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Organic fertilization of peach trees: implication on nitrogen availability, root growth and carbon distribution within plant

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ABSTRACT

In the last years, sustainable horticulture has been increasing; however, to be successful this practice needs an efficient soil fertility management to maintain a high productivity and fruit quality standards. For this purpose composted organic materials from agri-food industry and municipal solid waste has been used as a source to replace chemical fertilizers and increase soil organic matter. To better understand the influence of compost application on soil fertility and plant growth, we carried out a study comparing organic and mineral nitrogen (N) fertilization in micro propagated plants, potted trees and commercial peach orchard with these aims: 1. evaluation of tree development, CO_2 fixation and carbon partition to the different organs of two-years-old potted peach trees. 2. Determination of soil N concentration and nitrate-N effect on plant growth and root oxidative stress of micro propagated plant after increasing rates of N applications. 3. Assessment of soil chemical and biological fertility, tree growth and yield and fruit quality in a commercial orchard. The addition of compost at high rate was effective in increasing CO_2 fixation, promoting root growth, shoot and fruit biomass. Furthermore, organic fertilizers influenced C partitioning, favoring C accumulation in roots, wood and fruits. The higher CO_2 fixation was the result of a larger tree leaf area, rather than an increase in leaf photosynthetic efficiency, showing a stimulation of plant growth by application of compost. High concentrations of compost increased total soil N concentration, but were not effective in increasing nitrate-N soil concentration; in contrast mineral-N applications increased linearly soil nitrate-N, even at the lowest rate tested. Soil nitrate-N concentration influenced positively plant growth at low rate (60- 80 mg kg^{-1}), whereas at high concentrations showed negative effects. In this trial, the decrease of root growth, as a response to excessive nitrate-N soil concentration, was not anticipated by root oxidative stress. Continuous annual applications of compost for 10 years enhanced soil organic matter content and total soil N concentration. Additionally, high rate of compost application (10 t ha^{-1} $year^{-1}$) enhanced microbial biomass. On the other hand, different fertilizers management did not modify tree yield, but influenced fruit size and precocity index. The present data support the idea that organic fertilizers can be used successfully as a substitute of mineral fertilizers in fruit tree nutrient management, since they promote an increase of soil chemical and biological fertility, prevent excessive nitrate-N soil concentration, promote plant growth and potentially C sequestration into the soil.

CONTENTS

1. INTRODUCTION

World annual fertilizer consumption steadily rose from 30 million t in 1960 to 143 million t in 1990, arriving up to 170 million in the past few years (Taiz and Zeiger, 2010). Global nitrogen (N) fertilizer application rate increased almost twenty fold in the last 50 years, reaching almost 100 million t per year; though, an important portion of this is lost under field conditions (Glass, 2003). In fact, global estimate indicates that nearly 50% of N fertilizer applied is removed by crops, 2 to 5% is stored in the soil and the residual 45-48% has a negative effect on the environment, by being leached to aquatic systems or emitted into the atmosphere (Galloway et al., 2004). The excessive use of mineral fertilizers and the development of intensive agricultural practices have contributed to reduce organic matter (OM) concentration in most Mediterranean soils, leading to increased risk of erosion and fertility losses (Melero et al., 2007). In many Italian soils the OM concentration is lower than 1.5% (Ungaro et al., 2002).

Recently, sustainable agriculture is increasing; however, to be successful this practice needs the increasing of environment friendless practices implies an efficient soil fertility management, because soil quality determines the sustainability and productivity of agroecosystems (Prasad and Power, 1997; Melero et al., 2007).

1.1. Soil Organic Matter

Soil OM plays a vital role on the properties of soils and represents the greatest terrestrial reservoir in the global carbon (C) cycle. It contains approximately 1500 Gt of organic C that is twice the amount present in the atmosphere (Oades, 1995; Amundson, 2001)

1.1.1. Organic Components

Soil OM includes a wide variety of microorganisms, animals and plant tissues in different stages of decomposition (Dell'Agnola et al., 1993; Wolf and Snyder, 2003). According to the decomposition stages, soil OM can be divided into two groups: the first one, called litter, usually lies on soil surface, consists in degraded materials in which the anatomy of the plant substance is still visible, and has major effects in soil physics characteristics (e.g. affecting soil structure, decreasing bulk density, etc.). The second one, a complex known as humus, includes a major portion of soil OM, consists of completely decomposed and unrecognizable materials that play major roles in physicochemical properties of soils, like soil aggregation, aggregate stability and cation exchange capacity (CEC) (Bot and Benites, 2005;

Tan, 2011). Humus is a complex mixture of dark-brown, amorphous and colloidal substances modified from the original plant material or synthesized by various soil organisms (Prasad and Power, 1997). Although more stable than the organic materials from which it is derived, humus is transitory in nature, easily degraded by oxidation and broken down by soil microorganisms that use it as food and energy source (Wolf and Snyder, 2003; Tan, 2011). Humus has different properties such as complex formation with clay or other silicate surfaces, storage and release of soil N, buffer capacity, anion and CEC, adsorption of pesticides and other agricultural chemicals (Prasad and Power, 1997). Humus consists of non-humic and humic substances, the latter being the major part (table 1.1). The non-humic compounds are proteins, amino acids, starch and sugars that are directly released from fresh residues cells; usually this portion of humus is strongly influenced by weather conditions, soil moisture, growth stage of vegetation and addition of organic residues, thus it is easily decomposed (Bot and Benites, 2005).

Table 1.1. Percentage of the different components of humus (Stevenson and Cole, 1999).

Type of material	**Usual range (% by weight)**
Non-humic substances	
Lipids	1 – 6
Carbohydrates	5 – 25
Proteins/peptides/amino acids	9 – 16
Other	trace
Humic substances	up to 80

On the other hand, humic substance consists of a series of highly acid, yellow to black colored polyelectrolytes (Stevenson and Cole, 1999), abundant in carboxyl groups, with weak acidic phenolic groups, free-radicals that can fix small molecules by both hydrogen bounding and nonpolar interactions (MacCarthy, 2001). Humic substances exhibit hydrophobic and hydrophilic characteristics (MacCarthy, 2001) that confers them the ability to form complexes with metal ions, oxides, hydroxides, mineral and organic compounds, thereby affecting nutrient availability (Bot and Benites, 2005). Based on their solubility, the humic compounds are classified into three groups: fulvic acids, humic acids and humin.

Fulvic acids are soluble in water under all pH conditions; they have the lowest molecular weight and a common light yellow to yellow-brown color. They are produced in the earlier stages of humus formation, with high oxygen (O_2) and low hydrogen (H) and N

content, and are easily attacked by microorganisms (Wolf and Snyder, 2003; Bot and Benites, 2005; Tan, 2011). Humic acids are soluble in water with a pH > 2; they have a medium molecular weight and their common color is dark brown to black; they have high C and N content and are semi-resistant to microbial action (Bot and Benites, 2005; Tan, 2011). Humin is not soluble in water at any pH; is commonly black and has the highest molecular weight. It is inert and the most resistant to decomposition (Wolf and Snyder, 2003; Bot and Benites, 2005).

1.1.2. Factors affecting the organic matter content of soil

The OM concentration in the soil depends on inputs and mineralization rate. Plants are the main source of OM, that is supplied as leaves, other plant residues, and rhizodeposition (Dell'Agnola et al., 1993). Rhizodeposition is considered the largest source of OM because it can be physically protected by soil clay matrix and also because it consists mainly of roots that have a resistance to mineralization due to the great concentration of lignin and phenolic compounds (Oades, 1995; Bolinder et al., 1999; Wolf and Snyder, 2003). The continuing addition of plant residues to the soil surface enhances the biological activity of soil and C cycling processes in the soil (figure 1.1).

The rate of accumulation and mineralization of organic compounds depends on many factors, such as climate, parental material, topographic position, biota and human activity (Amundson, 2001; Trumbore, 2009).

Temperature and precipitation are the most significant factors (Prasad and Power, 1997) that affect soil OM. Temperature is a key factor controlling the rate of decomposition since it affects the activity of soil micro-organisms, that present a $Q_{10} \geq 2$ (Amundson, 2001).

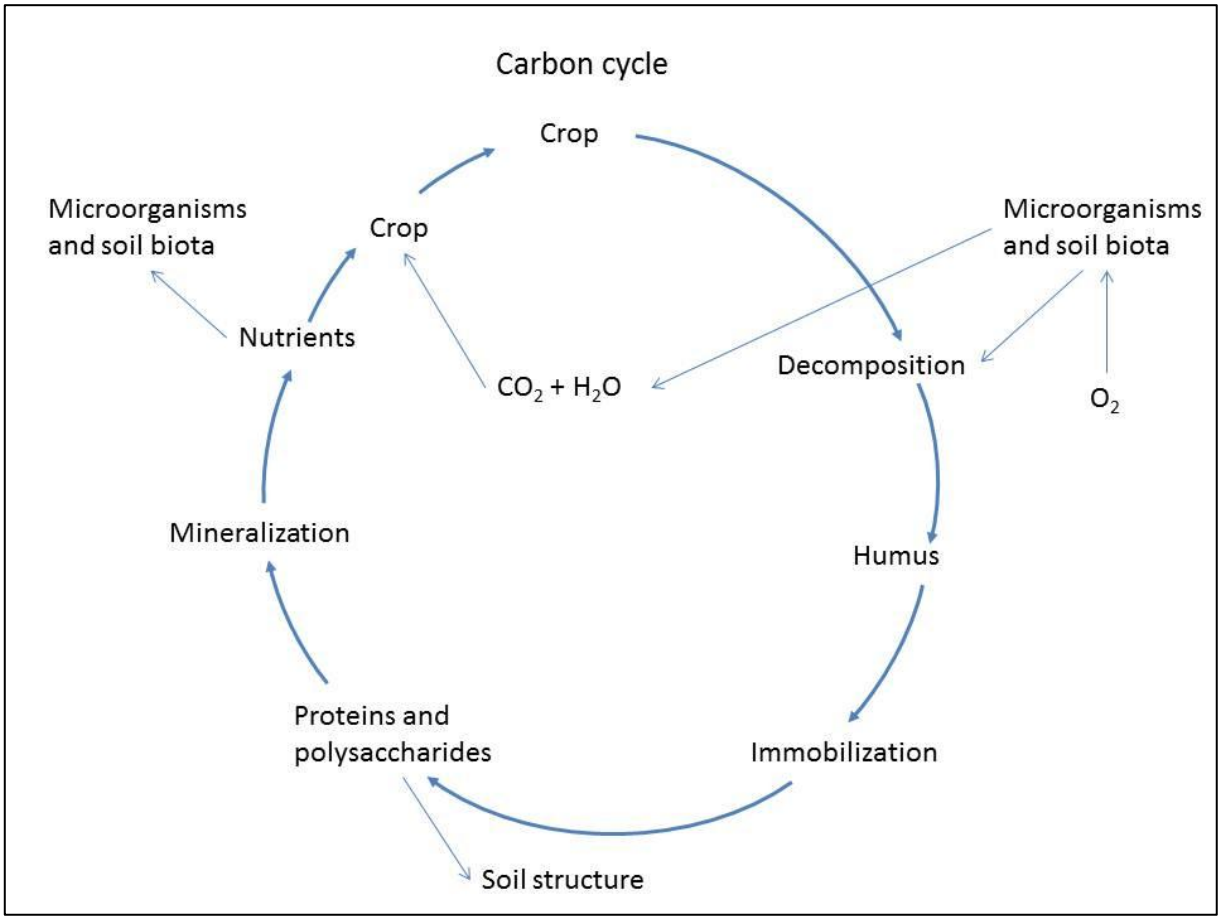

Figure 1.1 Soil carbon cycling process (Bot and Benites, 2005).

Soils in cooler climates commonly present a higher concentration of OM (figure 1.2) because of the reduced microbial activity for most of the year; in contrast, in tropical climate, decomposition occurs rapidly (Wolf and Snyder, 2003; Bot and Benites, 2005). Precipitations play an important role in soil OM accumulation, which increases with mean annual precipitation (figure 1.2) (Bot and Benites, 2005). Intermediate ranges of moisture (close to field capacity) stimulate soil OM decomposition; on the other hand, water saturation leads to anaerobic conditions that increases soil OM residence times (Amundson, 2001).

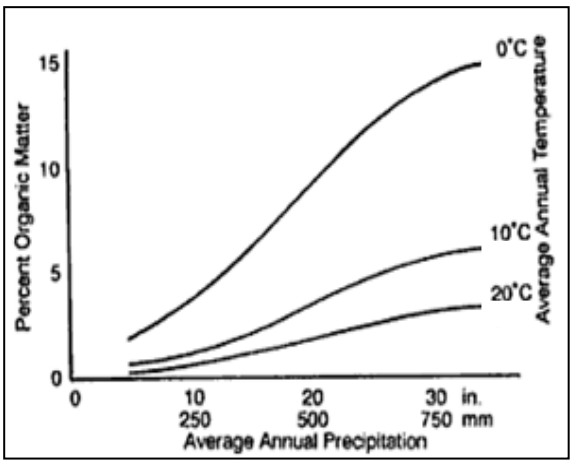

Figure 1.2 Typical trend of organic matter accumulation in the A horizons of grassland soils as influenced by average precipitation and temperature (Troeh and Thompson, 2005).

Topography produces a strong effect on the amount of organic matter in the soil by modifying the microclimate

and vegetation; it also affects water and soil movement (Troeh and Thompson, 2005). Organic matter usually accumulates at the bottom of hills, because of the higher moisture than in the mid or upper slope positions and also because OM is transported to the lowest point through runoff and erosion (Bot and Benites, 2005).

Parental material influences OM accumulation in soil, mainly by affecting soil texture. Sandy soils permit more rapid decomposition than finer-textured soils, because they are usually warmer and better aerated. In contrast, soil clay content is positively correlated to the organic content (Oades, 1988; Troeh and Thompson, 2005), because the clay fractions protect soil OM from microbial action by making aggregates that adsorb OM on their surfaces (Wolf and Snyder, 2003; Bot and Benites, 2005).

Recent estimate assesses that agricultural practices reduce OM accumulation in soils decreasing the original soil C content of about 30% (Amundson, 2001). Tillage, for example, disturbs soil aggregates and exposes OM to decomposition; tillage also rises soil temperature and consequently microbial activity (Amundson, 2001). On the other hand, the use of fertilizer, especially N, and pesticides can increase microorganisms activity and enhances decomposition of soil OM (Bot and Benites, 2005).

1.1.3. Effect of OM on soil properties

Soil OM influences chemical, physical and biological properties of soils; thereby it is imperative to keep it at optimal content in order to have a healthy soil.

1.1.3.1. Physical properties

Physical properties, especially soil structure, are closely related to the amount and quality of soil OM. Soil structure describes the manner in which soil particles are arranged into larger units; this property affects soil aeration and water infiltration, thus affecting root growth (De Nobili and Maggioni, 1993; Wolf and Snyder, 2003). Soil OM improves soil structure and consequently enhances water infiltration and increases air porosity, allowing better movement of water and air through the soil. Organic matter also reduces soil bulk density, because it is much lighter than a similar volume of other soil components, and it also increases aggregate stability that benefits pore space (Wolf and Snyder, 2003).

Additionally OM can retain a larger amount of water, thus affecting soil temperature; in fact, the soil gets warm and cool slower with elevated water content. Soil temperature is also affected by soil color, because it affects the solar energy absorption. The dark color of OM increases soil temperature (De Nobili and Maggioni, 1993).

1.1.3.2. Biological properties

Soil OM influences the number and kinds of organisms (microfauna and microflora) that are present in a soil (Wolf and Snyder, 2003). Organic matter plays a key role in the protection of soil enzyme activity, which has an ephemeral existence if they do not find a suitable organic substrate (Sequi and Nannipieri, 1989; Pérez-Piqueres et al., 2006; Hargreaves et al., 2008). Enzymes such as urease and phosphatase, for example, take advantage from the presence of OM and make it possible reactions of hydrolysis even under unfavorable conditions for microbial life by preventing the leaching of N and phosphorus (P) (Perucci, 1992; Giusquiami et al., 1995). Several authors have observed that after application of OM, soil enzyme activity increased, along with microbial activity (García-Gil et al., 2000; Lee et al., 2004; Böhme et al., 2005). On the other hand, OM soil applications as compost can influence microbial community composition and enhance the competition and/or antagonism among microbes, leading to a decrease of plant pathogens (Pérez-Piqueres et al., 2006).

1.1.3.3. Chemical properties

Soil OM plays an important role in soil pH neutralization especially in sub alkaline soils, where OM decomposition produces acid compounds that decrease pH. Moreover, it affects plant nutrition directly or indirectly; in the first case OM increases the availability of nutrient through mineralization by enhancing microorganisms activity (De Nobili and Maggioni, 1993; Bot and Benites, 2005). The indirect effect is related to the production of chelates, which are substances that bound several metallic elements, such as iron (Fe), copper (Cu) and zinc (Zn), making them available over a wide ranges of pH. Moreover soil OM reduces the immobilization of P, aluminium (Al), Fe and manganese (Mg), leaving them available for plant uptake (Wolf and Snyder, 2003).

On the other hand, OM increases CEC of soil that is the capacity of soils to adsorb and exchange cations (Tan, 2011). An elevated CEC in the soil increases the retention capability of cations such as ammonium (NH_4^+), potassium (K^+), calcium (Ca^{++}) and magnesium (Mg^{++}), increasing the mineral reservoir of nutrients available for plant growth (De Nobili and Maggioni, 1993; Wolf and Snyder, 2003).

1.1.4. Organic fertilization

The accumulation of OM in the soil is a slow process, it may take from decades to centuries; however, its degradation is very fast, due mainly to human activity. According to the European Commission (*COM2006/231)* "the decrease in organic matter content in soils is considered a threat and an element of land degradation"; moreover, the Kyoto protocol indicates that soil plays an important role in the storage of C, so that it is necessary to protect soil OM and increase its content.

In the Mediterranean climate, the high summer temperatures along with the intensive cultivation of land are responsible for an elevated consumption of soil OM through a high annual mineralization rate (Perucci et al., 1997).

Due to continuous decomposition in cultivated soils, it is necessary to restore adequate level of OM. A way to supply OM into agricultural systems is the addition of exogenous organic material, such as manure, compost and peats, biosolids, etc.

The use of fresh, immature organic matter should be used carefully because it competes with roots for O_2 (Sweeten and Auvermann, 2008), may reduce soil mineral N availability due an intense microbial activity (De Nobili, 1999; Micciulla and Benedetti 1999), and might release compounds toxic for plants such as acetic acid (Sweeten and Auvermann, 2008). Thus, organic amendments must be stabilized through a composting process before their application.

1.1.4.1. Manure

Manure is organic matter deriving from animal faeces and urine usually mixed with plant material (such as wheat straw), which has been used as bedding for animal. Common forms of animal manure include farmyard manure (FYM) or farm slurry (liquid manure). Agricultural manure in liquid form, known as slurry, is produced by more intensive livestock rearing systems, where concrete or slats are used, instead of straw bedding. Animal wastes vary in chemical composition, physical form and quantities produced and the major factors affecting this variability are 1) the digestive physiology of animal species; 2) the composition and form of the diet; 3) the stage of growth and productivity of the animal; and 4) the management system of waste collection and storage (Azevedo and Stout, 1974). The waste from different species has different physical characteristics, for example, sheep, equine and poultry waste contains less moisture than waste from dairy, beef and swine due to differences in the physiological mechanisms for water retention and excretion (table 1.2).

Table 1.2. Nitrogen, P, K and Ca composition of different manures (Fontenot et al., 1983).

Animal manure	**Dry matter (%)**	**N (% D.W.)**	**P (% D.W.)**	**K (% D.W.)**	**Ca (% D.W.)**
Poultry	25	4.4	1.7	1.9	1.9
Swine	9	5.2	1.5	3.2	2.0
Beef	12	2.0	0.4	1.2	1.1
Dairy	14	2.5	0.6	2.4	1.5
Sheep	26	4.4	0.6	3.0	1.7

Particle-size distribution of fresh manure depends on the diet and digestion process of the animal. Particle density increases as waste degradation develops during storage and increased settling of solids occurs in dilute slurries, particularly with wastes containing high ash contents. The relatively higher fibre content in waste from ruminants, higher N content of poultry and swine waste, lower P content in waste from ruminants and higher Ca content of cage layer waste are common illustrations. Depending upon the duration and method of storage, the chemical composition of waste can change considerably during storage. Microbial decomposition of the waste occurs with the partial biodegradation of OM and the transformation of nutrients into a less complex organic and/or inorganic form. Nutrient losses during storage can result from leaching and runoff in open lot systems or from volatilization. In general, pH in waste becomes more acidic in anaerobic pits, whereas, the pH of waste becomes more alkaline in dilute anaerobic lagoons and aerobic treatment systems (Fontenot et al., 1983).

Normally, 75-90% of major nutrients that are fed to livestock pass directly to the animal into the manure. How good these nutrients can be returned to the soil, depends on the way the manure is stored and handled. There can be huge loss (24 - 83%) of NH_4^+ as a result of drying manure and ammonia (NH_3) volatilisation (Fontenot et al., 1983).

Several authors indicated that manure applications enhanced soil properties such as porosity and hydraulic conductivity (Wong et al., 1999), decreased bulk density (Wong et al., 1999; Celik et al., 2004) and decreased heavy metal bioavailability (Walker et al., 2004). They also increased total OM, macro and micro nutrients availability (Wong et al., 1999; Celik et al., 2004) and crop dry weight yields (Wong et al., 1999).

1.1.4.2. Compost

Compost is the product of decomposition of organic substance made by bacteria, fungi and actinomycetes (Ruol and Santon, 2009), with a final formation of new compounds (humo-like and humic substances). The controlled biological decomposition of organic material leads to the production of stabilized products that can be used as fertilizer. Compost can be obtained from different materials, such as wastes from fruit and vegetable processing, winery industry as well as municipal solid waste (MSW), and the wastes from the management of park and urban green areas. The chemical composition of compost is related to the starting material (table 1.3; Wolf and Snyder, 2003); predominance of vegetal fraction leads to a higher C:N ratio compared with a compost obtained mainly from animal manure. According to this characteristic it is possible to define the final use of compost. Organic materials from agro-industry are, for example, potentially organic fertilizers with slow N release rate that allow a complete 'nutrient cycling': the breakdown of organic substances, the release of energy and matter captured by life processes and their use to stimulate the new growth.

Table 1.3. Selected chemical characteristics of a compost (Wolf and Snyder, 2003).

Test	**Range of analyses**
C:N	6:1 – 20:1
pH	5 – 8
Electric Conductivity	0.2 – 2 S/m
Total N	0.5 – 3 %
P	0.1 – 2.0 %
K	0.2 – 1.0 %
Ca	0.8 – 3.5 %
Mg	0.3 – 0.6 %
S	0.1 – 2.0 %

Composting is a natural aerobic biological process, during which micro-organisms degrade organic compounds drawing energy for their metabolic activities and producing H_2O, carbon dioxide (CO_2), NH_3, minerals, OM stabilized rich in humus (Sweeten and Auvermann, 2008; Sadik et al., 2010) and heat (temperature should be stabilized around 65°C). The purpose of the composting process is to obtain a stable material (not phytotoxic) used as

agricultural fertilizer. The stabilization process lasts around 90 days and at the end of the stabilization process, the initial weight is reduced by more than 50% and O_2 consumption and release are in equilibrium. Composting process is affected by several factors that alter microorganism activity, such as moisture, aeration, temperature, nutrient balance and pH (Sweeten and Auvermann, 2008). Moisture is an essential factor for microorganism survival and growth because it influences O_2 availability; several authors indicate diverse optimal ranges that goes from 40 to 70% (Wolf and Snyder, 2003; Sweeten and Auvermann, 2008), with low values decomposition rate slows down, whereas with high moisture content, the time required for compost to stabilize decrease and eventually anaerobic conditions occur (Sweeten and Auvermann, 2008). Thermophilic microorganisms that develop in a range between 37 and 70°C predominate in the composting process (Wolf and Snyder, 2003; Sadik et al., 2010). Under stable moisture and O_2 conditions, microorganism activity rises with temperature (Wolf and Snyder, 2003). The C:N ratio affects the biological activity rate; values between 20 and 30 are optimal. Higher values decrease the decomposition rate while lower levels result in high degradation rates, with NH_3 losses to the atmosphere (Sweeten and Auvermann, 2008; Sadik et al., 2010).

Compost is rich in humo-like substances, with nutrients, high physical properties, hygienically safe and free of viable weed seeds (Sweeten and Auvermann, 2008). The mature compost is dark in color, soft to the touch, with a temperature equal to or slightly greater than that of the external environment. It is important to use in field mature compost because poorly stabilized ones have problems during storage, marketing and use. In storage, immature compost can produce bad odors and develop toxic compounds and it may heat up in pallets during shipment. Continued active decomposition in the soil could reduce O_2 and availability of N in the root zone causing problem to plant growth. Mature compost should have finished the process of composting and should show the minimal negative effects on plant growth. As maturity can not be defined by a single parameter, there are several characteristics that can be taken into consideration for evaluating compost maturity (table 1.4). After the stabilization process, compost can be subjected to refining, crushing, dehydration, etc. in order to make it marketable (Cristoforetti, 1997).

Table 1.4. Possible system and parameters to classify compost maturity (Brinton, 2000).

	Very mature	**Mature**	**Immature**
Characteristics	Compost with no decomposition and no potential toxicity	Compost with little odor production, limited toxicity and minimal impact on soil N	Compost with intense odor production and high toxicity potential
Method			
O_2 uptake (O_2 hr^{-1})	< 0.5	0.5 – 1.5	> 1.5
CO_2 (C hr^{-1})	< 2	2-8	> 8
NH_4/NO_3 (N ratio)	< 0.5	0.5 – 3	> 3
Total NH_3 (ppm N)	< 75	75 - 500	> 500
Seed germination (% of control)	> 90	80-90	< 80

The technical advisory committee for Italian fertilizers identified three types of compost (Zorzi, 1997):

- **green compost:** product obtained through a process of transformation and stabilization of waste from maintenance of ornamental plants, crop residues and other wastes of plant origin, with the exception of algae and other marine plants;
- **mixed compost:** a product obtained through a process of transformation and stabilization of the organic fraction of municipal solid waste from waste collection, waste from animals including manure, residues of agro-industrial activities, sewage and sludge, and by the starting materials required for the green compost;
- **peaty compost:** product obtained by mixture of peat (> 30%) with green or mixed compost.

The concern related to compost use in agriculture is the potential increase of soil concentration of nitrate-N (NO_3^--N) and that can be easily leached through the soil profile. In addition, heavy metals such as lead (Pb), cadmium (Cd), Cu, Zn, etc., and various persistent organic toxins (Giusquiami et al., 1995), can be added to the soil with low quality compost application. Heavy metal limits have been defined in order to reduce soil pollution even if there are still differences among European countries (table 1.5).

Table 1.5 Heavy metal limits (mg kg^{-1} D.W.) for European countries and USA and normal concentration ranges for European soils (Brinton, 2000).

Element	Italy	Germany	France	Spain	USA	Normal soil concentration
Cadmium	1.5	1.5	8	40	39	0.3-0.7
Chromium	100	100	-	750	1200	5-100
Copper	300	100	-	1750	1500	3-20
Lead	140	150	800	1200	300	12-100
Mercury	1.5	1.0	8	25	17	0.05-0.4
Nickel	50	50	200	400	420	4-50
Zinc	500	400	-	4000	2800	14-125

Beside heavy metal there are other parameters for compost standards as for example the presence of non-organic matter (glass, plastic and metal) and stones (table 1.6).

Other important factors that should be taken into consideration in order to obtain a high quality product are pesticide, herbicide, weed, and salt content. Moreover, biological parameter (table 1.7) has to be carefully controlled to have safe compost.

Table 1.6 Maximum non-organic particles allowed in compost in various national standards (Brinton, 2000).

Country	Stone (% D.W.)	Non-organic matter (D.W.)
Australia	< 5% of > 5 mm size	< 0.5% of > 2 mm fraction
France	-	Max contamination 20%; < 6% of >5 mm fraction
Germany	< 5% of > 5 mm size	< 0.5% of > 2 mm fraction
Italy	-	< 3% total
Spain	-	Free of contamination
U.K.	< 5% of > 2 mm size	< 1% of > 2 mm; < 0.5% if plastic

Table 1.7 Biological parameter for a safe compost in Italy.

BIOLOGICAL PARAMETER	
Salmonella	No detect in 25 g
Total enterobacteriaceae	< 1 x 10^2 CFU
Faecal streptococci	< 1 x 10^3 (MPN g^{-1})
Nematodes	absent in 50 g t F.W.
Cestodes	absent in 50 g t F.W.

By incorporating recycled OM into the soil, a sequestration of C, that otherwise would follow disposal processes which potentially release CO_2 in the atmosphere, occurs.

In a 9-years-long trial, yearly application of 5 and 10 t ha^{-1} of compost made of municipal solid wastes, mixed with pruning material, from urban ornamental trees and waste material from agro-industry processes increased soil OM content (from 1.6 to 4.5%), total N, P, K and microbial biomass (Baldi et al., 2010). Similar results were observed by Herencia et al. (2007) in a greenhouse experience after 9-years of vegetal compost fertilization. In addition, several studies have demonstrated that compost application at high rates improved soil physical and chemical properties, such as bulk density, porosity, water holding capacity (Evanylo et al., 2008), microbial biomass C and enzymatic activity (Melero et al., 2007).

1.2. Nitrogen

Nitrogen is considered one of the main factors limiting plant growth. In fact, N is the most important element of plant composition after C, H, and O_2 (Touraine et al., 2001; Boukcim et al., 2006; Lea and Azevedo, 2006). Nitrogen is part of many plant cell components, such as amino acids, proteins, nucleic acids, chlorophyll and growth regulators (Below, 2002; Taiz and Zeiger, 2010). In the biosphere N is available as elemental di-nitrogen (N_2) gas, volatile NH_3 and N oxides (NO_x) in the atmosphere; or as organic (amino acids, peptides, etc.) and inorganic N (nitrate and ammonium) in the soil (von Wiren et al., 1997). The required plant-N concentration for optimal growth varies between 2 and 5% of the plant dry weight (Marschner, 1995).

A major part of plant N is acquired from the soil, where NO_3^- and NH_4^+ are the major sources; however, available soil-N supplies are often inadequate in agricultural soil (Novoa and Loomis, 1981; Marschner, 1995; Tischner, 2000; Follett, 2001), as a result, addition of N from chemical fertilizers is usually required to optimize plant growth.

1.2.1. Nitrogen cycle

Nitrogen is present in vast quantities in the atmosphere as N_2 representing 79% of dry air; however, N_2 does not impact environmental quality and directly available N for plant uptake and metabolism (Follett, 2001; Robertson and Vitousek, 2009). Acquisition of N from the atmosphere requires the breaking of an exceptionally stable triple covalent bound that can be possible through biological nitrogen fixation (BNF) or industrial nitrogen fixation. The BNF is catalysed by the metalloenzyme nitrogenase and consist in the reduction of N_2 to NH_3; there are few living microorganisms, symbiotic and non-symbiotic capable of this process, with those of the genus *Rhizobium spp.* that lives in symbiosis with legumes (Schulten and Schnitzer, 1998; Follet, 2001; Violante, 2005), the most important for agriculture. Industrially, N fixation occurs via the Haber-Bosch process, in which natural gas methane (CH_4) is burned to produce H, which then reacts with N_2 under high temperature and very high pressure. The quantity of N_2 fixated industrially is three or four times lower than BNF, which is about 17.2×10^7 t $year^{-1}$ (Violante, 2005; Robertson and Vitousek, 2009).

Nitrogen from microorganism fixation and decomposition of animal and plant residues becomes part of soil N (figure 1.3), which represents only a small fraction of total N on Earth. More than 90% of total soil N is contained in OM, and can be divided into two groups. The first one is composed by small molecules such as amino acids, nucleic acids and amino sugars, which are present in soil solution (dissolved organic N) in just a little quantity. The second group is relatively stable and not directly available for plants because the N is associated to larger, insoluble molecules or complexes. The availability of N from organic source depends mainly on the mineralization process, which is defined as the production of NH_4^+ from organic N (Follett, 2001; Myrold and Bottomley, 2008).

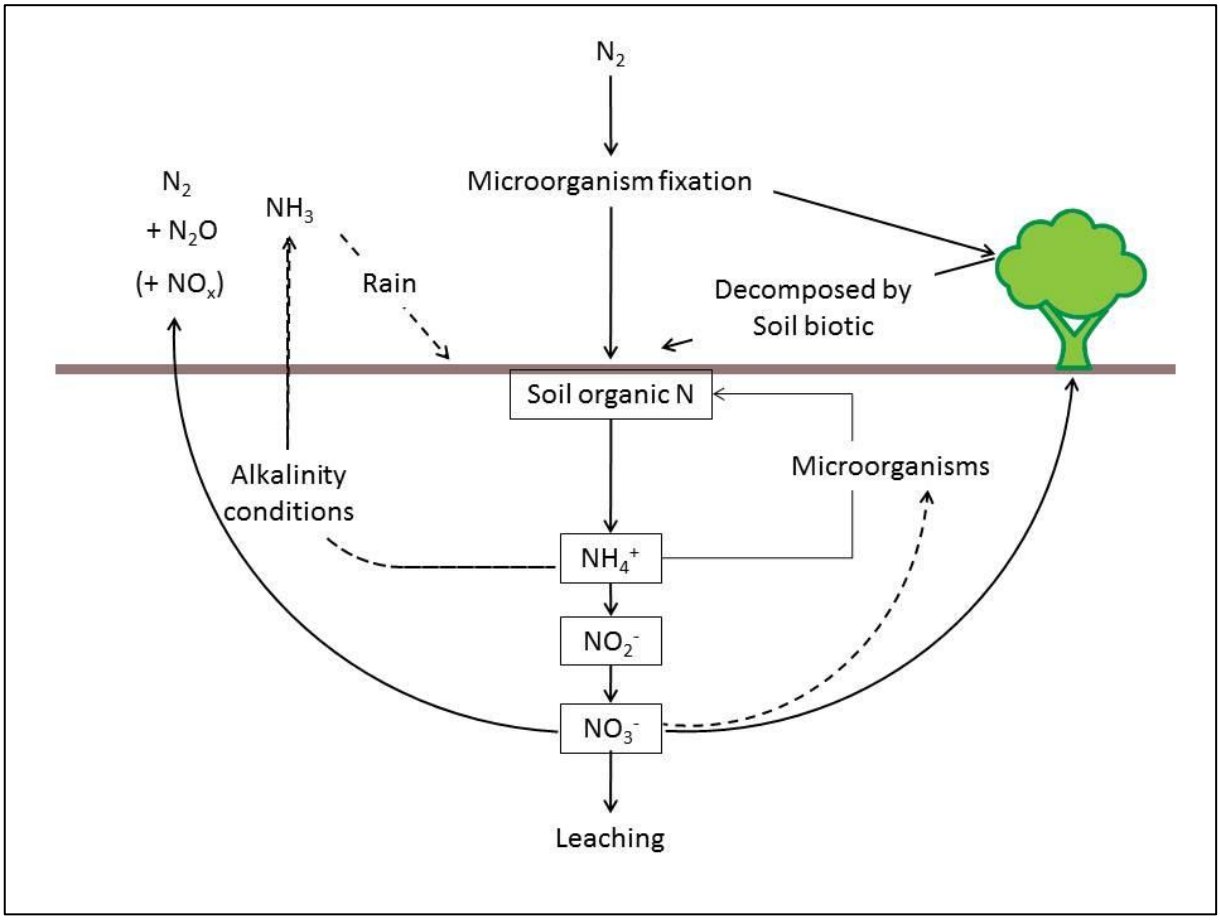

Figure 1.3 Nitrogen cycle in the soil-plant-atmosphere system. (Adapted from Violante, 2005).

Mineralization is a very slow process, mediated by heterotrophic microorganisms, which break down organic monomers and release NH_4^+. This process is affected by soil conditions, such as moisture and temperature; and also by C:N ratio (Wolf and Snyder, 2003; Agehara and Warncke, 2005; Myrold and Bottomley, 2008). Soil moisture regulates O_2 in soil and maximizes aerobic microbial activity with 50 to 70% of water holding capacity, therefore increasing mineralization rate. Cool soil temperatures inhibit N release from the soil OM (Wolf and Snyder, 2003; Agehara and Warncke, 2005). Low C:N substrate ratios (about 25:1) enhances NH_3 release; in contrast, greater C:N ratios are associated with immobilization of NH_4^+, process by which inorganic N is incorporated in organic forms (Sims, 1995; Schulten and Schnitzer, 1998; Myrold and Bottomley, 2008). Moreover, plant roots play an indirect role in N mineralization process by releasing root exudates which are potential sources of C and N of the rhizosphere, and by altering soil structure or water availability (Myrold and Bottomley, 2008).

The NH_4^+ released by mineralization process is oxidized to NO_3^-, using nitrite (NO_2^-) as an intermediate form, by a process called nitrification (Follet, 2001). This process is mediated by *Nitrosomonas sp.* and *Nitrobacter sp.* bacteria, the first transforms NH_4^+ to NO_2^-, and the second one converts NO_2^- to NO_3^- (Postgate, 1998). Several microorganisms uses NO_3^- and NO_2^- as O_2 source, transforming them in either N_2, nitrous oxide (N_2O), nitric oxide (NO) or other gaseous N oxide compounds through the denitrification process (Follet, 2001; Violante, 2005; Robertson and Vitousek, 2009).

1.2.2. Plant N nutrition and N accumulation in plants

Under agricultural soil conditions, NO_3^- is more abundant than NH_4^+ which is quickly oxidized by nitrifying bacteria; in fact NO_3^- can reach levels between 0.5 and 10 mM, while NH_4^+ concentration is 10 to 1000 times lower (Marschner, 1995; Daniel-Vedele et al., 1998; Yamaya and Oaks, 2004).

The availability of the different form of N for root uptake influences several plant physiological processes including N-assimilation, cation-anion balance, water relations, photosynthesis and secondary metabolism (Roosta et al., 2009). Preferential uptake of one or other forms depends on the species and environmental conditions (Marschner, 1995). Plants adapted to low soil redox potential, acid and wet soils have a preference for NH_4^+ ions, in contrast, plant that grows in high pH soils, use NO_3^- (Marschner, 1995; Bloom et al., 2003; Larcher, 2003). Ammonium is assimilated in the root, using C skeletons, thus reducing sugar content in roots; in contrast NO_3^- can be stored in plants without being assimilated in the roots (Marschner, 1995). In addition, NH_4^+ assimilation produces a strong rhizosphere acidification that retards plant growth by the release of one proton (H^+) per NH_4^+ taken up; whereas NO_3^- induces an increase in pH that might have negative effects on mineral nutrient acquisition and also on availability within the plants (Marschner, 1995).

In addition, the external concentration of both inorganic N forms influences plant growth and root uptake. At low concentrations, small differences between NO_3^- and NH_4^+ occur; however, when external concentration rise, NO_3^- is the most important source as NH_4^+ detrimentally affects plant growth (Marschner, 1995). It has been demonstrated that the contemporary availability of both NO_3^- and NH_4^+ -N for root uptake increases growth and crop productivity, because it is easier for the plant to regulate intracellular pH and also absorption and assimilation energy cost are reduced (Marschner, 1995; Below, 2002). Furthermore, the two forms of N have different soil mobility (Below, 2002), NH_4^+ is fixed to negative charged soil particles becoming relatively immobile; in contrast, soil particles repel

NO_3^- making it particularly mobile, hence it moves about 10 times faster than NH_4^+. Thus, high levels of NO_3^- in soils are unusually maintained, because plant absorption, soil leachings and microbial denitrification deplete soil nitrate (Crawford and Glass, 1998; Below, 2002; Jackson et al., 2008).

1.2.2.1. Nitrogen uptake

Plants acquire N from the soil mainly in the form of NH_4^+ and NO_3^-, but the spatial and temporal availability of these ions is highly heterogeneous (Bloom et al., 2003). Nitrogen absorption depends on the root system development (soil colonization) and N uptake capacity of the root (Bahrman et al., 2005). Uptake of inorganic N involves the movement across the plasma membrane, transport or storage within the plant, and finally assimilation into organic compounds (Below, 2002).

Nitrate uptake is an energy dependent process that consumes 1 to 3 moles of ATP per mole of NO_3^- taken. Its net absorption is the balance between apoplasm to cytoplasm influx and efflux in the reverse direction; with the latter being passive and increasing with decreasing of external NO_3^- concentration (Crawford and Glass, 1998; Daniel-Vedele et al., 1998; Touraine, 2004). Nitrate uptake occurs throughout the root surface, mainly in the sub-apical region, even though older root zones should contribute with an important part of total NO_3^- acquisition due their large size compared with the actively growing roots (Touraine, 2004; Baldi et al., 2010). In contrast NH_4^+ uptake does not require metabolic energy (Engels and Marschner, 1995; Glass, 2003).

Two kinetically distinct types of transport systems that co-exist in the plasma membrane of root cells have been identified for NO_3^- influx. The first one, called low affinity transport system (LATS) generally found in older root, is active at high external NO_3^- concentrations (>0.5 mM) with no saturation up to 50 mM. The second one, located close to the root tip, works at low external concentrations (<0.5 mM) and is referred as the high affinity transport system (HATS). Two different HATS have been suggested, one constitutive (cHATS) and the other one is induced (iHATS) by nitrate and nitrite (Tischner, 2000; Touraine et al., 2001; Glass, 2003; Touraine, 2004). High affinity transport system and LATS also exist for NH_4^+ (Engels and Marschner, 1995; Glass, 2003).

Nitrogen uptake can be influenced by internal factors, such as N and carbohydrate concentration, and by external factors, such as NO_3^- and NH_4^+ soil concentrations, temperature, O_2 levels, and rhizosphere pH. Plant species and developmental stage can also influence N uptake (Below, 2002). Ammonium uptake does not appear to be influenced by

NO_3^-, however NH_4^+ induces inhibition of NO_3^- uptake; in fact several studies have demonstrated that the presence of NH_4^+ in the growing media reduces NO_3^- influx into roots (Glass, 2003). Carbohydrate in the phloem sap may regulate NO_3^- uptake as demonstrated by a decline in NO_3^- uptake after blocking phloem translocation through stem girdling (Imsande and Touraine, 1994; Touraine et al., 2001). Low temperatures reduce plant demand for N, and generally increase availability of NH_4^+, because nitrification process is more sensible to low temperatures than ammonification (von Wiren et al., 1997; Glass, 2003). Soil pH plays an important role on N uptake with NH_4^+ uptake enhanced by neutral conditions and limited at low pH. Uptake of NO_3^- is faster at pH around 4-5 and is reduced at higher pH (Violante, 2005). Additionally, it has been suggested that plants absorb NH_4^+ faster than NO_3^- during early vegetative growth, whereas the opposite situation occurs as growth progresses and more NO_3^- is absorbed than NH_4^+, possibly due to the presence of incomplete functional systems for NO_3^- uptake and assimilation in young plants (Below, 2002).

1.2.2.2. Nitrogen assimilation

Nitrogen must be assimilated into organic forms to be used by plant. Ammonium ion is quickly assimilated in the roots because is toxic to plant tissues at relatively low levels, and translocated as organic compounds. In contrast, symplastic NO_3^- within roots can follow 4 destinations: 1) reduction to NH_4^+; 2) return efflux across the plasma membrane to the apoplasm; 3) storage in the vacuoles of root cells or 4) long distance transport through the xylem, to be stored or reduced elsewhere (Crawford and Glass, 1998; Below, 2002).

The reduction of NO_3^- to NH_4^+ can occur either in the root or in the shoot. It consists in two steps, the reduction of NO_3^- to NO_2^- through the enzyme nitrate reductase (NR) and the reduction NO_2^- in NH_4^+ by the enzyme nitrite reductase (NiR), figure 1.4 (Marschner, 1995). Nitrate reductase, considered the rate limiting step in the reaction, is located in the cytosol and uses electrons from NADH and/or NADPH to reduce NO_3^- (Engels and Marschner, 1995; Below, 2002; Taiz and Zeiger, 2010). Nitrite reductase, located in leaf chloroplast and root proplastid, oxidizes a reduced ferrodoxin (Fd) for the reaction; reduced Fd is derived from the photosynthetic electron transport (photosystem I) in leaves, and from NADPH generated by the oxidative pentose phosphate pathway in roots (Engels and Marschner, 1995; Taiz and Zeiger, 2010).

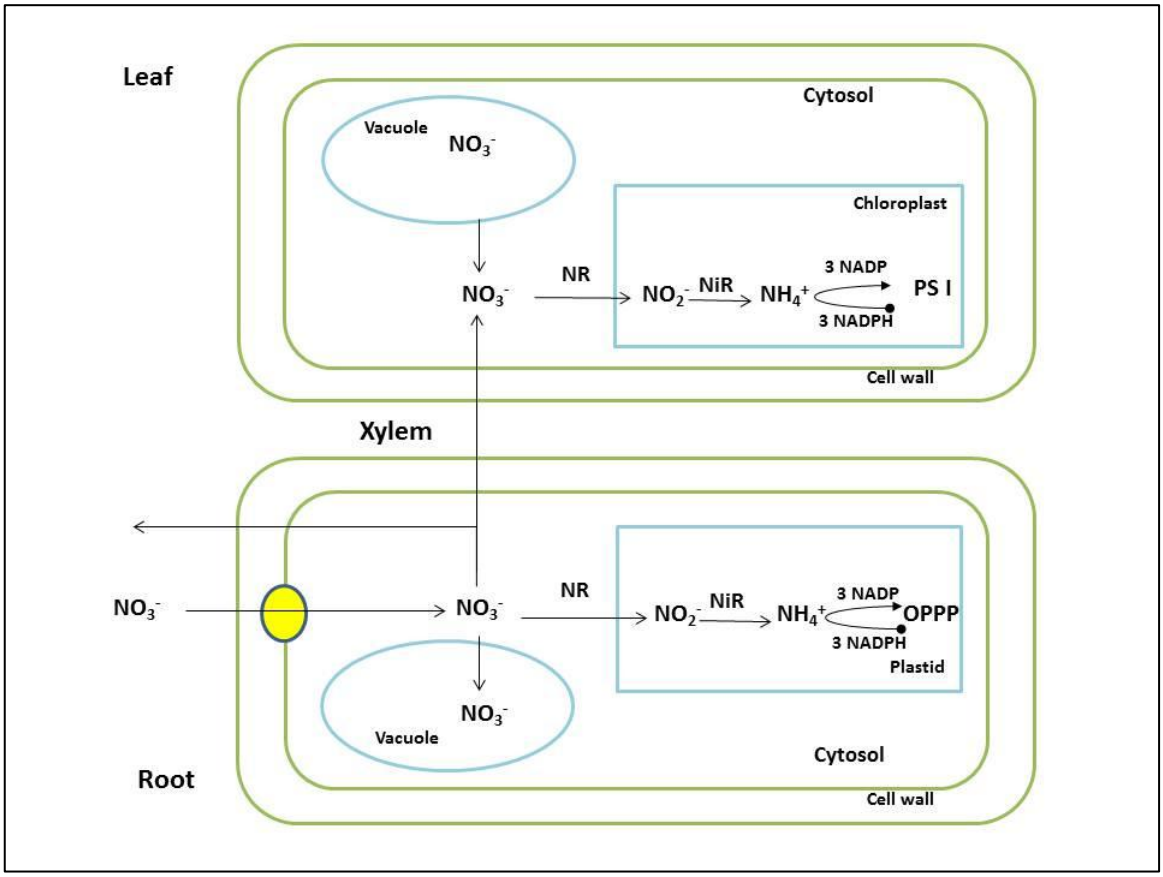

Figure 1.4 Schematic presentation of the pathway of NO_3^- reduction in root and leaf cells; NR = nitrate reductase, NiR = nitrite reductase, OPPP = oxidative pentose phosphate pathway, PS I = photosystem I. Adapted from Engels and Marschner, 1995.

The magnitude of NO_3^- reduction carried out in roots and shoots depends on the level of NO_3^- supply and on plant species. In general, with high external NO_3^- supply, a large quantity of the total N is translocated as NO_3^- to the leaves; in contrast, when NO_3^- supply is low, the most part of the NO_3^- is reduced in roots (Marschner, 1995). Herbaceous plants and temperate deciduous trees such as peach reduce large proportion of the NO_3^- in the roots, when external concentrations is not much higher than 1 mM (Marschner, 1995).

Ammonium is assimilated into essential amino acids by glutamate synthase cycles (figure 1.5), which consists in two successive reactions catalyzed by glutamine synthetase (GS) and glutamate synthase (GOGAT). In this system, NH_4^+ is transformed into glutamine via GS using one ATP and a divalent cation as a cofactor. Glutamine synthetase can be located in cytoplasm, root plastids and leaf chloroplasts. In root plastids, GS generates amide for local consumption and in leaf GS re-assimilates NH_4^+ produced by photorespiratory process (Engels and Marschner, 1995; Taiz and Zeiger, 2010). The amide group from glutamine is then transferred to 2-oxoglutarate by GOGAT, and can be located in root plastids

and leaf vascular bundles. The root-GOGAT accepts electrons from NADH whereas leaf-GOGAT accepts electrons from ferredoxin (Below, 2002; Taiz and Zeiger, 2010; Lea and Miflin, 2011). Also, NH_4^+ can be assimilated by an alternative and reversible way, which is catalyzed by glutamate dehydrogenase (GDH) and combines 2-oxoglutarate with NH_4^+. This reaction can be NADH-dependent when occurs in mitochondria or NADPH-dependent if it is localized in the chloroplasts of photosynthetic organs (Taiz and Zeiger, 2010).

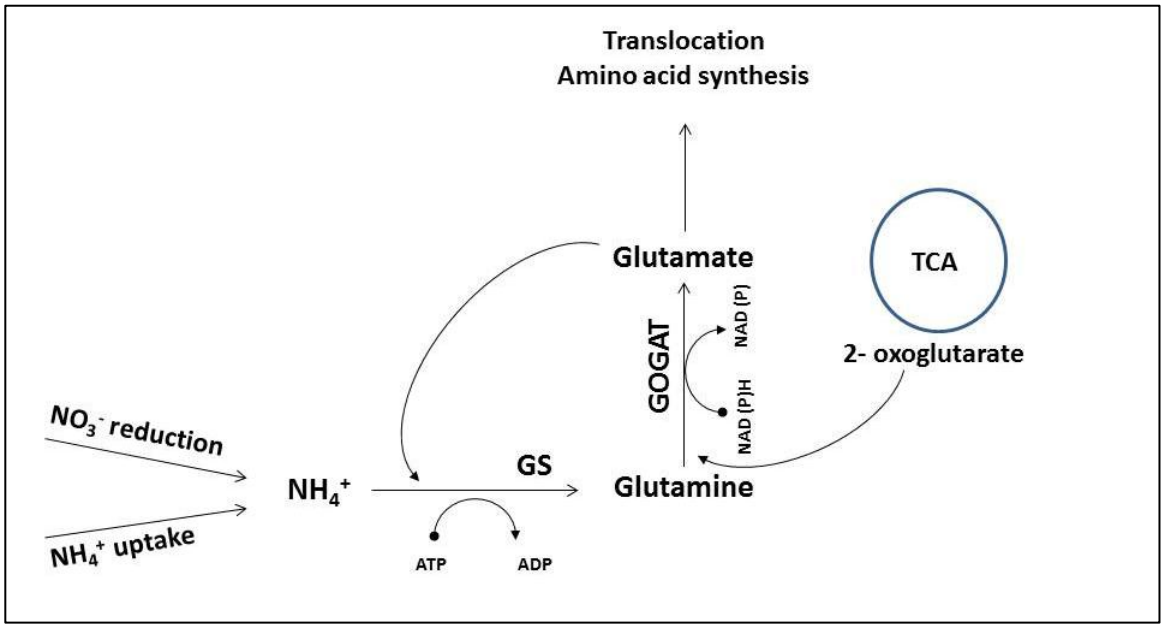

Figure 1.5 Schematic presentation of NH_4^+ assimilation; GS = glutamine synthetase, GOGAT = glutamate synthase, TCA = tricarboxylic acid cycle (Engels and Marschner, 1995; Below, 2002).

1.2.3. Nitrogen environmental impact

Human activities, fertilizer applications and fossil fuel combustion, have increase two-fold the amount of N in terrestrial ecosystems since the early 20th century (Hall and Matson, 1999; Wang et al., 2009). This increase of N input has resulted in substantial N pollution and ecological damage (Kramer et al., 2006). In agricultural systems, N fertilizers are the center of a sharp conflict between the need of maintain the food supply and the need of protect the environment (Sims, 1995). Nowadays, N fertilizer use is higher than 100 million t per year; however only 15% to 50% of total N supply is absorbed by fruit trees and extensive crops, respectively (Sanchez et al., 1995; Galloway et al., 2004; Robertson and Vitousek, 2009). Nitrogen losses from the ecosystems is mainly as inorganic forms through soil leaching, denitrification to N_2, volatilization NH_3 and fluxes of N_2O and NO_x to the atmosphere (Robertson and Vitousek, 2009).

1.2.3.1. Leaching N

Nitrogen losses by leaching generally are, NO_2^- and NO_3^-, that is quantitatively the most important, because its high solubility and mobility in the soil. In general, leaching of NO_3^- is caused by any descending movement of water through the soil profile and the magnitude of the leakage is positively correlated with soil NO_3^--N concentration and the volume of leaching water (Sims, 1995; Kramer et al., 2006). Agriculture systems are considered as the most important anthropogenic source of NO_3^- to aquifers and groundwater (Burkart and Stoner, 2001). In fact, it has been observed that agricultural areas often exhibit seasonal concentrations greater than 10 mg NO_3^--N L^{-1}, whereas in natural background levels commonly NO_3^--N is less than 2 mg L^{-1} (Keeney and Hatfield, 2001). The high nitrate levels in drinking water and food may increase the risk of methaemoglobinaemia, that is particularly high for babies (Sims, 1995; Keeney and Hatfield, 2001), and increase the occurrence of stomach cancer (Forman et al., 1985; O'Riordan and Bentham, 1993). On the other hand, excess of NO_3^- can contribute to the eutrophication (excess of nutrients availability) of natural water systems, hence, enhances growth of aquatic organism, with an increase of turbidity and a reduction of dissolved O_2, that affect the metabolism and growth of aerobic species, causing a condition referred to as hypoxia (Sims, 1995; Follett, 2001; Keeney and Hatfield, 2001; Robertson and Vitousek, 2009).

1.2.3.2. Ammonia volatilization

Ammonia plays a relatively positive role in atmospheric chemistry because it serves to neutralize about 30% of the H^+ ions in the atmosphere, and in general is deposited as NH_3^+, or as NH_4^+ in rainwater or aerosols (Robertson and Vitousek, 2009). Among the global NH_3^+ emissions to the atmosphere, about 65% is emitted from agricultural systems, through volatilization process (Mosier, 2001). Ammonia volatilization refers to the loss of NH_3^+ from the soil to the atmosphere, and is considered the second major pathway by which N is lost from agriculture system (Sims, 1995; Robertson and Vitousek, 2009). Volatilization is influenced by NH_4^+ concentration in soil solution and soil pH; most losses occur when NH_4^+ is abundant and pH increases (Follett, 2001; Robertson and Vitousek, 2009). In general, NH_3^+ volatilization increases when: soil CEC is low, soil temperature increase, urea is used as fertilizer and high N organic wastes are decomposed on the soil surface. Volatilization decreases in the presence of growing plants (Follett, 2001).

1.2.3.3. Denitrification

Denitrification is the reduction of NO_3^- to gaseous form of N. The general sequence is as follows:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

This sequence is catalyzed by chemoautotrophic bacterial, which normally are aerobic, but under anaerobic conditions they can use reduced N oxides as electron acceptors in alternative to O_2 (Peoples et al., 1995; Sims, 1995).

Around 0.5% of fertilizer-N applied to agriculture systems is emitted to the atmosphere as NO (Mosier, 2001). Nitric oxides (NO_x), released mainly as nitric oxide (NO), plays an important role in troposphere chemistry; reacts with atmospheric oxidants such as ozone (O_3), hydroxyl radicals (OH) during oxidation of carbon monoxide (CO), CH_4, and non-methane hydrocarbons (Hall and Matson, 1999; Mosier, 2001). Elevated NO_x concentration lead to the production of O_3, due to the oxidation of atmospheric hydrocarbons such as CO. In contrast when the concentration is low, O_3 is destructed reducing the ability of the stratosphere as a barrier to ultraviolet radiation (Sims, 1995; Robertson and Vitousek, 2009). Additionally, hydroxyl radical in the atmosphere are involved in the removal of greenhouse gases, thereby NO_x contribute indirectly in atmospheric warming (Peoples et al., 1995).

Nitrous oxide (N_2O) is a powerful greenhouse gas, 300 times more active than CO_2, that in the troposphere absorbs thermal radiation and has a long residence time in the atmosphere (Kramer et al., 2006; Bronson, 2008; Robertson and Vitousek, 2009). Nitrous oxide is not one of the most abundant greenhouse gas, nevertheless, its emission rise with an increase of N availability, playing a considerable role in the agricultural contribution to climate change (Kramer et al., 2006; Robertson and Vitousek, 2009). In fact, according to the Group on Agriculture of the European Climate Change Programme, almost 51% of the total N_2O emissions come from agricultural activities (Favoino and Hogg, 2007).

1.2.4. Negative effect of N on plant growth

Nitrogen availability affects the biomass production and plant productivity (Bloom et al., 1993). Increasing of both NO_3^--N and NH_4^+-N supplies, stimulate roots biomass, and increase significantly the branching of axial root and the elongation of lateral roots (Boukcim et al., 2006). Baldi et al. (2010) observed a positive correlation between NO_3^--N concentration and the median peach root lifespan. However, many reports indicate a possible negative effect of high mineral N soil concentrations on root development. In fact, different studies found

that high NO_3^--N concentrations had a strong inhibitory effect on roots elongation of tomatoes (Bloom et al., 1993), *Arabidopsis* (Zhang et al., 1999) and maize (Tian et al., 2008). Scheible et al. (1997) observed a negative correlation between high levels of leaf NO_3^--N and total root growth that were associated with inhibition of starch synthesis and turnover in the leaves and decreased transport of sucrose to the roots. Also, several authors indicated that high soil mineral N concentration might affect negatively root lifespan due to an increase of root metabolic activity (Tjoelker et al., 2005; Withington et al., 2006) that could increases production of reactive oxygen species (ROS) such as superoxide radicals (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\bullet$), which are capable of unrestricted oxidation of various cellular components (Mittler, 2002; Misra and Gupta, 2006; Taiz and Zeiger, 2010).

1.3. Aim of the thesis

In the last years, composted organic materials from agri-food industry and municipal solid waste have been used as a sustainable source to replace chemical fertilizers and increase soil OM. Nevertheless, the influence of them on soil fertility and plant growth are poorly understood. Continuous application of compost can potentially affect NO_3^- -N concentration and/or induce toxic effect to root. Among the few reports available on the effects of organic fertilizer species root growth of woody species, Baldi et al. 2010 observed that the use of compost in peach fruit management increases root proliferation and lifespan. This response can have negative implication on tree C partitioning; in fact if the higher root growth is accompanied by a decrease in above ground C investment, a lower fruit production might be expected.

The aims of the present study were to evaluate the effects of organic and mineral N fertilizer on: 1) CO_2 fixation, tree development and C partition to the different organs of peach trees; 2) soil N concentration and NO_3^--N effect on root and shoot growth and root oxidative stress; and 3) soil chemical and biological fertility, tree growth and yield and fruit quality in a commercial orchard.

For this purpose, three trials were conducted:

- Carbon assimilation and partitioning in potted peach trees;
- Root oxidative stress, root morphology and growth of micro propagated plant of fertilized with increasing rate of soil applied, mineral or organic N;

- Assessment of the sustainability of annual compost fertilization as an alternative of mineral fertilizer in a commercial orchard.

2. EFFECT OF ORGANIC FERTILIZATION ON GROWTH AND CARBON PARTITIONING OF PEACH TREES.

2.1. Materials and methods

2.1.1. Plant materials and treatments

The experiment was carried out in 2009 at the experimental station of the University of Bologna, in Cadriano (44° 35' N, 11° 27' E) on 28 two-years-old peach trees (*Prunus persica* L. Batsch) cv. 'Orion', grafted on GF 677 rootstock (*Prunus persica* x *Prunus amygdalus*). Plants (figure 2.1 A) were potted in May 2008 in 40 liter containers filled with a clay loam Bathicalci Eutric Cambisols soil (FAO, 1990) and sand at rate of 3:1; and were fertilized as in a complete randomized block design (with seven replicates) as follows:

- mineral, fertilized in April 2009 with mineral 0.357 g of N pot^{-1}, 0.238 g of P pot^{-1} and 0.952 g of K pot^{-1} as granular fertilizer labeled as 15-10-40 (mineral control);
- cow manure, at a rate of 800 g DW pot^{-1} (cow manure);
- compost at a rate of 800 g DW pot^{-1} (compost 800);
- compost at a rate of 2400 g DW pot^{-1} (compost 2400).

Organic fertilizers were mixed with the soil before potting. Cow manure (table 2.1) was cow stable dung and wheat straw bedding, after 3 month stabilization, and provided by a local livestock farm. Compost (table 2.2) was obtained from domestic organic wastes (50%) mixed with pruning material from urban ornamental trees and garden management (50%) after 3 month stabilization. During the experiment trees, were grown outside, under a shelter net that reduced photosynthetic active radiation (PAR) by 30%, to protect from hail storm, and were watered daily by drip emitters.

Table 2.1 Selected chemical characteristics of cow manure used in the experiment.

Characteristic	**Value**
Dry matter (%)	28.5
Total N (%)	2.75
Total P (%)	1.96
Total K (%)	2.38
Total organic C (mg kg^{-1})	37.4
C.E.C. (meq 100 g^{-1} D.W.)	66.6
Humic and fulvic acids (%)	11.24

Table 2.2 Selected chemical characteristics of compost used in the experiment.

Characteristic	**Unit**	**Value**
pH		8.2
Conductivity	dS/m	1.21
Humidity	% m/m	31.8
Ashes	% D.W.	50.1
Organic matter	% D.W.	49.9
Total – N	% N D.W.	1.75
Cadmium	mg/kg D.W.	0.7
Chrome VI	mg/kg D.W.	< 0.50
Mercury	mg/kg D.W.	< 1.0
Nickel	mg/kg D.W.	23.5
Lead	mg/kg D.W.	50.4
Cooper	mg/kg D.W.	85.6
Zinc	mg/kg D.W.	177
Plastic materials < 10 mm	% D.W.	absent
Plastic materials > 10 mm	% D.W.	absent
Other inert materials <10 mm	% D.W.	absent
Other inert materials >10 mm	% D.W.	absent
Salmonella	MPN/g	absent

2.1.2. ^{13}C plant enrichment and partitioning

In May 2009, tree canopies were enclosed in a transparent plastic chamber (figure 2.1 F) in order to label plants with ^{13}C enriched CO_2 ($^{13}CO_2$). Before the enrichment, each pot was enclosed in a plastic bag to avoid soil and roots contamination with ^{13}C (figure 2.1.B).

The plastic chamber, 10 m long, 4 m width and 2 m high was made of a high-density polypropylene sheet with a PAR reduction of 10%. Temperature was controlled by placing inside the chamber almost 30 kg of ice along with two fans, set at the opposite side of the chamber, in order to make air circulation and avoid any temperature gradients. An infrared gas analyzer (EGM – 4; PP system, Hitchin, UK) was used to monitor CO_2 evolution inside the chamber.

The $^{13}CO_2$ pulse was carried out inside the chamber by dissolving 20 g of barium carbonate ($Ba^{13}CO_3$ -99 atom %, Sigma) in 350 ml of 85% lactic acid to produce 101.5 mM of $^{13}CO_2$. The trees remained inside the chamber for about 90' until the CO_2 concentration was constantly < 200 ppm, indicating the absence of leaf net fixation. During the time course of the pulse, the temperature ranged between 30 °C and 36 °C.

Before ^{13}C feeding, 4 plants (one per treatment) were harvested to evaluate natural ^{13}C abundance of the different organs (leaves, shoots, fruits, wood and roots). Immediately (T0) and 7 days (T7) after the $^{13}CO_2$ pulse, six leaves per tree were collected for isotopic ratio determination. Nineteen days after the pulse (T19) and at the end of plant growth (T185), 3 trees per treatment were harvested and separated into different organs. At T19 trees were divided in leaves, shoots, shoot apexes (portion with no fully expanded leaves), whole fruits, wood and roots. At T185, trees were separated in fallen leaves, twigs (lignified shoot), whole fruits (harvested at maturation in august) wood and roots. All plant material was oven-dried at 60°C for 96 hours, weighed and ground to a fine powder. Carbon concentration and ^{13}C enrichment were determined by an elemental analyzer (EA 1110, Carlo Erba, Milan, Italy) instrument coupled with a Finningan Delta plus (Bremen, Germany) mass spectrometer. The ^{13}C enrichment was calculated according to Wu et al. (2009) as follows:

$$\delta^{13}C\ (‰) = [(R_{sample} / R_{standard}) - 1] \times 1000 \qquad \text{Eq. (1)}$$

$$R_{sample} = {}^{13}C/{}^{12}C = [(\delta^{13}C/1000) + 1] \times R_{standard} \qquad \text{Eq. (2)}$$

$$F = {}^{13}C/ ({}^{13}C + {}^{12}C) = R/ (R+1) \qquad \text{Eq. (3)}$$

$$\text{Atom \% excess} = (F_{labeled} - F_{unlabeled}) \times 100 \qquad \text{Eq. (4)}$$

$$\text{New } {}^{13}C \text{ content} = (\text{Atom \% excess}/ 100) \times \text{dry mass} \times [C] \qquad \text{Eq. (5)}$$

$$\text{Relative Partitioning (\%)} = \frac{\text{(New } ^{13}\text{C content in the organ)}}{\text{(New } ^{13}\text{C in all the sampled organs)}} \times 100 \qquad \text{Eq. (6)}$$

where the $\delta^{13}C$ (‰) value is calculated from the measured C isotope ratios of the sample and standard reference material (Eq. 1). The absolute ratio (R) of a sample is determined by Eq. 2, where $R_{standard}$ (Vienna PeeDee Belemnite (PDB) carbonate) is 0.0112372. Atom % excess is an index to determine the enrichment level of a sample (Eq. 3 and 4). The new ^{13}C content is determined in the different organs according to dry matter and C concentration (Eq. 5). The partitioning of new ^{13}C in the plants is expressed as a percentage of the ^{13}C in the organ divided by total ^{13}C in the plant (Eq. 6).

The amount of C found in the leaves, immediately after $^{13}CO_2$ pulse (T0), was considered the only one in the tree (no mobilization to other organs occurred yet). The total amount of labeled C fixed by tree with the $^{13}CO_2$ pulse was consequently obtained by multiplying the values of the single leaf by the total leaf biomass measured at T19.

2.1.3. Canopy analysis

On 27 May and 15 June 2009, CO_2 assimilation rate was measured in the morning (from 9 to 11 a.m.) on two healthy, fully expanded and well exposed leaves per tree with an infrared gas analyzer (ADC- LCA2, Hoddenson, Herts, UK). On the same day, leaf chlorophyll was measured by the portable SPAD 502 (Minolta, Co. Ltda, Ramsey, NJ, USA) on 25 young, healthy and fully expanded leaves per tree.

2.1.4. Soil analysis

At each plant harvest, soil samples were taken to evaluate NO_3^- -N and ammonium NH_4^+ -N concentration, and microbial biomass C. Nitrate-N and NH_4^+-N were extracted from 10 g fresh weight (FW) of sieved (2 mm) soil in 100 ml of 2M KCl solution and shaken at 90 rpm for 1 h. After soil sedimentation, the supernatant was collected and stored at -20°C until analysis (Auto Analyzer AA-3, BRAN + LUEBBE, Norderstadt, Germany). Microbial biomass C was measured using the substrate induced respiration (SIR) method (Anderson and Domsch, 1978). Fifty grams of fresh soil were sieved (diameter of 2 mm), placed in 500 ml glass jars and equilibrated at room temperature for at least 24 h. The samples were then mixed with 200 mg of glucose and incubated at 22°C for 3 h. CO_2 evolution was measured by an infrared gas analyzer (EGM-4; PP system; Hitchin, UK); CO_2 data were converted into microbial C according to Anderson and Domsch (1978).

Figure 2.1 a) Peach trees of cv. 'Orion'/GF 677 used in the experiment; b) pots enclosed in black plastic bags to avoid direct contamination of roots and soil with ^{13}C; c) plant growth after (left to right): compost 2400, compost 800 and mineral fertilizer application; d) fan positioned inside the plastic chamber; e) ice bags used to avoid excessive increase of temperature inside the chamber; f) infrared gas analyzer for monitoring CO_2 evolution inside the chamber.

2.1.5. Statistical analysis

At each sampling time, data were statistically analyzed as in a factorial experimental design with 2 factors: soil fertilization (4 levels: mineral control, cow manure, compost 800 and compost 2400) and tissues (6 or 5 levels according to the sampling time). When analysis of variance showed an effect of treatment statistically significant ($P \leq 0.05$), means were separated by Student Newman-Keuls (SNK) test; when interaction between factors was significant, 3 times standard error of means (MSE) was used as the minimum difference between two means statistically different for $P \leq 0.05$ (Saville and Rowarth, 2008).

2.2. Results

2.2.1. Biomass production

At T19 and T185, compost 2400 treated trees showed the highest plant biomass as compared with the other treatments, while cow manure and compost 800 did not affect tree growth compared to mineral fertilization (figure 2.2).

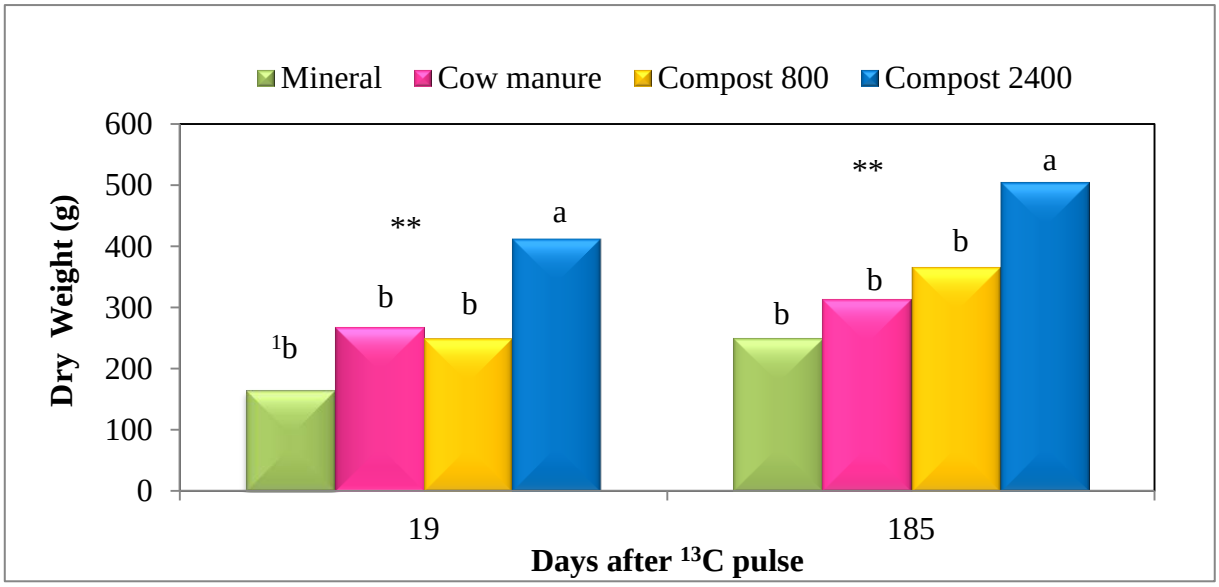

Figure 2.2 Effect of fertilization treatment on whole tree weight as measured 19 and 185 days after ^{13}C pulse. **: effect significant at $P \leq 0.01$. [1]Means followed by the same letter are not statistically different ($P \leq 0.05$).

In both dates, treatment and plant organ significantly interacted with organ weight. At T19, compost 2400 treated plants exhibited a higher leaf, wood and root biomass (table 2.3) than the other treatments. Cow manure induced a wood biomass lower than compost 2400, similar to compost 800 and higher than mineral control. Root biomass of compost 800 and

cow manure treated trees were similar each other and both were higher than mineral control plants (table 2.3). At T185, no significant differences between treatments were observed in weight of fallen leaves and twigs (table 2.4). Compared to mineral fertilized control, no effect of treatment on fruit weight was observed, however fruits from cow manure fertilized trees were smaller than those treated with compost 800 and 2400 (table 2.4). Wood biomass was increased by organic fertilization as compared with mineral control, while root biomass of compost 2400 treated plants was higher compared to the other treatments. Unlike cow manure, compost 800 induced a higher root development than mineral control (table 2.4).

Table 2.3 Effect of fertilization treatment and organ sample on organ biomass (g DW) 19 days after $^{13}CO_2$ pulse.

Treatment	**Leaves**	**Shoot apex**	**Shoots**	**Fruits**	**Wood**	**Roots**
Mineral	18.4	3.00	4.11	11.4	43.9	83.6
Cow manure	31.2	3.25	4.00	9.05	88.8	134
Compost 800	31.7	3.76	4.13	10.5	72.6	128
Compost 2400	53.2	3.31	7.62	28.4	118	203
Interaction			**** (3 SEM = 29)*			

*** Interaction between treatment and tissue significant at $P \leq 0.001$. Values differing by 3 standard error of means (SEM) are statistically different.

Table 2.4 Effect of fertilization treatment and organ sample on organ biomass (g DW) 185 days after $^{13}CO_2$ pulse.

Treatment	**Fallen leaves**	**Twigs**	**Fruits**	**Wood**	**Roots**
Mineral	16.3	10.3	42.9	74.0	121
Cow manure	20.4	9.38	17.9	113	153
Compost 800	25.5	9.00	72.6	121.4	162
Compost 2400	42.4	23.0	67.1	145	228
Interaction			** (3 SEM = 36)*		

* Interaction between treatment and tissue significant at $P \leq 0.05$. Values differing by 3 standard error of means (SEM) are statistically different.

2.2.2. Leaf ^{13}C partitioning

There was no treatment effect on ^{13}C content in single leaf immediately after the pulse (T0); however, seven days (T7) after the ^{13}C enrichment, mineral fertilized plants had a higher amount of labeled C per leaf, than organic-fertilized plants (figure 2.3). From T0 to T7 leaf ^{13}C decreased in all treatments, in detail in compost 2400 treated trees it decreased of about 77%, in cow manure of around 75%, in compost 800 of about 74%, and in mineral treated plants of about 58% (figure 2.3).

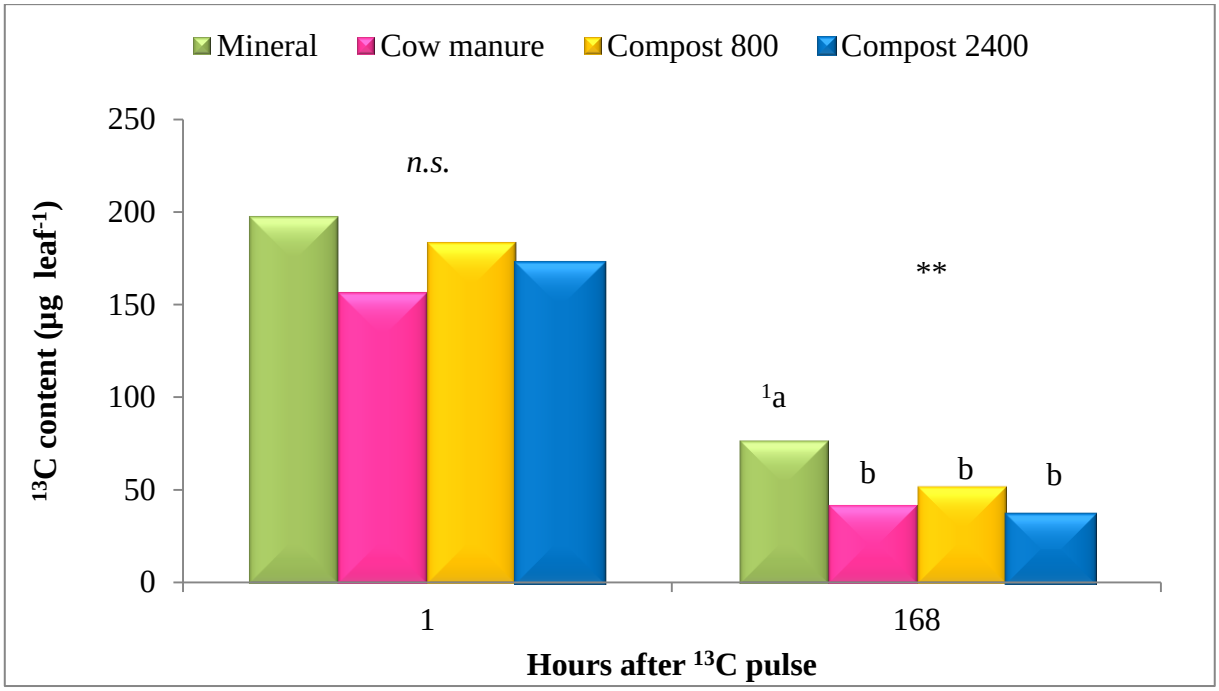

Figure 2.3 Effect of fertilization treatment on 13C content in the single leaf, immediately (1 hour) and 168 (7 days) hours after 13C pulse. n.s., **: effect not significant or significant at P≤0.01, respectively. 1Means followed by the same letter are not statistically different (at P≤0.05).

2.2.3. Organ C concentrations and ^{13}C content

Nineteen days after 13C labeling (T19), root C concentration was higher in cow manure treated plants, followed by mineral control and compost 800 and 2400 (table 2.5). No significant differences were induced by treatments on C concentration of other organs (table 2.5). At T185, C concentration in fallen leaves was not affected by treatment; the application of compost at high rate decreased twigs C concentration, if compared to the other treatments. Fruit C was increased by application of compost 800 followed by compost 2400, mineral

control and cow manure. Wood and root C concentration was enhanced by application of organic fertilizer (table 2.6).

Table 2.5 Effect of fertilization treatment on carbon concentration (%) in different organs of peach tree at 19 days after ^{13}C pulse.

Treatment	Leaves	Shoot apex	Shoots	Fruits	Wood	Roots
Mineral	45.8	44.0	42.4	40.3	44.0	44.0
Cow manure	45.3	44.3	41.0	40.3	44.6	46.4
Compost 800	45.3	42.6	41.3	41.8	43.9	40.8
Compost 2400	44.2	43.5	41.8	41.8	44.6	41.4
Interaction			** (3 SEM = 2.5)*			

* Interaction between treatment and tissue significant at $P \leq 0.05$. Values differing by 3 standard error of means (SEM) are statistically different.

Table 2.6 Effect of fertilization treatment on carbon concentration (%) in different organs of peach tree in December, 185 days after ^{13}C pulse.

Treatment	Fallen leaves	Twigs	Fruits	Wood	Roots
Mineral	43.8	48.2	40.3	46.3	35.8
Cow manure	43.5	46.8	39.5	49.7	41.3
Compost 800	43.0	48.2	45.2	49.8	43.6
Compost 2400	44.5	43.0	42.6	49.9	42.7
Interaction			**** (3 SEM = 3.1)*		

*** Interaction between treatment and tissue significant at $P \leq 0.001$. Values differing by 3 standard error of means (SEM) are statistically different.

The ^{13}C enrichment found in the different tree organs decreased with time (table 2.7 and 2.8). At T19, mineral fertilized plants showed the highest ^{13}C enrichment in leaves, followed by compost 800 and cow manure treated trees; compost 2400 had the lowest values (table 2.7). Cow manure induced a significantly higher ^{13}C enrichment in shoot apex than mineral and compost 800 plants, but similar to compost 2400 treated plants; no significant differences were found for the other tissues (table 2.7).

Table 2.7 Effect of fertilization treatment on ^{13}C enrichment (‰) in leaves, shoot apex, shoots, fruits, wood and roots of peach trees 19 days after ^{13}C pulse.

Treatment	Leaves	Shoot apex	Shoots	Fruits	Wood	Roots
Mineral	0.378	0.116	0.225	0.120	0.025	0.027
Cow manure	0.201	0.283	0.143	0.159	0.035	0.043
Compost 800	0.269	0.177	0.182	0.176	0.043	0.037
Compost 2400	0.128	0.222	0.148	0.174	0.045	0.044
Interaction			*** (3 SEM = 0.10)*			

** Interaction between treatment and tissue significant at $P \leq 0.01$. Values differing by 3 standard error of means (SEM) are statistically different.

At T185, compared with the other treatments, cow manure treated plants exhibited the higher ^{13}C enrichment in fallen leaves, while the other treatments had similar values. In twigs, the highest ^{13}C enrichment was found in mineral control and cow manure treated plants, while compost 800 and compost 2400 had the lowest content. Fruits of compost treated trees presented higher ^{13}C enrichment than cow manure and mineral control plants (table 2.8). Compost 2400 treated plants had the highest ^{13}C enrichment in wood and roots, followed by compost 800 and cow manure (that showed similar values) and mineral treated trees that had the lowest ^{13}C enrichment (table 2.8).

Table 2.8 Effect of fertilization treatment on ^{13}C enrichment (‰), 185 days after pulse (December) in leaves, twigs, fruits, wood and roots of peach trees.

Treatment	Fallen leaves	Twigs	Fruits	Wood	Roots
Mineral	0.112	0.114	0.035	0.019	0.014
Cow manure	0.132	0.094	0.039	0.030	0.031
Compost 800	0.118	0.085	0.060	0.025	0.032
Compost 2400	0.112	0.068	0.064	0.041	0.043
Interaction			*** (3 SEM = 0.02)*		

*** Interaction between treatment and tissue significant at $P \leq 0.001$. Values differing by 3 standard error of means (SEM) are statistically different.

Immediately after the pulse, total assimilated ^{13}C was higher in compost 2400 treated trees compared to the other treatments, and then it decreases in all treatment with time (figure

2.4). At T185, between 26% to 43% of the total assimilated ^{13}C was found in the plants, with compost 2400 treated plants retaining the most ^{13}C, followed by cow manure, compost 800 and mineral treated plants (figure 2.4).

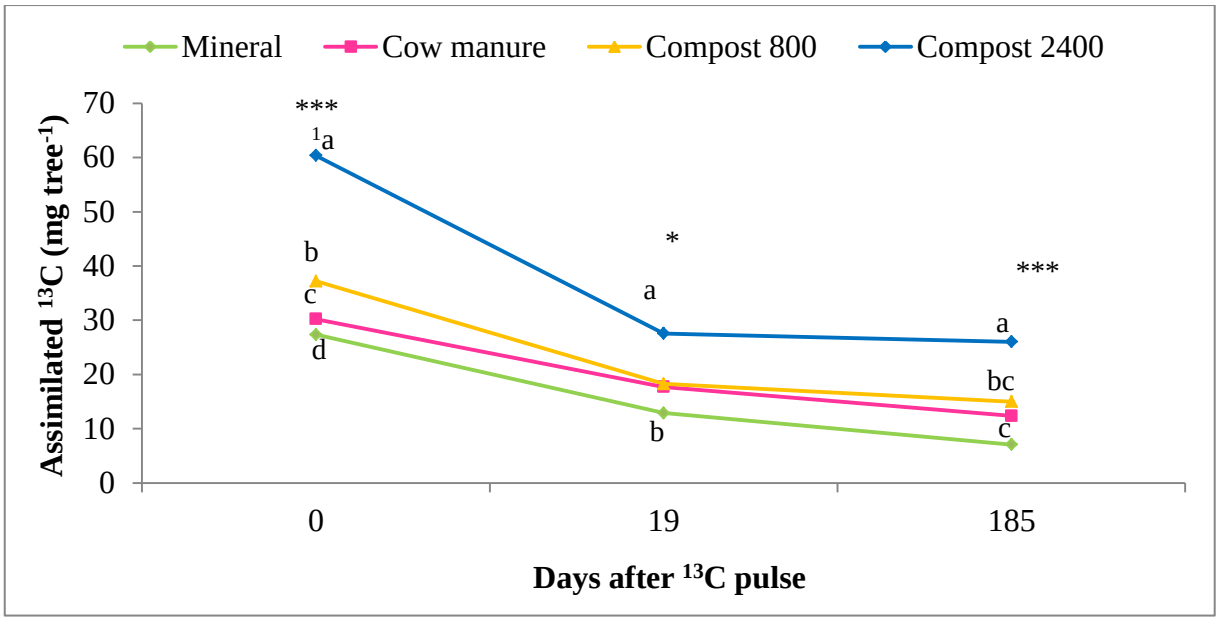

Figure 2.4 Effect of the fertilization treatment on total ^{13}C fixed and found in the plant during the time course of the experiment. *, ***: effect significant at $P \leq 0.05$ and $P \leq 0.001$, respectively. [1]Means followed by the same letter are not statistically different (at $P \leq 0.05$).

2.2.4. Relative ^{13}C partitioning

At T19, compost 2400 treated trees partitioned a higher amount of C to fruits as compared with the other treatments (figure 2.5). Mineral control plants presented 54% of the total fixed ^{13}C in the leaves, compost 800 and cow manure treated plant had 40% and 35% respectively, and compost 2400 plants showed the lowest percentage (25%). No significant differences were observed among treatments on shoot apex and total shoot percentage of labeled C partitioning. Cow manure and both compost treatments promoted a higher partitioning of C to the wood and roots than mineral fertilized trees (figure 2.5).

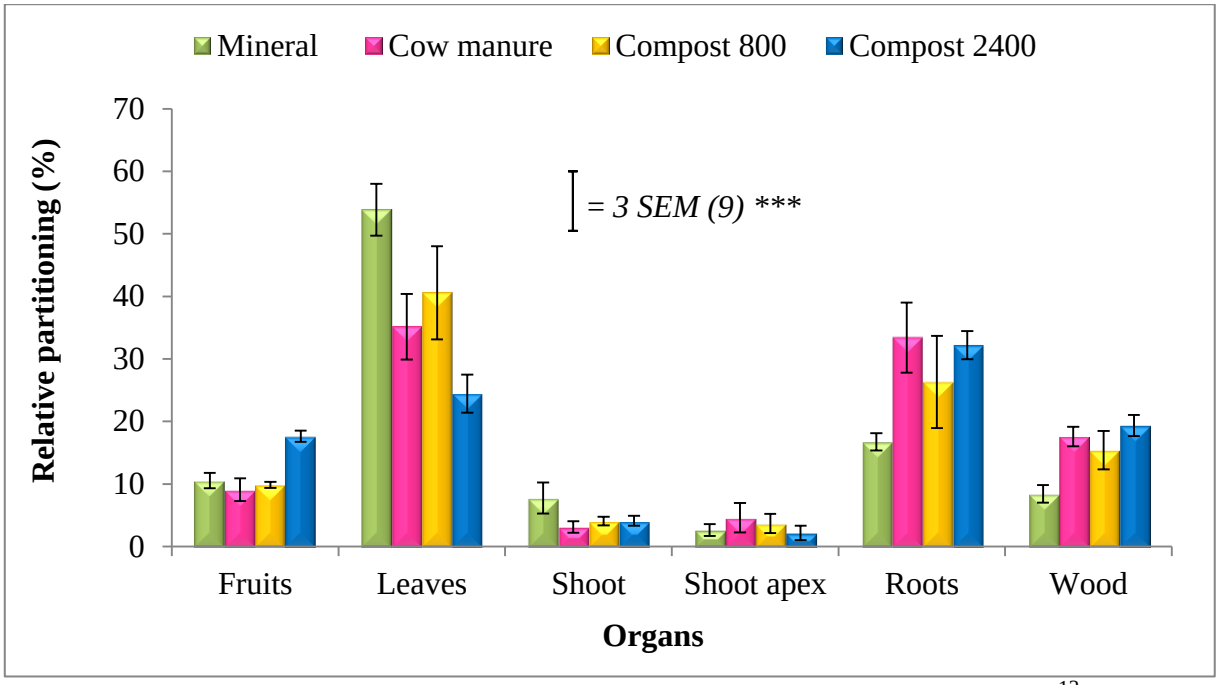

Figure 2.5 Effect of the fertilization treatment on relative partitioning of fixed ^{13}C 19 days after ^{13}C pulse. Bars indicate ± standard error. *** Interaction between treatment and organ significant at $P \leq 0.001$. Values differing by 3 standard error of means (SEM) are statistically different.

At the second harvest, compost 800 treated plants showed the highest percentage of ^{13}C in fruits, mineral and compost 2400 plants were intermediate, and cow manure had the lowest effect (figure 2.6). At T185, application of compost 2400 induced the lowest percentage of ^{13}C in fallen leaves; in contrast mineral fertilization promoted the highest percentage of ^{13}C. Mineral treated plants showed a higher ^{13}C percentage in lignified shoots than organic-fertilized plants. Cow manure, compost 2400 and compost 800 treated trees had higher percentage of ^{13}C in roots than mineral control. Percentage of ^{13}C in wood was higher in cow manure than the other treatments (figure 2.6).

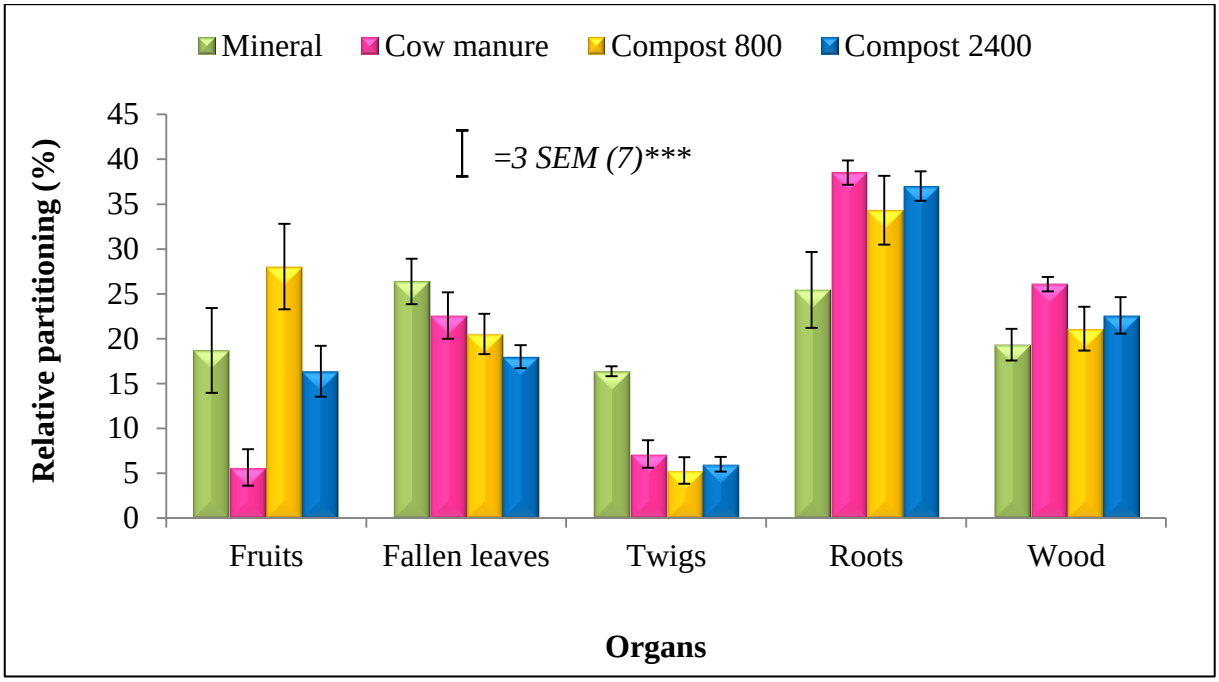

Figure 2.6 Effect of fertilization treatments on relative partitioning of labeled C to the different tree organs, at the end of the season (185 DAP). The fruit were sampled at harvest (67 DAP). Bars indicate ±standard error. *** Interaction between treatment and organ significant at $P \leq 0.001$. Values differing by 3 standard error of means (SEM) are statistically different.

2.2.5. Leaf chlorophyll and CO_2 assimilation rate.

The day of the ^{13}C pulse (May 2009), compost 2400 treated plants showed SPAD values similar to mineral control and higher than compost 800 and cow manure. At T19 (June 2009), mineral control trees showed the highest SPAD values, followed by compost 2400, compost 800 and cow manure (table 2.9).

Table 2.9 Effect of fertilization treatment on leaf chlorophyll (SPAD unit)

Treatment	**May-09**	**Jun-09**
Mineral	35.2a	35.8a
Cow manure	29.6b	31.5c
Compost 800	30.7b	31.6c
Compost 2400	33.6a	34.0b
Significance	***	***

***: effect significant at $P \leq 0.001$. Values followed by the same letter are not statistically different (at $P \leq 0.05$).

On May 2009, mineral and compost 800 treated plants showed a higher photosynthetic activity than compost 2400, and similar to cow manure treatments (table 2.10). On June 2009, no significant differences were found among treatments.

Table 2.10 Effect of fertilization treatment on leaf CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1})

Treatment	**May-09**	**Jun-09**
Mineral	6.23a	7.15
Cow manure	4.85ab	8.32
Compost 800	5.88a	7.00
Compost 2400	3.83b	7.30
Significance	*	*n.s.*

n.s.,*: effect not significant or significant at $P \leq 0.05$, respectively. Values followed by the same letter are not statistically different (at $P \leq 0.05$).

2.2.6. Soil fertility

Nineteen days after the $^{13}CO_2$ pulse (T19), NO_3^--N concentration in soil was not affected by treatments; however, NH_4^+-N concentration was higher in compost 2400 as compared with other treatments (figure 2.7).

Compost 2400 treated plants increased microbial biomass in the soil as compared with mineral, cow manure and compost 800 plants (figure 2.8).

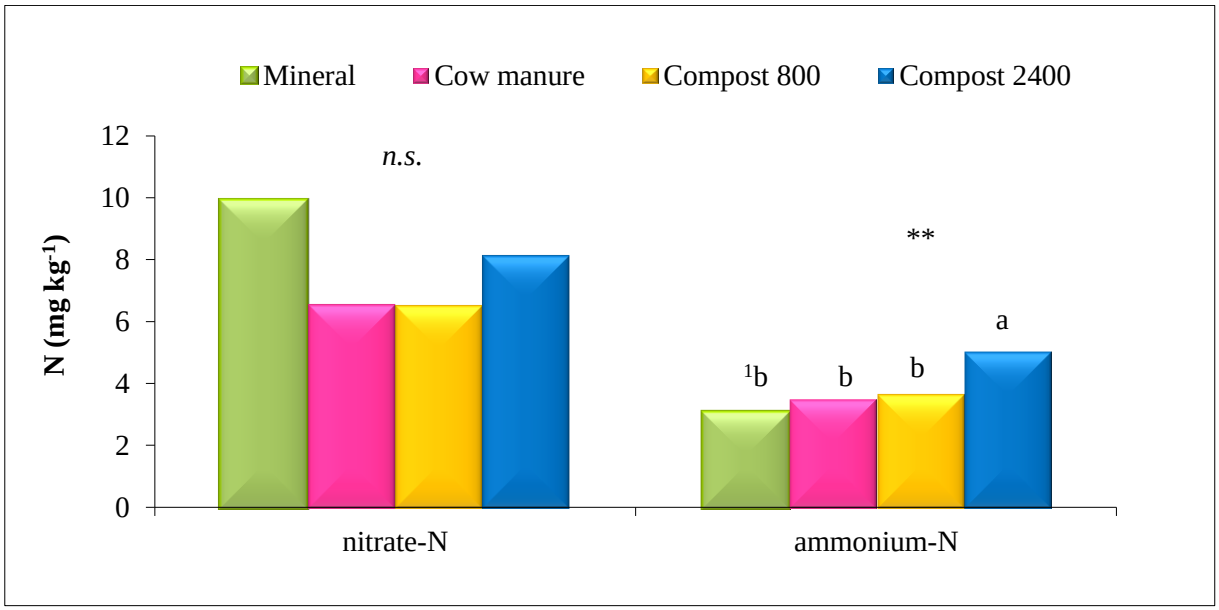

Figure 2.7 Effect of fertilization treatment on nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) soil concentrations. n.s., **: effect not significant or significant at $P \leq 0.01$, respectively. [1]Means followed by the same letter are not statistically different (at $P \leq 0.05$).

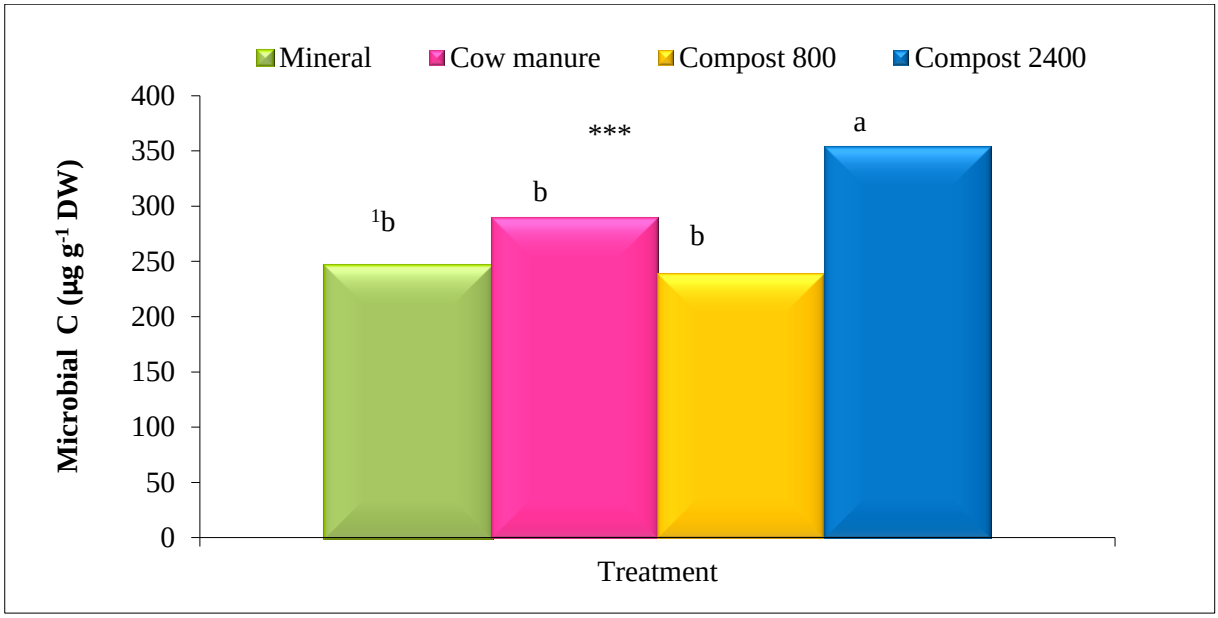

Figure 2.8 Effect of fertilization treatment on soil microbial biomass. ***: effect of treatment significant at $P \leq 0.001$. [1]Means followed by the same letter are not statistically different (at $P \leq 0.05$).

2.3. Discussion

Our data show that organic fertilizer applied at the highest rate (2400 g pot^{-1}) enhanced plant growth as compared with mineral fertilization. It is important to stress that this effect was observed not only in root, but also in wood, leaves and fruits, indicating a general positive effect of compost management on canopy net CO_2 fixation. Several authors indicated that organic amendment applications improve soil properties, such as: 1) nutrient availability (Melero et al., 2007; Baldi et al., 2010), 2) porosity (Aggelides and Londra, 2000; Celik et al., 2004), 3) microbial biomass and activity (Ferreras et., 2006; García-Gil et al., 2000; Melero et al., 2007; Tu et al., 2006); reduce bulk density (Aggelides and Londra, 2000) and penetration resistance (Aggelides and Londra, 2000); thus enhancing plant growth. In this study, microbial biomass was stimulated by application of compost at the highest rate. Since soil microbial activity regulates plant nutrient availability through the solubilisation of soil minerals and mineralization of OM (Grayston et al., 1997; García-Gil et al., 2000), therefore it is expected a close relationship between microbial biomass and soil fertility. Among nutrients, compost application was effective in promoting soil NH_4^+-N concentration, that is the form of N that is absorbed and assimilated by root with minimum energy expenses (Bloom et al., 1992). Studies on the effect of N ions on plant growth showed a higher response when at least part of N was supplied as NH_4^+-N. In tomato root growth the best ratio between NO_3^--N and NH_4^+-N was established as 3 (Bloom et al., 1993), our results show a

ratio of 3, and lower than 2 for mineral and compost fertilized soils, respectively. We can speculate that each species has an optimum ratio between NO_3^--N and NH_4^+-N, and that compost application could have supplied to peach root the ratio that allowed a better tree growth, than mineral fertilized trees. Moreover, it is possible that the addition to the soil of organic fertilizer has induced a positive priming effect releasing in the soil higher N quantity. Priming effect is defined by Kuzyakov et al. (2000) as "strong short term change in the turnover of soil OM caused by comparatively moderate treatments of the soil", the positive priming effect occurs when the added substance causes an acceleration of soil OM decomposition with an extra release of CO_2, mineral N and other nutrients that all together can have contributed to improve tree performances.

Leaves through photosynthesis produce large amount of C that is exported to the tree sinks (Leonardos and Grodzinski, 2002; Marchi et al., 2005) such as fruits, shoots, roots, buds, etc. In this study, leaf ^{13}C export was affected by fertilizer, because seven days after pulse, organic treated plants presented the lowest content of leaf ^{13}C, meaning a faster translocation of C to the other tree organs (i. e. shoot apex, root and wood), compared to mineral fertilized trees. These data are supported by the values of ^{13}C enrichment found in leaves 19 days after ^{13}C pulse, when mineral control trees showed a higher value, compared to organic fertilized plants. Carbon compounds assimilated in the leaves are partitioned to the organs according to the tree phenological stage (i.e. the timing of organ initiation and growth) (Wardlaw, 1990; Hendrix, 2002), the distance from the leaves (Jordan and Habib, 1996), so that sinks closer to the leaves seem to attract more C compounds than those located far away from the source (like trunk and roots). Thus storage in roots begins in late summer, when shoot and fruits has concluded their growth. However, in this work, C partitioning did not always follow these trends. In fact, in June (19 days after ^{13}C pulse), mineral fertilized plants presented more than half of the total ^{13}C of the tree still allocated into the leaves, in contrast, organic fertilized trees showed the highest percentage of ^{13}C in the permanent organs (roots and wood). In December (185 days after ^{13}C pulse), organic fertilized plants showed an important percentage of ^{13}C in roots, whereas mineral control trees showed higher partitioning in fallen leaves and lignified shoots. This response can be explained by the rate of growth of the roots that was higher in compost treated trees as compared to mineral fertilized plants, that was probably responsible for the higher percentage of labeled C partitioned to the growing root. From our results it seem that fertilizer affected tree growth, and growth affected C partitioning within tree.

As expected, the amount of ^{13}C found within the plant decreased over time, with a loss that 185 days after the pulse ranged between 57% (compost 2400) and 74% (mineral) of the total ^{13}C fixed, with the highest losses of C between May and June. This C was the results of tissue respiration as well as root rhizodeposition. Interestingly organic fertilized plants presented higher C recovery than mineral control plants, probably because the better soil conditions did not promote a high root exudation to improve nutrient uptake. The high loss rate found in late spring is probably the result of a high metabolic activity in this time of the year with the consequent high respiration rate.

Fertilizer source altered the C relative distribution at the end of the season (December), when all organic treated plants showed a higher percentage of ^{13}C in wood and root and lower ^{13}C enrichment in leaves and twigs compared to mineral fertilized plants. This result can be explained by stronger sink strength of roots in organic treated plants as shown by the higher biomass production and C concentration. In fact, mineral fertilized plants recycled a lower percentage of C, meaning the presence of weaker sinks.

3. RESPONSE OF ROOT GROWTH AND OXIDATIVE STRESS TO INCREASING CONCENTRATION OF SOIL MINERAL AND ORGANIC NITROGEN

3.1 Materials and methods

3.1.1. Plant materials and treatments

The experiment was carried out on 176 micro propagated rootstocks of GF 677 hybrids *Prunus persica* x *Prunus amygdalus*, between May and September 2009 at the Cadriano experimental station (44° 35' N, 11° 27' E) of the University of Bologna, Italy. Plants were potted in May 2009 in 4 liter containers filled with a clay loam Bathicalci Eutric Cambisols soil (FAO, 1990) and sand at rate of 2:1 and were fertilized as in a completely randomized block design with the following mineral or organic N rates:

- unfertilized control (0 mg of N kg^{-1} of soil);
- 200 mg kg^{-1} of N;
- 500 mg kg^{-1} of N;
- 1000 mg kg^{-1} of N.

The different rates of soil N were obtained by application of urea (mineral N) at: 2.2 g, 5.4 g and 10.8 g pot^{-1} for 200, 500 and 1000 mg kg^{-1}, respectively. Organic N was applied as compost at 76, 190, and 380 g pot^{-1}. Compost (table 2.2) was mixed with the soil before potting and was the same used in experiment 1. To prevent any risk of N leaching plants were protected from the atmospheric precipitations and were manually irrigated.

Eight, 37 and 94 days after fertilization (DAF) trees were harvested and separated in shoot (leaves + axes) and roots. At each harvest, shoot length was measured, and roots were cleaned with distilled water to eliminate the adherent soil, a white root sample was taken for enzyme analysis and electrolyte leakage. Shoot and remaining roots were oven-dried at 60˚C for 96 hours and weighted. Additionally, at 8 DAF, roots were photographed to measure the total root length through the software WinRHIZO Tron MF (Regent Instrument Inc., Quebec, Canada).

3.1.2. Stress- related analysis

To determine the root stress and oxidative damage eventually associated to the level of N in the soil, 1500 mg of white roots were sampled. A sub sample of 500 mg was used to measure the electrolyte leakage according to Huang et al., 2005. The roots were immersed in a beaker with 40 ml of deionized water and electrical conductivity (EC) was measured immediately (EC_i), after 30 minutes (EC_{30}) and after boiling for 5 minutes (EC_f). Membrane leakage was estimated as a percent of total electrolytes in the root:

$$\text{Eletrolyte leakage (\%)} = 100 \times (EC_{30} - EC_i) / (EC_f - EC_i)$$

The rest of white root (1000 mg) were immediately frozen in liquid nitrogen and then stored at -80˚C until analysis.

Superoxide dismutase (SOD) and catalase (CAT), enzymes associated with reactive oxygen and reactive nitrogen species, and total protein were assessed. Enzymes were extracted from frozen root samples finely ground in liquid nitrogen using a mortar and pestle previously chilled with liquid nitrogen. The frozen root powder was immediately used for the enzyme determination. All procedures for enzyme activity and determination were carried out at 0 ˚C in an ice bath unless otherwise stated. The frozen powders were homogenized with 2.5 ml of the corresponding extraction buffer: for CAT, the roots were suspended in cold 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 5% (w/v) polyvinylpolypyrrolidone (PVPP). For SOD, the buffer was made of cold 100 mM sodium potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 5% (w/v) PVPP. The slurries were kept for 30 min in an ice bath and then centrifuged at 15.000 rip x g for 30 min at 4°C. Aliquots of 1.5 ml from the supernatants of CAT and SOD were desalted in disposable NAP^{TM}25 columns (Amersham Biosciences AB, Uppsala, Sweden) and a 2.5 ml of eluate was recovered from each sample and utilized for enzyme assay and total soluble protein determination.

Catalase activity was determined at 20°C according to Aebi (1984). The reaction medium contained 10 mM H_2O_2 in 50 mM NaK phosphate buffer, pH 7.0 and 100 µl of enzyme eluate in a total volume of 1.2 ml; CAT activity was estimated by the decrease in absorbance of H_2O_2 at 240 nm and was expressed according to Havir and McHale (1987), where one unit of CAT activity corresponded to the amount of enzyme that decomposes 1 µmol of H_2O_2 per minute.

Total SOD activity was determined according to Madamanchi et al. (1994) modified by Masia (1998). For each sample assay, six tubes were set up containing 10, 20, 40, 60, 80 and 500 µl of the enzyme extract. The reaction medium contained 2 µM riboflavin, 10 µM L-methionine, 50 µM nitro blue tetrazolium (NBT), 20 µM KCN, 6.6 µM Na_2EDTA, 10 to 500 µl of the enzyme eluate and 65 µM Na-phosphate buffer, pH 7.8, to give a total volume of 3.0 ml. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT to blue formazan. Tubes were thermostated at 25 °C for 10 min. in absence of direct light. The reaction was started by exposing the mixture to four white fluorescent lamps (Leuci, 15 WTS preheat, daylight 6500 °K) in a box with aluminium-foil-coated walls. The blue colour developed in the reaction was spectrophotometrically measured at 560 nm (A560). One unit of total SOD activity will be defined as the amount of enzyme required to produce 50% inhibition of NBT photoreduction.

Total protein was determined in an aliquot of eluate resulting from the desalted supernatant used from CAT and SOD determinations. The aliquots were added to 2 ml 20% thiobarbituric acid (TCA) solution and left overnight at 4°C; the denatured and flocculated proteins were suspended then centrifuged at 14.000 x g for 20 min and the supernatant discarded. The protein pellet was suspended in 2.0 ml 0.5 M NaOH. After complete solution the supernatant was used to determine total soluble proteins with a commercial kit (BCA protein assay reagent kit, Pierce, Rockford, IL, USA) according to Smith et al. (1985). Bovine serum albumine was used as calibration standard.

Starch was determined according to Rasmussen e Henry (1990) on a 0.2 g dry root sample weighted accurately (0.2 g) into a culture tube (16 x 100 mm). At each tube 2.5 ml of the acetate buffer and 20 µl Termamyl (α-amylase) were added and tubes were incubated in a boiling water bath for 30 min; and mixing samples 3 times with a vortex mixer. Tubes were then removed, allowed to cool to room temperature and then 10 µl amyloglucosidase were added and samples were incubated overnight at 60 °C. The following morning tubes were centrifuged at 2500 rpm for 5 min and 0.5 ml of supernate was diluted in 10 ml of distilled water. Only 0.2 ml of the diluted supernate was transferred to small tubes (15 x 85 mm); 5 ml of glucose oxidase solution were added to tubo and samples were incubated in a water bath for 15 min at 40 °C, removed and allowed to stand at room temperature for 60 min. Finally, the samples absorbance was read at 505 nm and the absorbance of blank was read against distilled water. Starch was calculated using a calibration curve obtained with increasing concentration of potato starch treated as samples

3.1.3. Organ N concentrations

Total N concentrations in leaves and roots were determined by Kjeldahl method (Schuman et al., 1973) by mineralizing 0.5 g of ground tissue with 12 ml of a 95:5 (v/v) H_2SO_4:H_3PO_3 mixture, at 420 °C, for 180 min, distillation with 32% (v/v) NaOH and titration with 0.2 M HCl.

3.1.4. Soil analysis

At harvest time, soil samples were taken to evaluate NO_3^--N and NH_4^+-N concentration, as previously described (see 2.1.4) and total N. Total N concentrations were determined by Kjeldahl method (Schuman et al., 1973) as previously described (see 3.1.3).

3.1.5. Statistical analysis

Data were statistically analyzed as in a factorial experimental design with 2 factors: source of N (2 levels: mineral and organic) and rate of application (4 levels: 0, 200, 500 and 1000 ppm). When analysis of variance showed statistical significance ($P \leq 0.05$), means were separated by Student Newman-Keuls (SNK) test; when interaction between source and rate of N was significant, 3 times standard error of means (MSE) was used as the minimum difference between two means statistically different at $P \leq 0.05$ (Saville and Rowarth, 2008). Pearson correlation analysis was performed to evaluate the relation between leaf and root N concentration, NO_3^-, NH_4^+ and total N.

Polynomial contrast analysis was carried out to evaluate the function that best described the response to increasing N application rate of soil nitrate-N, ammonium-N, and total N concentrations, shoot and roots biomass production, and leaf N and root N concentrations.

3.2. Results

3.2.1. Soil N concentration

At all dates, source and rate of N application significantly interacted with soil NO_3^--N concentration. Mineral fertilizer application rate always increased soil nitrate-N concentrations linearly ($P \leq 0.001$) (figure 3.1). In contrast, no significant differences were induced by the rate of organic N (figure 3.1)

Rate of application and fertilizer type significantly interacted with soil NH_4^+-N concentrations. Eight DAF, compost treated soils did not show any NH_4^+-N increase, while in

mineral N fertilized soil the concentration of NH_4^+-N increased linearly with the rate of N application (figure 3.2). The same trend was observed at 37 DAF and 94 DAF ($P \leq 0.001$) (figure 3.2). In these harvest dates, the application of organic N promoted a linear increase of soil NH_4^+-N concentration, with $P \leq 0.01$ and $P \leq 0.05$ at 37 and 94 DAF, respectively (figure 3.2).

At the first harvest, soil total N concentration was affected by N source and application rate, with no interaction between the 2 factors (figure 3.3). Compared with mineral treated soils, compost fertilized soil showed, on average, higher values of total N, at the same time the rate of 1000 mg kg^{-1} presented the highest N concentration, followed by 500 mg kg^{-1}, control and 200 mg kg^{-1} (figure 3.3 a). At 37 DAF and 94 DAF there was positive interaction between N source and fertilization rate; mineral 500 and mineral 1000 treatments had similar effect, lower than compost 1000 and higher than control and mineral 200. At the end of the experiment compost 1000 and compost 500 showed higher N concentrations than mineral and other compost rates (figure 3.3 c). Organic N application rates were always linearly related to soil total N concentration, see figure 3.3 ($P \leq 0.001$) in all harvests; while mineral N treatment showed this relation only at second ($P \leq 0.001$) and third harvest ($P \leq 0.05$).

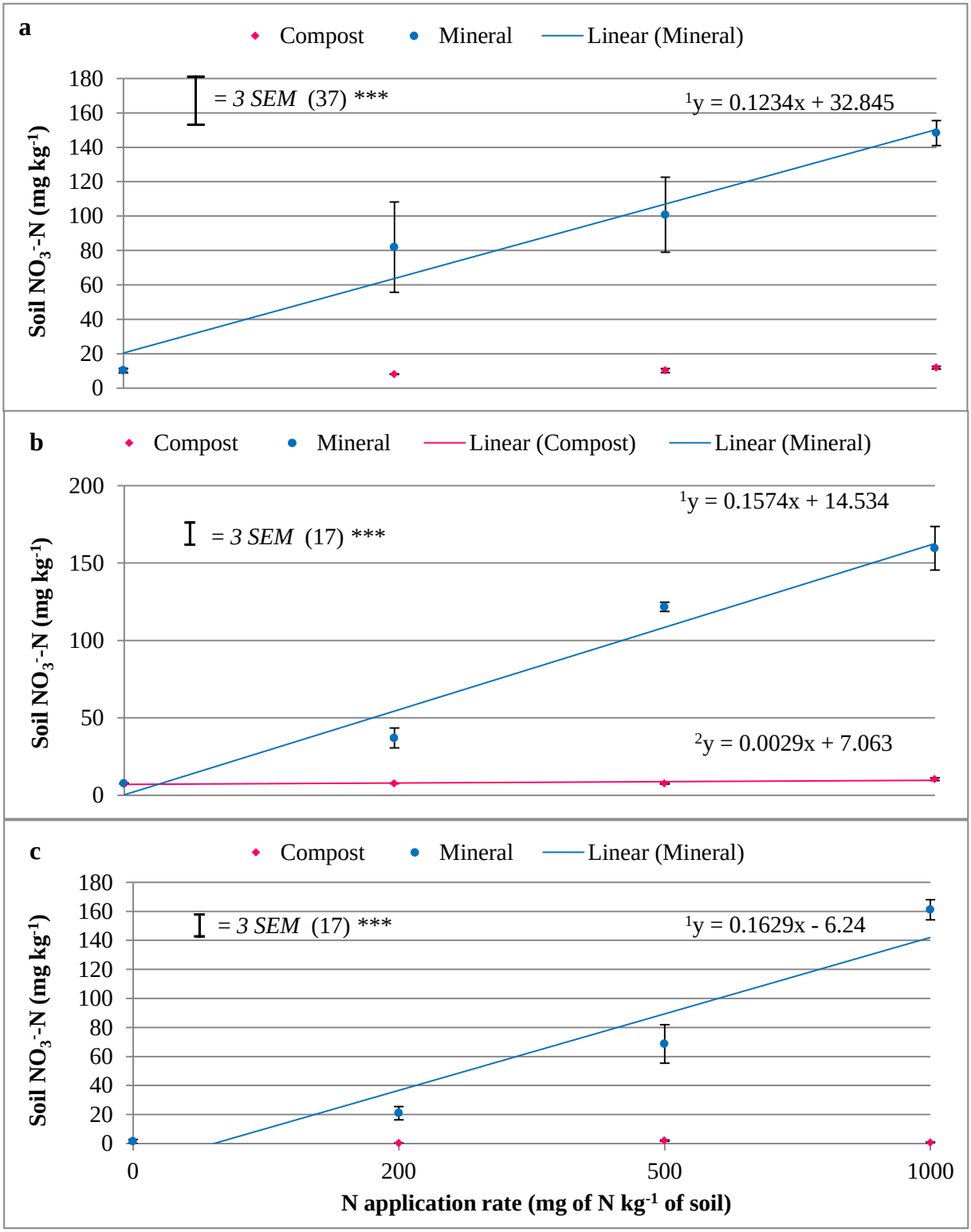

Figure 3.1 Effect of source and rate of N application on soil nitrate-N (NO_3^--N) concentration at 8 (a), 37 (b) and 94 (c) days after fertilization. *** Interaction between source and rate was significant at $P \leq 0.001$. Values differing by 3 standard error of means (SEM) are statistically different. [1,2]Mineral and compost trend function, respectively.

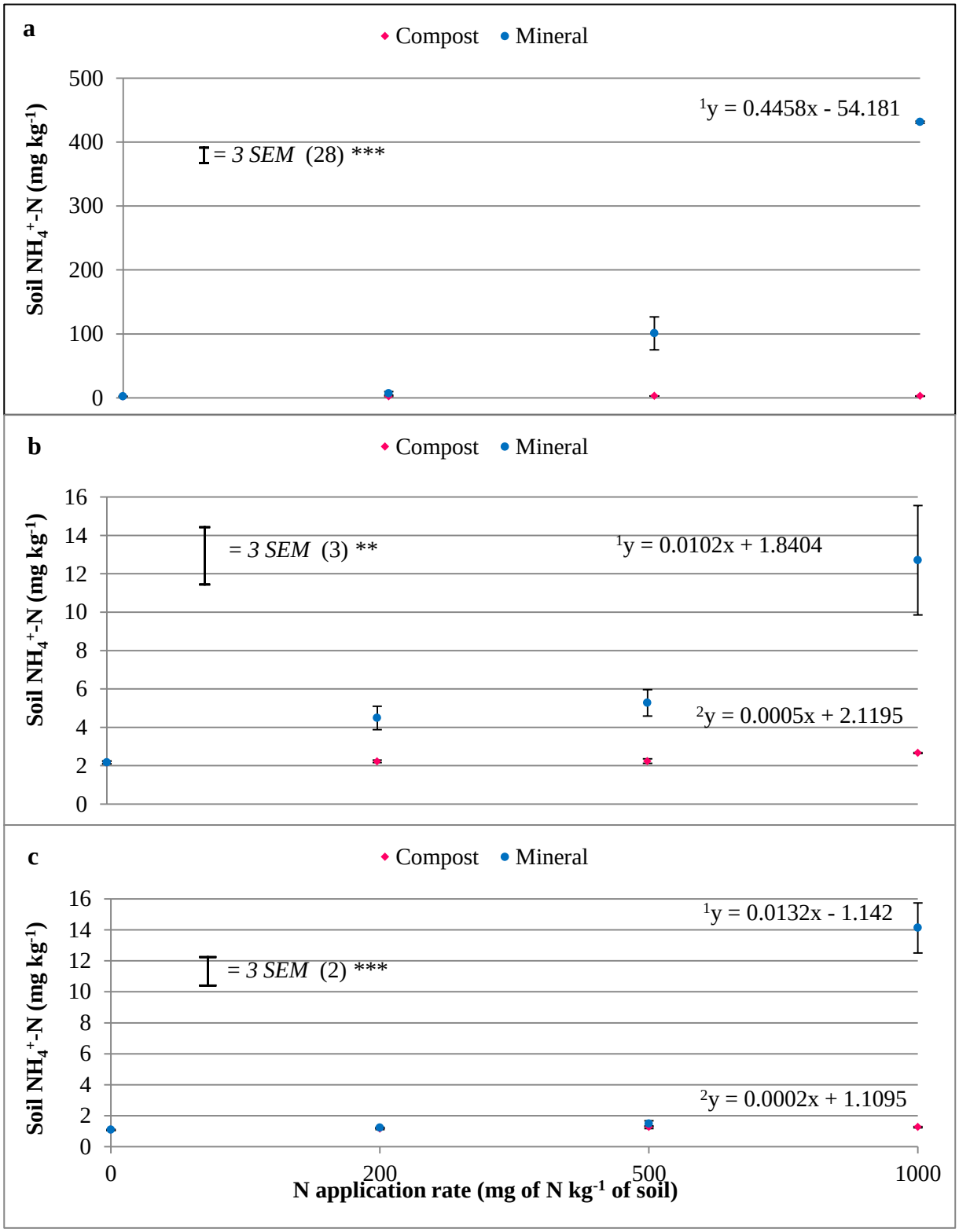

Figure 3.2 Effect of source and rate of N application on soil ammonium-N (NH_4^+-N) concentration at 8 (a), 37 (b) and 94 (c) days after fertilization. **, ***: Interaction between source and rate was significant at $P \leq 0.01$ and $P \leq 0.001$, respectively. Values differing by 3 standard error of means (SEM) are statistically different. [1,2]Mineral and compost trend function, respectively.

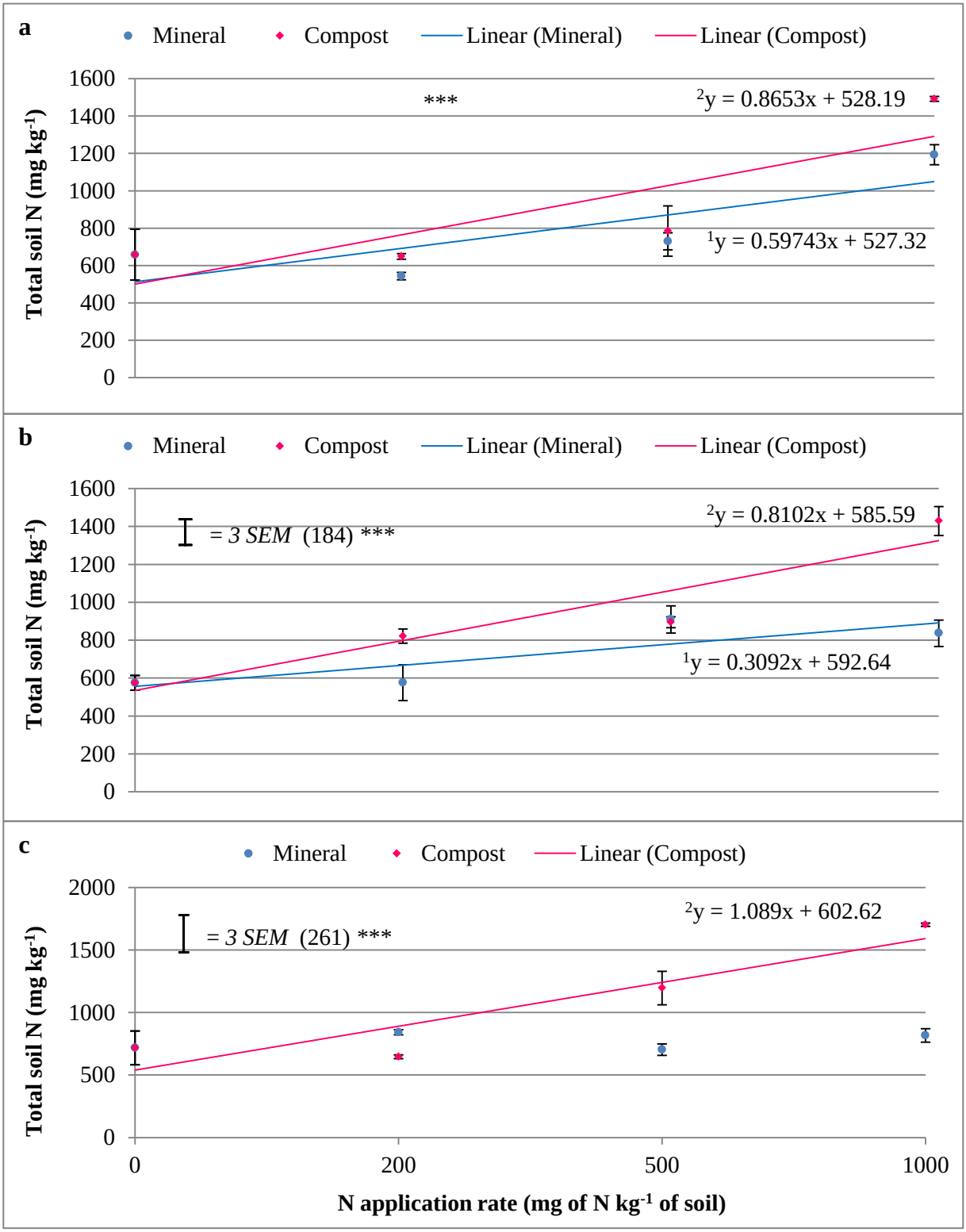

Figure 3.3 Effect of source and rate of N application on soil total-N concentration at 8 (a), 37 (b) and 94 (c) days after fertilization. 8 DAF no statistic interaction between source and rate of N application was observed and only application rate was statistically significant at $P \leq 0.001$. ***: Interaction between source and rate was significant at $P \leq 0.001$, 37 and 94 DAF. Values differing by 3 standard error of means (SEM) are statistically different. [1,2]Mineral and compost trend function, respectively.

3.2.2. Root length and biomass production

Source and rate of N significantly interacted with root length at 8 days after fertilization (table 3.1). Mineral 200 and 1000 and compost 200 mg N kg^{-1} were similar to unfertilized plants; compost 1000 showed the highest root length (table 3.1; and figure 3.4).

Table 3.1 Effect of source and rate of N application on total root length (cm) at 8 days after fertilization (DAF).

	8 DAF	
N Rate (mg kg^{-1})	Mineral	Compost
0	382	
200	509	512
1000	320	661
Interaction	** (3 SEM)= 187*	

* Interaction between source and rate of N application significant at $P \leq 0.05$. Values differing by 3 standard error of means (SEM) are statistically different.

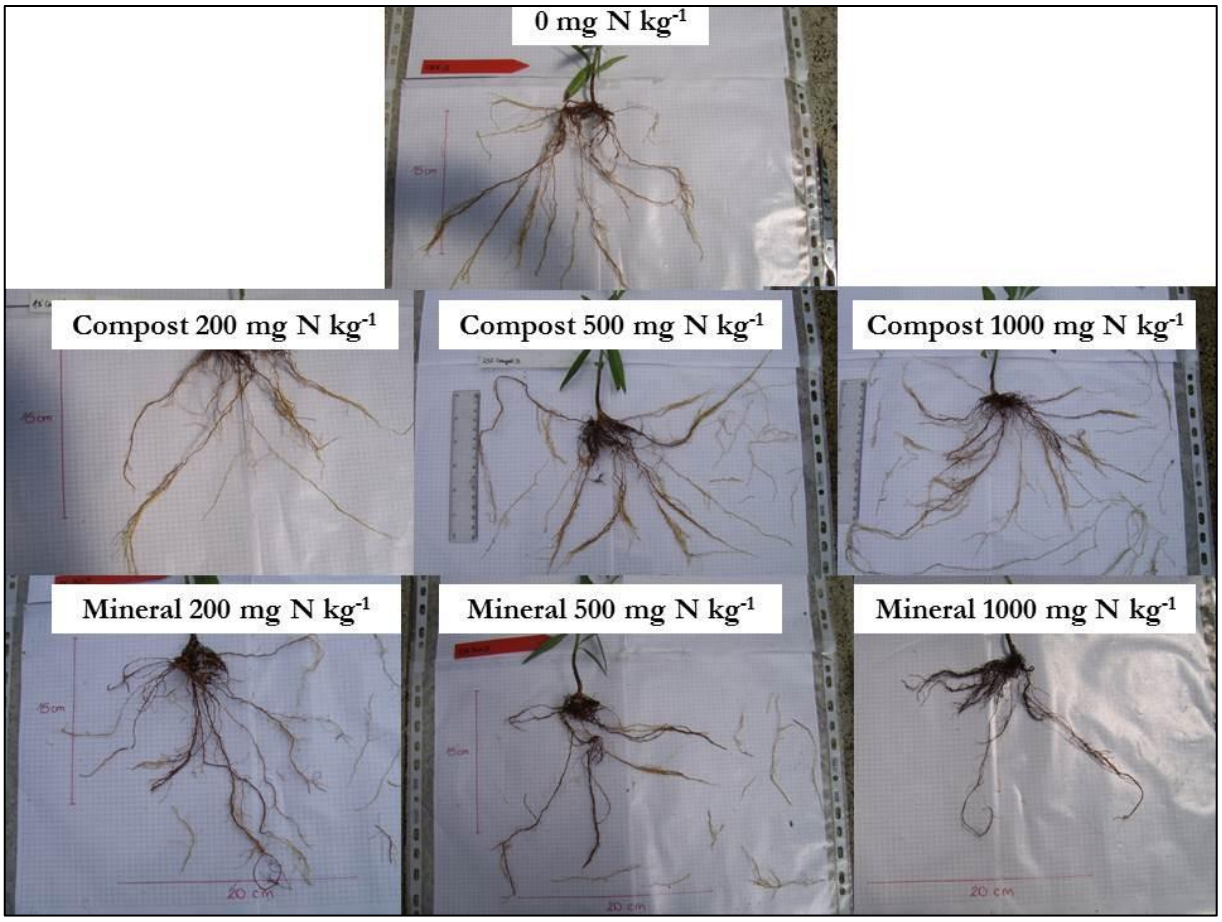

Figure 3.4 Effect of source and rate of N application on root length at 8 days after fertilization.

Source and rate of N significantly interacted with shoot growth at all times of harvesting. Eight days after fertilization, N application of mineral 200 and compost 1000 induced the highest shoot biomass (figure 3.5 a). The application of 200 mg kg^{-1} of mineral N induced an increase of shoot biomass compared to the untreated control, higher rate negatively affected shoot growth. Only the application of 1000 mg kg^{-1} of organic N brought about an increase of biomass compared to the untreated control. At 37 and 94 DAF, N application of mineral N at 200 and 500 mg N kg^{-1} showed the highest shoot biomass, with mineral 200 being higher at 37 DAF and mineral 500 higher at 94 DAF (figure 3.5). At both dates, compost treatments showed the lowest shoot growth, with little or no effect of increasing rates. At all harvest times shoots dry weight increased linearly and according to a second degree function as a response of organic ($P \leq 0.001$) and mineral fertilizer ($P \leq 0.001$) application rates, respectively (figure 3.5).

In the first and second sampling day, rate and source of N significantly interacted with root growth. Eight DAF, no significant differences were induced by compost treatments. Among rate of mineral N, the higher root dry weight was found as a response of application of 200 mg N kg^{-1} compared to untreated control (figure 3.6 a). In the second harvest, root biomass was increased by application of 200 and 500 mg of N kg^{-1} of mineral N, but not of organic N (figure 3.6 b). At last sampling no interaction between factors was found; mineral N was more effective than organic N in promoting root growth, with organic N application rate that showed not effect (figure 3.6 c). Roots biomass production showed a different response to increasing rate of N application, according to the source of N. Compost treated plants showed a linear and third degree trend at 37 DAF and 94 DAF (both with $P \leq 0.001$), respectively; in contrast, mineral treated plants presented a cubic trend at 8 DAF ($P \leq 0.05$) and second degree trend at 37 DAF ($P \leq 0.05$) (figure 3.6).

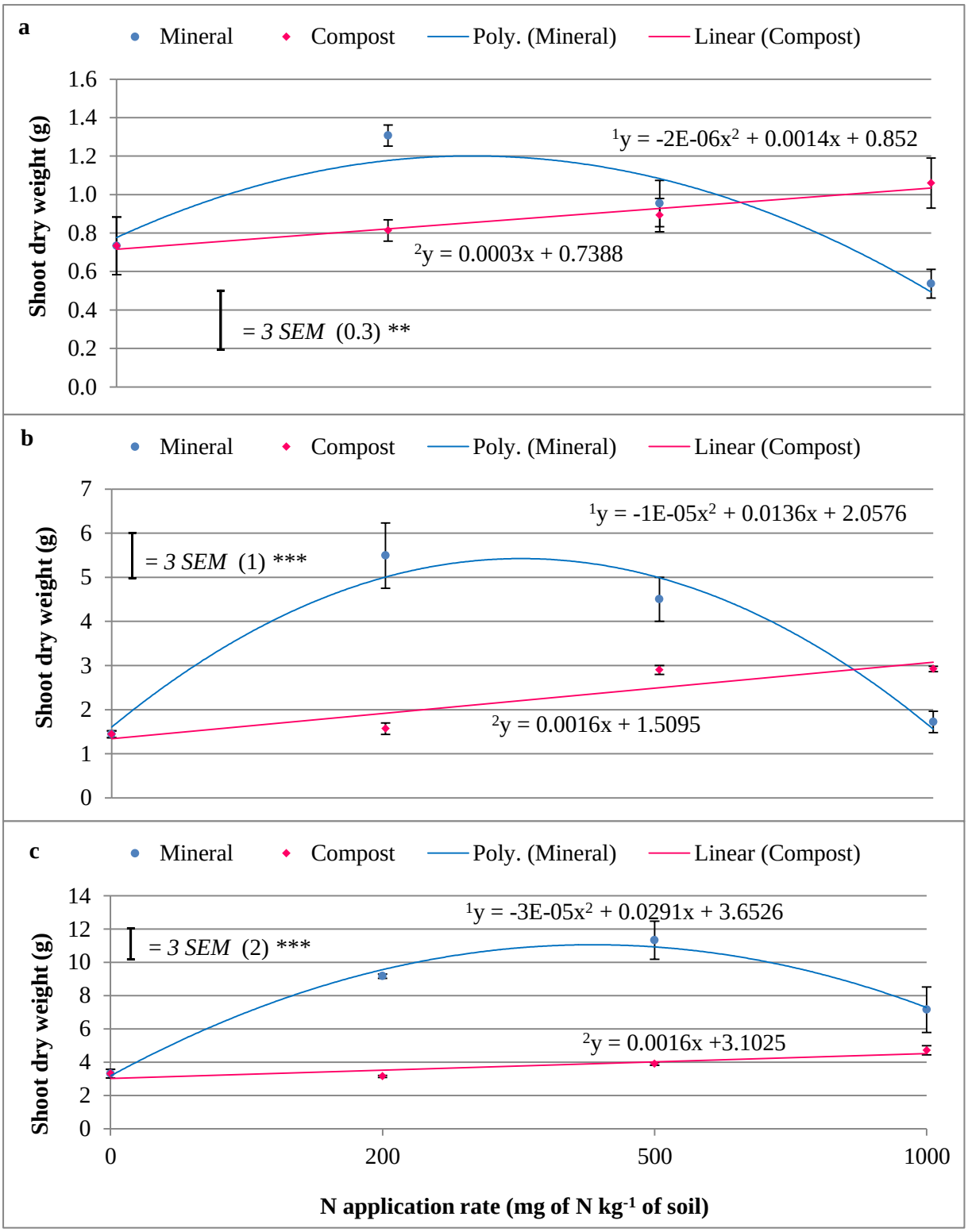

Figure 3.5 Effect of source and rate of N application on shoot biomass (g) at 8 (a), 37 (b) and 94 (c) days after fertilization. **, ***: Interaction between source and rate was significant at $P \leq 0.01$ and $P \leq 0.001$, respectively. Values differing by 3 standard error of means (SEM) are statistically different. [1,2]Mineral and compost trend function, respectively.

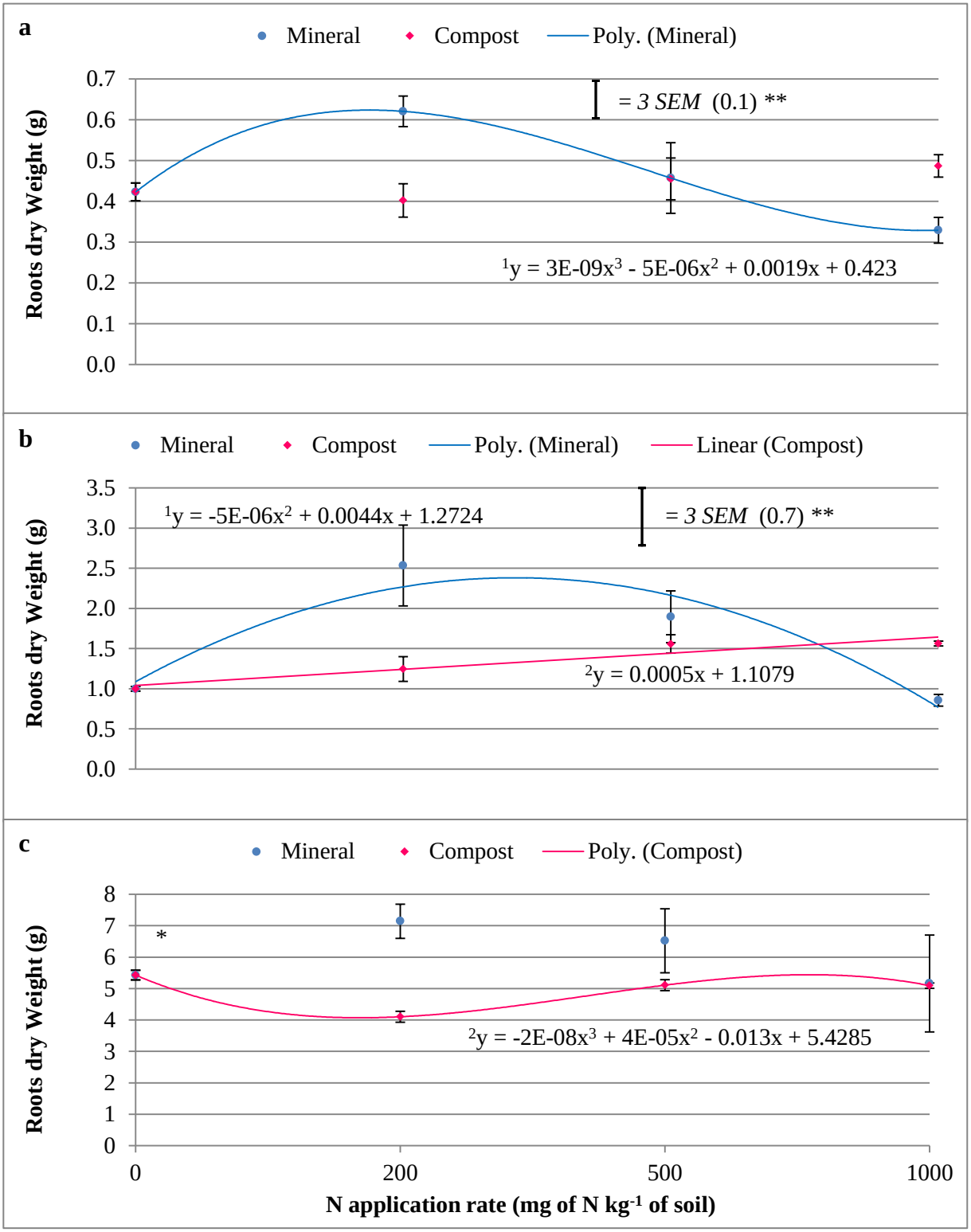

Figure 3.6 Effect of source and rate of N application on root biomass (g) at 8 (a), 37 (b) and 94 (c) days after fertilization. 94 DAF no statistic interaction between source and rate was observed and only source of N was statistically significant at $P \leq 0.05$. ** Interaction between source and rate was significant at $P \leq 0.01$. Values differing by 3 standard error of means (SEM) are statistically different. [1,2]Mineral and compost trend function, respectively.

3.2.3. Leaf and root N concentrations

Rate and source of N significantly interacted with leaf N concentration in all sampling dates. At 8 DAF all rates of mineral N were effective in increasing leaf N concentration that was similar in 200 and 500 mg N kg^{-1} treated plants. At the same time organic N had no effect, with the exception of compost 200 that showed the lowest leaf N concentration (table 3.2). Thirty-seven DAF, compost 200 and 1000 were similar to control plants; all mineral treatments increased leaf N concentration that was similar after the application rate of 500 and 1000 mg N kg^{-1}. At 94 DAF all compost treated plants and mineral 200 had similar concentration compared to unfertilized control; mineral 1000 showed the highest values followed by mineral 500 (table 3.2).

Leaf N concentration was linearly related to increasing rate of mineral N application at all harvest time ($P \leq 0.001$). In contrast, organic fertilized plants presented a cubic trend ($P \leq 0.001$) at 8 DAF and second degree trend ($P \leq 0.05$) at 94 DAF (figure 3.7)

Table 3.2 Effect of source and rate of N application on leaf N concentrations (%) at 8, 37 and 94 days after fertilization (DAF).

	8 DAF		**37 DAF**		**94 DAF**	
N RATE (mg kg^{-1})	Mineral	Compost	Mineral	Compost	Mineral	Compost
0	2.30		1.87		2.13	
200	3.08	1.56	2.41	1.82	2.18	2.06
500	2.97	2.11	2.75	2.20	2.94	2.01
1000	3.38	2.44	2.79	2.11	3.33	2.25
Interaction	**** 3 SEM= 0.25*		*** 3 SEM= 0.26*		**** 3 SEM = 0.35*	

, * Interaction between source and rate of N significant at $P \leq 0.01$ and $P \leq 0.001$, respectively. Values differing by 3 standard error of means (SEM) are statistically different.

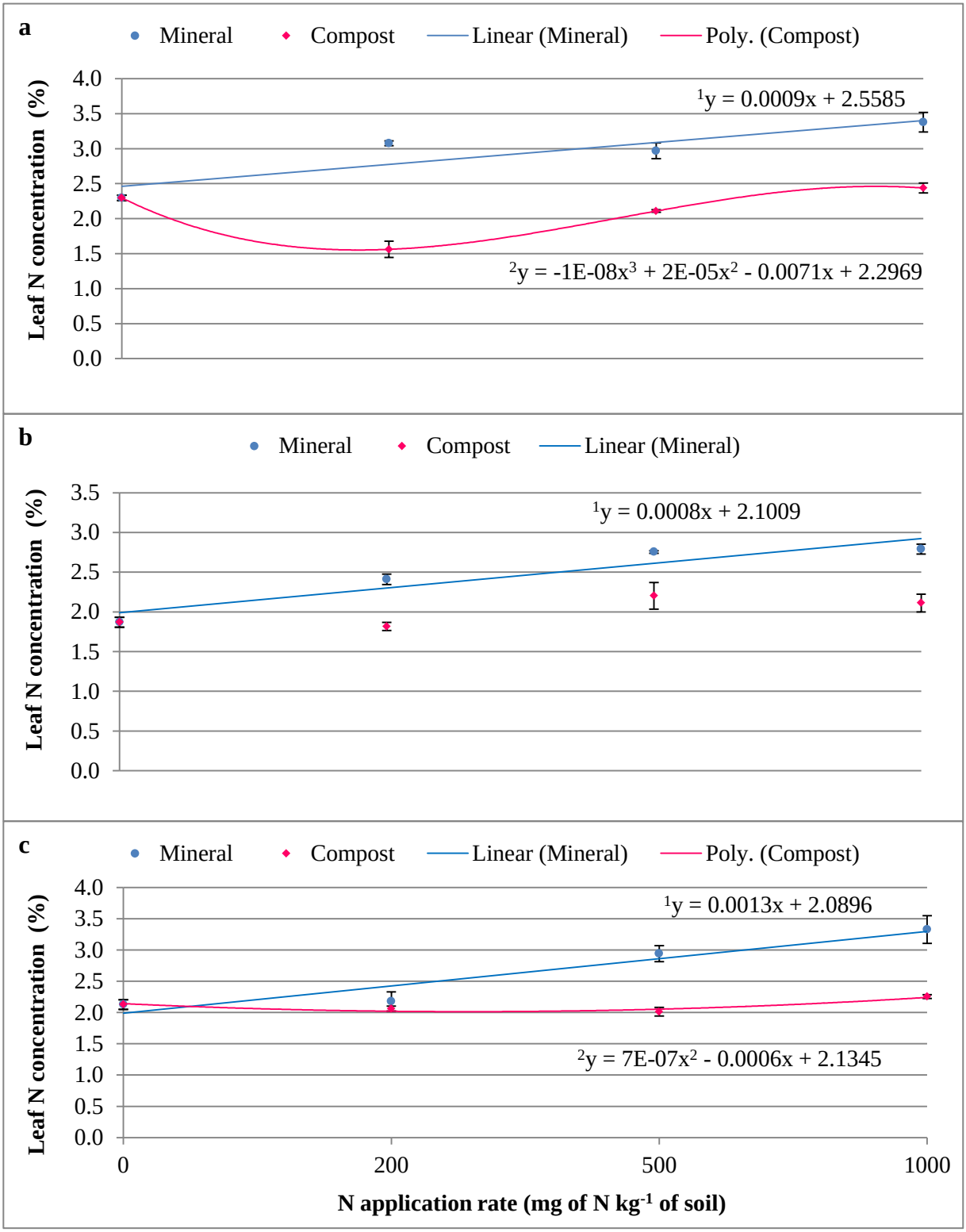

Figure 3.7 Effect of source and rate of N application on leaf N concentration (%) at 8 (a), 37 (b) and 94 (c) days after fertilization, according to polynomial analysis. [1,2]Mineral and compost trend function, respectively.

At all dates, source and rate of N application significantly interacted with root N concentration (table 3.3). Compost treated plants showed a similar root N concentration to unfertilized plants, and lower than mineral fertilized trees at all evaluation times. Eight days after fertilization, all mineral treated plants showed similar root N concentration, higher than control and all compost treatments (table 3.3). At 37 DAF mineral fertilization induced an increase of N concentrations compared to control and compost plants; application of mineral N at 200 and 500 mg N kg^{-1} promoted the highest values, followed by mineral 1000. In the last harvest, mineral 1000 showed the highest root N concentration, followed by mineral 500 and 200 that had similar concentrations (table 3.3).

Root N concentration was related to increasing rate of mineral N application. Eight and 37 DAF, a second degree equation best described this relation ($P \leq 0.05$ and $P \leq 0.001$, respectively), whereas at 94 DAF a linear trend $P \leq 0.001$ was observed (figure 3.8). In contrast, organic fertilized plants did not show a clear trend.

Table 3.3 Effect of source and rate of N application on root N concentrations (%) at 8, 37 and 94 days after fertilization (DAF).

	8 DAF		**37 DAF**		**94 DAF**	
N RATE (mg kg^{-1})	Mineral	Compost	Mineral	Compost	Mineral	Compost
0	1.26		1.15		0.94	
200	1.87	1.18	1.69	1.11	1.31	1.04
500	1.74	1.19	1.75	1.17	1.37	1.01
1000	1.66	1.03	1.52	1.07	1.66	1.08
Interaction	**3 SEM = 0.37*		****3 SEM = 0.16*		*** 3 SEM = 0.19*	

*, **, *** Interaction between source and rate of N significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively. Values differing by 3 standard error of means (SEM) are statistically different.

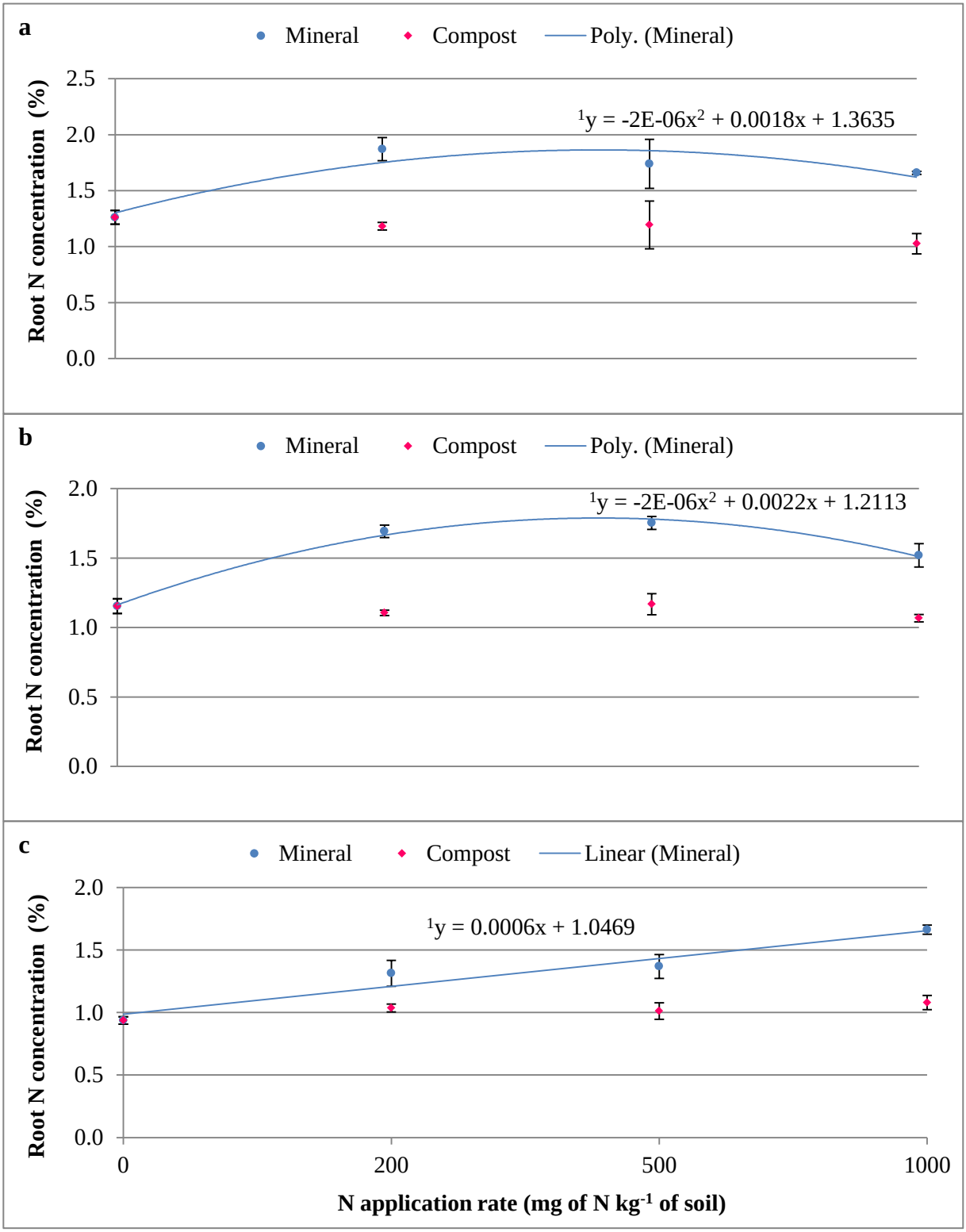

Figure 3.8 Effect of source and rate of N application on root N concentration (%) at 8 (a), 37 (b) and 94 (c) days after fertilization, according to polynomial analysis. [1,2]Mineral and compost trend function, respectively.

Soil NO_3^--N and NH_4^+-N concentrations were linearly relate to with leaf N concentration (figure 3.9) with a Pearson correlation coefficient higher for NO_3^--N than NH_4^+-N (table 3.4), in contrast, root N concentration was correlated only with soil NO_3^--N. Soil total N was not related to organ N concentration, or to NO_3^--N and NH_4^+-N. Root N concentration was related with leaf N concentration with a Pearson coefficient of 0.67 (table 3.4).

Table 3.4 Correlation coefficient (r) and significance between leaf and root N concentrations, nitrate-N, ammonium-N and soil total-N concentrations.

	Leaf-N	**Root-N**	**Soil NO_3^--N**	**Soil NH_4^+-N**	**Soil total-N**
Leaf-N	-	0.67	0.84	0.47	0.02
		***	***	***	*n.s.*
Root-N	0.67	-	0.68	0.29	-0.31
	***		***	*	*
Soil NO_3^--N	0.84	0.68	-	0.45	-0.08
	***	***		***	*n.s.*
Soil NH_4^+-N	0.47	0.29	0.45	-	0.18
	***	*	***		*n.s.*
Soil total-N	0.02	-0.31	-0.08	0.18	-
	n.s.	*	*n.s.*	*n.s.*	

n.s., *, **: not significant, significant at $P \leq 0.05$ or at $P \leq 0.001$, respectively.

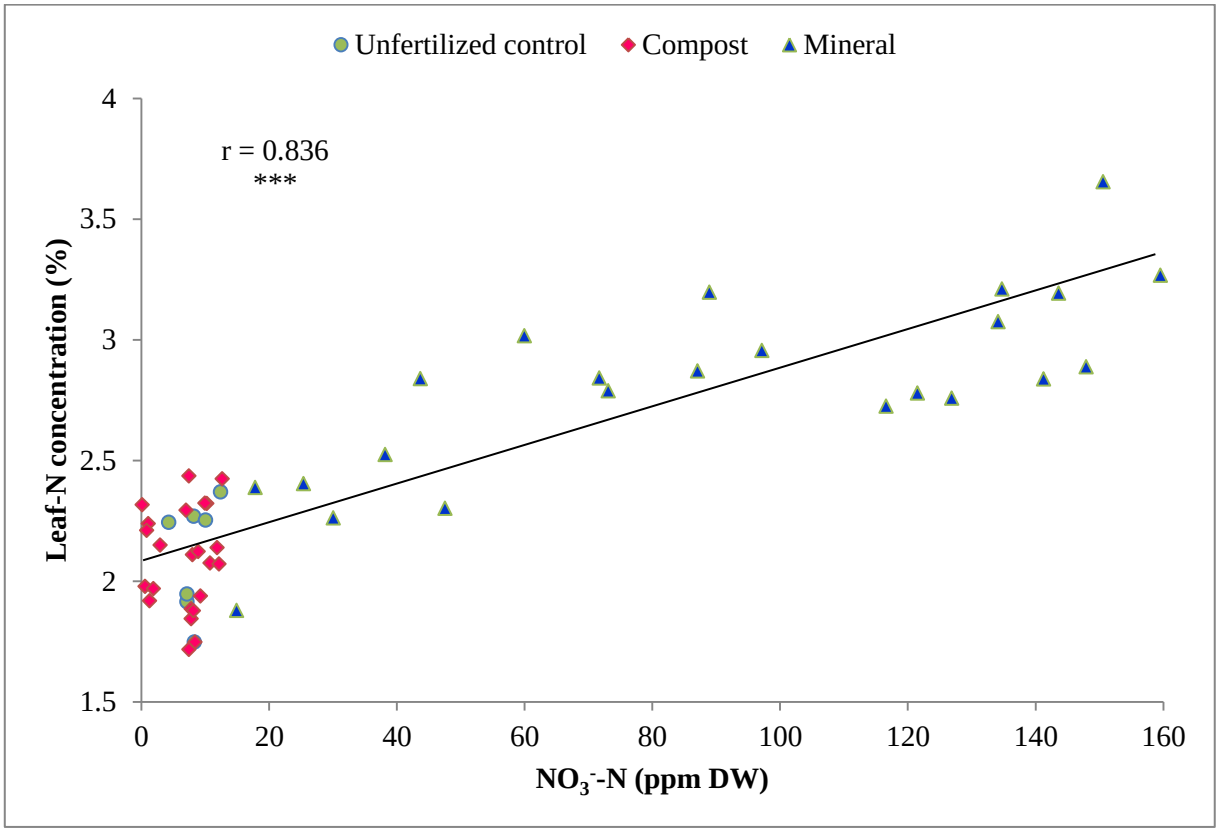

Figure 3.9 Correlation between soil NO_3^--N concentration and leaves N concentration (r: Pearson correlation coefficient; ***: linear correlation significant at $P \leq 0.001$).

3.2.4. Root stress evaluation

Source of N and rate of application did not significantly interact with root CAT activity. Eight days after fertilization CAT activity was not affected by source of N and was significantly higher in control plants than in fertilized trees (table 3.5). At 37 and 94 DAF no significant differences were observed among type of fertilizer and rates of N application. SOD activity was not influenced by the source of N at any of the sampling day. If considering the different N rate applications, 37 DAF SOD activity was increased by application of 200 mg N kg^{-1}, no matter the source of N (table 3.6). No significant differences were observed at 8 and 94 DAF.

Table 3.5 Effect of source and rate of N application on root CAT activity (unit mg^{-1} soluble proteins) at 8, 37 and 94 days after fertilization (DAF).

SOURCE OF N	**8 DAF**	**37 DAF**	**94 DAF**
Mineral	112	169	313
Compost	103	213	229
Significance	*n.s.*	*n.s*	*n.s*
N RATE (mg kg^{-1})			
0	186a[1]	132	169
200	84.1b	173	222
500	89.6b	226	311
1000	61.3b	232	372
Significance	**	*n.s*	*n.s*
Interaction	*n.s.*	*n.s*	*n.s*

n.s., **: effect of treatments not significant or significant at $P \leq 0.01$, respectively. [1]Values followed by the same letter are not statistically different (at $P \leq 0.05$).

Table 3.6 Effect of source and rate of N application on root SOD activity (unit mg^{-1} soluble proteins) at 8, 37 and 94 days after fertilization (DAF).

SOURCE OF N	**8 DAF**	**37 DAF**	**94 DAF**
Mineral	52.1	108	217
Compost	50.3	103	304
Significance	*n.s*	*n.s.*	*n.s.*
N RATE (mg kg^{-1})			
0	49.2	71.4b[1]	222
200	41.2	150a	305
500	67.0	107b	268
1000	46.5	94.5b	214
Significance	*n.s.*	**	*n.s.*
Interaction	*n.s.*	*n.s*	*n.s*

n.s., **: effect of treatment not significant or significant at $P \leq 0.01$, respectively.[1]Values followed by the different letter are statistically different (at $P \leq 0.05$).

The concentration of starch in roots was not affected by source of N (table 3.7). In the first harvest, root starch concentration was higher in control plants, compared to application

of 200 and 1000 mg N kg^{-1}; 500 mg kg^{-1} had a similar effect (table 3.7). No significant differences were found among factors in the other dates.

Table 3.7 Effect of source and rate of N application on root starch concentration (mg g^{-1} D.W.) at 8, 37 and 94 days after fertilization (DAF).

SOURCE OF N	**8 DAF**	**37 DAF**	**94 DAF**
Mineral	53.2	46.7	40.0
Compost	52.5	44.1	36.7
Significance	*n.s*	*n.s*	*n.s*
N RATE (mg kg^{-1})			
0	61.7a[1]	48.3	39.5
200	46.0b	44.3	39.7
500	55.1ab	45.4	37.5
1000	48.6b	43.6	36.6
Significance	*	*n.s*	*n.s*
Interaction	*n.s.*	*n.s*	*n.s*

n.s., *: effect not significant or significant at $P \leq 0.05$, respectively.[1]Values followed by the different letter are statistically different (at $P \leq 0.05$).

Thirty-seven DAF, in general the application of mineral N increased electrolyte leakage compared to compost application, while no differences were observed 8 DAF (table 3.8). Considering the rate of application, 8 DAF the application of 200 mg kg^{-1} N increased electrolyte leakage compared to 1000 mg kg^{-1} and unfertilized plants, no matter the source of N; 500 mg kg^{-1} had intermediate value. At 94 DAF there was a positive interaction between source and rate of N application; at this data mineral N at 200 and 500 mg N kg^{-1} showed the highest electrolyte leakage

Table 3.8 Effect of source and rate of N application on root electrolyte leakage (%) at 8, 37 and 94 days after fertilization (DAF).

	8 DAF	**37 DAF**	**94 DAF**	
TREATMENT			Mineral	Compost
Mineral	32.2	21.8a	-	-
Compost	29.7	16.1b	-	-
Significance	*n.s.*	*	-	-
N RATE (mg kg^{-1})				
0	28.4b[1]	17.7	21.5	
200	36.5a	18.5	28.0	17.9
500	32.1ab	18.0	31.8	17.9
1000	26.8b	21.4	23.1	16.1
Significance	*	*n.s.*	** 3 SEM = 6.20*	

n.s., *: effect not significant or significant at $P \leq 0.05$. [1]Values followed by the same letter are statistically different (at $P \leq 0.05$). At 94 DAF interaction between source and rate of N application a significant at $P \leq 0.05$. Values differing by 3 standard error of means (SEM) are statistically different.

3.3. Discussion

Soil total N concentrations in organically fertilized soils were higher than mineral fertilized plots. Other authors observed the same behavior when compared soil amended organically with mineral fertilized soils (Burger and Jackson, 2003; Kramer et al., 2006; Herencia et al., 2007). Since the rate of N application was the same for mineral and organic N source, we conclude that the organic fertilizer reduced the loss of N in the environment compared to mineral fertilizations. The N applied through compost is not immediately available for plant use and must be mineralized by soil microorganisms, thus resulting in a gradual release of inorganic N, which can be used for plants (Burger and Jackson, 2003; Herencia et al., 2007). The question is whether or not the mineralization rate can meet tree requirement. Soil nitrate-N concentration in compost fertilized tree was always included between 2 and 12 mg kg^{-1} that are considered optimal for peach growth (Tagliavini et al., 1996). Since peach trees seem to remove 10 mg kg^{-1} of N, corresponding to 50 kg N ha^{-1}, should meets tree requirements in all the phenological stages. Peach tree is considered to remove 100-150 kg N ha^{-1} year^{-1} according to tree yield.

This means that even high rate of OM application, does not produce a high mineralization rate, on the contrary it feeds soil microbial population, which decrease the risk

of an excessive production of NO_3^--N. The soil system reaches equilibrium, so that microbial biomass immobilizes the excess of NO_3^--N in the soil making it available at a low rate. Independently of the rate of N application, mineral fertilized soil had higher NO_3^--N concentrations compared with organic fertilization, which resulted in excess of tree demand increasing the risk of environment in the time because there is positive correlation between nitrate leaching and soil nitrate pools (Kramer et al., 2006). This NO_3^--N is available for plant use, but it can be more easily lost from the root zone because it is not adsorbed by soil particles and consequently is susceptible to leaching; for this reason high concentrations of inorganic N, can have detrimental environmental impacts (Keeney and Hatfield, 2001; Below, 2002; Robertson and Vitousek, 2009).

Shi and Norton (2000) showed low NH_4^+-N concentrations (< 1 mg kg^{-1}) in soil amended with compost. Similar results were obtained in this study, where independently of the rate of N application, compost treated plots showed very low NH_4^+-N. Compared to organically fertilized soil, mineral fertilization increased NH_4^+-N concentrations in the soil. This increase was greater in the first harvest, due to the rapid hydrolyzation of urea by free and microorganisms-bound urease; in fact, under Mediterranean conditions urea fertilizer can be completely hydrolyzed to ammonium within less than 5 days (Engels and Marschner, 1995).

Mineral fertilized plants presented higher leaf and root-N concentrations than compost treated plants. Similar results were found in apple leaves (Kramer et al., 2006) and in a crop rotation system (Herencia et al., 2007). Previous studies showed that fine root-N concentration is positively correlated with soil NO_3^- availability (Hendricks et al., 2000). Similar response was observed in this study, where either leaf and root-N concentrations were positively correlated with soil NO_3^--N concentrations better than with NH_4^+-N. These results bring evidence to the fact that soil fertility in term of N in calcareous soils can be increased by increasing NO_3^--N concentration.

In general, the response curve of plant growth to nutrient supply has three zones. In the first one, defined as deficient range, biomass production increases with increasing nutrient supply; in the second region, plant growth reaches a maximum and remains unaffected by nutrient amount (adequate range); finally, in the last region, plant biomass falls with increasing nutrient supply, indicating toxic range (Marschner, 1995). Shoot and root biomass production of mineral fertilized plants showed this trend. In fact at the first harvest, an adequate range was found at 200 mg of N kg^{-1} rate of N application, which presented a soil NO_3^--N concentration of 57 mg kg^{-1}, in contrast, 500 and 1000 mg of N kg^{-1} rate of N

application (with a NO_3^--N of 95 and 156 mg kg$^-$1, respectively) were toxic to plant growth; similar trend was observed at the second harvest; however, in the last harvest, the adequate range was 500 mg of N kg^{-1}, which had a soil NO_3^--N concentration of 75 mg kg^{-1}. In contrast, compost fertilized trees showed increases in biomass with increasing rate of N application because NO_3^--N in soil responded not linearly to compost application. These results suggest that soil NO_3^--N concentration higher than 95 ppm induced a toxic effect, in fact, there are different studies that showed a negative effect on plant growth at high soil and organ NO_3^--N concentration (Bloom et al., 1993; Scheible et al., 1997; Zhang et al., 1999; Linkohr et al., 2002; Wang et al., 2004). Moreover, mineral fertilized plants at rate of 1000 mg N kg^{-1} showed the lowest total root length with the highest NO_3^--N soil concentration. There are a number of evidences that high soil NO_3^--N concentration inhibited root growth. In *Arabidopsis*, Zhang et al. (1999) indicated that NO_3^--N concentration greater than 10 mM had a strong inhibitory effect on lateral root production, similar results were reported by Stitt and Feil (1999). As a possible explanation it has been postulated that NO_3^--N altered levels of phytohormones, such as cytokinin, auxins and abscisic acids, which were involved in root growth (Walch-Liu et al., 2005; Tian et al., 2008).

The soil NO_3^--N concentration that allowed the best growth of plant was higher than that discussed in commercial orchard and proposed by Tagliavini et al. (1996) who suggests 15 mg kg^{-1}. The value found in this study is 57, this discrepancy is probably the result of the different kind of trees investigated. Adult bearing tree, with a well establishes internal cycle of N, probably need less N per year than non-bearing young fast growing trees such those used in this experiment. In addition we used as a parameter to evaluate the effect of N soil availability the vegetation growth which is not the goal of a commercial orchard.

Higher soil N concentration probably negatively affect root life spans by increasing metabolic activity (Tjoelker et al., 2005; Withington et al., 2006; Guo et al., 2008). The enhancement of metabolism may require the presence of additional defense mechanisms against reactive oxygen species (ROS) such as CAT and SOD (Oliveira-Medici et al., 2004). In cotton plants, N application significantly increased the root CAT activity, but decreased SOD activity (Liu et al., 2008). Misra and Gupta (2006) observed higher SOD activity in NO_3^--fed plants than in NH_4^+ treated plants; and at the same time, higher CAT activity in NH_4^+-fed plants compared with NO_3^- supplied plants. Oliveira-Medici et al. (2004) observed in maize the highest CAT activity in roots grown at high N concentrations, whereas in barley, CAT activity was high in roots grown at the lowest N concentration. In the present study, only at the first harvest a significantly lower CAT activity was found on fertilized plants, with

unfertilized plants showing the highest values. Similar trend was observed for SOD activity, which only at second harvest presented significant differences, with highest values at 200 mg kg^{-1}. Catalase and SOD activities showed unclear trends, suggesting that unfertilized plants and 200 mg kg^{-1} treated plants would be more stressed than the other plants due to either by lack of mineral nutrients for the plant growth (in untreated plants) or by optimal plant growth that could lead a high metabolic activity. For this reason CAT and SOD were not a good tool for evaluate a possible root oxidative damage.

Scheible et al. (1997) found that nitrate accumulation in shoot leads to a strong inhibition of starch synthesis and turnover in leaves, decreasing level of sugar in root. In this study, at the first harvest, untreated plants showed higher starch content compared with the other treatments, indicating that increasing rate of N application might influence negatively starch accumulation in roots.

Electrolyte leakage can be associated to cell damage, and has been demonstrated that it is correlated with antioxidative enzyme synthesis (Mckay and White, 1996; Bajji et al., 2001). Elevated values indicate a high membrane leakage and so a high stress level. Our results are not completely clear, since an increase of electrolyte leakage was observed in plants fertilized with mineral N at a rate of 200 and 500 mg N kg^{-1} 8 and 94 days after fertilization. The magnitude of the response was however low, testifying a relatively mild stress. The level of nitrate N was probably low compared to the ability of the roots to adapt or this parameter does not indicate the real NO_3^- -N induced root stress.

4. EFFECT OF ORGANIC FERTILIZATION ON SOIL FERTILITY, TREE NUTRITIONAL STATUS AND PRODUCTIVITY

4.1. Materials and methods

4.1.1. Plant materials and treatments

The study was carried out in an experimental farm located in the south-eastern part of the Po valley of Italy (44° 27' Nord; 12°13' Est) on a nectarine (*Prunus persica*, Batsch var. *nectarina* (Ait) Maxim.) orchard. The trees of the variety Stark RedGold, grafted on hybrid GF 677 (*Prunus persica* x *Prunus amygdalus*), were trained as in a delayed vasette system and planted on January 2001, at a distance of 5 m between the rows and 3.8 m between trees along the row. Soil tillage was carried out in a 2 m wide tree row, while the alleys were covered with spontaneous grass. From June to September, trees were regularly watered with a drip irrigation system to return the weekly evapotranspiration rate, calculated on the basis of the data of the class A PAN evaporimeter of the local meteorological station. The key characteristics of the Calcaric Cambisol (FAO, 1990) soil of the orchard are summarized in table 4.1. The following treatments were compared, since orchard plantation in 2001, as in a randomised complete block design with four replicates:

- unfertilized control;
- mineral fertilization including phosphorus (P at 100 kg ha^{-1}) and potassium (K at 100 kg ha^{-1}) applied only at planting and N (70 kg ha^{-1}) split in May (60%) and September (40%). In 2004, N supply rate was increased to 120 kg ha^{-1}, and from 2006 to 130 kg N ha^{-1};
- compost at a rate of 5 t D.W. ha^{-1} $year^{-1}$;
- compost at a rate of 10 t D.W. ha^{-1} $year^{-1}$.

Fertilizer application in treatments 3 and 4 was split, as for mineral N fertilization in May (60%) and in September (40%). Compost was tilled into the soil at 25 cm of depth and applied only on the 2-m wide tree row, consequently on a hectare surface it was applied to 4,000 m^2 out of 10,000 m^2. Compost was the same used in the previous trials (table 2.2).

Table 4.1 Chemical and physical soil characteristics of the soil at the beginning (2001) of the trial (average of 4 replicates ± standard deviation).

Properties	
Sand (%)	6.7 ± 1.5
Silt (%)	67 ± 1.41
Clay (%)	26.2 ± 1.71
pH	7.8 ± 0.05
Ca carbonate (%)	30.5 ± 1.29
Active lime (%)	12.5 ± 1.29
Organic matter (%)	1.63 ± 0.13
K extractable (mg kg^{-1} D.W.)	182 ± 33.7
P Olsen (mg kg^{-1} D.W.)	18.5 ± 2.38
C.E.C.[1] (meq $100g^{-1}$ D.W.)	10.1 ± 1.95
Electrical conductivity (µS cm^{-1})	200 ± 8.2

[1]CEC: cation exchange capacity

4.1.2. Soil analysis

Soil OM and total N were measured yearly at the end of vegetative season. To assess the effect of treatments on soil NO_3^--N, NH_4^+-N and moisture, soil cores were collected at a depth of 0-40 and 40-80 cm four times a year (before spring fertilization, 40 days after spring fertilization, in mid-July and 40 days after late summer application). Nitrates and ammonium-N were extracted as previously described (cap. 2.1.4). Microbial biomass C was measured as described in cap. 2.1.4 on soil samples collected in the same dates of nitrate-N determinations and at the depth of 4-20 cm.

4.1.3. Canopy analysis

In July a sample of 40 leaves per plot were collected for mineral analysis, rinsed, oven-dried, milled and analysed for N, P, K, Ca, Mg, Fe, Mn, Zn and Cu. Total N concentrations were determined by Kjeldahl method (paragraph 3.1.3). Metal concentration in leaves was determined by atomic absorption spectrophotometry (Varian AA200, Mulgrave, Victoria, Australia) on samples previously mineralized by US EPA Method 3052 (Kingston, 1988) by treating 0.3 g of dry leaves in an Ethos TC microwave lab station (Milestone, Bergamo, Italy). Phosphorous concentration was determined according to Saunders and Williams, 1955 as follows: 0.5 g (DW) leaf samples were mineralized with 11 mL of 96%

(v/v) sulphuric acid and 4 mL of 35% (v/v) hydrogen peroxide, neutralized with 5 M NaOH and enriched with 30 mL of a mixture of 0.1 M ascorbic acid, 32 mM ammonium molybdate, 2.5 M sulphuric acid and 3 μM potassium antimonyl tartrate to develop a phospho-molybdic blue colour; P was spectrophotometrically quantified at 700 nm.

In September 2010 plastic net were positioned around one tree per block, in order to collect all abscised leaves; periodically leaves were collected and weighted.

4.1.4. Fruit production

Yield of the central 4 trees of each plot was recorded at commercial harvest; fruit average weight and precocity index were calculated. Precocity index was calculated according to the following formula:

$$P.I. = (dd1*kg1) + (dd2*kg2) + (dd3*kg3)/ kg1 + kg2 + kg3$$

where: dd = number of days between the day before the first harvest and each of the following harvest days (1, 2, and 3); kg = amount of peach harvested at each sampling date. Moreover 20 additional fruits were used for the determination of skin color, fruit firmness, pH, acidity and soluble solid content. Fruit firmness was determined by a pressure tester (Effe.Gi, Ravenna, Italy) fitted with an 8-mm-diameter plunger on two side of the fruit previously peeled. Two slices from each fruit were cut and homogenized; the juice obtained from each sample of 20 fruits was used for the determination of solid soluble concentration (SSC) by a digital refractometer (PR-1, Atago Tokio, Japan), acidity and pH by a Compact Tritator I (Crison, Barcellona, Spain). Two additional slices were collected, lyophilized, milled and used for mineral analysis as described previously.

4.1.5. Statistical analysis

Soil NO_3^--N and NH_4^+-N were analysed as in a factorial design with soil depths and treatments as factors. Otherwise data were analysed as in a complete randomised block design and when analysis of variance showed a statistical effect of treatments ($P \leq 0.05$), means were separated by Student Newman Keuls test.

4.2. Results

4.2.1. Soil fertility

In 2010, the application of compost at high rate increased soil OM if compared to other treatment (table 4.2). After 10 years, the application of 10 t ha^{-1} $year^{-1}$ of compost increased soil OM by 222 %, compost at 5 t ha^{-1} $year^{-1}$ increased OM by 104 if compared to the untreated control (table 4.2). Total N concentration was increased by compost application at the rate of 10 t ha^{-1} compared to the other treatments that showed similar N concentrations (table 4.2). Soil OM and total N were significantly correlated (figure 4.1). Soil pH resulted lower in soil treated with compost at both rates only if compared with unfertilized control (table 4.2).

Table 4.2 Effect of fertilization treatment on total N and organic matter and pH in the soil at the beginning of the trial (2001) and in 2010.

TREATMENT	Total N (‰)		Organic matter (%)		pH	
	2001	**2010**	**2001**	**2010**	**2001**	**2010**
Control	1.05	1.12 b[1]	1.67	1.93 b	7.8	8.08 a
Mineral	1.07	1.24 b	1.65	1.98 b	7.8	8.00 ab
Compost 5 t ha^{-1}	1.05	1.87 b	1.62	3.30 b	7.8	7.95 b
Compost 10 t ha^{-1}	1.05	2.83 a	1.63	5.25 a	7.8	7.93 b
Significance	-	***	-	**	*n.s.*	*

ns, *, **, ***: effect not significant or significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively.
[1]Means followed by the same letter are not statistically different ($P \leq 0.05$).

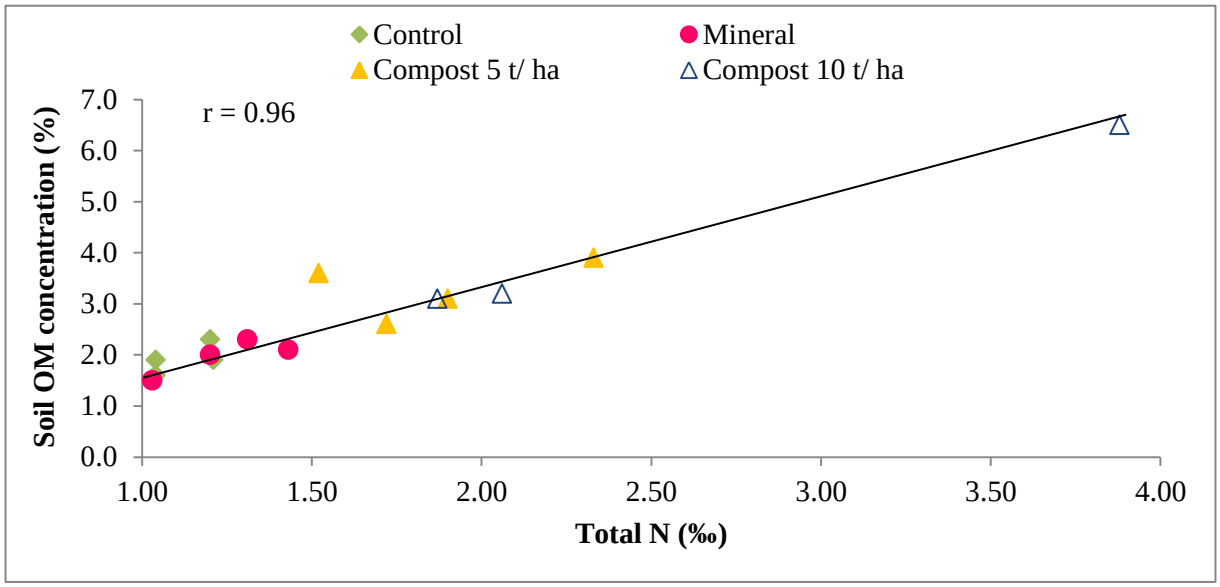

Figure 4.1 Correlation between soil organic matter (OM) and total N. (r: correlation coefficient: ***: linear correlation significant at $P \leq 0.001$).

In 2010 no significant interaction between soil depth and fertilization treatment was observed for mineral N concentrations. In May the application of mineral fertilizer and compost increased, in the soil profile of 0-80 cm, NO_3^--N concentration. In July, while compost application (at both rates) showed intermediate values. The application of compost at 10 t ha^{-1} increased nitrate-N availability at the end of the season (figure 4.2).

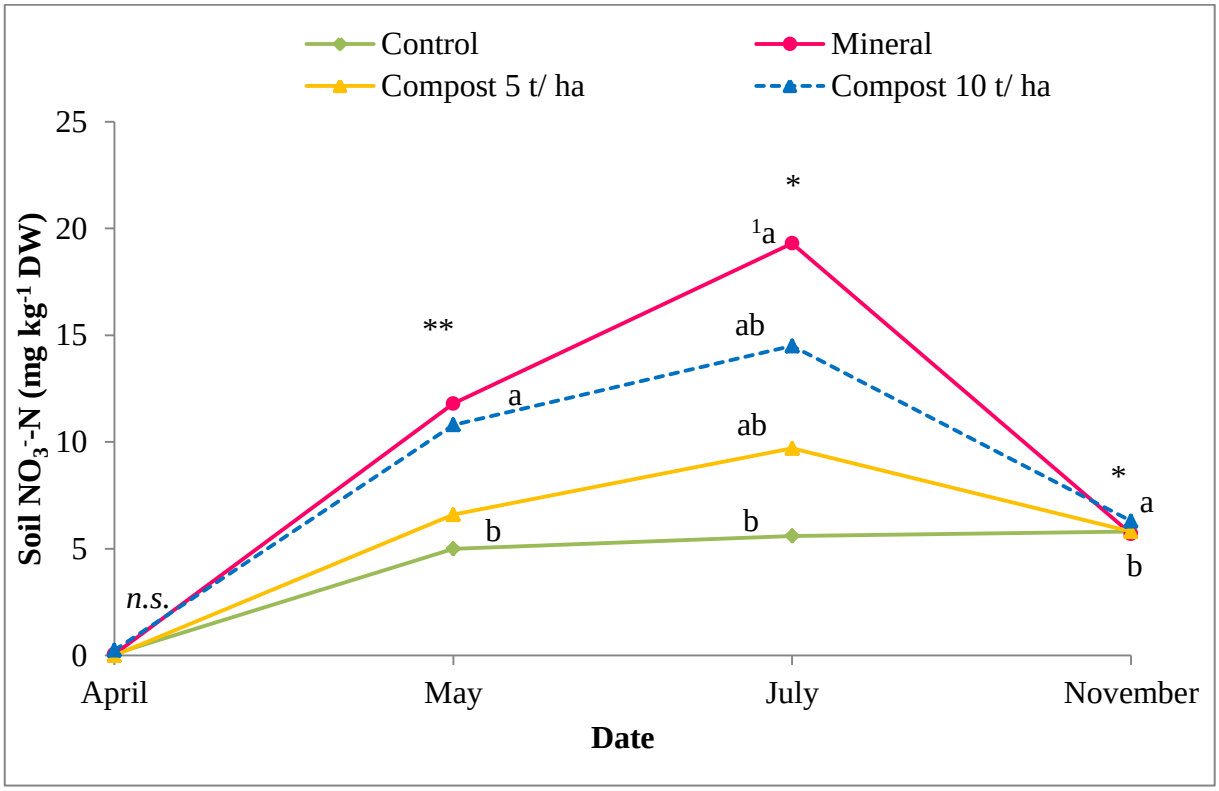

Figure 4.2 Effect of fertilization treatments on NO_3^--N concentration in soil as observed in 2010 at 0-80 cm of depth. ns, *, **: effect not significant or significant at $P \leq 0.05$ and $P \leq 0.01$, respectively. [1]Means followed by the same letter are not statistically different ($P \leq 0.05$).

Soil ammonium-N concentration was increased by the application of compost at the highest rate in April (table 4.3) and May. In July and November, low concentrations (less than 1 mg kg^{-1} DW) of NH_4^+-N were measured (data not reported). Depth did not influence NH_4^+-N concentration in soil.

Table 4.3 Effect of fertilization treatments on NH_4^+-N concentration in soil as observed in 2010.

TREATMENT	**April**	**May**
Control	4.0 b[1]	5.9 b
Mineral	7.1 b	5.7 b
Compost 5 t ha^{-1}	6.2 b	7.7 ab
Compost 10 t ha^{-1}	10.5 a	10.3 a
Significance	**	*
DEPTH (cm)		
0-40	8.5	8.5
40-80	5.3	6.3
Significance	*n.s.*	*n.s.*

ns, *, **,: effect not significant or significant at P ≤0.05, P ≤0.01, respectively. [1]Means followed by the same letter are not statistically different (P ≤ 0.05).

No significant interaction between treatment and depth was observed for soil moisture (table 4.4). The application of compost at high rate increased soil water content in April. In May, July and November no significant differences between treatments were observed. In April and November soil moisture was higher in the shallower soil layer, while in May and July it was higher among 40 and 80 cm of depth (table 4.4).

The application of compost at the highest rate increased soil microbial biomass in April and May, followed by compost at 5 t ha^{-1} (table 4.5), which promoted a higher microbial C, compared to untreated and mineral fertilizer treated soil (table 4.5). No significant differences were observed in July and November.

Table 4.4 Effect of fertilization treatments on soil moisture (%) as observed in 2010.

TREATMENT	April	May	July	November
Control	19.4 b[1]	15.5	14.4	17.5
Mineral	19.8 b	16.9	12.0	16.9
Compost 5 t ha^{-1}	20.1 b	16.4	14.1	17.2
Compost 10 t ha^{-1}	22.2 a	17.7	14.1	17.7
Significance	**	*n.s.*	*n.s.*	*n.s.*
DEPTH (cm)				
0-40	21.1	17.3	12.6	19.1
40-80	19.7	15.9	14.7	15.6
Significance	**	**	**	**

ns, *, **: effect not significant or significant at $P \leq 0.05$, $P \leq 0.01$, respectively. [1]Means followed by the same letter are not statistically different ($P \leq 0.05$).

Table 4.5 Effect of fertilization treatment on soil microbial carbon (µg C g^{-1} DW) in 2010.

TREATMENT	April	May	July	November
Control	276 d[1]	321 c	299	218
Mineral	345 c	355 c	342	280
Compost 5 t ha^{-1}	435 b	494 b	528	269
Compost 10 t ha^{-1}	588 a	733 a	548	337
Significance	***	***	*n.s.*	*n.s.*

ns, ***: effect not significant or significant at $P \leq 0.001$, respectively. [1]Means followed by the same letter are not statistically different ($P \leq 0.05$).

4.2.2. Leaf chlorophyll and mineral nutrient concentration

In 2010, trees treated with mineral fertilizer showed a higher leaf chlorophyll content than compost 5 t ha^{-1} and control; the application of compost at 10 t ha^{-1} showed SPAD values not different from mineral and compost 5 t ha^{-1} but higher than control (table 4.6). Leaf area of mineral plants was similar to compost (both rate) and higher than untreated control (table 4.6); specific leaf weight was higher in control plants if compared with compost at high rate and mineral fertilized trees (table 4.6).

Table 4.6 Effect of fertilization treatment on leaf chlorophyll, leaf area and specific weight.

TREATMENT	Leaf chlorophyll (SPAD unit)	Leaf area (cm^2/leaf)	Specific weight (mg/cm^2)
Control	38.0 c[1]	43.4 b	7.6a
Mineral	42.8 a	49.0 a	6.9b
Compost 5 t ha^{-1}	40.3 b	45.8 ab	7.2ab
Compost 10 t ha^{-1}	41.4 ab	47.4 ab	6.9b
Significance	***	*	*

ns, *, ***: effect not significant or significant at $P \leq 0.05$, $P \leq 0.001$, respectively. [1]Means followed by the same letter are not statistically different ($P \leq 0.05$).

Leaf N concentration of plants treated with compost at both rate was lower than that in mineral fertilized trees but higher than control (table 4.7). No significant differences among treatments were observed for P, K and Ca; magnesium leaf concentration was higher in control plants (table 4.7).

Table 4.7 Effect of fertilization treatment on macronutrient leaf concentration in 2010.

TREATMENT	N (% D.W.)	P (% D.W.)	K (%D.W.)	Ca (%D.W.)	Mg (%D.W.)
Control	2.7 c[1]	0.5	1.8	2.4	0.42 a
Mineral	3.4 a	0.4	1.8	2.3	0.38 b
Compost 5 t ha^{-1}	3.0 b	0.3	1.9	2.2	0.38 b
Compost 10 t ha^{-1}	3.2 b	0.6	2.0	2.2	0.37 b
Significance	***	*n.s.*	*n.s.*	*n.s.*	**

ns, **, ***: effect not significant or significant at $P \leq 0.01$, $P \leq 0.001$, respectively. [1]Means followed by the same letter are not statistically different ($P \leq 0.05$).

No significant differences between treatments were observed in micronutrient leaf concentration (table 4.8), with exception of Mn that was higher in mineral treated plants as compared with the other treatments.

Leaf chlorophyll (SPAD unit values) was correlated to N concentration in leaves (figure 4.3).

Table 4.8 Effect of fertilization treatment on micronutrient leaf concentration in 2010.

TREATMENT	Fe (ppm D.W.)	Mn (ppm D.W.)	Cu (ppm D.W.)	Zn (ppm D.W.)
Control	59.3	27.9 b[1]	6.8	30.5
Mineral	59.9	35.6 a	8.0	32.5
Compost 5 t ha^{-1}	58.6	29.3 b	7.3	33.1
Compost 10 t ha^{-1}	59.6	31.4 b	7.7	32.8
Significance	*n.s.*	**	*n.s.*	*n.s.*

ns, **: effect not significant or significant at $P \leq 0.01$, respectively. [1]Means followed by the same letter are not statistically different ($P \leq 0.05$).

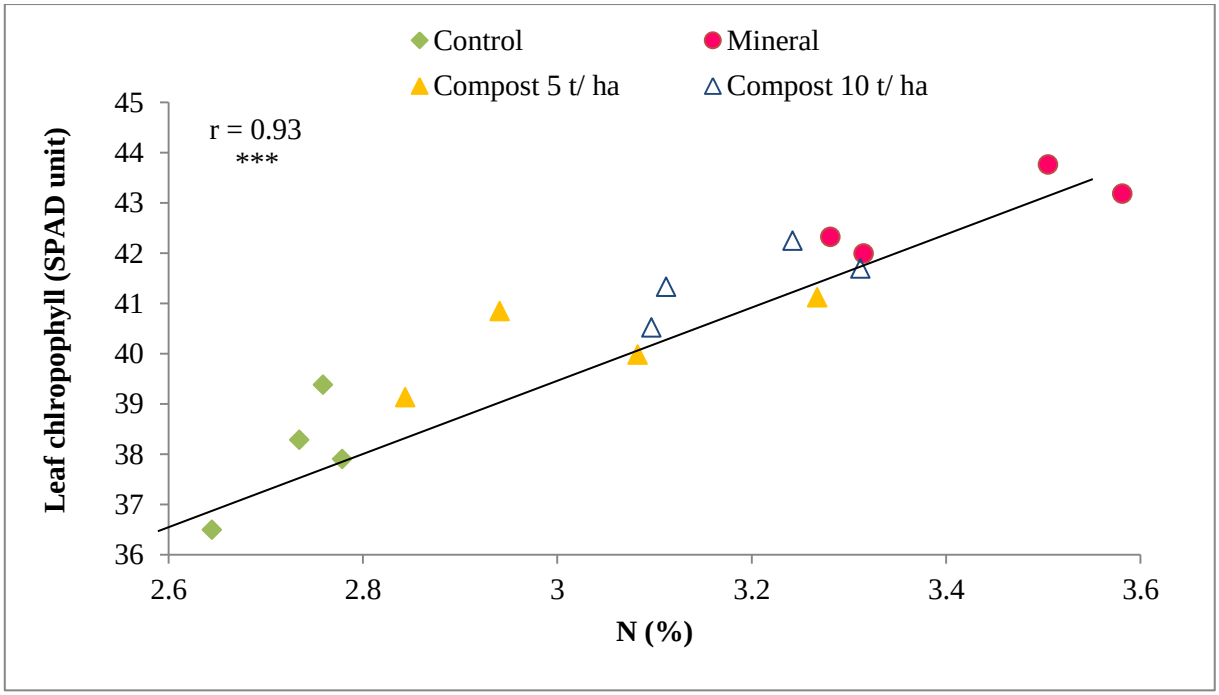

Figure 4.3 Correlation between leaf N concentration and leaf chlorophyll**.** (r: Pearson correlation coefficient: ***: linear correlation significant at $P \leq 0.001$).

4.2.3. Fruit quality and plant productivity

Ten years of different fertilizers management did not modify tree yield, however, in 2010 the application of compost at high rate and of mineral fertilizer increased fruit size and precocity index if compared with untreated control (table 4.9).

Acidity was increased by the application of compost at high rate and mineral fertilizer, no significant differences were observed for fruit SSC, pH and firmness (table 4.10).

Table 4.9. Effect of fertilization treatment on plant yield, fruit weight, precocity index (PI) in 2010.

TREATMENT	Yield (kg tree^{-1})	Fruit weight (g)	PI (days)
Control	44.5	148 b[1]	3.0 c
Mineral	43.3	170 a	5.2 a
Compost 5 t ha^{-1}	47.7	164 ab	4.2 b
Compost 10 t ha^{-1}	49.2	172 a	4.8 ab
Significance	*n.s.*	**	***

ns, **, ***: effect not significant or significant at $P \leq 0.01$, $P \leq 0.001$, respectively. [1]Means followed by the same letter are not statistically different ($P \leq 0.05$).

Table 4.10 Effect of fertilization treatment on acidity, soluble solid content (SSC) and firmness of fruit in 2010.

TREATMENT	Acidity (g L^{-1})	SSC (° brix)	pH	Fruit firmness (kg)
Control	10.0 b[1]	13.0	3.5	2.0
Mineral	11.3 a	13.0	3.5	2.6
Compost 5 t ha^{-1}	10.9 b	13.1	3.5	2.2
Compost 10 t ha^{-1}	11.3 a	13.2	3.5	3.8
Significance	*	*n.s.*	*n.s.*	*n.s.*

ns, **: effect not significant or significant at $P \leq 0.01$, respectively. [1]Means followed by the same letter are not statistically different ($P \leq 0.05$).

Fruit N concentration was increased by the application of mineral fertilizer and compost 10 t ha^{-1} year^{-1} (table 4.11). Also fruits from compost 5 t ha^{-1} year^{-1} treated trees showed higher N concentration than control fruits, but lower than mineral and high compost. No significant differences among treatments were observed for the other macro and micronutrient concentrations (table 4.11 and 4.12).

Table 4.11 Effect of fertilization treatment on macronutrient fruit concentrations in 2010.

TREATMENT	N (% D.W.)	P (% D.W.)	K (% D.W.)	Ca (ppm D.W.)	Mg (ppm D.W.)
Control	0.6 c[1]	0.4	1.2	326.5	544.3
Mineral	1.0 a	0.2	1.2	242.2	567.5
Compost 5 t ha^{-1}	0.8 b	0.3	1.2	269.2	551.4
Compost 10 t ha^{-1}	0.9 ab	0.3	1.3	232.2	575.6
Significance	**	*n.s.*	*n.s.*	*n.s.*	*n.s.*

ns, ***: effect not significant or significant at $P \leq 0.001$, respectively. [1]Means followed by the same letter are not statistically different ($P \leq 0.05$).

Table 4.12. Effect of fertilization treatment on micronutrient leaf concentrations in 2010.

TREATMENT	Fe (ppm D.W.)	Mn (ppm D.W.)	Cu (ppm D.W.)	Zn (ppm D.W.)
Control	17.7	3.2	5.9	10.6
Mineral	18.6	3.1	6.6	10.9
Compost 5 t ha^{-1}	17.3	2.9	6.3	10.5
Compost 10 t ha^{-1}	17.6	2.8	6.9	9.9
Significance	*n.s.*	*n.s.*	*n.s.*	*n.s.*

ns, **: effect not significant or significant at $P \leq 0.01$, respectively. Means followed by the same letter are not statistically different ($P \leq 0.05$).

No significant differences among treatments were observed in the weight of abscised leaves (table 4.13).

Table 4.13 Effect of fertilization treatment on abscised leaves.

TREATMENT	Abscised leaves (kg)
Control	2.74
Mineral	4.19
Compost 5 t ha^{-1}	2.92
Compost 10 t ha^{-1}	3.78
Significance	*n.s.*

ns: effect not significant.

4.3. Discussion

The most important effect of distribution of compost at a rate of 10 t ha^{-1} $year^{-1}$ for ten years in this commercial orchard was the increase of OM that jumped from 1.63 to 5.25%. It must be stressed however that this value refers to a volume of soil of 0.25 m depth by an area of 2/5 of that occupied by orchard since, OM was incorporate in a 2 m width strip. It is thus expected a lower effect in term of the whole volume of soil occupied by an annual crops. Related to this increase also a higher total N was observed after application of compost at the highest rate (10 t ha^{-1} $year^{-1}$) that allowed restoring soil fertility after 10 years of experimentation as also reported by other authors (Sanchez et al., 1989). Despite the improvement of soil chemical properties, NO_3^--N availability remained in the range of 5 to 20 mg kg^{-1}, concentration that is considered optimal for peach nutrition (Tagliavini et al., 1996). In general compost application is reported to increase soil pH, due to the mineralization of C, which produces OH^- ions (Eghball, 2002; García-Gil et al., 2004; Butler and Muir, 2006; Melero et al., 2007; Hargreaves et al., 2008). Nevertheless, in this study, both compost application rates prevented soil pH to increase. This can be explaine considering the relatively high native soil pH and the beneficial effect of soil OM to bring soil pH to neutrality.

On the other hand, several authors indicated that application of OM affects soil biological properties, for example increasing microbial activity (Leifeld et al., 2002; Pérez-Piqueres et al., 2006; Chang et al., 2007; Diacono and Montemurro, 2010). In this case, compost at high rate increased soil microbial C since the beginning of the experiment (Baldi et al., 2010).

Kramer et al. (2006) and Herencia et al. (2007) observed that compost-fertilized plants presented lower leaf N concentration as compared with mineral supplied plants, similar results were observed in this study; in contrast, leaf chlorophyll content was similar between mineral and compost-treated plants. Additionally, a positive correlation between SPAD values and leaf N concentration were observed, such as previously demonstrated by Tagliavini et al. (1996) and Porro et al. (2000).

It is known that N fertilization stimulates plant yields by increasing assimilate availability (Saenz et al., 1997); however after 10 years of different treatment, tree yield was not affected. Fruit N concentration was increased by the application of mineral fertilizer and compost, as well as fruit weight, indicating a relation between N application and peach size as observed by Rader et al. (1985).

Soluble solid concentration usually do not respond to fertilizer application (Stylianidis and Syrgiannidis, 1995), rather high application of N can reduce fruit firmness even if, in the

present experiment, we did not find this effect on fruit firmness but only a delay of fruit ripening, shown by higher acidity and precocity index, after treatment with mineral fertilizer and 10 t ha^{-1} $year^{-1}$ of compost. Probably the higher mineral N soil availability delayed fruit maturation; in peach this is better determined by considering the precocity index rather than fruit firmness, because fruit harvest is managed through several pickings, to remove fruit at the most uniform maturity stage.

5. CONCLUSIONS

The results reported in these studies show that the addition of compost at high rate (60 g kg^{-1}) is effective to increase CO_2 fixation and to promote not only root growth, but also shoot and fruit biomass, indicating that compost application does not subtract C from the fruit to promote root growth. Furthermore, organic fertilizer change C partitioning, favoring C accumulation not only in roots, but also wood.

The highest rate of compost application used in the pot trial, corresponds to several hundreds of tons per hectare, which is possible to obtain after continuous application through all the orchard lifetime. This high concentration of soil OM is rather responsible for increasing a number of soil fertility indexes, including microbial biomass. However, this rate was not effective to increase nitrate-N soil concentration, indicating the soil capacity to maintain low level of mineral N is relevant, even with high level of OM.

Nitrate-N release was in contrast the most promptly effect of urea-N applications even at the lowest (200 mg kg^{-1}) rate tested. This fertilization strategy, from one hand is effective to remediate N deficiency swiftly, from the other hand has a potential in environment pollution, related to nitrate-N leaching with the draining water.

The data here presented on plant growth confirms previous reports about the best nitrate-N soil concentration for peach trees. The highest root and shoot dry weight was found at nitrate-N soil concentration of 57 mg kg^{-1}. Any increase above this value was ineffective to promote growth. In our trial, the decrease of root growth, as a response to excessive nitrate-N soil concentration, was not anticipated by root oxidative stress. Probably the stress analyses evaluated in our experiment were not the most suitable for the hybrid peach x almond rootstock here investigated.

The quality of compost over the mineral N was observed also in commercial orchard conditions. The application of compost at 10 t ha^{-1} $year^{-1}$ under our conditions for 10 years allowed to storage in the soil around 2.7 t of C per year.

The high C assimilation rate was clearly the results of a larger leaf area, that probably was promoted by the better root environment conditions, characterized by a general higher fertility. We believe that the possible improved availability of nutrient in the soil alone, can not explain the boost of plant biomass production; probably a combination of biochemical factors were responsible for the plant response, involving a possible ‘priming effect’ of organic material added to the soil, along with an increase of the population of the so called

plant growth promoting rhizobacteria, that together increased efficiency of root uptake and availability of exogenous bioregulators and finally plant growth.

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