216.

Ryan M.H., Small D.R., Ash J.E., 2000. Phosphorus controls the level of colonisation by arbuscular mycorrhizal fungi in conventional and biodynamic irrigated dairy pastures. *Australian Journal of Experimental Agriculture* 40:663- 670.

Sanders F.E., Tinker P.B., 1971. Mechanism of absorption of phosphate from soil by Endogone mycorrhizas. *Nature* 233:278-279.

SAS Institute, 2008. SAS/STAT Users' Guide. Release 6.08. Ed. SAS, Cary, NC.

Scagel C.F., 2005. Inoculation with ericoid mycorrhizal fungi alters fertilizer use of highbush blueberry cultivars. *HortScience* 40:786-794.

Schüssler A., Schwarzott D., Walker C., 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* 105:1413-1421.

Shetty K.G., Hetrick B.A.D., Schwab A.P., 1995. Effects of mycorrhizae and fertilizer amendments on zinc tolerance of plants. *Environmental Pollution* 88:307-314.

Sieverding E., 1991. Vesicular-Arbuscular Mycorrhiza management in tropical agroecosystems. Deutshe Gesellschaft Technische Zusammenarbeit (GTZ) GmbH, Eschborn, Germany, 371 pp..

Smith S.E. and Read D.J., 1997. Mycorrhizal Symbiosis. 2nd edn. Academic Press, London

Tisdall J.M., 1991. Fungal hyphae and structural stability of soil. *Australian Journal of Soil Research* 29:729-743.

Treseder K.K. and Allen M.F., 2002. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi, a model and field test. *New Phytologist* 155:507-515.

van Bruggen A.H.C., Semenov A.M., 2000. In search of biological indicators for soil health and disease suppression. *Applied Soil Ecology* 15:13-24.

Withers P.J.A., Edwards A.C., Foy R.H., 2001. Phosphorus cycling in UK agriculture and implications for phosphorus loss from soil. *Soil Use and Management* 17:139-149.

Wright S.F., Franke-Snyder M., Morton J.B., Upadhyaya A., 1996. Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. *Plant and Soil* 181:193-203.

Wright S.F., Green V.S., Cavigelli M.A., 2007. Glomalin in aggregate size classes from three different farming systems. *Soil and Tillage Research* 94:546-549.

Wright S.F., Upadhyaya A., 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil* 198:97-107.

Chapter 7
General conclusions

Intensive agriculture management determined a worsening of soil fertility in farms sited in the Piana of Sele River in Campania Region, in South Italy, where under plastic cover crops were cultivated for long-term,. A clear decrease in organic carbon content and a strong depletion of enzyme activities were mainly detected in the twenty soils monitored in this study. As no organic amendments were provided to these soils since several years, these results confirmed that one of the most relevant causes of soil degradation, in terms of fertility declining, are the loss and the consequent deficiency of organic matter.

In the second phase of the present study an experimental design was set up in order to compare the effects of different organic amendments on soil chemical and biochemical properties of two farms selected among those previously monitored. All the used organic amendments determined positive effects on soil chemical properties, and in particular on the recovery of organic carbon in both studied soils. However, a better response was obtained in the Farm 1, where soil clayey nature contributed to maintain high the achieved OM levels after one year, confirming the determinant role of soil geopedologic properties. Also in the Farm 2, characterized by a sandy soil, a significant OM recovery was observed, but not so stable as in the Farm 1 over time.

Nevertheless it was not possible to discriminate between the organic amendments utilized in this study in terms of better performance neither to establish the more suitable dose inducing the higher advantages to soil properties. The presence of wood scraps in the amendment mixtures certainly contributed to maintain high the organic C content. More recalcitrant materials favoured a slight increase of C/N ratio and, as their decomposition slowly occurred, guaranteed a continuous organic carbon restoring in soils in long-term. Organic amendments modified the enzyme activity of treated soils and the use of enzymes as indicators of the change in soil quality was particularly useful. Through statistical analysis, chemical as well as biochemical

properties of both soils were separated in clusters in according to samplings, indicating a ready and positively effects of organic amendments.

Spectroscopic studies performed on humic and fulvic acids extracted from differently amended soils gave interesting information about the chemical changes occurred in OM upon the treatment. ^{13}C CPMAS NMR analysis showed changes in functional group composition of soil OM. More labile OM fractions, such as carbohydrates, lipids decreased through the experiment, in particular, the faster OM degradation in F2 soils was confirmed. On the other hand, recalcitrant hydrophobic molecules, such as lignin and lignin like compounds, were preserved highlighting the important role of woody fractions mixed in the used amendments in the OM mineralization rate.

The enrichment of amendment mixture with wood scraps was determinant choice, that led to results in disagreement with previous research, in which the OM loss was found in study also by the addition of high compost doses. However it will be very interesting to follow the second year of this ongoing study and the further results.

Finally the study carried out on the relationship between red guano and lupine flour, as organic fertilizers, and indigenous AMF in lettuce plants in a Chilean soil confirmed the positive effects of these amendments on soil biodiversity and, in particular, on AMF development, although the nature of the used organic fertilizers had a crucial role. Among studied lettuces the cultivar Milanesa showed the best performace in terms of growth upon organic fertilization and no negative effects on the indigenous AMF development.

In conclusion, it needs to think about the long-term implications of farming practices and the broad interactions and dynamics of agricultural systems. A key goal is to understand agriculture from an ecological perspective — in terms of nutrient and energy dynamics, and interactions among plants, microorganisms, insects, etc. in agroecosystems — then balance it with economic and social needs. In according to sustainable agriculture it is possible to optimize skills and technology to achieve long-term stability of the agricultural management, environmental protection, and

consumer safety, by minimizing adverse impacts to the immediate and off-farm environments while providing a sustained level of production and soil fertility.

Acknowledgements

Molteplici sono le persone che in una maniera o nell'altra hanno contribuito a questa tesi, con piccoli o grandi aiuti, spesso con parole o suggerimenti, alle volte inconsciamente. Il mio ringraziamento va a tutti loro.

Se è vero che questi sono stati tre anni caratterizzati da eventi speciali e, se alla fine questa tesi in qualche maniera sarà speciale, è grazie alla persona che mi accompagna dall'inizio della mia carriera universitaria, che con i Suoi consigli, le sue meticolose revisioni e l'immancabile appoggio scientifico e morale è stata la tutor di questo percorso scientifico. Un sentito ringraziamento alla prof. Maria A. Rao.

Un ringraziamento va anche alla prof. Liliana Gianfreda, è anche grazie al suo aiuto se questa tesi è giunta al termine, ma soprattutto è grazie a Lei che ho vissuto la mia splendida esperienza in Cile. Parlando di Cile, non posso non ricordare la prof. Maria de la Luz Mora Gil, coordinatrice di questo dottorato in Cile, a cui va il mio grazie per l'accoglienza e la disponibilità dimostrate.

Se il fantastico mondo delle micorrize è entrato a far parte dei miei studi di dottorato è grazie al prof. Fernando Borie e al dr. Pablo Cornejo Rivas, che mi hanno sempre supportato ed aiutato durante le mie ricerche presso il Departamento de Ciencias Químicas, Universidad de la Frontera, facendomi sentire come a casa. Cosi come Sebastian, Alex, Gustavo e Maria José, che oltre che ad essere compagni di lavoro, sono stati i migliori compagni di vita che avrei potuto trovare dall'altra parte del mondo.

Gli studi del mio dottorato mi hanno condotto in ambiti della ricerca del tutto nuovi, come quelli della risonanza magnetica nucleare. Se oggi ho ampliato le mie conoscenze sull'NMR e la rilassometria e questi argomenti sono entrati a far parte di questo lavoro, è grazie all'aiuto insostituibile del prof. Pellegrino Conte e della dr. Anne E. Berns, grazie sinceramente.

Come le opere di una certa importanza, anche questa ha visto partecipi un gran numero di persone, senza le quali il risultato non sarebbe stato sicuramente lo stesso, perché quando i grandi cadono, tocca ai piccoli guidare. Allora non posso

dimenticare la dr. Scelza, prima amica e poi compagna di lavoro, senza la quale le mie ore trascorse in laboratorio sarebbero state sicuramente più vuote, grazie per il tuo in mancabile appoggio.

Se parlo di laboratorio, non posso tralasciare tutte quelle persone che rendono il DiSSPAPA unico per me, trasformando le noiose ore lavorative, in momenti unici. Grazie, allora, a Barbara, Bianca, e Pietro, che mi hanno accompagnato oltre che nella vita lavorativa, anche e soprattutto nella vita al di fuori del laboratorio, spero sinceramente che tutto questo non termini mai.

Come non ringraziare Massimo, per il suo in mancabile supporto abitativo, e per le bellissime ore trascorse insieme, ma soprattutto come non ringraziare Imma e Barbara, le mie compagne di vita per quasi sei mesi, mai avrei immaginato di poter trascorrere esperienza più entusiasmante con voi, grazie per avermi supportato e "sopportato" nei momenti più difficili.

Parte dei risultati prodotti in questo lavoro sono stati resi possibili da persone che con semplici consigli o parole mi hanno aiutato, tra queste non posso dimenticare Lina e Anna, che hanno partecipato a pieno nel mio lavoro di ricerca, a Diana, Donato e Riccardo, che mi hanno aiutato nei momenti più complessi di questi studi.

Ci sono persone per le quali la ricerca è passione, che è affascinante sentire parlare e che mi hanno contagiato con la loro passione per la ricerca, dandomi sempre nuovi motivi per andare avanti e nuove idee per raggiungere risultati sempre più alti, grazie a tutti Voi.

Per ultima, ma non per importanza, la mia famiglia, che mi ha sempre supportato nelle mie scelte e mi ha sempre dato una spalla a cui appoggiarmi nei momenti più difficili, grazie di esistere.

Se è vero che il mio dottorato qui si conclude, si chiude un cerchio che ho iniziato a tracciare tre anni fa, in questi tre anni mille eventi, positivi e negativi, si sono susseguiti, è difficile trovare l'inizio e la fine di tutto questo, ma spero sinceramente che possa essere l'inizio di una nuova avventura, perché solo attraverso le difficoltà si possono raggiungere grandi traguardi, "Per Aspera Ad Astra".

In conclusione, se ho dimenticato qualcuno in questo lungo elenco di ringraziamenti, che non se la prenda a male, perché "Cento volte al giorno ricordo a me stesso che la mia vita interiore e esteriore sono basate sulle fatiche di altri uomini, vivi e morti, e che io devo sforzarmi al massimo per dare nella stessa misura in cui ho ricevuto." (A. Einstein).