University of Trás-os-Montes e Alto Douro

Carbon and nitrogen management for the sustainability of organic horticulture

PhD thesis

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Supervisors:

Professor Luís Miguel Cortêz Mesquita de Brito

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Vila Real, 2016

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The subjects expressed in this thesis are

the exclusive responsibility of the author

For my sons Janeca, Ruisinho, Maricotas, Margarida and Francisquinho with all my love

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Abstract

Improved fertilizer recommendations based on experimental data are required to increase organic crop yields. With this aim a three year field organic crop rotation was set up in a sandy loam soil with a cover crop of rye consociated with vetch over the autumn/winter for green manure, followed by potato and lettuce (1st year), Swiss chard and turnip (2nd year), and Portuguese cabbage and carrot (3rd year). This rotation was arranged as a randomized block design with green manure (GM), GM with 20 and 40 t ha^{-1} farmyard compost manure (C20 and C40), GM with 1 and 2 t ha^{-1} of commercial organic fertilizer (CF1 and CF2) and a control treatment without fertilizers, to access crop yield and N uptake. Nitrogen (N) mineralization was determined by field incubation to ressemble the environmental conditions in the field, having been found a positive correlation between the sum of mineralized N and crop yield during the growth period of the crops, showing that field incubation maybe a good indicator to predict fertilization. N uptake and crop yields were compared to N mineralization in order to develop recommendations to adjust N mineralization to crop needs.

Lettuce, turnip and carrot yields were significantly increased 90%, 115% and 56% respectively for C40 compared to CF2, probably because of the rapid N mineralization of the commercial fertilizer during the previous crop, decreasing N availability for the 2nd crop of the season whereas compost N mineralization took place during all crop rotation decreasing the risk of N losses. Differences on potato yield were not found between the treatments C40, CF1 and CF2, probably due to the long potato growth season (124 days) which allowed a better efficiency use of the mineralized N from the compost in which N remineralization increased after 71 days N immobilization. In contrast, Swiss chard, with a much shorter growing season (54 days), presented a yield increase for CF2 compared to C40. Probably, it would be advantageous to delay S. chard plantation due to the period of 31 days N immobilization with C40 to increase synchronization between N mineralization and N uptake. Portuguese cabbage yield, unlike Swiss chard, did not increase for CF2 compared to C20 and C40 in the 3rd year crop rotation, even with the same short growth period (56 days), probably due to the effect of continuous compost and green manure application that increased N availability in the long-term.

The amount of total N and extractable P and K in soil decreased for GM, C20, CF1 and CF2 whereas these nutrient contens were similar for C40 after 3 years of organic fertilization. The pH increased from 4.9 to 6.7 after liming and decreased to 6.1 in the end of the experiment.

Total C did not increase after three years of accumulated application of organic fertilizers and there were no significant differences between treatments. However, the permanganate oxidizable carbon content increased for C40, indicating greater impact on C sequestration. Briefly, the application of 40 t ha^{-1}farmyard manure compost and rye consociated with vetch as green manure during three years of organic horticultural crop rotation has the potential to (i) enhance crop yields; (ii) decrease the risk of N losses; and (iii) increase C sequestration.

C and N mineralization were determined by laboratory incubation to assess C and N dynamics after organic fertilizers incorporation into the soil and to argue about N mineralization differences in field and laboratory incubations. Compost and green manure N mineralization rate, accumulated mineralized N and the pattern of N released were different in field and laboratory incubations. However, N mineralization rate and the accumulated mineralized N were similar with commercial fertilizer incorporation in both incubations.

Keywords: compost, field incubation, laboratory incubation, nitrogen mineralization, organic agriculture

Resumo

Os ensaios de campo no modo de produção biológico são necessários para melhorar as recomendações de fertilização e aumentar as produções neste modo de produção. Com estes objectivos realizou-se uma rotação trianual, num solo franco-arenoso, com uma cultura de cobertura outono/invernal de centeio e ervilhaca para adubação verde nos três anos de rotação, seguida de batata e alface (1º ano), acelga e nabo (2º ano), e penca e cenoura (3º ano). Esta rotação foi delineada com um arranjo de blocos casualizados com adubo verde (GM), GM com 20 e 40 t ha^{-1} de compostado (C20 e C40), GM com 1 e 2 t ha^{-1} de adubo orgânico comercial e um tratamento de controlo sem aplicação de fertilizantes para determinar a acumulação de azoto (N) e a produção das culturas. A mineralização de N foi determinada através de uma incubação de campo tendo sido encontrada uma correlação positiva entre o somatório da mineralização de N nos vários períodos de incubação e a produção das culturas durante o período de crescimento destas, prevendo que a incubação de campo pode ser um bom indicador para a fertilização das culturas.

A produção de alface, nabo e cenoura aumentou 90%, 115% e 56% respectivamente com o tratamento C40 em comparação com os tratamentos CF1 e CF2 em virtude da rápida mineralização de N do adubo comercial ocorrida na cultura anterior, diminuindo a disponibilidade de N para a 2ª cultura da estação. Pelo contrário a mineralização de N do composto ocorreu ao longo da rotação diminuindo o risco de perdas de N. A produção de batata foi semelhante nos tratamentos C40, CF1 e CF2 devido à maior disponibilidade de N do compostado na cultura de ciclo-longo da batata (124 dias) na qual a remineralização de N aumentou após 71 dias de imobilização. Pelo contrário, a produção de acelga aumentou com o tratamento CF2 em relação ao tratamento C40 devido ao curto período de crescimento da acelga (54 dias). Provavelmente seria vantajoso atrasar a plantação da acelga devido ao periodo de imobilização de N de 31 dias com C40 de modo a aumentar a sincronização entre a mineralização de N e a absorção de N pela acelga. No 3º ano de rotação as produções idênticas da couve penca nos tratamentos CF2 e C40, apesar desta ser uma cultura de ciclo-curto (56 dias), poderá dever-se à aplicação continuada de compostado e adubação verde que aumentou a disponibilidade de N.

O teor total de N, P e K disponíveis no solo foram semelhantes no início e no fim da rotação com o tratamento C40. Pelo contrário, estes teores de nutrientes diminuíram com os restantes tratamentos após 3 anos de fertilização. O pH aumentou de 4.9 para 6.7 após a aplicação da calagem no ínicio da rotação, diminuindo para 6.1 no fim do ensaio. O C total não aumentou após 3 anos de aplicação de fertilizantes orgânicos, e não se verificaram diferenças significativas entre os vários tratamentos. No entanto, o permanganato de C oxidável aumentou com o tratamento C40 em relação aos restantes tratamentos, revelando o potencial do tratamento C40 para aumentar o sequestro de C no solo. Resumindo, a aplicação de 40 t ha^{-1} de composto e centeio consociado com ervilhaca durante uma rotação de 3 anos em horticultura biológica demonstrou potencial para: (i) aumentar a produção das culturas; (ii) diminuir o risco de perdas de N; and (iii) aumentar o sequestro de C no solo.

A mineralização de C e N foram determinadas numa incubação de laboratório para avaliar as dinâmicas de C e N dos fertilizantes orgânicos incorporados no solo e comparar as diferenças de mineralização de N durantes as incubações no campo e no laboratório. A taxa de mineralização de N, a acumulação de N e as curvas de mineralização de N do compostado e adubo verde foram diferentes nas incubações de campo e laboratório. No entanto, a taxa de mineralização e a acumulação de N com o adubo orgânico comercial foram idênticas em ambas as incubações.

Palavras-chave: agricultura biológica, composto, incubação de campo, incubação de laboratório, mineralização de azoto

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List of abbreviations

C/N	Carbon/nitrogen ratio
C20	20 t ha^{-1} farmyard manure compost
C40	40 t ha^{-1} farmyard manure compost
CF1	1 t ha^{-1} commercial organic fertilizer
CF2	2 t ha^{-1} commercial organic fertilizer
DM	Dry matter
EC	Electrical conductivity
FYM	Farmyard manure
GM	Green manure
OA	Organic agriculture
OM	Organic matter
SOM	Soil organic matter

List of symbols

Al	Aluminium
Al_3^+	Aluminium ion
C	Carbon
Ca	Calcium
CO_2	Carbon dioxide
K	Potassium
Mg	Magnesium
N	Nitrogen
NH_3	Ammonia
N_2O	Nitrous oxide
NH_4^+	Amonium ion
NO_3^-	Nitrate ion
NO_2^-	Nitrite ion
O_2	Oxigen
P	Phosphorus

1. INTRODUCTION

1.1 A brief summary of organic agriculture history

Organic agriculture (OA) is a modern way of agricultural management that combines best environment practices, a high level of biodiversity, the preservation of natural resources and the application of high animal welfare standards in order to minimize the negative effects on the environment and to increase human health or animal health (Council regulation EC 834/2007). Organic agriculture aims to maintaining or enhancing soil fertility, feeding the crops through the soil eco-system and not through soluble fertilizers applied to the soil in accordance with the Council Regulation (EC) 834/2007 and the Commission Regulation (EC) 889/2008.

Organic farming emerged in Europe as a response to intensified farming and industrial agriculture, which uses synthetic fertilizers, chemical pesticides, monocultures into large areas and the separation of animal husbandry from plant production (Rembialkowska et al., 2012). The decade of 1960 was of great social and political upheaval worldwide and public awareness of environmental threats including those resulting from agriculture. The most dramatic threat came from the use of pesticides and was revealed by the *Silent Spring* book from Rachel Carson (Carson, 1962). Diclorodifeniltricloroetano (DDT) and other organochlorines were banned from the market in the early 1970s because of the harm they did to birds. Another cause for concern was the risk of methaemoglobinaemia (blue baby disease) caused by high nitrate contents found in drinking water supplies. The increased use of synthetic fertilizers caused several agricultural problems. The crop cultivars at that time were not yet adapted to higher nitrogen contents and weakened plants were easily attacked by pathogens and insects, whereas acid mineral fertilizers acidified the soil (Lockeretz, 2007).

Rudolf Steiner in German introduced the principles that lead to biodynamic agriculture in 1924. Steiner presented the farm as a living organism based on interactions between crop production and animal husbandry well adapted to environmental conditions (Steiner, 1958). From 1925 to 1930, the English Sir Albert Howard developed in India an aerobic composting technique and concluded that soil fertility based on a humus-rich soil was the precondition for healthy plant and animals. A key scientist of organic agriculture in France was Claude Aubert who wrote *L'agriculture biologique* (Aubert,

1970) which became a fundamental book for organic farming. Jerome Rodale from the United States started a movement which advocated the principle of small organic farms as cells of a sustainable society. Jerome Rodale started the magazine “Organic gardening and farming and his book “Pay dirt” was published in 1945 (Lockeretz, 2007).

Although the concept of organic farming already existed over the 1980s years, it was only since the implementation in 1993 of the Council Regulation (EEC) nº 2092/91, that it became the focus of significant attention from consumers and farmers. There was convergence of European policy goals with those of the organic movements, particularly with respect to ameliorating the impacts of intensive production on the environment and promoting higher animal welfare as well as food quality standards. Nowadays, the new emphases on climate change and global recession present new challenges for organic farming development.

Organic farming's reliance on livestock is seen by some as a weakness (Balmford et al., 2005); but by others as a way of significantly reducing emissions of green-house gases associated with manufacture and use of nitrogen fertilizers, and also providing opportunities for sequestration of soil organic carbon (Meisterling et al., 2009). On the other hand, integrated organic systems may have similar productivity to conventional production systems, while being less dependent on inputs from non-renewable resources (Stolze et al. 2009). The small and familiar organic farms almost always produce higher output levels per unit area than larger conventional farms (Chappell et al., 2011) and the improvement of biological activity and biodiversity in organic systems provide an efficient use of resources (Mäder et al., 2002). Therefore, organically manured crop rotation with organic fertilizers from the farm itself can be a realistic alternative to conventional farming systems (Sandhu et al., 2010) and in developing countries, the potential of organic farming, to directly enhance food productivity has been recognized (Ericksen et al., 2009).

The most recent survey on certified organic agriculture (FIBL IFOAM, 2014) reports that 37.5 million hectares of agricultural land were managed organically by 1.9 million producers. The countries with greatest organic agricultural land are Australia (12 million ha), Argentina (3.6 million ha) and the United States (2.2 million ha). Currently 0.9% of the agricultural land in the world is used for organic agriculture. In ten countries more than 10% of the agricultural land is managed organically (for example, Falkland islands, 36.3%; Liechtenstein, 29.6%; Austria, 19.7%). In Europe, there are 11.2 million ha managed organically by more than 320.000 farms (2.3% of European agricultural land). The countries with the largest organic agricultural area in Europe are Spain (1.6 million ha), Italy (1.2 million ha) and Germany (1 million ha). In Portugal 6% of the agricultural land (201.054 ha) is managed organically, but the area used for organic horticulture (764 ha) is less than 0.5% of the organically managed land. Organic farming is gaining worldwide acceptance and, looking at the future of organic farming, there is clearly a need for more research and investment to explore the potential of organic farming (Gomiero et al., 2011).

1.2 Organic and conventional agriculture

Conventional farming has played a significant role in improving food and fiber productivity to meet human consumption demands, but has become excessively dependent on high-yield varieties, increased irrigation and use of pesticides and fertilizers. As a result of desertification, salinization, soil nutrient depletion and harmful residues in soil, water and food became concerning environmental issues (Baker et al., 2002; Kibblewhite et al., 2008). The practices of intensive agriculture also led to the degradation of several ecosystem services such as biological control of insect pests with natural enemies, biological control of diseases by soil microorganisms that naturally suppress of soil-borne diseases, pollination of crops, availability of nutrients by soil microbial activity and storage of carbon in soils (Sandhu et al., 2010). These last authors evaluated the role of land management practices on 14 organic and 15 conventional fields in the maintenance or enhancement of ecosystems services in agricultural land, and showed that organic agriculture was more sustainable than conventional agriculture to maintain ecosystem services. Organic farming systems also increases flora and fauna biodiversity compared to conventional systems (Krebs et al., 1999; Gomiero et al., 2011).

Organic and conventional farming systems differ in soil physical and chemical properties. For example, bulk density decreases in organically managed soils (Bulluck et al., 2002; Ge et al., 2013). The pH value usually decreases in soil managed with synthetic fertilizers because many of these are acidifying mineral fertilizers (Bulluck et al., 2002). The pH value also tends to decrease with organic management because of soil microbial mineralization activity (Ge et al., 2011). However, the incorporation of organic matter (OM) mitigates the undesirable effects of the aluminum ion, and so the plants are less susceptible to soil acidity (Varennes, 2003).

Many authors reported that carbon (C) and nitrogen (N) contents increased in organically managed soils (Marinari et al., 2006; Evanylo et al., 2008; Nett et al., 2010). The total C increased 27.9% in organically managed soil with cattle manure application compared to 8.6% in conventionally managed soil after 22 years of crop production (Pimentel et al., 2005). Soil organic carbon content was 8% higher in the organic system compared with the conventional system after 27 years, in soils amended with equivalent amounts of organic carbon (Birkhofer et al., 2008), and labile C increased 76% under organic compared to conventional management (Marinari et al., 2007).

Drinkwater et al. (1998) reported a 15 years' study in which they compared net balances of carbon and nitrogen in a conventional system with two different methods in organic system: one in which the crop biomass was fed to cattle and returned to the field as manure, and another that received C directly through incorporation of green manure. There was a significant increase in carbon stored in soil from manure (12 kg C ha^{-1}) and green manure (6.6 kg C ha^{-1}) incorporation compared to the conventional system (2.2 kg C ha^{-1}). These authors found a significant increase in soil N content after manure incorporation and green manure incorporation, whereas in the conventional system soil N content decreased due to greater leaching. On the contrary, Marinari et al. (2006) found no differences in soil C content between organic and conventional management after 7 years. This might have been a consequence of organic management practices which might induce decomposition of native soil organic matter, the so-called priming effect (Kusyakov et al., 2000) or a high short-term decomposition of organic matter caused by mechanical weed control used more often in organic agriculture than in conventional agriculture. Therefore, the N use efficiency in organic and conventional systems may be contradictory. Mäder et al. (2002) found over a 21 year experience that the input of fertilizers (N, P and K), energy and pesticides in the organic systems was reduced by 34%, 53% and 97%, respectively, although mean crop yield was only 20% lower, indicating a more efficient use of resources of crop production. On the contrary, Aronsson et al. (2007) found that the yield of cereals in the organic systems was 15% to 50% less than in conventional systems, leading to a less efficient use of N.

Organically managed soils exhibit greater biological activity than conventionally managed soils (Mäder et al., 2002; Marinari et al., 2006). Mäder et al. (2002) reported an increase of the amount of earthworms, by a factor of 1.3 to 3.2 in the organic plots as compared to conventional ones, whereas the density of arthropods, carabids, staphlinids and spiders in organic plots was almost twice than in conventional plots. Microbial communities are also different between organic and conventional systems (Chu et al., 2007). Over a 4 year horticultural rotation, Gunapala et al. (1998) observed that bacteria were more important than fungi in organic compared to conventional soils. This was explained by the type of organic input. The major source of C for soil organisms in conventional system were soil humus and the exudates of roots, while the organic system received large inputs of manure and cover crops. Arbuscular mycorrhizal symbiosis, that is believed to ameliorate plant mineral nutrition, enhance water stress tolerance and contribute to a better soil aggregation, was also enhanced 30% to 60% in the organic farming systems compared to the conventional ones (Mäder et al., 2000), whereas root length colonized by mycorrhizae in organic farming was 40% higher than in conventional systems (Mäder et al., 2000).

High soil respiration rates were found for organically managed soils compared to conventionally managed soils, indicating higher soil microbial activity in organic farming soils (Ge et al., 2011). Indeed, Pimentel et al. (2005) found that soil respiration was 50% higher in organic system soils compared to the conventional system. In a long-term agricultural experiment (21 years) microbial biomass was 43% higher in organic systems compared to conventional systems (Fliebach et al., 2007). In this experience the metabolic quocient (qCO_2) that related soil respiration to microbial C biomass was 43% lower in organic systems compared to conventional systems. This means that microorganisms in organic systems need less energy to maintain their biomass than in conventional systems. A higher microbial C biomass and lower qCO2 indicates that organic soil management better conserves soil organic carbon (Mäder et al., 2002).

Pesticides may affect soil biological activity, including bacterial groups with important functions in soil nitrogen transformations (Johnsen et al., 2001). *Rhizobium* fixing N in symbiosis with bean (*Phaseolus vulgaris L.),* Azospirillum and Azotobacter free-living N fixing bacteria and the nitrifying bacteria may be severely affected by pesticide applications (Martinez-Toledo et al., 1992; Ramos and Ribeiro, 1993).

Nitrogen leaching from organic systems is usually lower than from the conventional systems (Benoit et al., 2014). Drinkwater et al. (1998) showed that N losses due to leaching during 15 years was on average 13 kg N ha^{-1} after application of manure and green manure, which corresponds to 35% lower than in conventional systems (20 kg N ha^{-1}). Based on a system modeling approach, Hansen et al. (2000) showed that N leaching in organic soils varied from 19 to 30 kg ha^{-1} in loamy soils and 36 to 65 kg ha^{-1} in sandy soils. Whilst in conventional soils, N leaching varied from 32 to 48 kg ha^{-1} in loamy soils and 90 to 111 kg ha^{-1} in sandy soils. These authors further concluded that N leaching was lower in organic systems due to lower input of mineral N and the use of catch crops. The lower potential risk of N leaching in organic agriculture appears to improve agricultural sustainability while maintaining similar crop yields in comparison to conventional agriculture (Poudel et al., 2002).

Yield differences between organic and conventional farming are quite controversial. Soils on organic farming can be as productive as conventional farming after 5 years of organic production, probably due to beneficial effects on soil properties of long-term organic amendments (Bulluck et al. 2002; Pimentel et al., 2005). Maize yield in a two year rotation with soybean fertilized with hairy vetch residues before sowing maize was not significantly different from conventional management after 6 years (Drinkwater et al., 2000). However, Seufert et al. (2012) reported for 316 studies with comparisons between organic and conventional yields, with 34 different crops in 62 farms that the average organic yields were 25% lower for organic compared to conventional yields. These authors also suggested that the yields varied substantially with crop types. For example, yields of oilseeds were only 11% smaller in organic farming than in conventional farm, whereas organic cereals and vegetables yields were 26% and 33% lower, respectively, compared to conventional farming (Seufert et al., 2012). These differences were explained by the slower release of mineral N from organic sources which often do not keep up with the high crop N demand during the peak growing period (Pang and Letey, 2000). Badgley et al. (2006) examined 293 examples from all over the world to compare yields of organic and conventional systems. These authors found out that the average ratio of organic/non organic yield was slightly below 1 in the developed world and above 1 in developing countries. This same study concluded that organic agriculture has potential to fulfill food demand of a growing population at low cost, low input and locally available eco-technologies.

The large number of variables affecting mineral composition of plants makes it difficult to reach a definite conclusion about the effect of organic practices on mineral composition. However, it was observed that organic crops usually show higher phosphorus, iron, magnesium, vitamin C and dry matter contents and lower total nitrogen and nitrate content than conventional crops (Worthington, 2001; Herencia et al., 2011). Polyphenol content and antioxidant capacity tended to increase with organic farming management (Faller and Fialho, 2010), thus increasing the palatability of organic fruits and vegetables (Rembialkowska et al., 2012). Moreover, long-term addition of organic fertilizers increased soil content of vitamin B_{12} (Mozafar, 1994). This last author measured B_{12} content in the seeds of soybean, barley grains and in the leaves of spinach in plants grown in soils amended with cow manure (10 g kg^{-1}) and found that the soil amendment did not alter B_{12} in soybean seeds, but increased the B_{12} content in barley grains by more than threefold and in spinach leaves by close to twofold. This may offer special benefits to people living on strict vegetarian diets who are known to be in danger of B_{12} deficiency.

A meta-analysis comparing environmental impacts of organic and conventional farming in Europe was performed using a wide range of indicators: soil organic matter, nitrogen leaching, nitrous oxide emissions, ammonia emissions, phosphorus losses, land use, energy use, greenhouse gas emissions, eutrophication potential, acidification potential and biodiversity (Tuornisto et al., 2012). This study concluded that the key goals in conventional farming should be improvement of soil quality, nutrients recycling and biodiversity protection, whereas in organic farming the main points to improve are nutrient management and increasing yields.

1.3 Soil fertility in organic agriculture

1.3.1 Crop rotation

In the early Middle Ages, crops in Europe were often organized in a three year rotation, in a system utilizing one third fallow (Duby, 1962). Later, by the 14th century, fallow rotations were replaced by rotations with forage leguminous, thus allowing production during the three consecutive years (Duby, 1962). Unfortunately, during 1950s and 1960s it was believed that synthetic fertilizers and pesticides could replace crop rotation without loss of yield, opinion that changed in the subsequent years. Nowadays, crop rotation is one of the building blocks of organic farming systems. Careful design of crop rotation may ensure soil fertility for maintaining productivity and prevent problems with weeds, pests and diseases (Mourão, 2007). Moreover, the nutrient management under crop rotation target the needs of crops rather than focusing on individual crops because application of OM has strong residual benefits over several crop cycles (Singh et al., 2001).

The benefits of crop rotation to increase crop yields and the sustainability of agricultural systems has been reported (Yusuf et al., 2009). A 2-year crop rotation with maize and soybean increased 5% to 20% maize yields when compared with continuous maize production or maize after fallow (Bullock, 1992). A 4-year crop rotation consisting of fababean, barley and barley intercropped with pea increased mineral N and microbial N content compared to barley monoculture, indicating that leguminous contributed with N to the crop rotation through biological N fixation and addition of N enriched crop residues (Chang and Juma, 1994). Reeves et al. (1997) compaired maize yields for 50 years, between continuous cropping and 3 crop rotations: corn-oat, corn-oat-soybean and corn- oat- red-clover- alfalfa, and found that maize yields were lowest in the monoculture and highest with the corn- oat- red-clover- alfalfa rotation because of the presence of leguminous.

Long rotations with pasture or hay improve soil physical, chemical and biological properties, whereas short rotations such as maize-soybean may result in the degradation of soil properties (Bullock, 1992). For instance, a rotation including two years of pasture per each year of cereal was necessary to maintain soil structural stability (Reeves et al., 1997). Furthermore, Studdert et al. (1997) reported that rotations consisting of 7 years of short rotations require a minimum of 3 years of pasture to maintain soil properties in order to meet the goals of sustainable agriculture. Other authors indicate that short rotations with leguminous may also enhance physical and chemical properties (Liang et al., 2004; Kayser et al., 2010). A 4-year crop rotation with corn, oats and alfalfa (2 years), increased the content of soil organic matter and aggregate stability compared to a continuous corn production after 43 years (Drury et al., 2004). Furthermore, a 2-year crop rotation with oat, pea, hairy vetch and potato increased soil organic C from 23.9 to 25.9 g kg^{-1} (Grandy et al., 2002). In order to maintain and increase soil fertility, a crop rotation should include crops with different nutritional needs. For example, leafy crops, leguminous crops and root crops require high levels of N, P and K, respectively (Mourão, 2007). A crop rotation should also explore the whole soil depth by using crops with different root lengths (Monfort, 1985).

Crop rotation can help to reduce the amount of weeds (Marshall et al., 2003). In fact, Koocheki et al. (2009) found that weed density in continuous winter wheat (6300 seeds m^{-2}) was higher than weed density in rotations with maize or sugar beet (5000 seeds m^{-2}). Total weed density was higher in continuous maize (39 plants m^{-2}) compared with maize grown in rotation with spring barley (29 plants m^{-2}) (Demjanova et al., 2008). Importantly, a crop rotation must take into account which crops increase weed emergence and the ones that prevent their growth in order to alternate them. For example, potato and spinach cover the soil preventing weed growth, as opposed to carrot and onion (Monfort, 1987).

Crop rotation may control diseases caused by soil pathogens (Hwang et al., 2009). This can be explained because crop rotation favors the development of antagonistic bacteria (Gorlach-Lira and Stefaniak, 2009). These authors found that a 6-year crop rotation increased the number of mycoantagonistic actinomycetes that inhibit the growth of *Fusarium oxysporum* in sugar beet (*Beta vulgaris*) and winter rape (*Brassica napus*). In addition, two year rotation of spring barley and potato and a three year rotation of barley, red clover and potato significantly reduced the levels of *Rhizoctonia solani* in potato (Peters et al., 2003). Crop rotation also reduced the risk of Fusarium wilt (*Fusarium oxysporum*) damage in lettuce (Scott et al., 2012); it has also been reported that pests are reduced with crop rotation mostly because of the interruption of food supply, being crop rotation most effective against pest species with narrow host (Mazzi and Dorn, 2011).

1.3.2 Soil organic matter

Soil organic matter (SOM) is an essential reservoir of carbon, nutrients and energy in the life cycle (Craswell and Lefroy, 2001), and the mineralization of SOM can be a major source of mineral N in agricultural soils. It has been estimated that 1% to 3% of the organic N is mineralized annually (Soltner, 1996). This can represent 30 to 90 kg N ha^{-1} if the OM content of the soil is approximately 3%. Furthermore, SOM is the most important indicator of soil quality and agriculture sustainability because of its impact on soil physical, chemical and biological properties (Reeves et al., 1997).

Regarding physical characteristics, the role of OM in the formation of stable aggregates has a major influence on water holding capacity, aeration and resistance to root growth (Benbi et al., 1998). Mamman et al. (2007) set up a laboratory experiment in which soil samples with three soil OM and moisture content levels were pressured with four different compactive efforts and found out that soil bulk density and penetration resistance decreased with increasing OM content. Therefore, OM addition alleviates the problems of soil compaction. Concerning chemical characteristics, OM incorporation into the soil increased total N, P and K contents (Bayu et al., 2006) and increased soil cation exchange capacity (Yilmaz and Alagoz, 2010). Besides, OM serves as a buffer in ameliorating the adverse effects of inorganic pollutants through their complexation with humidified OM. For example, organically complexed forms of Al are less harmful to plants than Al^{3+} (Varennes, 2003). SOM is the driving force for biological activity as the primary source of energy and nutrients for many soil organisms. A direct effect of the biological activity is seen through the formation of soil pores as a consequence of faunal activity, root and fungal growth. Other effects of the biological activity are changes in organic compounds that result from biological breakdown and mineralization and immobilization of nutrients (Craswell and Lefroy, 2001).

Organic matter is a key resource to restore fertility in depleted soils. For example, coarse-textured sandy soils with low OM content are difficult to manage as they easily lose nutrients by leaching and are easily susceptible to erosion (Burt et al., 2001). However, soil fertility can be restored by increasing OM content. Manure showed the potential to restore soil fertility of the depleted sandy soils by increasing SOM content, supplying nutrients, and increasing water-holding capacity after three years of manure application (Zingore et al., 2008). Nevertheless, addition of OM to soils may have some negative effects. From 14 field experiments studying the long-term-effect of manure application, Edmeades et al. (2003) showed that, because the ratio of nutrients in manures is different from the ratio of nutrients removed by the crops, excessive accumulation of some nutrients, particularly P and N can arise from the long-term use of manures. These authors also reported that the use of manures can result in soils becoming excessively enriched in P, K, Ca and Mg in the top soils and in nitrate, Ca and Mg in the subsoils. Moreover, applying manure on a N basis, leads to soils enriched with P because the N/P ratio in manure is lower than the crop's requirements (Sikora and Enkiri, 2004).

Intensification of agricultural practices has increased the use of mineral fertilizers compared to the application of organic materials, with subsequent decline in soil OM content (Wells et al., 2000). To meet increasing food demand without accelerating land degradation, including the decline in organic matter, is one of the greatest challenges that the human race has to face in the 21st century (Craswell and Lefroy, 2001). A comparison, made between two different ancient agricultural sites (154 years of agricultural production) from USA and Peru, showed that agriculture in USA was based predominantly on corn as opposed to Peru that cultivated a diversity of crops, including corn, potato, small grains, faba bean and alfalfa. After 154 years, the OM content of USA farms was 46% lower in cultivated soils compared to uncultivated soils. In striking contrast, in farms from Peru, the OM content in cultivated soils was 30% higher than in uncultivated soils (Reeves et al., 1997). Nevertheless, other long term-studies have shown that most continuous cropping systems result in a decrease of OM content (Reeves et al., 1997). In their review, Reeves et al. (1997) reported that a rotation with corn, oat and leguminous crops with lime and manure fertilizer decreased 11% of the OM soil content compared to uncultivated sites after 70 years. However, the dynamics of OM are quite different depending on soil type, climate and crop management. According to Craswell and Lefroy (2001), humans are capable of maintaining and improving soil quality under continuous cultivation by wise soil management practices that include manure additions, inclusion of pasture in rotations, perennial crops and no-tillage systems.

Crop rotations and tillage practices influence the quantity of soil organic C and N. The soil C content increased 19% in a rotation with cereals and legumes in no-tillage management compared to conventional tillage management (Hungria et al., 2009). Similarly, no-tillage system (uses herbicides for fallow weed control) increased total organic C by 2 t ha^{-1} (6%) comparatively to conventional tillage (uses tillage for fallow weed control and heavy cultivator followed by rodweeding and packing before sowing) after 8 years in continuous wheat cultivation in a soil with approximately 4% OM. However, after 16 years of no-tillage in a wheat-fallow rotation, total organic C was only 1.6 t ha^{-1} higher than in conventional tillage (Larney et al., 1997). Larney et al. (1997) suggested that fallow periods stimulated decomposition by providing favorable moisture and temperature conditions, limiting the build-up of organic C. Hence, intensification of cropping practices by fallow elimination and tillage reduction may build-up soil OM content. However, in OA the use of no-tillage system is difficult to achieve because the weeds have to be controlled and a higher number of tillage operations are required for weed control (Sartori et al., 2005). Moreover, without a significant input of C from crop residues or manures, tillage conservation can only decelerate the loss of soil organic matter.

Hungria et al. (2009) reported that C and N biomass increased in no-tillage system in comparison to a conventional system after 14-year rotation with cereals and legumes. They also reported an increased metabolic coefficient (microbial respiration/microbial biomass) in the conventional tillage system, which is indicative of a lower efficiency in the use of soil C by microbial population in this system. This is probably due to the fact that no-tillage systems favors fungi over bacteria and fungi apparently have lower maintenance energy requirement than bacteria (Haynes, 1999). Although the content of organic matter increased in no tillage systems compared to conventional systems, yields tended to decrease in minimum tillage systems because there is no disruption of soil below 20 cm leading to a reduction in soil aeration and temperature and consequently net N mineralization decreases. As an example, Jackson et al. (2004) found lower lettuce and broccoli yields when minimum tillage was used continuously for two years compared to conventional tillage. These authors suggested that, for improving soil quality and assuring profitability, farmers should utilize a combination of conventional and minimum tillage with frequent additions of organic matter.

Application of organic amendments to soil, such as animal manures (Haynes and Naidu, 1998), composts (Amlinger et al., 2003) and green manures (Thorup-Kristensen et al., 2003), is a current organic agricultural practice used to replenish C losses and improve soil biological and chemical properties (Goyal et al., 1999). However, it is not easy to manage N supply in organic farming systems, mainly because N availability depends crucially on the synchronization between N release from organic sources and crop uptake (Ranells and Wagger, 1996).

1.3.3 Compost

1.3.3.1 Compost characteristics

Animal manures have been traditionally used in soils as fresh material, because of their high N content. However, application of animal manures may cause serious environmental problems, such as plant toxicity due to high electrical conductivity and ammonia contents, soil N immobilization and nutrient loss through leaching as a result of inopportune timing of application (Brito, 2001; Wong et al., 1999). Degradation of animal manure in soils leads to high CO_2 production which creates reducing conditions in the soil, due to depletion in O_2 level (Bernal et al., 1998a). However, composting may reduce environmental problems by transforming organic wastes into safer and more stabilized material, therefore eliminating the risk of dissemination of pathogenic organisms and reducing viable weed seeds (Gómez-Brandon et al., 2008). Compost has also advantages over raw manure in terms of storage, handling, and uniform application with little odor.

Composting is a biological process of aerobic decomposition, which degrades labile organic matter into carbon dioxide, water vapor, ammonia and mineral salts. This process results in a sanitized and stabilized organic material containing humic substances which can be used to improve and maintain soil quality and fertility (Larney and Hao, 2007). The chemical composition of composts is quite variable because animal manures present great variability in composition (Morvan et al., 2006). This variability depends on animal type, protein and fiber contents of their diet and type of bedding (Larney and Hao, 2007). Moral et al. (2005) studied the chemical characteristics of animal manures from horse, cow, pig, sheep, goat, rabbit, chicken and ostrich. The average OM content ranged from 39.6% to 69.7%, with horse and cow manure presenting the highest and lowest OM content, respectively. The organic N content ranged from 1.5% to 2.9%, with only chicken and pig manures presenting values above 2%. Chicken manure showed the lowest C/N ratio (11.1) and horse manure the highest (20.8). Composting mixtures are mainly based on the C/N ratio of the blend because microorganisms require an energy source (C) which is related to N assimilation for their development and activity. The most adequate C/N ratio is approximately 30. High C/N ratios make the process of composting very slow; on the contrary, in a situation of low C/N ratio, inorganic N is quickly produced and can be lost by volatilization or leaching.

Aeration is a key factor for composting because it removes excessive moisture and CO_2 and at the same time provides O_2 for biological processes. The ideal moisture content should be between 50% and 60%. When the moisture content exceeds 60-70% the composting process tends to be anaerobic. The optimum temperature range for composting is between 40 ºC and 65 ºC. Temperatures above 55 ºC are required to eliminate pathogenic microorganisms, but when the temperature elevates above 65 ºC general microbial activity declines rapidly (Brito, 2007). There are four important stages in the composting process. (i) the initial mesophilic phase where mesophilic bacteria and fungi degrade simple compounds such as sugars, amino acids and proteins, thus quickly increasing temperature; (ii) the thermophilic phase where thermophilic microorganisms degrade fats, cellulose, hemicellulose and some lignin, at which destruction of pathogens occur; (iii) cooling phase, which is marked by a fall in temperature to values close to the ambient air temperature; and (iv) the maturation phase, which is a lengthy period of stabilization intended to produce highly stabilized and humidified mature compost free of phytotoxicity.

1.3.3.2 Compost maturity

A maturated compost was described by Bernal et al. (1998b) as stable OM without phytotoxic compounds and plant or animal pathogens. Physical characteristics such as colour, odour and temperature may give a general idea of the decomposition stage of the OM but not full information about the degree of maturation. To this end, chemical methods are widely used. A decrease in the C/N ratio with the progress of composting has been widely reported due to relative increased C losses as CO_2 during decomposition of organic matter compared to N losses (Nolan et al., 2011; Zhu, 2007). Moreover, a significant reduction of C/N ratio is due to the decomposition of easily degradable organic C, whose concentration decreases with composting age (Bernal et al., 1998b). As a result, there is a negative correlation between compost maturity and C/N ratio throughout the composting process. However, due to the variability in the C/N ratio of starting materials, one should be cautious on using this ratio as an indicator of composts' maturity (Nolan et al., 2011). For example, when wastes rich in N are used as source material for composting, like sewage sludge, the C/N ratio can fall within the defined values for a stable compost even though it may still be unstable whereas woody material may be stable with a high C/N ratio.

The ratio NH_4^+-N/NO_3^--N is also associated with compost maturation. As the maturation phase of composting progresses, this ratio declines due to a decrease in NH_4^+ concentration and an increase in the NO_3^- concentration via nitrification (Gao et al., 2010; Zhu, 2007). Compost maturity can also be assessed by its microbial activity. As a consequence of the abundance of easily biodegradable compounds, an immature compost has a strong demand for O_2 and high CO_2 production rates due to intense microbial activity and respiration. For this reason, O_2 consumption or CO_2 production can also be used as indicative measurements of compost stability and maturity (Bernal et al., 1998b).

The EC values increased with increasing composting age of cattle manure (Atallah et al., 1995). These authors found that EC values increased from 3.7 to 3.9 dS m^{-1} after 29 days composting time and from 3.1 to 7.0 dS m^{-1} between 21 and 89 days composting time. However, EC of cattle slurry solid fraction decreased from initial values of 1.9 to 2.0 dS m^{-1} to values between 0.6 and 1.4 dS m^{-1} in maturated composts (168 days), probably due to the decrease in NH_3 concentrations and by the precipitation of mineral salts (Brito et al., 2012a). The cation exchange capacity (CTC) increases with humification, for example, in a sewage sludge cotton waste mixture CTC increased from 535 mmol kg^{-1} before composting to 1244 mmol kg^{-1} in maturated compost (Bernal et al., 1998a). High salt concentrations may cause phytotoxicity problems on crop seed germination and seedling emergence; thus, the EC value is important in evaluating the suitability and safety of compost for agricultural proposes (Brito et al., 2012a).

1.3.3.3 Short and long term effect of compost on crop yield

The compost should maintain or increase SOM content and at the same time, allow a sufficient release of mineral N to increase crop yield (Parkinson et al., 1999). The beneficial effects of compost on crop production are directly related to the application rate of compost. Wong et al. (1999) showed that the application of manure compost up to 50 and 25 t ha^{-1} increased crop yields of maize (*Z. mays*) and Chinese cabbage (*B. chinensis*) respectively. This suggests that the nutrient requirement for maximum growth of Chinese cabbage might be less than that of maize. Compost use as soil amendment usually increases yields in the first year of application, but under certain circumstances the compost tends to increase yields only after successive compost applications, mainly because compost acts as a slow-release source of N (Hartl and Erhart, 2005).

A fertilization with 23 t ha^{-1} year^{-1} of biowaste compost during 10 years of crop rotation with potato and cereals did not increase yields in potato and winter rye during the first two years of compost application, but, at the end of ten years, there was a 10% increase in the yield of potato and cereals (Erhart et al., 2005). These authors suggested that the low yield response in the first years of compost application may be due to the dry climate conditions and to the C/N ratio (averaging 23) that did not favour N mineralization. D'Hose et al. (2012) also showed that crop yields of beet, maize, Brussels sprouts and potato only increased in the fourth year of farm compost (C/N ratio = 18.2, total N = 7.8 g kg^{-1} and OM = 260 g kg^{-1}) application. These authors suggested that the crop yields did not increase in the first three years of compost application because OM content and total N were rather low in comparison to the reference values of farm composts. In another investigation, the application of municipal solid waste and wood scraps compost decreased vegetable crop yields by 25% in the first year, followed by a 39% increase in the next two years (Bonanomi et al., 2014).

Yield increases after successive compost application may be explained by successive accumulation of residual organic N in soil (Sorensen and Amato, 2002). Chalhoub et al. (2013) showed that after the application of municipal solid waste compost, farmyard manure compost and a compost with green wastes, wood chips and sewage sludge during 8 years soil organic N increased 9%, 18% and 27%, respectively, compared to the control. Long-term application of composts may contribute to high yields and increase soil C and N, as well as to build a self-sustainable soil nutrient cycle that provides a wide range of nutrients for plant growth (Hepperly et al., 2009; Hose et al., 2014). However, composts with higher biodegradability show higher proportion of N recovery by crops during the year following their application, whereas more stabilized composts increase long-term N availability mainly through the increase of soil organic N content (Chalhoub et al., 2013).

1.3.3.4 Compost effects on soilborne pathogens

Organic amendments may be useful for management of soilborne diseases such as damping-off (*Pythium ultimum*, *Rhizoctonia solani*, *Phytophtora* spp.), and wilts (*Fusarium oxysporum and Verticilium dahliae*) (Martin, 2003). For example, plant waste and bark compost decreased root rot disease caused by *Fusarium oxysporum* (Cheuk et al., 2003), whereas vermicompost produced from animal manures supressed the pathogen *Phytophtora nicotianae* (Szczech et al., 2001). However, pathogens populations either increase or decrease when composts are applied, and vary according to the type of compost and pathogen. From 120 essays involving 18 composts and 7 pathogens, Termorshuizen et al. (2006) found disease suppression in 54% of all cases, no significant suppression in 42% and disease enhancement in 3.3%. The majority of composts suppressed *Pythium* and *Phytophthora* but only about 20% were able to suppress *Rhizoctonia solani* (Hoitink and Boehm, 1999).

The suppressive efficacy of composts is difficult to predict because composts are highly heterogeneous materials due to the material origin, composting methodologies, and stage of maturity. Actually, decomposition is a crucial process for pathogen suppressiveness. As decomposition proceeds, the availability of easily degradable carbon sources progressively decreases, thus creating microbiostatic conditions that may lead to an increase of suppressiveness by favouring competitive saprophytes. In the last stage of decomposition, the microbial community has already exhausted carbon resources, which then may cause a reduction of suppressiveness (Bonanomi et al., 2010). Suppressiveness is often pathogen specific and may occur in different ways. Composts induce the production of fungitoxic compounds such as organic acids or ammonia, or they provide substrates for organisms that enhance antagonism through competition (Hoitink and Boehm, 1999).

1.3.4 Green manure

1.3.4.1 Green manure characteristics

Green manures are cover crops that are incorporated into the soil for the main purpose of fertilizing the subsequent crop through the mineralization of the cover crop biomass. Both before and after soil incorporation, cover crops offer many benefits to the soil that improve its fertility. For instance, cover crops have proved to be effective in reducing soil erosion (Kuo and Jellum, 2000), and improving soil structure by increasing aggregate stability (Dabney et al., 2001). The uptake of available N by winter cover crops also reduce soil NO_3^--N over winter, thereby lowering the potential for leaching. (Kuo and Sainju, 1998; Baggs et al., 2000; Salmeron et al., 2011). One important criterion for selection of cover crops aiming at reducing NO_3^--N leaching is their ability to uptake N at low temperatures, at which N mineralization may occur, and cereal cover crops, most notably rye, have demonstrated a superior ability to reduce residual mineral N (Kuo et al., 2001).

Rye and oat used as cover crop after corn reduced N leaching by 58% and 26%, respectively (Kaspar et al., 2012). The use of perennial ryegrass (*Lolium perenne*) as catch crop also reduced leaching by 30% to 38% on sandy soils (Askegaard et al., 2005). Rye sown after potato reduced N leaching by 60% after the recommended manure application rate (113 kg N ha^{-1}), but only by 35% with twice the recommended application rate (Torstensson and Aronsson, 2000). Spontaneous regenerated vegetation was as effective as a sown cover crop such as rye, ryegrass, barley and wheat in retaining N (Baggs et al., 2000), although development of weed population. Therefore, cover crops or spontaneous regenerated vegetation instead of fallow, increases carbon flux into the soil and provides food for soil microorganisms (Dabney et al., 2001).

When cover crops are incorporated into the soil as green manure an increase in soil quality was found, reflecting the improvement of biological, physical and chemical properties (Dabney et al., 2001; Sainju et al., 2006). In fact, the incorporation of red clover (*Trifolium pratense*) and rape (*Brassica napus*) into the soil, increased soil microbial biomass, soil respiration and soil enzymatic activities (Tejada et al., 2008; Elfstrand et al. 2007). This increase in soil microbial biomass and soil respiration can be attributed to the incorporation of easily degradable materials and exogenous microorganisms (Blagodatsky et al., 2000; Tejada and Gonzalez, 2006). The latter degrade organic matter through the production of extracellular enzymes and, for this reason following application of green manures to soil an increase in soil enzymatic activities was registered (Goyal et al., 1999; Kautz et al., 2004).

Leguminous, cereals and crucifers are commonly used as green manures. Leguminous green manures supply to the soil important amounts of N in relation to non-leguminous (Doltra et al., 2011), mostly due to N_2 fixation (Baggs et al., 2000; Kumar and Goh, 2000). The ability of N fixation varies widely depending on crop and weather conditions. For example, Faba bean (*Vicia faba*) fixed 413 kg N ha^{-1} during a maize/bean rotation (Fan et al., 2006) whereas vetch (*Vicia sativa*) fixed 227 kg N ha^{-1} during 5 months in winter and early spring, and, white clover (*Trifolium repens*) was more effective at fixing N (327 kg N ha^{-1}) than pea (*Pisum sativum*) (286 kg N ha^{-1}) (Kumar and Goh, 2000). Vetch is more tolerant to frost than many forage leguminous and more productive than Faba bean under water stress conditions (Rochester et al., 2005).

Kuo and Jellum (2000) showed that even though hairy vetch (*Vicia villosa*) produced comparatively less above ground biomass than rye (Secale cereal) or ryegrass (Lolium multiflorum), its higher total N content led to higher total N accumulation in the above ground biomass. In fact, the lower wheat grain yields obtained under non-leguminous residues when compared to leguminous ones were related to lower N addition (64 and 72 kg N ha^{-1} for ryegrass and oat residues, respectively, and 223 and 141 kg N ha^{-1} for white clover and field pea, respectively) (Kumar and Goh, 2002). On the other hand, the incorporation of leguminous crops into the soil may lead to a faster release of mineral N and potential N leaching (Hepperly et al., 2009). For example, incorporation of vetch residues may result in a very rapid net release of mineral N, at a stage at which the subsequent crop is still minimal, thus increasing nitrate leaching potential (Drinkwater et al., 2000).

Combining species of leguminous with non-leguminous cover crops can improve the synchronicity between N release and the subsequent crop demand, therefore decreasing N leaching (Rosecrance et al., 2000). Maize yield after the incorporation of oats (*Avena sativa*) consociated with vetch (*Vicia sativa*) or after vetch monoculture was similar; however, soil residual N was lower with oats consociated with vetch, meaning that synchronicity between maize N uptake and N released from oat and vetch incorporated before maize was improved in comparison to N release from vetch monoculture (Restovitch et al., 2012). Furthermore, there are only slight reductions in N release from leguminous consociated with non-leguminous compared to leguminous monocultures (Rosecrance et al., 2000). Whereas non-leguminous cover crops are more effective in reducing N leaching (Rosecrance et al., 2000), leguminous cover crops are capable of increasing soil N availability because they have the ability to provide substantial amounts of biologically fixed N (Wagger et al., 1998). Therefore, a combination of leguminous and non-leguminous cover crop could both enhance soil productivity and reduce NO_3^--N leaching (Kuo et al., 2001; Kuo and Sainju, 1998). Another advantage of mixing vetch with cereals is to provide structural support for vetch growth, and to improve crop light interception and weed growth reduction.

The proportion of leguminous and cereals to be used in order to increase yields of the subsequent crop is very important. For example, in a mixture of rye or annual ryegrass with residues of vetch, the proportion of the former should not exceed 60% in order to increase N availability to the subsequent crop because if the proportion of rye and annual ryegrass increases, total N content decreases and the C/N ratio increases (Kuo and Sainju, 1998). Moreover, Clark et al. (1994) reported that rye consociated with vetch (sowing rate: 21 kg vetch ha^{-1} and 47 kg rye ha^{-1}) could reduce leachable N while maintaining maize yields comparable to vetch monoculture.

1.3.4.2 The short and long term effect of green manure on crop yields

Changes in yield induced by different green manures depend on a complex interaction between soil, plant and environmental factors (Kumar and Goh, 2000). In a one-year study, yield reduction was observed when non-leguminous cover crops were incorporated into the soil (Kumar and Goh, 2002; Salmeron et al., 2011). This can be explained by the high C/N ratio that leads to immobilization of the soil N, thus reducing yield (Mary et al., 1996). Inversely, incorporation of leguminous residues may increase N addition and probably the yield of the subsequent crop increases (Kumar and Goh, 2002). However, the lack of impact of leguminous green manures on subsequent crop yield is not an uncommon finding (Baggs et al., 2000; Baijukya et al., 2006). For example, maize yield increased after fallow compared to the soil incorporation of vetch and pea (Dupont et al., 2009) but, in another experiment it did not increase after fallow compared to vetch incorporation (Salmeron et al., 2011). In a meta-analysis with different leguminous and non-leguminous green manures, Tonitto et al. (2006) reported that on average the subsequent crop yield after incorporation of non-leguminous cover crops did not statistically differ from conventional management without cover crops. In addition, these authors found that subsequent crop yield after incorporation of leguminous cover crops was reduced by an average of 10% because the leguminous biomass was inadequate and that a leguminous cover crop should provide more than 110 kg N ha^{-1} to achieve crop yields equivalent to the ones obtained in conventional systems.

The impact of repeated cover crops over the long term may differ from annual effects. The contribution of catch crops to the long-term mineralization can increase general soil fertility (Kuo and Jellum, 2000) with only 26% to 40% of N (depending on species) in catch crops being mineralized in the first year after incorporation (Thomsen et al., 2001) and the remaining N increasing the soil organic N after few years (Thorup-Kristensen et al., 2003). In addition, soil mineral N immobilized during decomposition of a catch crop with a high C/N ratio will be mineralized later, during the decay of the newly formed microbial biomass. Several studies indicate that the repeated use of non-legume catch crop produce an initial decrease in yield but a positive effect in later years (Torstensson and Aronsson, 2000; Kumar and Goh, 2002; Constantin et al., 2011). Kuo and Jellum (2000) also observed that both winter rye and annual ryegrass over an 8-years period improved maize biomass production due to increased soil organic N. The repeated use of green manuring with catch crops including leguminous, increases crop yields compared to non-leguminous alone. Loes et al. (2011) found that cereal yield increased 22% and 24% with clover consociated with rye and clover alone, respectively, after 4 years compared to rye. Kuo et al. (2001) also found that N uptake in maize increased 176%, 138% and 166% after 5 years with vetch, rye/vetch and ryegrass/vetch, respectively, when compared to rye incorporation. Moreover, continuous spring incorporation of green manures leads to higher yields than continued autumn incorporation probably due to increased leaching after autumn ploughing (Hansen and Djurhuus, 1997).

1.3.4.3 Effects on pathogens and weeds

Pathogen populations either increase or decrease when cover crops are incorporated into the soil (Bonanomi et al., 2010). Vetch incorporated into soil increases damping-off (*Pythium*) of lettuce if the crop is planted immediately after incorporation, but if lettuce is planted after the vetch green manure has been colonized by soil microorganisms the disease is suppressed (Hoitink and Boehm, 1999). Supressiveness is pathogen specific and it occurs either by the release of certain organic compounds with toxic effects or by stimulating microbial activity resulting in an increasing competition between antagonistic and pathogenic organisms (Thorup-Kristensen et al., 2003). For example, the use of mustard (*Brassica hirta*) or sudangrass (*Sorghum sudanense*) as cover crop before potato crop enhanced growth of microbial fungus that are antagonistic to soil borne pathogens (*Verticilium dahliae, Pythium, Fusarium*) (Collins et al., 2006). An example of the activity of certain substances to eliminate pathogens is the use of Brassicae plants as cover crop to suppress pea root disease (*Aphanomyces euteiches*). Briefly, Brassicae plants contain glucosinolates and water soluble toxic substances such as oxazolidine that suppress soil borne pathogens like *A. euteiches* (Hossain et al., 2012). A green manure of vetch (*Vicia sativa*), lupin (*Lupinus luteus*) and rye (*Secale cereale*) or mustard (*Sinapsis alba*) before potato decreased the effect of potato scrabes (*Streptomyces scabies*) (Schmid and Henggler, 1989). *Verticilium dahliae* was also controlled following 2-3 successive years of sudangrass green manure (Davis et al., 2010). Furthermore, catch crops may exert a nematicidal effect through their decomposition compouds (Thorup-Kristensen et al., 2003). For instance, the incorporation of mustard (*Brassica foliosa*) before potato, carrots, lettuce and tomato crops can decrease the development of nematodes in these crops (Monfort et al., 1987).

Cover crop effects on weeds largely depend on cover crop species and management. Weeds may be suppressed by direct competition and allelopathy by the release of allelochemicals from leaves, flowers, seeds, stems, and roots of living or decomposing plant materials (Weston, 1996). These allelochemicals are released into the soil rhizosphere by decomposition of the residues, volatilization and root exudation (Weston, 2005). For example, rye has shown potential for releasing allelopathic compounds (Barberi et al., 2002) reducing weed biomass by 25% (Zotareli et al., 2009). This observation was further validated by Monfort (1987) who reported that the use of rye as cover crop prevents the growth of Bermuda grass (*Cynodon dactylon*). Another example is sorghum whose incorporation before maize reduced the amount of weeds by about one fourth (Einhellig et al., 1989) because it contains sorgoleone, a compound that reduces weed emergence (Duke et al., 2000). The weed suppressive ability of leguminous is usually lower because of the stimulatory effect on weed emergence of N released from cover crop residues especially when they are ploughed into the soil (Barberi et al., 2000). Incorporation of oat (*Avena sativa*) before pepper reduced about 97% the aboveground weed biomass being more suppressive than hairy vetch (*Vicia vilosa*), (Radicetti et al., 2013). Another study analysed the effect of incorporation of hairy vetch, ryegrass, rye and common vetch before pepper crop on the reduction of weed emergence, and concluded that Hairy vetch was capable of reducing weed density more than the other species (70%) (Isik et al., 2009). Mohler and Teasdale (1993) reported that hairy vetch and rye did not differ in their effect on weed emergence and growth in an early stage of the growing season, but hairy vetch decomposed more rapidly and allowed greater emergence later on. When cover crop residues are left as mulch, weed suppression results mostly from allelochemicals and physical effects. Strip placement of hairy vetch residues as mulch assured an effective weed control and high yield in tomato and pepper production (Campiglia et al., 2010; Radicetti et al., 2013).

1.4 Carbon mineralization

1.4.1 Organic matter mineralization and CO_2 emission

Green plants, to produce plant tissue, absorb the nutrients from the soil except for the carbon which is fixed from the air though the process of photosynthesis. Plant and animal wastes return to the soil and are processed by microorganisms. Part of the organic carbon metabolized by microbes is incorporated into their cells, and part is oxidized to produce energy and is evolved as CO_2. This process is called C mineralization and it is essential for maintaining soil quality and fertility because it provides C necessary as an energy source for heterotrophic soil microorganisms (Tian et al., 2011; Hassan, 2013). The balance between photosynthetic assimilation of C and C loss by plant respiration and C evolved from the soil after plant residues decomposition and mineralization by soil microorganisms explains C sequestration in the soil. Carbon dioxide emission contributes to the global atmospheric CO_2 content, being fertilizer production, transport and application, the factor that most affect agriculture contribution to CO_2 content in the atmosphere and therefore for the greenhouse effect. Measurements of soil CO_2 emissions can provide useful insights into soil C cycling and evaluation of potential C sequestration (Franzluebbers et al., 1995). Green manure and animal manures added to soil have a wide range of C compounds that vary in rates of decomposition. For example, the percentage of organic C evolved in a 30 days incubation was higher from alfalfa (55%) and sorghum (53%) residues compared to corn (29%) and soybean (33%) residues (Ajwa and Tabatabai, 1994). These authors also observed higher C recovery from pig (58%), chicken (52%) and cow manure (48%) compared to horse manure (25%) during the same period.

Commonly, after the incorporation of the organic amendments into the soil, there is a rapid increase in C mineralization during the initial stages of decomposition, followed by a slower release stage. For example, CO_2 evolution during the first 5 days of incubation of chicken and pig manures, accounted for 50% of total CO_2 evolved in a 30 days' period (Ajwa and Tabatabai, 1994). Carbon mineralization rates of animal manures depend on the type of animal feed. For example, pig manure showed low fibrous content and high N content, as opposed to horse manure that contained large amounts of fibrous materials and a low N content (Ajwa and Tabatabai, 1994). After the readily decomposed organic compounds are utilized, a relatively complex organic fraction is metabolized, leading to a small but steady emission of CO_2 (Bernal et al., 1998a; Rochette et al., 2006). This complex organic fraction, which is hard to decompose, persists for long periods, and is a constitutive part of soil humus (Troeh and Thomson, 2005).

1.4.2 Biotic and abiotic factors that drive carbon mineralization

1.4.2.1 Composition of organic fertilizers

The amounts of mineralized organic C vary among plant residues and animal manures depending on their C and N content and C/N ratio (Mafongoya et al., 2000; Antil et al., 2011), and on the environmental conditions. The total amount of CO_2 released from alfalfa, sorghum, soybean and corn treated soils was increased for the crops with higher N content in a 30 days' incubation (Ajwa and Tabatabai, 1994). The amount of CO_2 released increased from composted sewage sludge followed by cattle manure compost, hen cattle manure compost and sewage sludge during 168 days of incubation because the increasing amount of C content (Antil et al., 2011). The rate of decomposition of plant residues increases when the plant C/N ratio decreases. For example, CO_2 emission from common vetch (C/N ratio = 25) increased, and at a faster rate than CO_2 emission from ryegrass (C/N = 71) (Mancinelli et al., 2013). Moreover, peanut green manure with low C/N ratio (C/N = 17) showed fast C decomposition compared to rice straw (C/N = 62) (Hassan, 2013).

In accordance to the ability of microbial exoenzymes to break down organic polymers into smaller units, the chemical composition of C can be divided in two classes of compounds. One class comprises carbohydrates and proteins which are labile components composed by low molecular weight chemicals that are often water soluble, and thus, more accessible to microbial assimilation to act as energy and nutrient sources; on the other class, lipids, lignin and humic substances which are considerably more difficult to decompose (Ahn et al., 2009). The two most common methods to quantify available soil C are water extraction and acid-hydrolysis. This soluble C is well correlated with C recovery. For example, Antil et al. (2011) found that C recovery from cattle manure compost as well as from hen + cattle manure compost was well correlated with soluble C during the first 4-6 weeks of incubation. Moreover, Morvan et al. (2006) studied 47 animal wastes and also found positive correlations between C mineralization and their content in soluble C after 224 days of incubation.

There is a great variability in the composition of animal manures mostly due to the protein and fibre content of the animal, the kind of bed material and the composting process. Therefore, there has been a need to understand how C mineralization correlates with certain biochemical characteristics. For instance, alfalfa residues with high N content, low C/N ratio and lignin content showed high C mineralization rates (Raiesi, 2006), as opposed to rice straw which had high C/N ratio and high content of cellulose and lignin, thus making it resistant to microbial attack (Zhu, 2007). Polyphenols inhibit C mineralization by inhibiting microorganisms and inactivating enzymes (Batzer and Sharitz, 2008). However, polyphenol content was reduced by composting; for example, during composting of olive mill wastewater with poultry manure and grape marc with cattle manure, giving composts without a phytotoxic character (Bustamante et al., 2008; Rigane et al., 2015). Morvan et al. (2006) characterized 47 animal manures during a 224 days' laboratory incubation and found a positive correlation between C mineralization and the fractions of hemicelluloses and a negative correlation between C mineralization and lignin fraction. Moreover, after 3 days of incubation, C mineralization was greater with hen manure than with cow manure because the former is known to contain high amounts of cellulose, hemicelluloses and NH_4^+-N and low amounts of lignin; whilst the latter contain more lignin than cellulose and hemicellulose and low amounts of inorganic N (Ribeiro et al., 2010). Indeed, a high initial NH_4^+-N content may increase C mineralization. As an example, CO_2 evolved from 4 different animal manures after 10 weeks of incubation, was positively correlated with the initial NH_4^+-N content, suggesting that ammonium supplied with manure stimulated microbial activity during the incubation period (Calderon et al., 2005).

1.4.2.2 Rate of application of organic fertilizers

Carbon evolved from organic wastes depends on their composition but is also affected by the rate of application. Actually, relative C recovery from organic fertilizers decreased at higher application rates (Hadas et al., 1996; Antil et al., 2011; Huang and Chen, 2009). For example, C recovery during 168 days of incubation from increasing amendment rates (2% to 4% of soil weight) of cattle manure compost, hen cattle manure compost and sewage sludge compost decreased from 16 to 11%, 37 to 27%, and 28 to 19%, respectively (Antil et al., 2011). In a different study, C recovery also decreased from 13% to 8% by increasing the application rate of cattle manure compost (from 5 to 15% of soil weight) after 33 weeks of incubation (Hadas et al., 1996).

1.4.2.3 Temperature and soil moisture

Temperature and soil moisture influences microbial activity and, consequently, the rate of C mineralization. An increase in temperature leads to increased enzymatic and physiological activity of microorganisms, contributing to higher CO_2 emission from soils (Zhou et al., 2007). For example, evolution of CO_2 increased from 36% at 5 ºC to 54% at 25 ºC of the initial C of fresh municipal solid waste compost during 112 days of incubation (Chodak et al., 2001). Furthermore, in maturated municipal solid waste compost these authors found that accumulated CO_2 evolution after 112 days increased from 1% of the initial C at 5 ºC to 6% at 25 ºC. However, increasing temperature increases OM decomposition at a faster rate for residues with low C/N ratio compared to plant residues with high C/N ratio. For example, Hassan (2013) found that the increase in C mineralization from soil with rice straw incorporation was lower compared to green manure incorporation when soil temperature was raised from 25 ºC to 35 ºC.

Soil moisture is another key factor that influences microbial activity and C mineralization. Soil moisture content severely limits CO_2 emission under dry and wet conditions (Mancinelli et al., 2010), being CO_2 emission greater for the soil near field capacity, as microbial activity increased (Abera et al., 2012). High soil moisture content may inhibit CO_2 emission because it increases diffusion resistance of CO_2 from the soil to the atmosphere and of O_2 from the atmosphere into soils, thus resulting in less CO_2 production (Ding et al., 2007). On the other hand, low soil moisture limits microbial and root respiration (Yuste et al., 2003). Murwira et al. (1990) found that during decomposition of cattle manure, C mineralization increased from 0% to 50% of water-holding capacity and decreased from 50% to saturation in a curvilinear response. Similarly, C mineralization from peanut green manure and rice straw also showed a significant decline from 50% to 100% of water holding capacity. However, the decline in C mineralization was smaller with green manure (Hassan, 2013). Abera et al. (2012)

also found that the effect of decreasing soil moisture in C mineralization was minimal with leguminous incorporation due to the presence of easily mineralizable substrates. C mineralization also increases rapidly with soils amended with leguminous. For instance, the CO_2 emitted from soils unamended and amended with clover increased linearly and exponentially, respectively, when water potential increased from -5.0 to -0.003 MPa (Quemada et al., 1997).

The effects of temperature and soil moisture on C mineralization are linked. Soil temperature is correlated with CO_2 emission when soil moisture is above the wilting point; whilst CO_2 emission is affected by soil moisture (between 23% to 81% of water filled pore space) only at temperatures above 18ºC (Dilustro et al., 2005; Ding et al., 2006). This indicates that at high temperatures, sufficient soil moisture content is necessary to support diffusion processes of sequential biochemical reactions to the sites of microbial activity. However if soil moisture values exceeds certain thresholds, which depend on soil properties, microbial respiration is limited by O_2 diffusion (Wu et al., 2010).

Drying and rewetting cycles can stimulate microbial activity and increase C mineralization through the disruption of macroaggregates (Denef et al., 2001). Butterly et al. (2010) studied the effect of drying and rewetting cycles in 32 soils during 90 hours of incubation. These authors concluded that, in comparison to constantly wet soils, in 12 soils the rate and the amount of mineralizable C increased with the effect of drying and rewetting cycles. For these soils a greater quantity of available C was accessible to microorganisms on rewetting. On the contrary, in 17 soils the final amount of mineralizable C was similar in the constantly moist soil compared to the soil with drying and rewetting cycles, but the rate of C mineralization was enhanced by the drying and rewetting cycles. Butterly et al. (2010) suggested that one possible reason for this occurrence was that the effect of the drying and rewetting cycles released a small amount of labile C, which was sufficient to alter the C rate but not the amount of C. Two soils showed a reduction in the mineralization rate, but no change in the amount of mineralizable C, and one showed no change in either the amount and rate of mineralizable C, possibly due to low C contents and small amount of microbial biomass in these soils. In the field, soils are subjected to several drying and rewetting cycles that probably change C dynamics. For instance, in an annual grassland, soil respiration increased from 376 µg C-CO_2 g^{-1} in continuously wet soils to 425 µg C-CO_2 g^{-1} after 12 dry/wet cycles (Xiang et al., 2008). A dry-wet cycle started with a rewetting period during three days followed by dry period of 7 days. Controversially, Mikha et al. (2005) found that after 96 days of incubation accumulated mineralized C was greater with constant water content compared to dry/wet cycles. These results indicate that the increased microbial activity that occurred after wetting was not sufficient to compensate for the reduction in C mineralization during the drying period.

1.4.2.4 Soil properties

Carbon mineralization rates of OM increase in coarse-textured soils compared to fine-textured soils. Carbon recovery from biowaste compost on loamy soil was lower and showed a delayed mineralization compared to sandy soil (Leifeld et al., 2002). Carbon recovery also decreased in clay soil compared to sandy soil for sewage sludge compost, cattle manure compost and hen/cattle manure compost (Antil et al., 2011). On the contrary, C mineralization was similar in clay and loam soils with leguminous residues probably due to easily mineralizable substrate (Abera et al., 2012).

Total N and C/N ratio of the soil also play an important role in C mineralization. For example, C mineralization from chicken manure, mungbean and wheat residue was increased in soils with lower C/N ratio and higher total N content (Khalil et al., 2005). Moreover, the decomposition of organic residues is affected by the initial soil pH. Carbon mineralization increased in soils with pH between 7.3 and 7.8 when compared to soils with pH between 4.3 and 5.7 (Khalil et al., 2005). This was probably due to a direct effect of pH on enzyme activity and microbial biomass (Haynes and Swift, 1988). These authors showed that lime additions increased the activity of protease and sulphatase enzymes that are involved in C mineralization.

1.4.2.5 Tillage

Tillage disrupts soil aggregates, exposing stable OM to microbial degradation. Therefore, the implementation of agricultural practices that reduce soil mobilization can provide an opportunity to increase C sequestration and agricultural sustainability. Accordingly, it would be expected that the advantages of the no-tillage system would increase soil organic matter and sequestration of atmospheric CO_2 (Rouw et al., 2010). However, the effects of management on soil C dynamics are complex and often quite variable. Cover cropping and organic N fertilization can either increase or maintain soil organic C in tilled soils (Sainju et al., 2006). For example, C mineralization was similar with conventional tillage with moldboard plowing to 20 cm depth and no-tillage after a 10 years crop rotation with rye, cotton and corn, fertilized before corn and cotton with poultry litter (Sainju et al., 2008). Carbon can even increase after four years under conventional tillage using disk to plough down to 20 cm the crop residues, when compared to a no-tillage system (Rouw et al., 2010). In this experiment, the maximum increase in C under conventional tillage occurs in a depth range between 20 and 30 cm. In this situation, after four years, the balance was positive for C under conventional tillage, because although aeration of soil by tillage accelerated microbial oxidation leading to increased C mineralization, at the same time, carbon losses were compensated for by the restitution of crop residues. The input of fresh crop residues has resisted to oxidation, being only partly decomposed by organisms whereas, no-tillage lacks the inputs of fresh organic material deep in soil.

1.4.3 Carbon mineralization from organic fertilizers

1.4.3.1 Compost

Composts C mineralization decreases with compost age, because only stable OM more resistant to microbial degradation remains in the compost (Atallah et al., 1995). For example, in a 107 days' period of incubation of farmyard manure (FYM) mineralized C decreased from 44% of total carbon added in FYM to 13% after composting FYM for 2 months (Morvan and Nicolardot, 2009). Bernal et al. (1998c) studied C mineralization in composts with different mixtures of organic wastes prepared with sewage sludge, animal manures, city refuse and plant residues for an incubation period of 70 days. Mineralized C ranged from 62.3% to 93.2% of total organic carbon in non-composted wastes, and was higher for sewage sludge (93.2%) and city refuse (80.1%), which had a high proportion of easily degradable organic compounds. However, these authors found that the percentage of mineralized C in mature composts ranged from 9.0% to 37.9%. Among them, the highest value (37.9%) was observed for a compost enriched in maize straw that had a low degree of maturity due to slow degradation of straw during the composting process.

Bernal et al. (1998a) showed that C mineralization was above 60% of added C 70 days after the incorporation of a blend (not composted) of sewage sludge with cotton into the soil, whereas C mineralization decreased to 22% and 20% when this compost was incorporated after the active and the maturation phase, respectively. These results suggest that the C remaining after the oxidative phase of the composting process is relatively resistant to microbial degradation. Therefore, mature composts show small amounts of evolved C after their incorporation into the soil as a result of C stabilization during the maturation phase of composting. So, to protect the soil and conserve its organic carbon content, mature composts with stabilized organic C are desirable compared to fresh OM (Bernal et al., 1998 c). In addition, non-composted manures may promote degradation of native soil OM (Bernal et al., 1998c).

1.4.3.2 Green manure

Incorporation of cover crops improve soil C accumulation by promoting C fixation during the growth of cover crops. Sainju et al. (2006) reported that soil total C increased in agricultural ecosystems after 3 years of cover crops application. Moreover, Miller and Dick (1995) compared two vegetable crop rotations, a traditional vegetable rotation with a winter fallow and another including a leguminous crop (red clover) as green manure and found that the latter maintained root activity throughout most of the year, improved soil aggregation (as the amount of macroaggregates increased in comparison to microaggregates), and enhanced buffering capacity of soil organic C within a relatively short period of time (two years).

Carbon mineralization reported by Wang et al. (2010) differed among different cover crop species, yet C sequestrated was higher in cereal cover crops than in leguminous cover crops. The quantities of C left in the residues were higher for both triticale (266 g m^{-2}) and ryegrass (223 g m^{-2}) compared to purple vetch (144 g m^{-2}) and white clover (72 g m^{-2}) because the C/N ratios of triticale (52) and ryegrass (41) were higher compared to white clover (14) and purple vetch (22). Therefore, C/N ratio is an important quality that influences C mineralization (Wang et al., 2010). However, the percentage of C mineralized from triticale, ryegrass, purple vetch and white clover was respectively 21, 22, 34 and 47% after 127 days of decomposition because the total C input was higher for cereals compared to leguminous cover crops.

The incorporation of green manure increase in short-term the available soil organic C via the decomposition of crop residues by microorganisms, which is indicated by the large amount of CO_2 released (Zhou et al., 2012). The amount of accumulated CO_2 released in a laboratory incubation during 30 days from soil with no amendments and soil incorporated with corn, soybean, sorghum and alfalfa was approximately 500, 3000, 3200, 5000 and 5200 mg kg^{-1}, respectively (Ajwa and Tabatabai, 1994). During the first 5 days of this incubation, the rate of CO_2 emission from alfalfa (*Medicago sativa* L.) and sorghum (*Sorghum vulgare* L.) was 50% and 40% of total CO_2 evolved in 30 days. The increase of available organic C enhances both growth and activity of soil microorganisms which may lead to an increase in soil fertility (Kautz et al., 2004). Moreover, the addition of fresh C from cover crops into soil can even improve the turnover rates of the resistant C fraction of soil (Zhou et al., 2012). Elfstrand et al. (2007) reported that incorporation into the soil of fresh leguminous cover crops enhances microbial biomass and enzyme activities more than other forms of organic matter.

1.4.4 Priming effect

Soils contain a considerable amount of OM, mainly as humic substances that soil microorganisms can utilize very slowly. On the other hand, for fast growth and reproduction, soil microorganisms must rely on inputs of fresh OM. However, the incorporation of fresh OM may contribute to decreased native SOM as a result of increased microbial activity which also attacks native SOM. This is the result of higher availability of energy produced from the decomposition of fresh OM. These short-term changes in soil organic matter induced by easily available C or N sources is the so-called priming effect (Kusyakov et al., 2000).

The dynamic of the priming effect involves a chain of mechanisms. In a first step, soil microorganisms switch to the freshly added substrate that contain easily utilizable C source. The second phase corresponds to an increase of the microbial activity as a direct consequence of the first phase. In the meanwhile, the available low molecular weight substrates act as an external inducer to activate inactive soil microorganisms (De Nobili et al., 2001). After consumption of the most labile substrates, microorganisms will look for and utilize the substrates of lower availability, producing extracellular enzymes which may promote SOM decomposition. Finally, the initial state of the soil will be reached as a consequence of the decline of microbial activity (Blagodatskaya and Kusyakov, 2008; Kusyakov and Bol, 2006).

Soil amendment with fresh organic material may change the activity of microorganisms and cause decomposition of SOM (positive priming effect) or immobilization of C and N in a very short time, generally from several days to weeks (negative priming effect) (Kusyakov et al., 2000; Fu et al., 2000).

The priming effect depends on the availability, composition and amount of the organic fertilizer incorporated into the soil. The priming effect is usually induced by fresh OM (Blagodatskaya and Kuzyakov, 2008). By increasing the chemical diversity of fresh OM incorporated into the soil, the diversity of the produced enzymes will be boosted and, consequently the probability of occurrence of priming effect (Fontain et al., 2003). For example, Mondini et al. (2006) demonstrated that compost, carbohydrates and amino acids added to soil showed priming effect in opposition to glucose.

The priming effect may be induced by organic or mineral fertilizer (Fangueiro et al., 2007) and plant residues (Fu et al., 2000; Bell et al., 2003). For example, application of crimson clover (*Trifolium incarnatum*) and wheat (*Triticum aestivum*) as green manure increased the decomposition of soil carbon, and caused a net loss of total soil carbon on short-term (Fu et al., 2000). In another study, Pascault et al. (2013) showed that the decomposition of SOM also increased with the incorporation of alfalfa and wheat. The priming effect occurred mainly during the first month of incubation and was higher with alfalfa incorporation compared to wheat incorporation. This was due to the difference in biodegradability that increased microbial biomass with alfalfa compared to wheat. Bol et al. (2003) also found a positive priming effect after slurry incorporation in grassland soils. In this experiment, the priming effect increased for soils with higher C content (5.1%) compared to soils with a lower C content (2.3%).

1.5 Nitrogen mineralization

1.5.1 Definition

The process of N mineralization in soil that makes N available to crops can be defined as the conversion of organic N into mineral forms which occurs through biochemical transformation mediated by microorganisms (Stevenson, 1985). Microbial activity gradually breaks down the complex organic molecules into simple inorganic ions that can be utilized by growing plants. This process consists of a sequence of enzymatic steps in which the enzymes and the substrate are provided by living and dead microbial biomass, respectively (Mengel, 1996). Thus, provision of substrate depends on soil and weather conditions that lead to greater dying of soil microbes. These conditions could be dryness, lack of oxygen or lack of chemical energy for microbial life. For example, crops provide organic carbon to rhizosphere microorganisms; upon depletion of this source, a lack of energy will induce the death of microorganisms followed by release of inorganic N (Mengel, 1996).

The process of OM decomposition into ions available to crops is divided into three phases: hydrolisys of complex organic compounds into simple organic molecules (for example, hydrolysis of proteins into aminoacides, amines and amides), ammonification and nitrification. Ammonification is the process of organic N mineralization into ammonium ions, which occurs when heterotrophic microorganisms attack amino acids and break the bond between a carbon atom and an amino group (NH_2). Most of the ammonium ions are adsorbed on cation-exchange sites, and held until they are either utilized by microorganisms and plants or oxidized to other forms. Nitrification is the process that transforms ammonia into nitrate. Nitrossomonas oxidize ammonia to nitrite and Nitrobacter oxidize nitrite to nitrate (Troeh and Thomson, 2005).

Nitrogen mineralization is part of the N cycle. The global biogeochemical N cycle occurs on three major reservoirs: Atmosphere, oceans and terrestrial ecosystem, between which exchanges occur. In terrestrial ecosystems about 96% of N is bound up in dead organic matter. The inorganic N needed by plants is released by microorganisms through decomposition of OM (N mineralization). On the other hand, the organic N and C that microorganisms need for biosynthesis, growth and energy is delivered to soil microorganisms through root exudates and plant death, thereby closing the cycle (Kinzig and Socolow, 1994).

Because N availability limits crop growth, efforts have been made to produce industrial N fertilizer, reducing N_2 to NH_4^+ to increase crop yields. The consequences of increasing soil N content are manifold. Firstly, nitrogen deposition in terrestrial ecosystems may cause differential growth of species because some species will assimilate the additional nitrogen more easily than others. This may reduce ecosystem biodiversity or change ecosystem function. Secondly, the fixed N may cause eutrophication of coastal waters. Thirdly, the flow of nitrous oxide (N_2O) from soils to the atmosphere destroys the ozone layer and is an important contributor to the greenhouse effect whereas the volatilization of NH_3 increases eutrophication, acidification and odour pollution. Lastly, the increased concentration of NO_3^- in the ground water has a direct impact on health because certain bacteria residing in vertebrate digestive tracts can convert nitrate (NO_3^-) into highly toxic nitrite (NO_2^-) (Kinzig and Socolow, 1994).

Nitrogen is mostly lost from the soil system through leaching, denitrification and ammonia volatilization. Nitrate is lost from an agricultural system when it is leached outside the root zone, which is influenced by soil type, N input and crop type. The impact of rainfall on N leaching is especially relevant in the weeks following N application (Goulding, 2005). In order to minimize N leaching it is important to synchronize N mineralization with crop needs (Bergstrom and Brink, 1986).

Denitrification is the biological conversion of nitrate (NO_3^-) to nitrogen gas (N_2), nitric oxide (NO) or nitrous oxide (N_2O). This process occurs in a situation of appropriate availability of electrons (carbon) and nitrate ions (NO_3^-) and insufficient oxygen, so that denitrifying bacteria use the nitrate rather than oxygen. Denitrification of soil nitrates increased with the incorporation of farmyard manure into the soil (5.7% of annual N input) (Mogge et al., 1999). Denitrification also increased after hairy vetch incorporation into the soil compared to rye-vetch mixture or rye alone. Rosecrance et al. (2000) found that 17%, 9% and 12% of total N loss was denitrified by vetch, rye-vetch mixture and rye, respectively. This high denitrification rates in vetch samples can be explained as a result of both high NO_3^- availability and high organic carbon availability (Rosecrance et al., 2000). In yet another example, the presence of available C in slurries favoured denitrification (Calderon et al., 2005). These authors found N losses of 14.7 to 39.2% of added N from slurries. Application of slurry is also one of the main emission sources of ammonia (Martinéz-Lagos et al., 2013). Ammonia volatilization depends on many factors such as type and characteristics of manure, rate and method of application and climatic conditions (Sommer et al., 2003). Ammonia volatilization is higher with slurry and fresh manure compared to composted manure. Brinson et al. (1994) found that NH_3 losses in 21 days ranged from 17% to 31% of the applied N for fresh poultry litter, and from 0% to 0.24% for composted poultry litter. This low NH_3 volatilization from composts is explained by the low NH_4^+ content of composts and low N mineralization rates. To minimize ammonia volatilization losses, manure should be immediately incorporated into the soil.

1.5.2 Abiotic and biotic factors that drive N mineralization

1.5.2.1 Composition of organic fertilizers

Soil amendment composition has a significant impact on organic N mineralization (Van Kessel and Reeves, 2002). The mostly used indicators for predicting N mineralization from soil amendments are their organic N content and C/N rate. For instance, Jensen et al. (2005) incubated 37 species of plants for 217 days and concluded that N mineralization was closely correlated to total plant N content. Morvan et al. (2006) studied N mineralization from 47 animal wastes during 224 days of incubation and found highly significant positive correlations between N mineralization and organic N content and C/N ratio. Organic amendments with high organic N content induce a fast release of N, as opposed to organic amendments with high C/N ratio that cause immobilization of inorganic N (Mengel, 1996). Therefore, the balance between mineralization and immobilization of N is strongly controlled by the C/N ratio of the organic amendments (Abera et al., 2013). Reddy (2008) reported that N released from green manures and poultry manure having C/N ratio of 12 to 14 was fast; whereas farmyard manure with a C/N ratio of 29 to 33 resulted in N immobilization during the first 6 weeks and subsequent release of mineral N. Addition of wheat straw with a C/N ratio of 79 resulted in N immobilization throughout the entire incubation period (16 weeks) (Reddy et al., 2008).

Organic N is mostly found in proteins, peptides and polymers of amino sugars; however only a small percentage of total N is easily mineralizable and contributes to the pool of soil mineral N. This easily mineralizable fraction, composed mainly by amino-N and polymers of amino sugars, and present in the microbial biomass, is a good indicator to predict N mineralization (Mengel, 1996). This fraction may be expressed by hot water extractable N (Curtin et al., 2006) or by $CaCl_2$ extractable N and is a reliable indicator of N mineralization in sandy soils (Apple and Mengel, 1993). This easily mineralizable organic N is affected by mineralization, immobilization, leaching and plant N uptake in the same way as mineral N but its pool size is more constant than that of mineral N (Murphy et al., 2000; Goulding, 2005).

The most important indicators to predict short-term N availability from organic amendments are the easily mineralizable organic N fraction and NH_4^+-N content (Gutser et al., 2005). Organic fertilizers with low contents of mineral N (NH_4^+-N and NO_3^--N) and high C/N ratios release nutrients slowly, whereas high mineral NH_4^+-N content usually leads to fast N mineralization (Antil et al., 2011). Indeed, Cooperband et al. (2003) found that ryegrass N uptake was higher after incorporation of compost aged for 16 days compared to composts aged for 60 days due to higher amount of mineralizable N from fresh compost.

The correlation between C/N ratio and N content from soil organic amendements with N mineralization has not always been clear (Kumar and Goh, 2003). Hence, many studies tried to find other biochemical characteristics to predict N mineralization. Some of these characteristics are lignin content, polyphenol content, lignin/N and polyphenol/N ratios (Singh et al., 2001). Kumar and Goh (2003) incubated 6 species of plants (white clover, peas, ryegrass, wheat, barley and oats), mushroom compost and dairy sludge and found that significant correlations occurred between N mineralization and C/N, lignin/N and polyphenol/N ratios after 110 days of incubation. Cordovil et al. (2005) also concluded that small lignin content in poultry manure was the main factor determining fast N mineralization.

1.5.2.2 Rate of organic fertilizers application

It is generally believed that N mineralization relative to the amount of added N is independent on the rate of compost application, depending mostly on the composition of compost (Hadas et al., 1996; Hadas and Portnoy, 1997; Antil et al., 2011). However, the proportion of applied organic N that was mineralized increased with the application rate of compost during a three year vegetable crop rotation in greenhouse probably due to increasing soil microbial population and soil biological activity (Chang et al., 2007) or, in contrast, decreased with increasing application rate when the amount of compost added was greater than 1.5% of the soil dry weight (Rees et al., 1993). This phenomenon is due to a lower degree of N mineralization, probably as a result of an anaerobic condition being developed in the soil (Rees et al., 1993). Therefore, the percentage of organic N mineralized from composts may depend on the rate of compost application but this relation is difficult to predict.

1.5.2.3 Temperature and soil moisture

Nitrogen mineralization depends on the activity of microorganisms that is closely associated to temperature and soil moisture. The majority of microorganisms is mesophylic and prefers temperatures between 25 ºC and 37 ºC, and so N mineralization rates increase at temperatures ranging within this interval (Wang et al., 2006). Nevertheless, N mineralization also occurs at low temperatures but the microbial activity is smaller and nutrients are released slowly, thus being difficult to adjust the availability of nutrients to the crops needs (Gill et al., 1995). Water availability also controls N mineralization. Maximal N mineralization occurs when soil moisture is close to field water holding capacity and then decline as soil water potential becomes more negative (Wang et al., 2006). For instance, after compost chicken manure incorporation, N mineralization was enhanced 13% after 12 weeks when temperature increased from 15 ºC to 25 ºC, and 21% when soil moisture increased from 50% to 90% of water holding capacity (Agehara and Warncke, 2005). Under waterlogged conditions mineralized N is lost possibly through denitrification reactions or leaching (Singh et al., 1988).

The effect of soil moisture on N mineralization may be less important compared to that of temperature, reflecting the adaptation of microorganisms to the moisture regime of the soil (Sierra, 1997). This author reported that the effect of temperature between 10 ºC and 25 ºC was higher than the effect of soil moisture between 1700 and 30 kPa on N mineralization. The effects of soil temperature and moisture are subject to positive interaction. For example, the response of N mineralization to soil moisture was higher at 20 ºC or 25 ºC than at 10 ºC (Sierra, 1997). Nitrogen mineralization is more responsive to a given factor when the level of the other was closer to optimal, as found by Wang et al. (2006) that at 15% soil moisture, N mineralization was 7.7 times greater at 35 ºC than at 5 ºC; but when soil moisture increased to 25%, N mineralization was 28.6 times greater at 35 ºC.

The peak of N mineralization from urea at 15 ºC occurred at 30 days after soil incorporation. However, at 30 ºC the maximum N mineralization occurred after 10 days

(Benitez et al., 2003). The period of N immobilization also decreases at high temperatures and low water potential (Sierra, 1997). The net release of NO_3^- from a not maturated solid waste compost occurred after 14 days at 25 ºC but only after 84 days at 5 ºC. (Chodak et al., 2001). Nitrogen immobilization rate was lower at pF2.5 than at pF3.9 irrespectively of organic sources and soil type (Abera et al., 2012).

As previously reported wetting and drying cycles in soil may increase N mineralization (Jarvis, 1996). The additional mineralization during wetting of soils depends on the duration of drying and wetting cycles. A short drying period accompanied by an extended wetting period would potentially increase N mineralization, however, the accumulated mineralized N during drying and wetting cycles is smaller compared with soil with optimum moisture (Mikha et al., 2005; Borken and Matzner, 2009). Weather conditions in early spring and summer affect N mineralization due to transitions between drying and wetting cycles. Kayser et al (2010) reported that in a 3-year rotation with grass red-clover, triticale, barley and maize, during the dry period of June and July, N mineralization was reduced. Subsequent rewetting of the soil in August led to strong mineralization after ploughing which continued throughout the winter time, thus leading to large residual soil nitrogen mineralization and leaching.

1.5.2.4 Soil properties

The decomposition rate of any organic material added to soil depend on its chemical composition and the factors that affect soil environment such as temperature, soil moisture, texture, pH, biological activity and the presence of other nutrients (Walpola and Arunakumara, 2009). N mineralization is faster in sandy soils than in clay soils (Mengel, 1996; Madrid et al., 2011). As a matter of fact, an increase in soil clay content from 10% to 40% caused a 10% reduction in net N mineralization rate from ryegrass green manure (Thomsen et al., 2001). This is due to the fact that sandy soils are characterized by higher air-filled porosity in comparison to clay soils, allowing greater aeration and consequently increased N mineralization (Rasiah and Kay, 1998). In addition, the activity and the C/N ratio of the microbial biomass increased in sandy soils compared to clay soils, thus leading to an increase in N mineralized per amount of microbial biomass (Hassink, 1994). On the contrary, a higher protection of the OM by humus-clay complexes render enzyme-protein interactions more difficult, decreasing N mineralization (Mengel, 1996).

The relationship between soil pH and net N mineralization is still not clear. Fu et al. (1987) showed that N mineralization decreased with decreasing soil pH from 8 to 4 in crop residues treated soils. This was probably due to lower microbial activity at low pH. On the contrary, Xiao et al. (2013) found that low soil pH (3.2 to 6.7) had little impact on N mineralization in crop residues treated soils and there was no a clear relationship between net N mineralization and soil pH. On the other hand, nitrification is more sensitive than ammonification to low pH (Khalil et al., 2005). Soil pH < 4.2 inhibited nitrification while ion ammonium accumulated in highly acidic soils. At soil pH > 4.5 nitrification rates increased, resulting in lower NH_4^+ concentration, as ammonium was more rapidly oxidized in high soil pH values (Xiao et al., 2013). For example, nitrification from sewage sludge compost in a laboratory incubation occurred during 56 days at low soil pH (5) as opposed to nitrification at high soil pH (7.1) that occurred within the first 3 days (Huang and Chen, 2009).

1.5.2.5 Tillage

Tillage increased soil N mineralization, while decreasing soil organic C and N (Kristensen et al., 2003). Moreover, increasing tillage intensity decreased C and N microbial biomass and the ability of soil to immobilize and conserve mineral N (Follett and Schimel, 1989; Franchini et al., 2007). This can be explained by increased soil aeration and enhanced exposure of OM to microbial degradation by the disturbance of soil structure (Kristensen et al., 2003). With time, tillage systems may result in soil OM decline, and consequently in crop yield decreases (Hungria et al., 2009). On the other hand, tillage practices with incorporation of crop residues into the soil can also increase soil organic N. Balota et al. (2004) found that during a two-year rotation with soybean (*Glycine max* L.), wheat (*Triticum aesativum* L.), maize (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.), N mineralization increased 78% in no-tillage systems compared to conventional systems. One explanation for this effect of no-tillage on N mineralization is that no-tillage systems provide more favourable habitat for microorganisms, since the size of macroaggregates is bigger compared to conventional tillage systems.

Soil tillage in the growing season not always improve synchronization between N mineralization and crop demand because N mineralization depends on the quality of the substrate (Thomsen and Sørensen, 2006). In a maize/hairy vetch rotation, the incorporation of hairy vetch before planting maize increased soil N mineralization compared to no-tillage, but the maximum mineral N content in soil occurred five to six weeks after tillage, long before maize fastest growing demand. However, mechanical weed control increased N mineralization of the vetch residues during the period of maximum N demand by the maize (Drinkwater et al., 2000).

1.5.3 Nitrogen mineralization from organic fertilizers

1.5.3.1 Compost

The compost acts as a slow and steady release source of N in the soil (Hartl and Erhart, 2005) and may have large residual effect (Benitez et al., 2003) due to formation of stable N compounds throughout the composting progression (Larney and Hao, 2007). Moreover, composted materials usually show lower mineralization rates than non-composted (Cordovil et al., 2007). For instance, N mineralization rates of 38% to 60% of total N supply for uncomposted manures in the first year were reduced to 6% to 20% upon composting (Benitez et al., 2003), with the residual compost N being mineralized at rates between 3% to 8% in the following years (Amlinger et al., 2003). Another study showed that the accumulated N released after 33 weeks of laboratory incubation was approximately 15% of added N for municipal waste compost (C/N = 15.5) and cattle manure compost (C/N = 11.2) (Hadas and Portnoy, 1997); and after 107 days of incubation, N recovery from composted cattle manure (C/N = 12.7) was 10% (Morvan and Nicolardot, 2009). Antil et al. (2011) also reported an N recovery of 15% from hen cattle manure compost (C/N = 7.1) during 168 days; however, analysis of N recovery from cattle manure compost (C/N = 9.2) by the authors revealed only a 5% rate probably due to a smaller inorganic N content.

Nitrogen mineralization rates may increase after a long period of time due to the residual effect of the compost. For instance, in the first year of a 21-year field experiment with waste compost the N mineralization rate was 16% of applied N, and during the final year this value rose to 40% of applied N due to the organic N accumulated by the successive application of compost during 21 years (Amlinger et al., 2003). Crop rotations with high nutrient demand crop (as maize) may increase N efficiency in comparison to other cereals. For example, in a 5-year rotation of maize, and wheat (one crop each year) the average compost N mineralization rate was 14% of applied N for wheat while for maize N mineralization increased to 32% of applied N (Amlinger et al., 2003). Nevertheless, crops with very high levels of N uptake, such as maize, would be difficult to fertilize without excessive organic N in soil and it would take several years after conversion to organic management to build-up the organic N in soil and reach appropriate yields (Pang and Letey, 2000).

The pattern of N release from compost varies greatly depending on the kind of compost used. N mineralization from composted pig manure increased rapidly up to 35 days, then progressively slowed down and stopped after 57 days in a laboratory incubation at 24 ºC and 60% of the soil water holding capacity (Cordovil et al., 2005). This behaviour of N mineralization reveals the existence of two organic fractions with different degrees of stability: labile organic N and recalcitrant organic N which are rapidly and slowly mineralizable, respectively. On the contrary, N mineralization from cattle manure and mushroom composts with similar laboratory conditions presented an initial phase of N immobilization during the first days (Kumar and Goh, 2003; Gil et al., 2011).

Nitrogen availability from composts depends on C/N ratio of raw materials, period of composting (immature or mature compost), timing of application (Amlinger et al., 2003), and environment conditions. It is considered that a C/N ratio of 25-30 for raw materials is the initial optimum ratio for composting (Bernal et al., 2009). The optimum initial C/N ratio depends on the kind of materials used as bed materials. The type of bed material optimizes compost initial properties such as air space, moisture content, mechanical structure and C/N ratio and has a determinant effect on N mineralization. For example, N mineralization from dairy manure composted with straw increased in comparison to compost from dairy manure with sawdust or pig manure blend with wood chips because straw biodegradability in soil is increased compared with sawdust or wood (Wang et al., 2004). This is explained by the increased cellulose content in sawdust and wood compared. Therefore, dairy residues composted with straw rather than other materials with increased C/N ratio would be more suitable for organic growers that rely on N provided by composts. However, regardless the fact that the application of fresh manure mixed with straw resulted in greater quantity of readily available N compared with composted manure with woodchips as bedding material, barley yield and N uptake after 8 years was not increased with the compost made with straw compared to the compost made with woodchips, as bedding material. Therefore, composted cattle manure and woodchips as bedding material may be used as an alternative to straw (Sharifi et al., 2014a).

Composts at early stages of composting release mineral N in soil faster than composts with increased composting time. For example, cattle and chicken manure compost (C/N = 14) with 68 days of composting time resulted in pick concentrations of mineral N in soil two weeks after incorporation, whereas 109 days of composting lead to peak concentrations of mineral N four weeks following incorporation (Levanon and Pluda, 2002). Furthermore, the amount of mineral N in soil also depends on the degree of maturity of the composts. For instance, the amount of mineral N in soil increased after incorporation of cattle and chicken manure compost (C/N = 14.4) at the end of the thermophilic period (115 days), in comparison to longer periods of composting (14 weeks) (Levanon and Pluda, 2002). However, the incorporation of compost in early stages of composting may cause N immobilization. For example, three cattle manure composts aged for 0, 21 and 47 days (C/N = 24.7, 18.1 and 12.7 respectively) promoted N immobilization, leading to a decrease in the amount of mineral N in soil, whereas an 89 days aged compost (C/N = 12.2) resulted in net N mineralization after incubation for 80 days (Atallah et al., 1995). A sewage sludge/cotton waste mixture incorporated before composting or at the end of active phase also promoted initial N immobilization, followed by N remineralization; on the contrary, the incorporation of the compost after 60 days of maturation phase promoted net N mineralization during the whole incubation period (Bernal et al., 1998a).

After composting, composts with final C/N ratio > 20 usually show an initial N immobilization or delay in N mineralization lasting several weeks as opposed to composts with low C/N ratio that exhibit net N mineralization immediately after soil incorporation (Probert et al., 2005). Nitrogen availability depends on compost characteristics, but also on the intensity of mechanical aeration through tillage or weed control and time of application before cropping or leaching phase (Amlinger et al., 2003). Therefore, it depends on the application method. For instance, incorporated manures caused more temporary N immobilization than applied on the surface. However, surface-applied manures showed greater $N0_3^-$-N concentration than incorporated manures which may lead to N losses especially during the early stage of the crops when N demand is low (Baitilwake et al., 2012).

A major advantage of maturated compost is probably its slow N mineralization in soil and the increased NO_3^--N content during the maturation phase of the compost. Another advantage of maturated compost is that it can be incorporated into soil at any time even when the crop is growing.

1.5.3.2 Green manure

One of the main objectives of using cover crops as green manures is to minimize excessive loss of N to the environment through N immobilization and to supply N to the succeeding crop through mineralization of the crop biomass (Clark et al., 1997; Thorup-Kristensen et al., 2003). Nitrogen mineralization from green manure and its effect on the subsequent crop may be highly variable. Nitrogen mineralization from a catch crop depends on the amount of N taken up by the catch crop, and the mineralized fraction that becomes available for the succeeding crop. If sowing of the catch crop occurs too late, there is a high risk that it will not grow enough to acquire sufficient N uptake capacity (Thorup-Kristensen et al., 2003). However, the poor effectiveness of mineralized N from the green manure is mainly attributed to the lack of synchrony between N release and crop N demand. If N from cover crops is not released in synchrony with the succeeding crop demand it may be lost through leaching (Sainju et al., 2006). This can arise under two circumstances: firstly, when mineral N supply comes too late for crop demand, such as in the case of slowly decomposing residues; and secondly when N supply occurs earlier than crop demand, which happens with fast decomposing residues. Thorup-Kristensen and Nielsen (1998) indicated that N availability for the succeeding crop also depended on the root depth of the succeeding crop. If the succeeding crop is deep rooted and leaching intensity is low, there is a high risk that the incorporation of the catch crop will have no effect on N availability for the succeeding crop. On the other hand, deep rooted crops like carrot and cabbage used N from leguminous cover crops more efficiently, whereas shallow rooted crops like onion and lettuce made better use of N left by non-leguminous crops (Thorup-Kristensen et al., 2003).

Nitrogen mineralization from green manures may vary from rates over 50% during the first few months (Breland, 1994) to immobilization at early stages of green manure decomposition (Constantin et al., 2011). The great variability in N mineralization, which can vary from negative effects to those equivalent to 200 kg N ha^{-1} (Thorup-

Kristensen et al., 2003), highlights how important it is to optimize the management of green manure to obtain best possible effects on crop growth.

The pattern and timing of green manures mineralization depends mainly on crop biomass quality, particularly N content and C/N ratio, and also on lignin and polyphenols contents, but it also depends on the timing and method of the green manure incorporation into the soil (Baggs et al., 2000). The C/N ratio of green manures largely determines the balance between mineralization and immobilization (Hadas and Portnoy, 1994; Tejada and Gonzalez, 2006). The lower the C/N ratio, the greater the net release of N. It is generally considered that C/N ratios < 25 favour net N mineralization (Clark et al., 1997; Kuo and Jellum, 2000). When cereals and crucifers with high C/N ratio (C/N > 30) were used as preceding cover crops before maize, N availability through maize cycle decreased, as opposed to the use of leguminous with lower C/N ratio (C/N between 10 and 20) (Salmeron et al., 2011). Likewise, vetch (C/N = 25) released N to the succeeding crop (pepper) whereas ryegrass (C/N = 71) caused N immobilization (Mancinelli et al., 2013). Controversially, Restovich et al. (2012) found that cover crops with C/N ratios < 25 limited N availability. Cabrera et al. (2005) reported that the C/N ratio break-point between N mineralization and immobilization may range between 15 and 40. This may be due to the presence of organic compounds with different degree of decomposition, to the higher root C/N ratio and to the biochemical composition of cover crops (Cabrera et al., 2005). The N immobilization period of time depends on the critical C/N ratio below which net N mineralization occurs (Kuo and Sainju, 1998). These authors found that in a mixture of rye and vetch N the period of immobilization was 10 weeks with a C/N ratio about 15 and increased to 30 weeks with 100% rye in which the C/N ratio was 23. Moreover, N immobilization may occur from the mixture rye and vetch with a C/N ratio about 10 (Kuo and Sainju, 1998).

The C/N ratio and N content of cover crops are dependent not only on the chosen crops (cereals, legumes or crucifers) but also on their developmental stage, which is associated to the moment of harvesting before incorporation into the soil as green manure (Restovitch et al., 2012). Late incorporations can lead to plant lignification, increasing C/N ratio which thereby leads to slower N mineralization or even immobilization during the succeeding crop (Clark et al., 1997; Thorup-Kristensen and Dresbøll, 2010). For example, postponed spring incorporation of rye increased C/N ratio with subsequent decreased N uptake by the maize (Thorup-Kristensen and

Dresbøll, 2010). In this particular case, the largest mineralization effect of rye occurred after early spring incorporation (Thorup-Kristensen and Dresbøll, 2010). On the contrary, due to increased cover crop N content, when rye was consociated with hairy vetch, maize N uptake was higher when incorporation was delayed (Clark et al., 1997). Besides, leguminous species that postpone incorporation will allow more time for biological N fixation to occur, and changes in the C/N ratio will be smaller than with non-legumes (Thorup-Kristensen et al., 2003).

In horticultural systems, planting of the following crop after incorporation of a high C/N residue such as rye or oats must be delayed at least 1 month (Baggs et al., 2000). On the other hand, these authors suggested that following the incorporation of a low C/N residue such as vetch, the following crop should be planted immediately after incorporation of green manure, due to faster release of N. However, catch crops can contain phytotoxic compounds, which may reduce germination of the subsequent crop (Dabney et al., 2001). So, probably, a short time delay between incorporation of leguminous green manure and planting of the succeeding crop would be convenient. For example, incorporation of hairy vetch consociated with rye 2 weeks before planting sorghum and cotton, increased soil N availability and cotton and sorghum biomass yields compared to rye incorporation. Kramer et al. (2002) compared N mineralization from vetch green manure with mineral fertilizers and concluded that mineralized N from vetch residues, which were incorporated into the soil before sowing maize, was not significantly available for maize during the early phase of the growing season. Afterwards, it gradually increased to maximum level between 70 to 80 days after incorporation into the soil. Therefore, N became available at a time coincident with the peak of N uptake by maize, thus suggesting better N synchronization than mineral fertilizers applied before the seeding of the maize and 36 days after seeding. Although the maize N uptake from fertilizers peaked at 4.3 kg N ha^{-1} per day between 70 and 80 days after sowing maize while N uptake from vetch reached only 0.6 kg N ha^{-1} per day during the same time period, the N uptake from fertilizers occurred early in the season compared to N uptake from vetch. Kramer et al. (2002) concluded that optimum yields can be achieved under organic management successfully matching N availability with crop uptake.

1.5.3.3 Commercial organic fertilizers

The main differences between composts and commercial organic fertilizers are that the latter have a trademark on the package, and should ensure fast N availability (as opposed to slow N releasing on-farm composts) (Wang et al., 2014). In addition, in Portugal the organic N content of commercial organic fertilizers must be above 3% of the total dry matter (Diário da République, 2015). The main characteristics of fertilizers that enable fast N release are: high total and mineral N content, low C/N rate, and high amount of available C and N (Stadler et al., 2006; Antil et al., 2011). These features are dependent on the kind of materials used, method and duration of composting. To reach these characteristics the organic N fertilizers are often made with materials rich in N like poultry manure, feather, guano and leguminous grain.

Poultry manure contains more nutrients per unit dry mass than swine or cattle manure, and the N from poultry manure is readily available to plants (Schomberg et al., 2011). For example, during 60 days of incubation, 69% of added N from raw poultry litter was mineralized (Sanchez and Mylavarapu, 2009). On the contrary, N mineralization of 107 samples of dairy cattle manure represented, on average, 12.8% of the organic N after 56 days' incubation (Van Kessel and Reeves, 2002). However, since N availability may be lower after composting, N mineralization from composted poultry litter aged for 1, 4 and 15 months was found 3 to 4 times smaller than N released from raw poultry litter (Cooperband et al., 2002). This can be partly explained by the higher amount of NH_4-N in raw manure (1.5%) compared to composted manures (0.02% to 0.29%). In addition, the value of poultry litter as N fertilizer also decreased after composting because of N losses. For instance, Tiquia and Tam, (2002) reported that 58% losses of initial N in poultry manure were attributed to NH_3 volatilization.

Stadler et al. (2006) evaluated N mineralization from commercial organic fertilizers produced with milled seeds of grain leguminous (pea, lupin and fababean) and industrially processed residues from plants (malted barley mixed with vinasse; residues from castor oil production mixed with vinasse) and microorganisms (residues of penicillin production), and found that the fertilizers based on leguminous grains had an N content from 3.0% to 4.0% and a C/N ratio from 9.9 to 13.3, whereas fertilizers composed by industrially processed residues had an N content from 4.7% to 8.5% and a C/N ratio from 5 to 8.6. These authors indicated that after 43 days of incubation, net N mineralization obtained from the industrial fertilizers (between 60% to 70% of added N) was above that found for milled grains (between 40% to 50% of added N), being N content a good indicator of N released. Furthermore, about 70% of total N mineralized during 43 days of incubation was mineralized during the first 15 days of incubation for all organic fertilizers.

Nitrogen mineralization from two commercial organic fertilizers produced by Italpolina, Rivoli, Veronese, Italy: (i) Dix10 composed mainly by chicken plumes (10% of N content); and (ii) Guano, composed by excrements of birds that had been accumulated for a long time in places near the sea where lack of rain allows natural drying (6% of N content), was evaluated by Marinari et al. (2010). These authors showed that the amount of mineralized N was 25% and 35% of total added N after 30 weeks of incubation and that the peak of N release was observed after 4 and 3 weeks upon treatment with Guano and Dix, respectively. Nevertheless, these authors suggested that Guano is likely to cover a longer period of crop N demand in comparison to Dix, thus preventing nitrate losses, due to a more gradual release of mineral N.

Many farms do not have manure due to the inexistence of livestock. Hence, the development of OA led to the emergence of commercial fertilizers certified for organic agriculture. Commercial organic fertilizers are often expensive. Rodrigues et al. (2009) draws particular attention to the rate cost/ benefit of commercial fertilizers because crop N recovery from organic fertilizers is usually low and the timing and amount of N that is released to the crop is very difficult to predict (Rodrigues et al., 2006). Brito et al., 2016) also reported that the commercial fertilizers are commonly recommended in situations of high crop N demand; however, these commercial fertilizers have potential disadvantages when are poorly maturated and present high electrical conductivity.

1.5.4 Synchronizing N mineralization and N uptake.

The amount of N released from organic fertilizers is often regarded as one of the limiting factors responsible for low crop productivity (Berry et al., 2002). Mineral N may remain in the soil and become part of the mineral N pool, or it may be immobilized by microbes or lost (Calderon et al., 2005). Actually, only 30-50% of applied N fertilizer is taken up by crops (Cassman et al., 2003). Ways to solve this problem will require significant increases in the efficiency of N use. Strategies to synchronize nutrient release from organic sources with plant demand are therefore needed

(Goulding, 2005). However, it is unclear whether the slow release of nutrients from organic compost or green manures can be adequately controlled to match crop demand. Manures with higher available N content usually result in higher yields, but these manures can result in an increased risk of polluting water and air. For example, denitrification and nitrate leaching losses with slurry are higher compared to farmyard manure (Chambers et al., 2000). Increasing the amount of incorporated manure can also lead to substantial N losses (Pang and Letey, 2000), and so the maximum amounts of N supplied via organic fertilizers is under strict regulation. In Portugal the maximum recommended application rate of organic N is 170 kg N ha^{-1} (Ministério da Agricultura, 1997).

Different cropping systems have different effects on N balance. For example, after farmyard manure application on permanent ryegrass there was accumulation of soil N, as opposed to an annual cropping system of maize and rye that showed a negative N balance (Salazar et al., 2005). This can be explained by increased N mineralization due to soil aeration while in permanent grass there is continuous organic matter accumulation.

Nitrogen mineralization depends on the organic fertilizer applied to the soil. For example, N mineralization from 33 animal wastes during 224 days of incubation ranged from 3 to 51% of added organic N (Morvan et al., 2006). Different N dynamics were observed by those authors: sludges showed net N mineralization for the entire incubation period and farmyard manure induced net N immobilization followed by net N mineralization. A deep knowledge of N mineralization and immobilization dynamics after incorporation of an organic amendment to soils is extremely useful to find out the appropriate timing and rate of application of organic amendments that enable farmers to maximize crop yield. For example, the incorporation into the soil of immature municipal solid waste compost (C/N = 23.5 and total N = 0.6%) resulted in an initial N immobilization for 12 weeks (Madrid et al., 2011) and the application of municipal solid waste compost maturated for 120 days (C/N = 18.1 and total N = 1.7%) presented an initial period in which the mineralization was very slow (100 days) but without N immobilization (Sanchez et al., 1997). Despite having different characteristics these two composts would have to be applied three months before sowing in order to synchronize N mineralization with crop needs. On the contrary, application of cattle (C/N = 3.6), sheep (C/N = 3.6), and poultry manure (C/N = 3.5) did not cause immobilization and the higher rates of N mineralization occurred during the initial 10/20 days (Abbasi et al., 2007). These authors recommended that manures should be applied at the time of planting, so that the initial mineralized N may be utilized by crops. In opposition, Azeez and Van Averbeke (2010) found that the highest rates of N mineralization of cattle, sheep and poultry manure were observed around 40 and 55 days after incorporation. In this case, manures should be applied 1 month before crop plantation.

Green manure is an important N source. However, N uptake from the succeeding crops is difficult to predict after green manure incorporation (Thorup-Kristensen et al., 2003). A possible strategy to improve N efficiency is to choose the most appropriate incorporation moment. For instance, incorporation of green manure in early spring increases leaching. On the other hand, if incorporation is performed in later spring, with elder plants, the plant C/N ratio increases which then decreases net N mineralization. Thus, cover crops grown in high rainfall areas on sandy soils should be incorporated later, whereas those on low rainfall areas should be incorporated earlier (Thorup-Kristensen and Dresbøll, 2010). Another way to improve N uptake efficiency could be to intercrop green manure between rows of the succeeding crop. However, N uptake efficiency of the main crop was not detected in a study conducted by Birgitta et al. (2001) in which strips of red clover (*Trifolium pretense* L.), intercropped with rows of leek, were either incorporated at planting time of leek, or at 2 and 4 weeks after planting leek. These authors found that leek N uptake did not increase with incorporation of red clover between rows either at planting or 2 weeks after planting, and it was even decreased after incorporation of red clover 4 weeks after planting (Birgitta, 2001). This was probably due to the fact that N uptake by the red clover strips kept soil mineral N at low level until time of incorporation and the distance between rows made it difficult for the leek to uptake the N present between rows. A number of factors still need to be optimized, such as sowing practices and design in intercropping leguminous and non-leguminous systems to increase the use of N_2 fixation and of the soil mineral N from OM mineralization (Bedoussac et al., 2014).

1.6 Carbon and nitrogen dynamics in soil

1.6.1 The role of C and N availability

Carbon and N cycles in soil are strongly linked because of the simultaneous assimilation of C and N by microorganisms (Mary et al., 1996). Soil microorganisms obtain from the OM, the N required for the synthesis of their own proteins as well as the organic carbon required as the building blocks of C skeleton and as energy source, consequently liberating CO_2 and plant nutrients (Mengel, 1996). Whilst in plants and animals there is a relatively wide range of C/N ratios, in microbes the C/N ratio ranges within a narrow interval (the average C/N ratio of bacteria and fungi falls between 4 and 10). So, the majority of C is utilized as energy and converted to carbon dioxide and nitrogen becomes part of the microbe tissue. Nitrogen is required into the microorganism's body tissue at a fixed rate in relation to the amount of carbon. Therefore, if plants have much higher C/N ratio compared to microorganisms needs, the soil microbes will use NO_3^- and NH_4^+ from the soil to build their own protein (N immobilization).

The organic N is mineralized by the microorganisms into mineral forms available to crops (N mineralization). The period of N mineralization usually increases for increased C/N ratio materials as opposed to small C/N ratio materials (Troeh and Thomson, 2005). Thus, mineralization and immobilization are soil microbial processes ruled by C availability. Indeed, easily mineralizable C has been considered the most significant variable to explain N mineralization (Kim et al., 2011), and even more important than the environmental conditions within mean daily temperature at 10 cm soil depth between 10ºC and 20ºC and water potential oscillating from field capacity to the beginning of water stress (-30 to -300 kPa) (Kim et al., 2011).

1.6.2 Carbon and N dynamics in soil after the application of organic fertilizers

The analysis of the processes of C and N mineralization and immobilization simultaneously is of great importance to predict N mineralization from composts. A high rate of C mineralization occurs in less maturated composts simultaneously with reduction of available N in soil probably due to N immobilization because of the great content of easily mineralizable carbon. The availability of N depends on the time of N immobilization, and the rate of N remineralization. However, after composting, as most labile C was mineralized, N immobilization probably does not occur. Morvan and Nicolardot (2009) found that N mineralization increased from net N immobilization of 35% with FYM to net N mineralization of 10% with compost FYM during 107 days of incubation because the content of available carbon decreased after composting (CO_2 evolution decreased from 43% to 12% of applied C after composting). Similarly, Bernal et al. (1998a) reported that N immobilization (4.3% of added N) occurred with a non-composted mixture based on sewage sludge and cotton waste after 70 days of incubation. However, N mineralization increased to 9% of total N after composting. In both experiments, the short duration of the incubation did not allow N mineralization from not maturated composts to increase, but, the organic N would probably continue to mineralize and afterwards N remineralization would occur.

The N mineralization from composts depends on the relation between N immobilization and remineralization and which one prevails. In less maturated composts if the rate of release of previously immobilized N increases, N mineralization in less matured composts may increase compared to maturated composts. For instance, C mineralization of less maturated municipal solid waste compost (C/N = 24) was 56% of added C during 91 days of incubation, indicated a high amount of easily mineralized C which led to a period of N immobilization of 14 days, followed by a period of N remineralization that corresponded to 26% of added N (Chalhoub et al., 2013). In the same experiment N mineralization of green wastes, wood chips and sewage sludge compost (C/N = 11) was only 3% of added N, although N immobilization did not occur because of the small amount of easily mineralized C (the amount C mineralized was only 16% of added C). This probably is due to the fact that municipal solid waste compost had a larger proportion of easily decomposed cellulose and mineral N and low proportion of lignin in comparison to green wastes, wood chips and sewage sludge compost. Therefore, the amount of N immobilized depended on the C/N ratio of the amendment but is more related to its biochemical composition. On the other hand, the differences on N remineralization arise from the type of amendment and the nature of the microorganisms involved in decomposition (Mary et al., 1996).

Incorporation of plant residues and cover crops as green manure enhances soil C mineralization because these residues are important sources of labile C, which is a link between soil C and N cycling (Hooker and Stark, 2008; Abera et al., 2012). Usually, in vegetable cropping systems, cover crops used as green manure have a low C/N ratio to avoid N immobilization and to release high amounts of mineral N to the subsequent crop. For example, as the proportion of rye increases in rye/hairy vetch mixtures, the proportion of available C also increased and the amount of available N decreased. Therefore, N immobilization increased because the increasing microbial biomass immobilized soil mineral N to decompose soluble C (Kuo and Sainju, 1998).

1.7 Fertilization of horticultural crops

1.7.1 Potato

Potato (*Solanum tuberosum* L.) is a *Solanaceae* that is grown as an annual crop even though its perennial character is provided by the tubers. Although potato content of protein is reduced, potato crop is an essential source of a large amount of proteins, carbohydrates and vitamin C to the Portuguese diet. The greatest potato yields are achieved with an average temperature between 15 ºC and 20 ºC. Temperatures above 30 ºC decrease the number of tubers and dry matter content and temperatures below 12 ºC delay potato growth and promote early formation of tubers. The potato crop prefers sandy or loam soils, being sandy soils better for early potatoes (Almeida, 2006). Its susceptibility to salinity is moderate (the higher value not associated with decreasing yields is 1.7 dS m^{-1}) (Maynard and Hochmuth, 1997). In terms of soil pH, potato grows well with pH between 5.0 and 7.0. However, it is important to note that a pH between 5.5 and 6.0 decreases the impact of the common scabies disease (Maynard and Hochmuth, 1997).

Potato yields increased with increasing rates of compost application (Ninh et al., 2014). For instance, potato yield increased from 17% to 36% with an increased rate of poultry manure application from 3 t ha^{-1} to 27 t ha^{-1} (Ninh et al., 2014). Also, potato yield increased 27% with cattle slurry application (138 kg N ha^{-1}) (Srek et al.,2012). Furthermore, after the application of 30 t ha^{-1} of farmyard cattle manure potato yield increased 50% (Balemi, 2012). In addition, this author assumed that the release of nutrients from farmyard manure occurs slowly in the year of application and that replacing 66.6% of the recommended inorganic fertilizer by 20 or 30 t ha^{-1} farmyard manure increased potato yield significantly. However, the response to cattle manure was not consistent throughout the growing seasons, revealing itself a very insecure source of available N when applied in the year before potato crop (Haase et al., 2007). Indeed, Palmer et al. (2013) reported that N content in composted cattle manure is low; therefore, to increase N supply to organic potatoes, higher available organic N fertilizers such as slurries and chicken manure should be used. Increased yields were obtained using cattle manure combined with chicken manure when compared to chicken or cattle manure alone, whereas the lowest yield was obtained with cattle manure alone (About-Hussein et al., 2003).

The release of N from different organic sources may affect the synchrony between available N released from organic amendments with plant demand (About-Hussein et al., 2003). For example, incorporation of a cover crop of rye and poultry manure enhanced potato yield from 40% to 210% compared to unamended plots (Nyiraneza and Snapp, 2006) whereas an annual green manure combined with municipal solid waste compost or composted paper mill waste satisfied potato N requirements (Sharifi et al., 2014b). Importantly, the use of leguminous in crop rotations increases the quantity of soil mineralizable N. For instance, potato N uptake increased for potato/pea/clover rotation compared to potato/oats/Italian ryegrass or potato/oats rotations because the potentially mineralizable N was greater after potato/pea/clover rotation compared to potato/oats/Italian ryegrass or potato/oats rotations, 35% and 22%, respectively (Sharifi et al., 2009). N availability also increased with potato following grass/clover when compared to wheat. However, it is relevant to highlight that growing potato after grass/clover increased the risk of potato damage from wireworms and slugs (Palmer et al., 2013).

A main challenge for organic potato production is nutrient management because optimal potato crop yields require a large N input although high levels of residual mineral N are left in the soil after crop harvest. Hence, agronomic practices are needed to match N mineralization from OM with potato crop N requirements. For this purpose, a model for potato growth (LINTUL-NPOTATO) was adapted from NPOTATO model (Wolf, 2000), and used to analyse how potato maturity and the timing of slurry application could affect N recovery, tuber yield and residual soil mineral N after slurry application into the soil before planting potato (Delden et al., 2003). These authors found that N recovery after slurry application was larger for mid-late (46%) than for an early cultivar (27%) with a concomitant increase on tuber yield of 3.9 t ha^{-1}. The residual mineral N was also lower for the mid-late than for the early cultivar due to larger N recovery in the former. Thus, a later maturing cultivar is a good option to improve crop yields and reduce N losses to the environment. Besides, a spring application of slurry is also preferable compared to autumn application because N uptake and tuber yield increased and winter N losses decreased (Delden et al., 2003). The NPOTATO model also suggests that the application of organic N with a large proportion of mineral N shortly after emergence, could improve potato yields in organic farming. This is probably due to the fact that potato is considered an inefficient crop in which concerns to using N and because potato N requirements appear to be critical early in the season (Westerman and Kleikopf, 1985).

One of the main advantages of using organic fertilizers is that they may influence soil biological activity and increase plant health by reducing potato plant diseases. For example, incorporation of rapeseed (*Brassica napus*) as green manure with conifer-based compost combined the disease suppressive effect of rapeseed with the positive benefits of compost on tuber yield (Bernard et al., 2014). These last authors reported that in the course of three years, tuber yield increased from 13% to 15% with compost application relative to non-amended plots. Another advantage is that a slow N release from composts decreases nitrate concentration in potato tubers. In an experiment carried out in Egipt, El-Sayed et al. (2015) reported that the application of 23.8 t ha^{-1} compost (C/N = 16; N = 1.5%) gave similar potato yields when compared to the plots that received recommended treatments with mineral fertilizers, and was also associated with low nitrate content and better storability. Importantly, a crop rotation including potato, may also restore OM instead of causing soil structural degradation and depletion of OM. For example, Grandy et al. (2002) reported that a green manure consisting of oat (*Avena sativa* L.), pea (*Pisum sativum* L.) and hairy vetch (*Vicia villosa* L.) incorporated before potato crop for two years increased soil C from 23.9 g kg^{-1} to 25.9 g kg^{-1}.

1.7.2 Lettuce

Lettuce (*Lactuca sativa* L.) is an annual herbaceous plant of the *Asteraceae* family. Lettuces have high content of vitamins A and C and provide minerals and fibre. Temperatures between 15 ºC and 20 ºC are ideal for the development of most lettuce varieties. On the contrary, temperatures above 24 ºC affect the formation of the head and cause stem elongation (Almeida, 2006). General changes in lettuce growth and development are correlated with sunshine duration. Seasonality affects lettuce growth more than nitrate concentration in leaves. Lettuces in the late spring season have higher yields associated with long sunshine duration that is known to promote the response of plants to fertilizers. On the contrary, in the late autumn season, lettuce yields decreased due to shorter sunshine duration that limit plant growth independently of N availability (Pavlou et al., 2007). In terms of soil characteristics, lettuce prefers loam or clay soils. Its tolerance to salinity is moderate (the higher value without register decrease in yield is 1.3 dS m^{-1}), and it is also sensible to soil pH (the optimum value ranges within 6.5 to 7.2) (Maynard and Hochmuth, 1997). Lettuce is not very demanding in nutrients, but nutrients must be easily available because lettuce grows fast and its roots are short in depth. Importantly, a large amount of N in soil may delay head formation and compact heads (Almeida, 2006).

There are several studies addressing the advantages of lettuce organic fertilization (Montemurro, 2010; Silva et al., 2010). Nitrogen can be easily provided by organic fertilization because lettuce is not very demanding in nutrients (Almeida, 2006). On sandy soils with low contents of OM, N mineral fertilization (64 kg N ha^{-1}) can be fully replaced by organic fertilization with cattle manure compost (45 t ha^{-1}) without compromising yields (Porto et al., 2008). In addition, organic fertilization resulted also in significant lower nitrate content compared to mineral fertilization (Porto et al., 2008). Similarly, during three successive crop seasons (late summer, late autumn and late winter) nitrate accumulation in lettuce leaves was greater with application of mineral fertilizers in comparison to composted sheep manure. Lettuce yield was similar for organic and mineral fertilization during late autumn and winter season, whereas lettuce yield increased during late summer with mineral fertilization (Pavlou et al., 2007). In another experiment in high tunnels, lettuce was grown in perlite with either a nitrate based soluble fertilizer and an organic solution with leaf compost amended with cotton seed meal. Here, the growth of lettuce was more responsive to the time of the year than to the fertilizer, although, lettuce grew faster with organic than nitrate based fertilizer. The fresh weight per plant at harvest with organic and mineral fertilization was 64 and 59 g, respectively, and, the relative growth rate with organic and mineral fertilization was 0.143 and 0.130 g day^{-1}, respectively (Gent, 2002).

Lettuce yields increased with increasing rates of organic compost application (Santos et al., 2001; Silva et al., 2010). For example, lettuce yield was significantly increased with cattle manure (C/N = 11 and N = 2%) and guano (C/N = 3 and N = 15%) amendments compared to the control; however, there were no significant differences in yield between soil amendments with cattle manure or guano (Manojlovik et al., 2009). The residual effects of OM incorporation in the second crop of the rotation is another advantage of OM fertilization. Santos et al. (2001) reported that increasing amounts of compost application increased residual effects in lettuce planted 80 and 110 days after compost application. It is relevant to emphasize that according to Maroto (1995) application of non-maturated compost should be carried out in the preceding crop.

Lettuce yield depends on soil N availability (Montemurro, 2010; Ribeiro et al., 2010). During a three year field experiment, Montemurro (2010) studied the effects of several organic fertilizers in N availability and lettuce yields. This author concluded that it is important to choose the right organic fertilizer to adjust N supply due to lettuce poor physiological N use efficiency (the amount of yield per unit of N fertilizer applied). Therefore, high amount of organic fertilizer may be required to achieve good yields with a concomitant increased in environmental risk. Fertilizer recommendation should consider crop N demand, soil mineral N content and other soil and weather conditions to avoid nitrate leaching but still increase crop yields. Compost recommendation needs to take into account the quantity of potentially mineralizable N so that lettuce uses N as efficiently as possible. This helps to regulate nitrate content in lettuce and control the quantity of residual mineral N in soil. For example, during a pot experience with lettuce, Ribeiro et al. (2010) reported that N mineralization with hen manure (53.4%) was higher compared to compost (4.5%). This higher N mineralization rate was explained by the lower C/N ratio of hen manure (10.2) compared to compost (16.8); the higher mineral N content of hen manure (5121 mg kg^{-1}) compared to compost (45 mg kg^{-1}); and by the low stability of the carbon present in hen manure when compared to the recalcitrant carbon fractions of compost that are more resistant to decomposition. In this experiment, higher lettuce yield was obtained with hen manure in comparison to compost. However, a mixture of compost and hen manure appears to be a good fertilization alternative, since it may reduce leaching and provide an amount of available N that allows maintaining lettuce yields. In addition, a mixture of compost and hen manure appears to be a good fertilization alternative because it reduces by half the amount of commercial fertilizer application (hen manure), and simultaneously cuts fertilization costs still maintaining lettuce yields (Ribeiro et al., 2010).

1.7.3 Swiss chard

Swiss chard (*Beta vulgaris* L.) belongs to the family of *Chenopodiaceae* and is a biannual plant easy to produce but not well known in Portugal. It has a high content of vitamins A and C, iron (Fe) and sodium (Na). Swiss chard tolerates both cool weather and heat. Its roots are long and prefer loam soils with high amount of OM. It is highly sensible to salinity and the optimum pH value ranged from 6 to 7 (Maynard and Hochmuth, 1997). Sensorial analysis showed that organically produced chard retained turgidity, colour and brightness longer than conventionally produced chard (Moreira et al., 2003).

Compost application significantly increased Swiss chard yields. For instance, Smith et al. (2001) reported that during a pot experiment in sandy soil, chard fresh weight increased 68% when the proportion of compost was increased from 25% to 50% of soil weight. These authors reported that a higher total leaf fresh mass was obtained in chard production when compost (made from market and garden refuse) had been turned as opposed to a situation in which it was not turned.

1.7.4 Turnip

Turnip (*Brassica rapa* cv. nabo) is a biannual plant and a member of the Brassicaceae family. Turnip has a high content of vitamins A and C and calcium. The optimum range of temperatures for turnip growth is between 15 ºC and 20 ºC. Dry weather and temperatures above 25 ºC decrease yields and affect root quality because it becomes fibrous (Almeida, 2006). Turnip prefers loam soils with high water holding capacity. The optimum pH range is between 6.5 and 7.0 and their sensibility to salinity is reduced (the higher value not associated with decreasing yields is 0.9 dS m^{-1}) (Maynard and Hochmuth, 1997).

Turnip is very sensible to the incorporation of compost, and thereby compost should be incorporated before the preceding crop (Maroto, 1995). Since N from organic fertilizers is generally more slowly released than from mineral fertilizers, it is particularly important to study the effect of organic fertilizers in successive crops. Hence, Termine et al. (1987) studied the effect of sheep manure compost, woodchip compost, blood meal and mineral fertilizers during a crop rotation of leek followed by turnip. Sheep manure and woodchip compost were incorporated only before leek, whilst blood meal and mineral fertilizers were applied before both leek and turnip because of their rapid release. Comparable yields were obtained with manure compost, blood meal and mineral fertilizers for the turnip crop because the slow release of N from manure compost allowed equal fertilization of both crops. However, the nitrate content in both crops was lower under application of sheep manure compost and woodchip compost than blood meal or mineral fertilizers. Hence, these authors suggested that sheep manure compost was better suited for that crop rotation since higher yields were obtained with a significantly lower nitrate accumulation in turnips and leeks.

1.7.5 Portuguese cabbage

Portuguese cabbage (*Brassica oleracea* var. tronchuda L.H. Bailey) is a biannual plant leaf cabbage of Brassicaceae family. Portuguese cabbage is a unique group of vegetables very important for Portuguese horticulture owing to their excellent adaptation to climatic conditions and good integration in the traditional small farming cropping systems (Silva, 1995). These leaf cabbages have higher content of vitamin A and calcium (Ca) than head cabbages. The presence of phytochemicals, phenolic compounds and organic acids, may exert a protective role against various diseases (Ferreres, 2005). For example, Sousa et al. (1997) reported its high potential to resist to downy mildew. These cabbages are cold season crops and more resistant to cold weather than cauliflower or broccoli, preferring a moist weather and temperatures between 15 ºC and 20 ºC. They have a shallow root system and prefer loam or clay soils. The optimum pH ranges between 5.5 and 6.8 (Maynard and Hochmuth, 1997).

Scientific reports on Portuguese cabbage fertilization are scarce. Rodrigues et al. (2009) studied the fertilization of Portuguese cabbage with organic fertilizers and found that the soil incorporation of an organic fertilizer based on poultry manure and feathers, before Portuguese cabbage resulted in an apparent N recovery of 14% to 16% of applied N. This value was very low considering the high N content (10%) and the low C/N ratio (4.7) of the organic fertilizer.

1.7.6 Carrot

Carrot (*Dacus carota* L.) is a biannual plant of the *Apiaceae* family. Carrot is a high fibre vegetable with high content of vitamins C, B_1 and B_2 and carotenoids. It is a cold season crop that grows between 5 ºC and 35 ºC, being the optimum temperature 18 ºC. Whilst temperatures above 21 ºC promote development of short roots, temperatures below 16 ºC promote long and thin roots. Carrot grows better in sandy loam soils because clay soils impairs root growth and increase the risk of diseases (Almeida, 2006) Carrot is sensible to salinity (the higher value that does not decrease yields is 1.0 dS m^{-1}) whereas its sensibility to pH is moderate with an optimum range of pH from 6.0 to 6.8 (Maynard and Hochmuth, 1997).

Reports about organic fertilization of carrot are scarce probably because the period of carrot growth is long and also because of the troublesome technical problem of weed management (Andrea et al., 2007). However, carrot is one of the most demanded crops by organic consumers (Stevens-Garmon et al., 2007). Carrot benefits from organic fertilization due to carrot’s slow N demand (Sorensen, 1993) and its deep rooting system that is capable of accessing N down to at least one meter depth (Thorup-Kristensen and Boogard, 1999). Furthermore, carrot has greater N uptake in response to the application of organic fertilizers in comparison to other crops because of its ability to uptake organic N directly from soil (Matsumoto et al. 2000).

Matsumoto et al. (2000) explained that N uptake by pimento and lettuce was higher from ammonium sulphate solution compared to organic rapeseed cake solution. In contrast, N uptake by carrot and Chinese cabbage from rapeseed cake solution was higher compared to ammonium sulphate solution (34%). One hypothesis to explain this refers that carrot rhizosphere soils promoted the activity of protease or other enzymes to improve N mineralization, but the differences in protease activity appeared to be negligible (Kanazawa et al., 1988). Another possibility was that carrot took up organic N directly from soil. Two steps are involved in the absorption of an organic molecule: first, the carrot ability to solubilize the organic molecules which could be related to root exudates. Secondly, the organic molecule must be able to penetrate through the cell wall. For example, a Protein-N with a molecular weight of 8000-9000 dalton (Da) that accumulated in the soil after the application of rapeseed cake solution seems to be direct uptake by Chinese cabbage because on the chromatogram it was detected in the xylem of Chinese cabbage as opposed to pepper. (Matsumoto et al. 2000).

Compost should not be incorporated into the soil immediately before sowing carrot, unless it is well maturated (Maroto, 1995). On the other hand, the incorporation of green manure before carrot is controversial. Green manure incorporation affects the amount and the depth distribution of available N in the soil. The incorporation of rye or ryegrass does not increase deep-rooted vegetable yields because they release the inorganic N in a top layer, in opposition to leguminous green manures that result in a more even distribution of N in the soil, thus promoting greater yields of deep-rooted vegetables like carrot (Thorup-Kristensen, 2006). These last authors suggested that the most ideal way to increase carrots' yield would be to grow mixtures of leguminous and non-leguminous green manures to have inorganic N in top and deep layers. However, incorporation of rye consociated with hairy vetch before carrot reduced root growth due to the negative allelopathic effects of rye (Thorup-Kristensen and Boogard, 1999).

The organic fertilizers not only increase yields but may also reduce the incidence of some diseases. For example, incorporation of poultry manure at a rate of 4 t ha^{-1} increased carrot yield when compared to the application of 0, 2 and 6 t ha^{-1}. Probably, the application of 6 t ha^{-1} in comparison to the application of 4 t ha^{-1} of poultry manure reduced yields due to the production of high toxic amounts of ammonia. The application of 6 t ha^{-1} promoted the highest reduction in nematode infestation due to the production of substances such as propionic, butyric and acetic acids (Kankam et al., 2015). These authors concluded that the best option for growers would be the application of 4 t ha^{-1} of poultry manure, since it reduces infestation levels and increases carrot yields simultaneously.

1.8 Objectives

The objectives of this study are: (i) to increase organic horticultural crop yields through fertilizer recommendation based on experimental data; (ii) to improve the management of SOM contributing to C sequestration and decreasing the environmental N pollution caused by N leaching and ammonia volatilization enhancing the sustainability of agricultural ecosystems; (iii) to increase synchronization between N mineralization and crop needs because N is usually the limiting nutrient and crops require it in great quantities; (iv) to find a correlation between N availability and crop yields with N mineralization determined by the incubation experiments.

For this purpose, a three year field organic crop rotation was set up with a cover crop of rye and vetch every year over the autumn/winter for green manure, followed by potato and lettuce (1st year), Swiss chard and turnip (2nd year), and Portuguese cabbage and carrot (3rd year). Although in Portugal there is a limited assortment of available horticultural crops, organic consumers seek for greater variety. Hence, there is a need to improve fertilization practices concerning the horticultural crops such as the Swiss chard. Moreover, production of traditional crops very well adapted to Portuguese climatic conditions such as the Portuguese cabbage should also be encouraged in organic agriculture.

In order to evaluate the effect of organic fertilizers incorporation into the soil, the nutrient accumulation in crops was determined. The field experiments were used to validate the N mineralization determined by laboratory and field incubations and estimated with the mineralization models. N mineralization was assessed by field incubation to resemble closing the fluctuating environmental conditions in the field as opposed to laboratory incubation where the temperature and soil moisture content are constant. Carbon mineralization was determined by laboratory incubation to evaluate C dynamics with green manure, farmyard manure compost and commercial fertilizer application.

2. MATERIALS AND METHODS

2.1 Study site description

2.1.1 Localization

The field experiences were carried out in the NW of Portugal, at the farm of Assento in Fafe, located at 41º 12’ N and 8º 20’ W, approximately at 300 m altitude.

2.1.2 Soil and climate

Soil formation had its origin from granite and it was classified as a sandy loamy Dystric Cambisol (IUSS, 2006) with 784 g kg^{-1} sand, 163 g kg^{-1} lime and 52 g kg^{-1} clay and a bulk density of 1.04. Soil chemical characteristics at the beginning of the experiment are shown on table 2.1.

Table 2.1 Soil chemical characteristics at the beginning of the crop rotation.

pH	EC	OM	N	Extractable phosphorus*	Extractable potassium*
	(dS m^{-1})	(g kg^{-1})	(g kg^{-1})	(mg P_2O_5 kg^{-1})	(mg K_2O kg^{-1})
4.9±0.02	0.03±0.01	49.0±2.4	1.9±0.2	118±7	215±19

*Egner-Riehm method

The weather had a strong Atlantic influence with mild temperatures and high average rainfall. The temperature increases from January to July and then decreases until December. According to data collected in the nearest weather station (Braga) for the period from 1961 to 1990, the average annual temperature was near 14 ºC, with July as the warmest month (20.6 ºC) and January (7.5 ºC) the coolest. For twelve days per year the minimum average temperature was below 0 ºC and for 25 days the maximum average temperature exceeded 25 ºC. The average annual precipitation is over 1500 mm. The driest month was July with 15 mm and the wettest month January with 166 mm. The weather is humid with average annual values of 81% relative humidity. Frosts occurred for 29 days a year, mostly during the month of January (9 days).

2.1.3 Soil and air temperatures

Soil and air temperature were automatically registered with two thermistores, one buried in soil at 10 cm depth and another below a reflector board at 30 cm height. The temperature was registered every hour with a Data Logger DL2 from Delta Devices and the average daily air and soil temperatures were calculated.

During the growth period of autumn/winter crops, from October until April, and for the three years of the crop rotation, the average daily air and soil temperature were 8.9 ºC and 9.2 ºC respectively. The maximum and minimum air temperatures were -3.3 ºC and 26.5 ºC, whereas the maximum and minimum soil temperatures were 3.4 ºC and 18.7 ºC respectively.

The average daily air and soil temperatures for the spring/summer crops in 2012, 2013 and 2014 are shown in figure 2.1. For the potato, Swiss chard and Portuguese cabbage, average daily air temperature was 15.5 ºC, 15.7 ºC and 15.1 °C, respectively. For lettuce, turnip and carrot, average daily air temperature was 18.0 ºC, 15.6 ºC and 14.6 ºC respectively. The minimum and maximum air temperature during the growth period of potato, Swiss chard and Portuguese cabbage were 0.7 ºC and 36.0 ºC, 1.8 ºC and 37.5 ºC, and 1.3 ºC and 33.9 ºC respectively. The minimum and maximum and air temperature during the growth period of lettuce, turnip and carrot were 6.5 ºC and 34.0 ºC, 7.8 ºC and 36.7 ºC, and 6.3 ºC and 36.5 ºC, respectively. Soil temperature ranges were smaller (approximately 4.7 ºC) compared to environmental air temperatures.

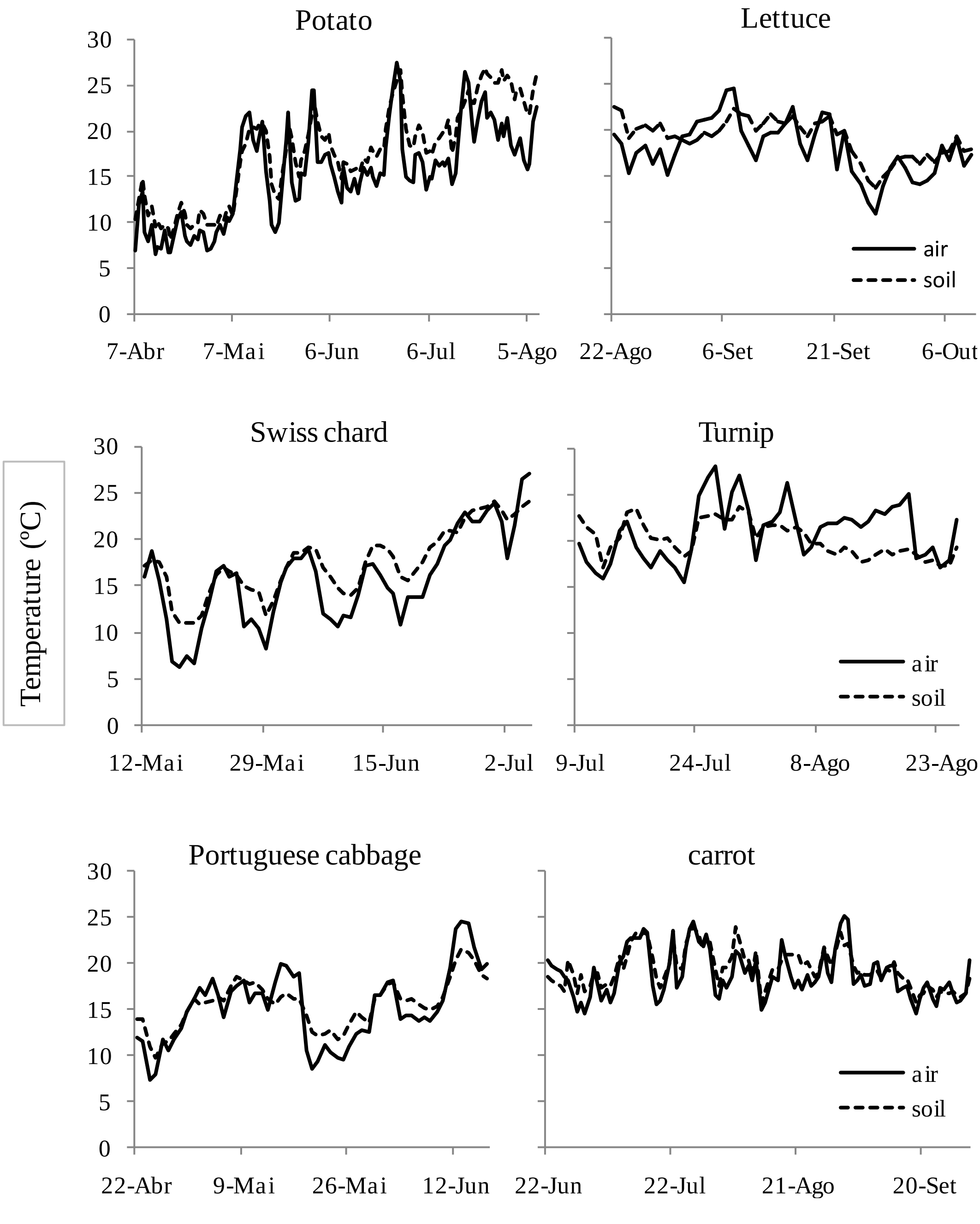

Figure 2.1 Air and soil daily mean temperature during the growth period of potato and lettuce (2012), Swiss chard and turnip (2013) and Portuguese cabbage and carrot (2014).

2.2 Field experience

2.2.1 Experimental design

A three year field crop rotation in organic agriculture was set up in 2011 with a cover crop of rye (*S. cereale* L.) consociated with vetch (*V. sativa* L. cv. barril) grown for green manure, over the autumn and winter, every year, followed by: (i) potato (*S.*

tuberosum L. cv. Desirée) from April to August and lettuce (*L. sativa* L. cv. Maravilla de verano) from August to October in 2012; (ii) Swiss chard (*Beta vulgaris* L. cv. Blonde a card) from May to July and turnip (*B. napus* cv. nabo 60 dias) from July to August in 2013, and (iii) Portuguese cabbage (*B. oleracea* var. tronchuda cv. penca da Póvoa) from April to June and carrot (*Dacus carota* L. cv. jarana F1) from June to October in 2014. The treatments were arranged as a randomized block design with four replicated plots per treatment. Fertilizer treatments incorporated before potato, Swiss chard and Portuguese cabbage included: (i) green manure (GM); (ii) and (iii) GM with 20 and 40 t ha^{-1} of farmyard manure compost (C20 and C40); (iv) and (v) GM with 1 and 2 t ha^{-1} of a commercial organic fertilizer (CF1 and CF2), and (vi) a reference treatment without fertilizers (T0). The experimental site with 360 m^2 was divided in 24 plots of 15 m^2 each with 3 m wide and 5 m long. The localization of the different experimental plots is shown in figure 2.2.

Block 1	Block 2	Block 3	Block 4
GMCF2	GM	GMC20	GMCF1
GM	0	GMCF1	GMC40
GMC40	GMCF2	GMCF2	GMCF2
0	GMC20	GMC40	0
GMC20	GMCF1	0	GMC20
GMCF1	GMC40	GM	GM

Figure 2.2 Diagram of the experimental plots with green manure (GM), compost (C) at the rates of 20 and 40 t ha^{-1} and commercial organic fertilizer (CF) at the rates of 1 and 2 t ha^{-1}.

2.2.2 Cultural practices

The different cultural practices carried throughout the three years of the organic crop rotation are scheduled in table 2.2.

Table 2.2 Schedule of cultural practices during the three years of crop organic rotation

Cultural practices	DAP/S*	Year	Month	Day
Gafsa phosphate application		2011	September	29
Lime application		2011	October	7
Green manure sowing		2011	October	20
Green manure harvest	158	2012	March	26
Soil incorporation of organic fertilizers		2012	March	28
Potato plantation		2012	April	6
Potato harvest	124	2012	August	8
Soil mobilization		2012	August	14
Lettuce plantation		2012	August	20
Lettuce harvest	51	2012	October	10
Soil mobilization		2012	October	21
Green manure sowing		2012	October	23
Green manure harvest	194	2013	May	5
Soil incorporation of organic fertilizers		2013	May	7
Swiss chard plantation		2013	May	12
Swiss chard harvest	54	2013	July	5
Soil mobilization		2013	July	8
Turnip sowing		2013	July	9
Turnip harvest	47	2013	August	25
Soil mobilization		2013	October	1
Green manure sowing		2013	October	6
Green manure harvest	189	2014	April	13
Soil incorporation of organic fertilizers		2014	April	16
Portuguese cabbage plantation		2014	April	22
Portuguese cabbage harvest	56	2014	June	17
Soil mobilization		2014	June	19
Carrot sowing		2014	June	22
Carrot harvest	101	2014	October	1

*DAP/S = Days after plantation or sowing

2.2.2.1 Soil amendments before the start of the field experience

Soil phosphorus content and the pH value were adjusted with soil amendments before the beginning of the crop rotation. The phosphorus was applied at the rate of 300 kg ha^{-1}

of Gafsa phosphate (26.5% P_2O_5 and 29% CaO). The liming was carried out with dolomite lime (88% $CaCO_3$ and 5% $MgCO_3$) and applied at the rate of 5 t ha^{-1}, 8 days after Gafsa phosphate application. These fertilizers were incorporated with a 20 cm spring loaded deep tine cultivator and the soil surface finished with a spike tooth harrow before sowing the cover crop for green manure.

2.2.2.2 Organic fertilizers

Farmyard manure compost

Composting piles were held in the previous year to use the compost for the spring/summer crops. Cow manure with wheat straw as bedding material, accumulated for 1 year, was removed from the cattle shed to build the composting piles to approximately 1.8 m wide, 1.5 m high and 6 m long (figure 2.3). The feedstock was composted for approximately 7 months and manually turned twice at 2 and 4 months after the start of composting to enable the entire pile to be homogenously composted (figure 2.4). The compost piles were covered with a double shade net to avoid rainfall or to avoid drying the external part of the piles.

a) b)

Figure 2.3 Stable with straw as bedding material (a) and pile of cow manure covered with a shed net (b).

a) b)

Figure 2.4 Turning compost pile (a) and compost application in the respective plots (b).

The characteristics of composts are shown on table 2.3. The OM content (between 56% and 59%) was within the range of farmyard manure compost with straw as bedding material (Hose et al., 2014). The composts were matured as indicated by the amount of NH_4^+-N (69, 338 and 81 mg kg^{-1}) below the maximum recommended value (400 mg kg^{-1} dm) for mature composts by Zucconi and de Bertoldi (1987). The ratio NH_4^+-N/NO_3^--N was also below to the suggested upper limit value of 1 for matured composts by Larney and Hao (2007) for the 1st year (0.5) and 3rd year (0.06) composts, but this rate was > 1 for the compost used for the 2nd year (15).

Commercial organic fertilizer

The commercial organic fertilizer (Dix traded by Crimolara) certified for organic agriculture was based on granulated fermented poultry manure, beet molasses and feathers. The commercial fertilizer characteristics are shown on table 2.4. The organic fertilizer was very rich in nitrogen, with a very low C/N ratio, but it was not matured once the amounts of NH_4^+-N (7272, 17557 and 5309 mg kg^{-1}) were far above the maximum recommended value (400 mg kg^{-1} dry matter) and the rates NH_4^+-N/NO_3^--N (3463, 14631 and 39) were extremely high compared to the suggested upper limit value of 1 for matured composts (Larney and Hao, 2007). The commercial fertilizer EC values (between 5.9 to 6.9 dS m^{-1}) were above the recommended value of 3 dS m^{-1} for composts used as soil amendments (Soumaré et al., 2002).

Table 2.3 Dry matter content (DM) and chemical characteristics of compost applied before potato (1st year), Swiss chard (2nd year) and Portuguese cabbage (3rd year) (mean ± standard deviation).

Characteristic	(Unit)	Year 2012	2013	2014
DM	(g kg^{-1})	259	232	247
pH (H_2O)		8.0 ± 0.05	9.1 ± 0.1	7.9 ± 0.02
EC	(dS m^{-1})	4.3 ± 0,3	3.6 ± 0.1	5.2 ± 0.8
C	(g kg^{-1})	328 ± 35	326 ± 49	313 ± 37
N	(g kg^{-1})	19.7 ± 0.7	23.1 ± 2.4	25.4 ± 1.5
C/N		17 ± 2.4	14 ± 2.7	12 ± 1.7
NH_4^+-N	(mg kg^{-1})	69 ± 16	338 ± 7	81 ± 3
NO_3^--N	(mg kg^{-1})	134 ± 38	23 ± 10	1334 ± 43
Soluble C	(g kg^{-1})	7.4 ± 0.5	9.2 ± 0.6	7.8 ± 0.5
Soluble N	(g kg^{-1})	1.4 ± 0.03	0.8 ± 0.03	1.2 ± 0.2
P	(g kg^{-1})	6.7 ± 0.2	5.5 ± 0.4	5.8 ± 0.3
K	(g kg^{-1})	44 ± 8.5	24 ± 3.8	29 ± 2.2
Ca	(g kg^{-1})	11 ± 1.2	21 ± 3.8	20 ± 4.1
Mg	(g kg^{-1})	8.0 ± 1.2	7.6 ± 0.9	5.0 ± 0.9
S	(g kg^{-1})	2.7 ± 0.3	2.3 ± 0.2	1.8 ± 0.1
Fe	(g kg^{-1})	6.5 ± 1.0	6.2 ± 0.6	5.4 ± 0.8
Cu	(mg kg^{-1})	30 ± 0.6	24 ± 1.5	23 ± 1.1
Zn	(mg kg^{-1})	187 ± 21	130 ± 7	142 ± 4
Mn	(mg kg^{-1})	528 ± 26	737 ± 34	730 ± 22
B	(mg kg^{-1})	21 ± 1.4	14 ± 0.2	11 ± 0.6

Nutrient contents are expressed on a dry matter basis

Green manure

A cover crop of rye consociated with vetch as green manure was sown in 20th October 2011 (120 kg ha^{-1} rye and 60 kg ha^{-1} vetch), 23rd October 2012 (100 kg ha^{-1} rye and 100 kg ha^{-1} vetch) and 6th October 2013 (75 kg ha^{-1} rye and 150 kg ha^{-1} vetch). The cover crop seeds were sown manually on each plot and superficially buried by gentle harrowing with a tine cultivator. The green manure was chopped when most of the vetch was flowering, using a breaker hammer attached to the tractor 158, 194 and 189 days after sowing in 2012, 2013 and 2014, respectively and left as mulch in situ (figure 2.5). After drying in the soil surface for 2 days, the green manure was incorporated into the soil with the compost and the commercial fertilizer in the respective plots by a rotary tiller. The green manure yields and nutrient contents are shown on table 2.5. The increased amount of vetch in comparison to rye enhanced green manure N content and consequently C/N rate decreased.

Table 2.4 Dry matter content (DM) and chemical characteristics of commercial organic fertilizer applied before potato (1st year), Swiss chard (2nd year) and Portuguese cabbage (3rd year) (mean ± standard deviation).

Characteristic	(Unit)	Year		
		2012	2013	2014
DM	($g\ kg^{-1}$)	915	917	905
pH (H_2O)		6.6 ± 0.1	6.9 ± 0.04	6.9 ± 0.01
EC	($dS\ m^{-1}$)	5.9 ± 0.02	6.8 ± 0.2	6.9 ± 0.2
C	($g\ kg^{-1}$)	461 ± 7	410 ± 3	423 ± 5
N	($g\ kg^{-1}$)	99.9 ± 1.5	97.4 ± 4.0	99.6 ± 5
C/N		4.6 ± 0.1	4.2 ± 0.2	4.3 ± 0.2
NH_4^+-N	($mg\ kg^{-1}$)	7272 ± 545	17557 ± 3095	5309 ± 150
NO_3^--N	($mg\ kg^{-1}$)	2.1 ± 1.0	1.2 ± 0.2	135 ± 9.4
Soluble C	($g\ kg^{-1}$)	42.7 ± 3	50.4 ± 1.7	52.2 ± 1.3
Soluble N	($g\ kg^{-1}$)	36.6± 2.8	20.6 ± 0.7	21.2 ± 0.3
P	($g\ kg^{-1}$)	8.8 ± 0.5	12.6 ± 0.5	12.8 ± 0.6
K	($g\ kg^{-1}$)	42 ± 3.5	28.1 ± 10.9	25.8 ± 9.5
Ca	($g\ kg^{-1}$)	9.3 ± 1.5	32.8 ± 3.4	32.8 ± 8.9
Mg	($g\ kg^{-1}$)	8.0 ± 2.6	3.1 ± 0.2	3.3 ± 0.3
S	(g kg)	9.3 ± 1.5	4.6 ± 0.3	4.2 ±0.2
Fe	(mg kg)	310 ± 14	454 ± 33	441± 23
Cu	(mg kg)	42 ± 1.8	39 ± 2	41 ± 1.7
Zn	(mg kg)	190 ± 10	286 ± 16	285 ± 25
Mn	(mg kg)	190 ± 4	274 ± 15	284 ± 36
B	(mg kg)	34 ± 1.2	15 ± 0.4	16 ± 0.5

Nutrient contents are expressed on a dry matter basis

a) b)

Figure 2.5 Green manure with flowering vetch (a) and chopping green manure using a breaker hammer before incorporation (b).

Table 2.5 Fresh weight, dry matter content (DM) and chemical characteristics of green manure applied before potato (1st year), Swiss chard (2nd year) and Portuguese cabbage (3rd year) (mean ± standard deviation).

Characteristic	(Unit)	Year		
		2012	2013	2014
FW	(t ha^{-1})	17.4 ± 1.3	28.1 ± 2.7	22.1 ± 2.4
DM	(g kg^{-1})	200 ± 4	239 ± 2	201 ± 5
C	(g kg^{-1})	460 ± 3.3	393 ± 18	399 ± 22
N	(g kg^{-1})	12.1 ± 1.9	13.3 ± 0.9	20.6 ± 2.2
C/N		38	30	20
$N\text{-}NH_4^+$	(mg kg^{-1})	271 ± 25	934 ± 81	1078 ± 12
$N\text{-}NO_3^-$	(mg kg^{-1})	147 ± 22	0 ± 0	0 ± 0
Soluble C	(g kg^{-1})	62.4 ± 3.8	78.4± 7.3	70.4± 1.5
Soluble N	(g kg^{-1})	3.2 ± 0.2	4.4 ± 0.5	6.1 ± 0.3
P	(g kg^{-1})	3.7 ± 0.1	3.1 ± 0.1	3.9 ± 1.1
K	(g kg^{-1})	29.7 ± 2.2	11.2 ± 1.1	23.6 ± 3.6
Ca	(g kg^{-1})	5.8 ± 0.4	5.0 ± 0.8	6.9 ± 1.1
Mg	(g kg^{-1})	1.6 ± 0.1	0.9 ± 0.1	1.9 ± 0.2

Nutrient contents are expressed on a dry matter basis

2.2.2.3 Horticultural crops

Potato

Nine days after incorporation of the organic fertilizers on 6th April 2012, potato, a long-cycle crop with 25-28 mm calibre was buried manually with a harrow in furrows with a space of 25 cm in-between plants and 60 cm apart. Hoe blade was carried out once, 38 days after planting and ridging was performed 52 days after planting to avoid weed competition (figure 2.6). Late blight (*Phytophtora infestans*) was controlled with two applications of Bouille bourdelaise (20 kg ka^{-1}) and cooper hydroxide (2 kg ha^{-1}). The potato beetle (*Leptinotarsa decemlineata*) was controlled with two applications of Spintor (Lusosem) based on the fermentation of the bacteria *Saccharopolyspora spinosa*. The potato tubers were harvested manually 124 days after planting and subsequently the leaves were removed from the experimental field.

a) b)

Figure 2.6 Potato crop ridging (a) and flowering potato, 52 days after planting (b) in the 1st year crop rotation .

<u>Lettuce</u>

Lettuce seeds were sown in polystyrene trays with 220 cells and 35 ml of volume per cell, containing substrate certified for organic agriculture (Tray mix from Bas Van Buuren) based on peat. Subsequently the cells were covered with vermiculite. After harvesting potato, soil preparation was carried out with a tine cultivator and lettuce, a short-cycle crop, was transplanted 6 days later on 20th August 2012 at 35 × 35 cm plant spacing, 24 days after sowing. The planting was performed in the ridges with 1.1 m wide and 5 m long with support of three threads placed 35 cm apart. Hoe blade was carried out twice to avoid weed competition, 18 and 33 days after planting. The lettuce was harvested 51 days after planting (figure 2.7).

a) b)

Figure 2.7 Lettuce crop seedlings (a) and planting lettuce (b) in the 1st year crop rotation.

Swiss chard

Swiss chard seeds were sown in polystyrene trays with the same substrate used for lettuce and, subsequently, the cells were covered with vermiculite. Five days after incorporation of the organic fertilizers on 12th May 2013, Swiss chard, a short-cycle crop, was transplanted to the experimental plots at a planting space of 40 × 40 cm, 25 days after sowing. Planting was carried out in the ridges with 1.2 m wide and 5 m long with the aid of three threads placed 40 cm apart (figure 2.8). Hoe blade was carried out twice, 23 and 39 days after planting to avoid weed competition Lettuce harvest was performed 54 days after planting.

a) b)

Figure 2.8 Swiss chard crop after the first weeding 23 days after planting (a) and before harvest (b) in the 2nd year crop rotation.

Turnip

After harvesting Swiss chard, soil preparation was carried out with a tine cultivator. On the following day, on 9th july 2013, the planting ridges were set up with 1.2 m wide and 5 m long. Thereafter, turnip, a short-cycle crop was sown with a manual sower with a space of approximately 20 cm in-between plants and 30 cm apart rows (figure 2.9). Hoe blade was carried out once, 20 days after sowing to avoid weed competition. The turnip was harvested 47 days after planting and the leaves were removed from the experimental field.

a) b)

Figure 2.9 Turnip crop sowing (a) and after weeding of turnip 20 days after sowing (b) in the 2nd year crop rotation.

Portuguese cabbage

Portuguese cabbage seeds were also sown in polystyrene trays with the same substrate used for lettuce and the cells were covered with vermiculite. Six days after incorporation of the organic fertilizers on 22nd April 2014, Portuguese cabbage, a short-cycle crop, was transplanted to the experimental plots at a planting space of 40 × 40 cm, 25 days after sowing. Planting was carried out in the ridges with 1.2 m wide and 5 m long with the help of three threads placed 40 cm apart (figure 2.10). Hoe blade was carried out twice, 18 and 39 days after planting to avoid weed competition. The harvest was carried out 56 days after planting.

a) b)

Figure 2.10 Portuguese cabbage crop irrigation after planting (a) and before harvest (b) in the 3rd year crop rotation.

<u>Carrot</u>

After harvesting Portuguese cabbage, soil preparation was carried out with a tine cultivator. Three days later, on 22nd June 2014, planting ridges were set up with 1.2 m wide and 5 m long. Then, carrot, a long-cycle crop was sown with a manual sower with a space approximately of 10 cm in-between plants and 30 cm apart rows. Weeding was carried out 4 times, 25, 40, 59 and 81 days after sowing to avoid weed competition. Hoe blade was held inter-rows and weeds were removed by hand in the row (figure 2.11).

All horticultural crops were irrigated with a system of mini-sprinklers placed at a distance of 6.5 m so that the water content in the soil was not limiting for plant growth. The carrot was harvested 101 days after sowing.

a) b)

Figure 2.11 Carrot crop after the third weeding 59 days after sowing (a) and before harvest (b) in the 3rd year crop rotation.

2.2.3 Sample collection

2.2.3.1 Soil samples

Soil samples were collected in the 0-20 cm top layer in the beginning of the crop rotation and in each experimental plot (n=5) after cropping potato, lettuce, S. chard, turnip, Portuguese cabbage and carrot. Fresh samples were used to determine the pH value, electrical conductivity and mineral N content. Then, soil samples were dried at 65 ºC in a thermoventilated oven until reaching a constant mass and sieved through a 2 mm mesh diameter to determine total C, total N, and K_2O and P_2O_5 content by Egner-Riehm method.

2.2.3.2 Compost and commercial organic fertilizer samples

Five samples were collected from compost piles immediately prior to soil incorporation. Each sample was based on 20 sub-samples collected randomly from the compost piles at different depths and mixed. Five samples were also collected randomly from the commercial fertilizer bag. Fresh samples were used to determine pH, CE and mineral N. Compost and commercial fertilizer samples were previously frozen for further process of freeze-drying treatment to reduce moisture content and subsequently all the samples were milled using a 2 mm sieve. These samples were used to determine total C, N, P, K, Ca, Mg, S, Fe, Cu, Zn, Mn and B contents and soluble C and N.

2.2.3.3 Green manure samples

Plants were manually harvested in each plot by using twice a 0.5 × 0.5 m quadrat for above ground biomass, to assess green manure yield, immediately before incorporation into the soil (table 2.6). These samples were dried at 65 ºC in a thermoventilated oven until reaching a constant mass to determine DM weight. Samples were milled at a micro laboratory mill and used to determine total N, P, K, Ca and Mg in each treatment. Plants samples were also manually harvested randomly from the whole area of the experience before incorporation into the soil to use for laboratory incubations. These samples were previously frozen for further process of freeze-drying treatment and subsequently all the samples were milled using a 2 mm sieve to determine total C, total N and soluble C and N.

Table 2.6 Crop sampling during the three years of organic rotation

Sampling	DAP/S*	Year	Month	Day
Green manure	158	2012	March	26
Potato	47	2012	May	23
	75	2012	June	20
	124	2012	August	8
Lettuce	23	2012	September	12
	37	2012	September	26
	51	2012	October	10
Green manure	194	2013	May	5
Swiss chard	30	2013	June	11
	42	2013	June	23
	54	2013	July	5
Turnip	26	2013	August	4
	40	2013	August	18
	47	2013	August	25
Green manure	189	2014	April	13
Portuguese cabbage	27	2014	May	19
	42	2014	June	3
	56	2014	June	17
Carrot	61	2014	August	22
	79	2014	September	9
	101	2014	October	1

*DAP/S = Days after plantation or sowing

2.2.3.4 Horticultural crop samples

The horticultural crop sampling was based on four plants of each replicate treatment (table 2.6). The plants were weighted to determine fresh weight and subsequently dried at 65 ºC in a thermoventilated oven until reaching a constant mass to determine DM weight. Then, these dry samples were milled at a micro laboratory mill and used to determine total N content. For the last sample of each crop, collected immediately before harvest, P, K, Ca and Mg contents were also determined.

2.3 Pot experiment

2.3.1 Experimental design

The objective of this experiment was to determine fresh weight, nutrient content and accumulated nutrients of lettuce through a pot experiment conducted simultaneously and at the same location of the field experience for comparing the results with those obtained in the field plots. If the results obtained in the field and in the pot experiment were similar, probably a pot experiment would be sufficient to predict the growth of lettuce in the field. A randomized block design pot experiment was set up with lettuce inside a green-house. The treatments used in the pot experiment were the same used in the field. Three pots (one for each harvesting date) were filled with 8 kg of soil collected randomly from the top (15 cm) layer of the soil of each experimental plot of the field experience (6 treatments x 4 blocks) after the potato harvest (figure 2.12).

Figure 2.12 Pot experiment inside a green house.

2.3.2 Cultural practices

Lettuce (cv. Maravilla de verano) was transplanted on 21st August 2012. The weeds were removed by hand immediately after emergence. Irrigation was performed frequently so that the water content in the soil was not limiting for plant growth. The harvest took place 48 days after transplantation.

2.3.3 Sample collection

2.3.3.1 Soil samples

Soil samples were collected at the beginning and at the end of the pot experiment. Fresh samples were used to determine pH, CE and mineral N. Then, soil samples were dried at 65ºC in a thermoventilated oven until reaching a constant mass and sieved through a 2 mm mesh diameter to determine total C and N, and, K_2O and P_2O_5 content by Egner-Riehm method.

2.3.3.2 Lettuce samples

Lettuces were collected 19, 30 and 48 days after planting. The plants were weighted to determine fresh weight and subsequently dried at 65ºC in a thermoventilated oven to constant mass to determine DM weight and DM content. Dried samples were milled at a micro laboratory mill and used to determine total N content. Plants collected with 48 days were also analysed for P, K, Ca and Mg contents.

2.4 Field incubation

One field incubation was set up each year, after organic fertilization for the spring/summer vegetables growth seasons, based on the methodology described by Raison et al. (1987). Briefly, samples were taken every 14 days during the incubation period except for the first period, which was only of 7 days. At the start of each incubation period, 5 pairs of samples were taken randomly from each plot using PVC cores with 15 cm long and 4 cm diameter to avoid soil disturbance, in which were perforated 6 holes with 6 mm of diameter to allow soil aeration. One sample from each pair of samples was frozen and the other was enclosed in a micro-perforated polyethylene bag and buried at 20 cm depth (figure 2.13). Net N mineralization was calculated by the difference between the amount of inorganic N (NH_4^+-N + NO_3^--N) of initial and incubated samples. Accumulated N mineralization per kg of soil was used as an estimation of mineralized N in the tilled layer. This value was provided by the sum of mineralized N in each period of incubation. The apparent net N mineralization of compost and of the organic commercial fertilizer was estimated by the difference between the net N mineralization in treatments C20, C40, CF1 and CF2 and the net N mineralization in treatment GM. The green manure apparent net N mineralization was calculated by the difference between the net N mineralization in treatments GM and T0. N mineralization rate (g kg^{-1} initial N) was calculated by the quotient between the accumulated mineralized N and the amount of N applied.

Figure 2.13 PVC cores enclosed in polyethylene bags for field incubation.

2.5 Laboratory incubation

The laboratory incubation was set up with samples of 60 g of soil (dry weight basis) mixed with the organic fertilizers of the last two years of the field experiment including four repetitions per treatment. The fertilizer treatments included: (i) green manure (GM); (ii) farmyard manure compost (C); (iii) commercial organic fertilizer (CF); (iv) farmyard manure compost with green manure (CGM); (v) commercial organic fertilizer with green manure (CFGM), and (vi) a reference treatment without fertilizers (T0). The compost was applied at the rate of 7.5 g kg^{-1} of dry soil dm (equivalent to 40 t ha^{-1} incorporated at the top 15 cm of the layer); the commercial fertilizer was applied at the rate of 1.4 g kg^{-1} soil of dry soil (equivalent to 2 t ha^{-1} incorporated at the top 15 cm of the layer) and green manure was applied at the rate of 3.9 g kg^{-1} dry soil (equivalent to 26 t ha^{-1} incorporated at the top 15 cm of the layer). Before the experiment, soil moisture was adjusted to 70% of soil water-holding capacity and pre-incubated for 15 days at 25 ºC.

The subsamples were placed in plastic containers and subsequently put into 1 L glass jars containing a vessel with 20 ml of NaOH (1M) solution to trap evolved CO_2 and a vessel with 40 ml of distilled water to avoid desiccation. The plastic containers were weighted every 15 days to adjust moisture content. The plastic containers and the jars sealed with air tight glass lids were incubated for 252 days at 25 ºC. During the first 14 days a vessel with 10 ml HCl (0.1M) was also placed into the 1 L glass jar to trap evolved NH_3. After 1, 3, 7 and 14 days the vessels with HCl (0,1M) were removed and replaced with fresh HCl. The trapped NH_4^+ was determined by molecular absorption spectrophotometry (San plus, Skalar) by Berthelot method. After 1, 2, 3, 5, 7, 10, 14, 21, 28, 35, 42, 49, 56, 70, 84, 98, 112, 126, 140, 154, 168, 182, 196, 210, 224, 238 and 252 days the vessels with NaOH solution were removed, stored for analyses and replaced with fresh NaOH. The trapped CO_2 was determined with an elemental analyzer with near infrared detector (NIRD) (Formacs, Skalar) The apparent loss of added organic fertilizers was estimated by subtracting total CO_2-C released in a given treatment from that measured in the reference treatment. The CO_2 emission was calculated by the quotient between the cumulative CO_2-C evolved and the C applied in each treatment. The soil subsamples were removed for N mineral analyses and replaced after 1, 3, 7, 10, 14, 21, 35, 56, 84, 112, 154, 196 and 252 days.

2.6 Laboratory analyses

2.6.1 pH value and electrical conductivity (EC)

The values of pH (H_2O) and pH (KCl) of fresh samples of soil and organic fertilizers were determined in the supernatant suspension of a 1:2.5 solutions with deionised water and 1 M KCl respectively. The pH was measured with a pH measurement electrode (632 pH meter, Metrohm) after stabilizing for 2 hours for deionised water and 1 hour for 1 M KCl solution.

The EC value of fresh samples was determined in a 1:5 deionised aqueous solution. The EC was measured with an electrical conductivity meter (Cond 3310, WTW) after mechanically shake this solution for two hours and filter through Whatman nº 42 filters.

2.6.2 Total carbon (C)

Total C in soil and organic fertilizers was determined by near-infrared detector (NIRD) in an elemental analyzer (Primacs SC analyser, Skalar) after combustion at 950 ºC of about 0.3 g dry sample. The OM content in the soil was calculated by multiplying C content by the factor of van Bemmelen (1.724) and in compost by a factor of 1.8 (Diário da République, 2015).

2.6.3 Total N, P and K

Total N and total P in compost, commercial fertilizer, green manure and crops were measured by molecular absorption spectrophotometry (San Plus System, Skalar) and K by flame photometry (PFP7 Flame Photometer, Jenway) after digestion with sulphuric acid (Temminghoff et al., 2000). Sulphuric digestion consisted of adding 2.5 ml of sulphuric acid and 1.5 ml of hydrogen peroxide to 0.3 g of dry samples in the digestion tubes. The digestion tubes were placed in the block digester (Raypa) set up for 50 minutes at 135 ºC and 90 minutes at 360 ºC.

2.6.4 Total Ca, Mg, Cu, Zn, Mn, Fe and S

Total Ca, Mg, Cu, Zn, Mn and Fe in compost, commercial fertilizer, green manure and crops were measured by atomic absorption spectrophotometry (iCE 3000 Series AA Spectrometer, Thermo Scientific) after nitro-perchloric digestion (Temminghoff et al., 2000). Total S was measured by molecular absorption spectrophotometry (San plus System, Skalar) after nitro-perchloric digestion. Nitro-perchloric digestion consisted of adding 3 ml of nitric acid and 1.5 ml of perchloric acid to 0.5 g of dry sample in the digestion tubes. The digestion tubes were placed in the block digester (Raypa) set up for 100 minutes at 105 ºC and 90 minutes at 195 ºC.

2.6.5 Boron (B)

Total boron in compost and commercial fertilizer was extracted from 1 g dry sample boiled at 550 ºC in an oven (Carbolite Furnaces) for two hours. After cooling, the

Azomthine-H method was performed to determine B content by molecular absorption spectrophotometry (Helios Omega UV-VIS, Thermo Scientific).

2.6.6 Soluble C and N

Soluble C and N in compost and commercial fertilizer were extracted from 4 g dry sample with $CaCl_2$ 0.01M 1:10 solution; soluble C and N in green manure were extracted from 2 g dry sample with $CaCl_2$ 0.01M 1:20 solution. The extracts were mechanically shacked for 2 hours (GFL 3016) and then filtered. Soluble C and N contents were measured by near-infrared proximity detector and chemiluminescence respectively in an elemental analyser (Formacs analyzer, Skalar) after combustion at 950 ºC.

2.6.7 Mineral N

Mineral N of fresh soil, compost and commercial fertilizer samples was extracted from 6 g fresh sample with KCl 1M 1:5 solution. The extracts were mechanically shaked (3016, GFL) for 1 hour and then centrifuged at 3000 rpm for 5 minutes (Mod. Digtor, Orto Alresa). NH_4^+-N and NO_3-N contents were determined by molecular absorption spectrophotometry in a segmented flow analyser system (San Plus system, Skalar) using Berthelot method and sulphonamide method after reducing NO_3^- to NO_2^-, respectively.

2.6.8 P_2O_5 and K_2O

The available contents of P_2O_5 and K_2O in the soil were determined by Egner-Riehm method. Phosphorus and K extraction was carried out by mechanical shaking 2 g of soil mixed with 40 ml of ammonium lactate-acetate solution for two hours and then filtering. Phosphorus and K contents in the extracts were measured by molecular absorption spectrophotometry in a segmented flow analyser system (San Plus system, Skalar) and by flame photometry (PFP7 Flame Photometer, Jenway) respectively.

2.7 Calculations

Crop nutrient accumulation (kg ha^{-1}) was calculated multiplying crop nutrient content (g kg^{-1}) by crop dry weight (t ha^{-1}). Apparent nutrient recovery (%) from compost, commercial organic fertilizer and green manure was calculated by the difference between nutrient accumulation in vegetable crops grown with and without these organic fertilizers application, divided by the amount of nutrients in each organic fertilizer. Total nitrogen efficiency (%) with compost and commercial organic fertilizer application during the crop rotation was calculated dividing the sum of accumulated N uptake from those fertilizers by the amount of organic N applied with compost and commercial fertilizer.

2.8 Statistical analysis and kinetic models

Analysis of variance (ANOVA) was performed by the general linear model procedure, and a probability level of $P<0.05$ was applied to determine statistical significance between treatment means. All statistical calculations were performed using SPSS v. 17.0 for windows (SPSS inc.). The N mineralization model developed by Bonde and Lindberg (1988) was fitted to the results. The first-order kinetic model with one mineralization pool was fitted to the N mineralization of compost, commercial fertilizer and green manure (equation 1).

$$N_m = N_0 [1-\exp(-k_1t-k_2t^2)] \quad [1]$$

Where N_m (mg kg^{-1}) is the accumulated mineralized N at time t; k_1 and k_2 are mineralization constant rates, whereas the amount of potentially mineralizable N is given by N_0 (mg kg^{-1}). The proportion of N_0 (g kg^{-1}) released during the growth period of each crop and in the end of each year of the crop rotation was calculated by the ratio between mineralized N (N_m) and potentially mineralizable N (N_0) estimated for the respective period. Nitrogen mineralization rate (g kg^{-1} initial N) was calculated by the quotient between Nm (mg kg^{-1}) and the total of organic N applied (mg kg^{-1}). The immobilization time period was determined based on the mineralization constant rates of the one pool model ($t = -k_1/k_2$) (Brito and Santos, 1996). Model equations were fitted by the non-linear least-square curve-fitting technique (Marquardt–Levenberg algorithm), to minimize the sum of the squared differences between the observed and predicted values of the dependent variable. The normality of data was checked with the test of Shapiro-Wilk, and the Levene test was used to assess homoscedasticity. The Pearson correlation test was performed to relate crop yield with the sum of mineralized N.

The first-order kinetic model with one mineralization pool was fitted to the C mineralization of compost, commercial fertilizer and green manure (equation 2).

$$Cm = C_0 [1-\exp(-k_1 t-k_2 t^2)] \quad [2]$$

Where Cm (mg kg^{-1}) is the accumulated mineralized C at time t; k_1 and k_2 are mineralization constant rates, whereas the amount of potentially mineralizable C is given by C_0 (mg kg^{-1}). Carbon mineralization rate (g kg^{-1} initial C) was also determined by the quotient between the accumulated evolved C (Cm) (mg kg^{-1}) and the total of C applied (mg kg^{-1}).

3. RESULTS

3.1 Field experience

3.1.1 Crop fresh weight

3.1.1.1 Green manure

In the first year of the crop rotation, fresh weight of green manure increased from 7 t ha^{-1} to 17 t ha^{-1} between 101 and 154 days after sowing, when the GM was incorporated into the soil. During this year there were no significant differences between plots on fresh weight of green manure because no organic fertilizers were yet incorporated in the respective plots. During the 2nd year of the crop rotation there were no significant differences between treatments on fresh weight of green manure 103 days after sowing. However, green manure yield (194 days after sowing) increased for C40 (31 t ha^{-1}) and CF2 (31 t ha^{-1}) compared to GM, C20 and CF1 (25 to 26 t ha^{-1}) (figure 3.1). The DM content (22 to 25%) of green manure before incorporation into the soil in this year was similar for all treatments.

During the 3rd year of the crop rotation, fresh weight of green manure increased for C40 in comparison to all other treatments at 105 days after sowing, and green manure yield (189 days after sowing) also increased for C40 (26 t ha^{-1}) compared to CF1 (19 t ha^{-1}) (figure 3.2). The DM content (20 to 22%) of green manure before soil incorporation in 2014 was not different between treatments.

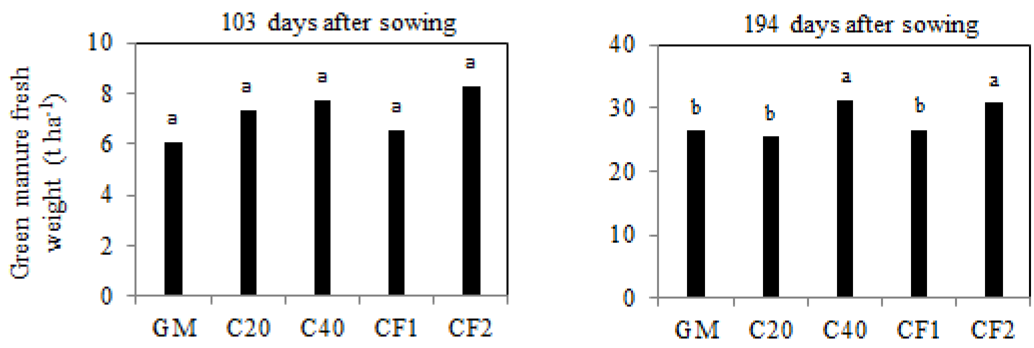

Figure 3.1 Fresh weight of green manure during the 2nd year of crop rotation (2013) for the control treatment (T0) and for the treatments with green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

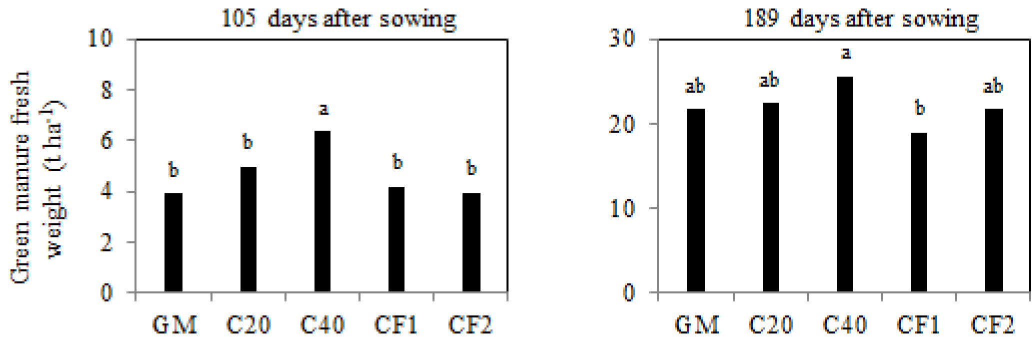

Figure 3.2 Fresh weight of green manure during the 3rd year crop rotation (2014), for the control treatment (T0) and for the treatments with green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

3.1.1.2 Horticultural crops

Fresh weight of potato tubers increased ($P<0.05$) for the treatment CF2 compared to all other treatments at 47 and 75 days after plantation, in the first year of the crop rotation. Potato yield (124 days after plantation) was not significantly different among treatments CF1, CF2 and C40 (35.9, 42.2 t ha^{-1} and 36.1 t ha^{-1}, respectively) ($P<0.05$). However, potato yield was significantly increased for CF2 compared to T0, GM and C20 (figure 3.3). The DM content of potato tubers was similar between experimental treatments (23 to 25%).

In this 1st year of the crop rotation, lettuce fresh weight increased significantly with compost application to the previous crop. This was true 23 days after plantation as well as for final lettuce yield (51 days after plantation), with significant increases for C20 and C40 (18 and 19 t ha^{-1} respectively) compared to all other treatments (8 to 11 t ha^{-1}) (figure 3.3). The DM content of lettuce was not significantly different between treatments (5 to 7%).

Over the 2nd year of crop rotation, fresh weight of Swiss chard 30 days and 42 days after plantation increased for CF2 compared to all other treatments, except CF1 at day 30. Swiss chard yield (54 days after plantation) increased for CF2 (46 t ha^{-1}) compared to all other treatments, and increased for C40 (36 t ha^{-1}) compared to T0, GM and C20 (figure 3.4). The DM content of Swiss chard was similar for all treatments (7 to 8%).

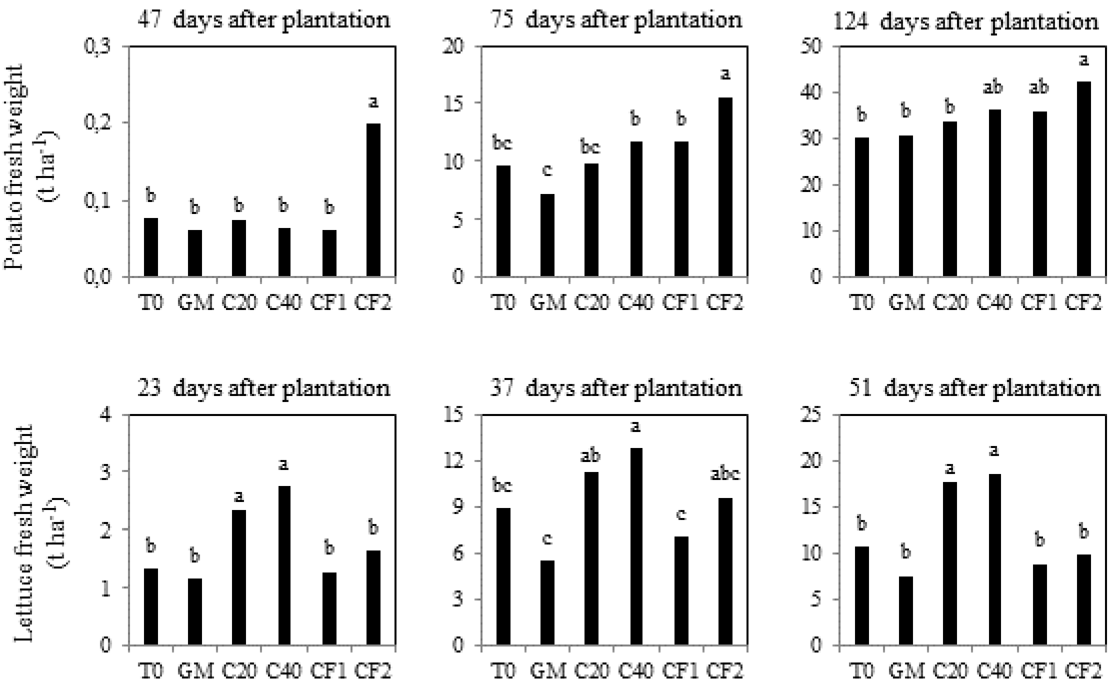

Figure 3.3 Fresh weight (t ha^{-1}) from potato and lettuce during the 1st year crop rotation for the control treatment (T0) and for the treatments with green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

Fresh weight of turnip increased significantly for C40 at day 26 after sowing compared to all other treatments. Although differences were not so clear at 40 days after sowing, turnip yield (47 days after sowing) was clearly enhanced for C40 (38 t ha^{-1}) in comparison to all other treatments (figure 3.4). There were no significant differences on DM content of turnip between treatments (9 to 11%).

In the 3rd year of crop rotation, fresh weight of Portuguese cabbage was not significantly different between C40 and CF2 at 27 and 42 days after plantation. However, fresh weight of P. cabbage was enhanced for C20, C40, CF1 and CF2 compared to T0 and GM at 27 days after plantation. Moreover, fresh weight of P. cabbage was also enhanced for C40 and CF2 compared to all other treatments at 42 days after plantation. There were no significant differences on cabbage yield (56 days after plantation) for treatments C20, C40 and CF2 (20 t ha^{-1}, 26 t ha^{-1} and 24 t ha^{-1} respectively). However, cabbage fresh weight increased for C40, C20, CF1 and CF2 compared to T0 and GM and also for C40 compared to CF1 (figure 3.5). The DM content of P. cabbage decreased from 12% in the unfertilized treatment to 9% with 40 t ha^{-1} compost.

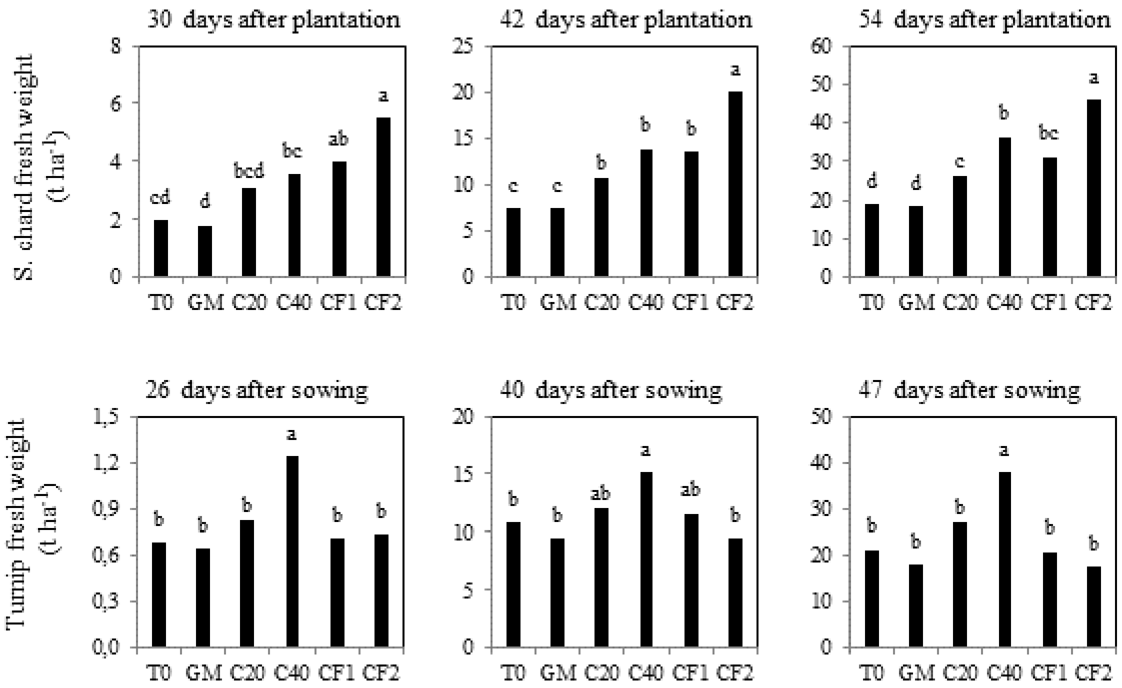

Figure 3.4 Fresh weight (t ha^{-1}) from S. chard and turnip during the 2nd year crop rotation for the control treatment (T0) and for the treatments with green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

Carrot fresh weight was clearly enhanced for C40 compared to all other treatments 61 and 79 days after sowing. Carrot yield (101 days after sowing) increase between treatments C20 and C40 (32 t ha^{-1} and 40 t ha^{-1}) was not significant. However, carrot yield increased significantly between treatment C40 and treatments T0, GM, CF1 and CF2 (figure 3.5). There were no significant differences on DM content of carrot between treatments (10 to 11%).

Overall, results show that in contrast to the first crop of the season, the application of 40 t ha^{-1} of compost and green manure (C40) increased crop yields by 90%, 115% and 56% for lettuce, turnip and carrot (second season crops), respectively, compared with the application of 2 t ha^{-1} of commercial organic fertilizer and green manure (CF2).

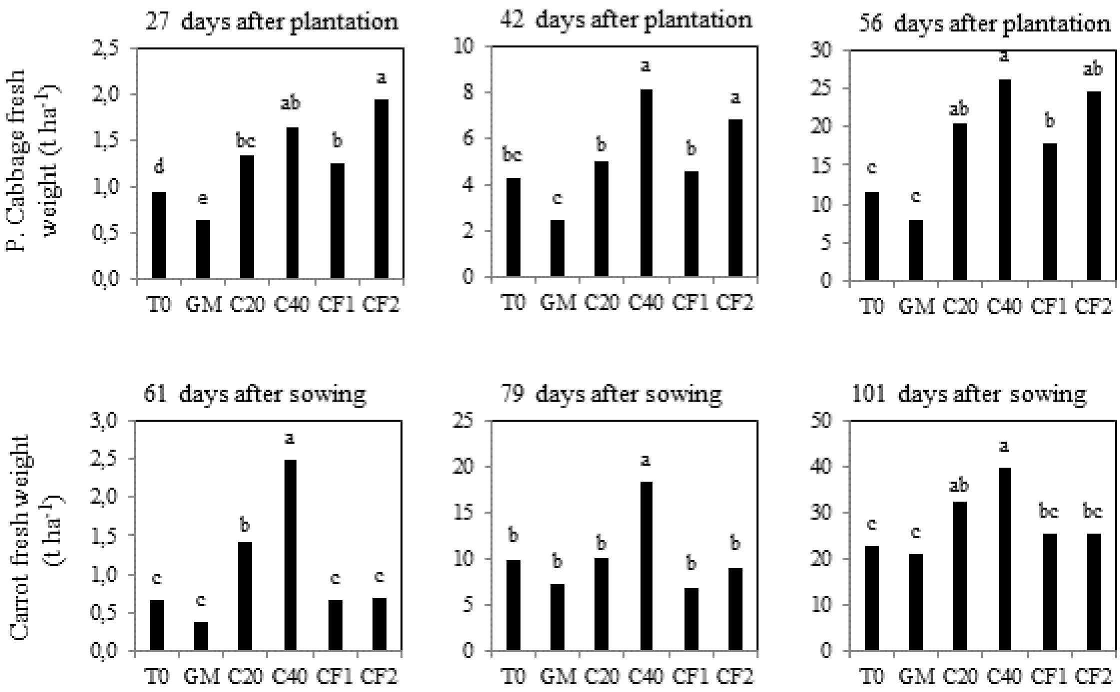

Figure 3.5 Fresh weight (t ha^{-1}) from P. cabbage and carrot during the 3rd year crop rotation for the control treatment (T0) and for the treatments with green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

3.1.2 Nutrient content

3.1.2.1 Green manure

In the 1st year of crop rotation, the nutrient content of green manure was similar for the different treatments since the organic fertilizers were still not incorporated in the respective plots (table 3.1). Over the 2nd (2013) and 3rd (2014) years of crop rotation, N, P, K, Ca and Mg contents of the green manure were also not significantly different among treatments except for occasional differences. For example, K content increased for C20 (12.3 g kg^{-1}) and C40 (12.4 g kg^{-1}) compared to GM (10.1 g kg^{-1}) and CF2 (10.0 g kg^{-1}) in 2013. Calcium and Mg content also increased for C20 (8.6 and 2.2 g kg^{-1} respectively) compared to CF2 (5.8 and 1.7 g kg^{-1} respectively) in 2014 (table 3.1). Therefore, N uptake and accumulation in crops depended on plant dry weight and not on plant nutrient content.

Table 3.1 Green manure nutrient content (g kg^{-1}) before experimental treatments application in 2012, and in response to green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2) in 2013 and 2014.

Year	Nutrient	GM	C20	C40	CF1	CF2	LSD
2012	N	22.2	20.1	21.2	20.3	21.0	6.0
	P	3.7	3.7	3.7	3.6	3.6	0.4
	K	32.0	28.1	28.8	32.0	27.4	5.7
	Ca	6.5	5.4	6.1	5.5	5.6	2.5
	Mg	1.7	1.6	1.7	1.6	1.5	0.4
2013	N	15.4	18.0	17.0	15.9	16.5	3.6
	P	3.0	3.3	3.0	3.2	3.1	0.5
	K	10.1	12.3	12.4	11.1	10.0	1.8
	Ca	4.6	6.5	5.1	4.5	4.6	2.6
	Mg	0.9	0.9	1.0	0.8	0.9	0.3
2014	N	27.6	22.3	23.4	28.8	22.2	9.9
	P	4.4	2.9	3.7	4.6	3.6	1.5
	K	28.4	19.1	21.0	25.0	24.7	6.3
	Ca	6.7	8.6	7.4	6.3	5.8	2.5
	Mg	1.8	2.2	2.0	1.8	1.7	0.4

LSD = Least significant difference ($P<0.05$)

3.1.2.2 Horticultural crops

In the 1st year of crop rotation, nutrient content in potato tubers was not significantly different between experimental treatments (table 3.2). Nutrient content in lettuce shoots was also not significantly different among experimental treatments except for occasional differences found for N and K. Nitrogen content in lettuce increased for C20 (32.3 g kg^{-1}) and GM (32.4 g kg^{-1}) compared to CF1 (23.8 g kg^{-1}). The highest K content was found in lettuce for T0 (20.7 g kg^{-1}) compared to the all other treatments (table 3.2).

Table 3.2 Crop nutrient content (g kg^{-1}) on the 1st year of crop rotation in response to green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

Crops	Nutrient	T0	GM	C20	C40	CF1	CF2	LSD
Potato	N	8.1	8.7	8.1	8.8	8.6	8.9	0.9
	P	2.3	2.2	2.5	2.5	1.8	2.2	0.7
	K	6.5	8.2	8.2	7.7	5.4	6.9	4.3
	Ca	0.6	0.5	0.2	0.5	0.3	0.6	0.4
	Mg	0.9	0.8	0.8	0.9	0.7	0.7	0.2
Lettuce	N	28.8	32.4	32.3	28.9	23.8	28.1	5.2
	P	3.7	3.6	3.3	3.5	3.2	3.4	1.3
	K	20.7	15.4	16.6	15.5	15.5	17.6	3.2
	Ca	7.1	7.5	7.3	6.8	6.3	8.5	1.6
	Mg	2.5	2.5	2.4	2.2	2.2	2.9	0.4

LSD = Least significant difference ($P<0.05$).

In the 2nd and 3rd years of the crop rotation nutrient content differences increased among treatments for N and K and occasionally for P (tables 3.3 and 3.4). For the first crop of the season, Swiss chard N content increased for CF2 (27.7 g kg^{-1}) in comparison to T0, GM, C40 and CF1, and Portuguese cabbage N content increased for CF2 (39.6 g kg^{-1}) compared to T0 (27.1 g kg^{-1}). On the contrary, K content in Swiss chard and cabbage increased for C40 (70.0 and 58.1 g kg^{-1} respectively) in comparison to commercial fertilizer application. Calcium content in chard was enhanced with commercial fertilizer compared to compost application. For the second crop of the season, turnip N content increased for CF2 compared to T0 in contrast to K content which increased between C40 and CF2 for turnip and carrot (tables 3.3 and 3.4).

Table 3.3 Crop nutrient content (g kg^{-1}) on the 2nd year of crop rotation in response to green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

Crops	Nutrient	T0	GM	C20	C40	CF1	CF2	LSD
S. chard	N	15.5	15.1	21.0	14.1	9.9	27.7	7.6
	P	2.5	1.9	3.1	2.7	1.1	2.6	1.2
	K	62.6	51.6	64.3	70.0	39.7	49.0	16.8
	Ca	9.1	10.5	7.2	7.3	10.8	12.2	2.8
	Mg	6.1	7.1	5.6	6.1	6.7	7.3	2.2
Turnip	N	23.7	27.2	24.8	26.8	25.3	30.4	5.7
	P	4.3	4.6	4.4	4.6	3.8	4.2	0.6
	K	33.8	38.0	39.2	44.8	33.6	34.6	6.3
	Ca	1.4	3.0	2.2	2.0	2.3	3.4	1.7
	Mg	0.7	1.3	0.9	0.9	0.9	1.0	1.3

LSD = Least significant difference ($P<0.05$).

Table 3.4 Crop nutrient content (g kg^{-1}) on the 3rd year of crop rotation in response to green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

Crops	Nutrient	T0	GM	C20	C40	CF1	CF2	LSD
P. cabbage	N	27.1	36.1	32.6	37.2	33.6	39.6	8.1
	P	4.2	4.1	5.1	5.9	3.9	4.8	1.1
	K	41.5	48.5	51.3	58.1	38.3	42.4	8.1
	Ca	26.4	35.5	30.8	26.0	31.6	35.1	9.2
	Mg	2.5	3.6	3.5	3.2	3.0	3.5	1.1
Carrot	N	11.3	10.1	12.2	13.0	11.6	11.6	2.3
	P	3.4	3.1	3.6	3.7	3.4	3.4	0.5
	K	24.5	22.9	34.8	38.6	29.1	24.5	7.6
	Ca	1.7	1.7	1.8	1.8	1.8	1.8	0.4
	Mg	0.9	0.9	0.9	1.0	1.0	0.9	0.2

LSD = Least significant difference ($P<0.05$).

3.1.3 Nutrient accumulation

3.1.3.1 Green manure

In the 2nd (2013) and 3rd (2014) years of crop rotation, N, P, K, Ca and Mg accumulation in green manure was not significantly different between treatments except for occasional differences. For example, in 2013, K and Mg accumulation increased for C40 in comparison to GM, C20 and CF1 and in 2014 Mg accumulation increased for C40 compared to GM, CF1 and CF2 (table 3.5).

Table 3.5 Green manure nutrient accumulation (kg ha^{-1}) in response to green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

Year	Nutrient	GM	C20	C40	CF1	CF2
2012	N	80.1 a	78.0 a	65.5 a	67.9 a	66.3 a
	P	13.5 a	14.3 a	11.4 a	11.8 a	11.5 a
	K	115.8 a	108.9 a	88.9 a	106.4 a	87.3 a
	Ca	23.5 a	20.9 a	17.8 a	17.3 a	17.7 a
	Mg	6.1 a	6.1 a	5.1 a	5.1 a	4.9 a
2013	N	100.4 a	103.9 a	121.6 a	100.2 a	127.0 a
	P	19.3 a	19.1 a	21.7 a	20.3 a	23.8 a
	K	65.2 b	70.6 b	88.7 a	70.9 b	77.4 ab
	Ca	29.3 a	37.2 a	36.8 a	27.5 a	35.5 a
	Mg	5.8 bc	5.1 c	7.4 a	5.2 c	7.0 ab
2014	N	117.5 a	100.4 a	116.7 a	108.2 a	97.4 a
	P	18.9 a	13.1 a	18.4 a	17.5 a	16.3 a
	K	122.0 a	85.1 a	104.1 a	91.3 a	112.2 a
	Ca	28.7 a	38.4 a	37.3 a	23.6 a	25.1 a
	Mg	7.9 bc	10.0 ab	10.4 a	6.7 c	7.4 bc

Mean values followed by different letters within the same parameter are statistically different ($P<0.05$).

3.1.3.2 Horticultural crops

Soil supplied with commercial fertilizer at the rate of 2 t ha^{-1}, in the 1^{st} year of crop rotation, enhanced N, Ca and Mg accumulation by potato compared to T0, GM or C20 treatments. Phosphorus accumulation by potato also increased with the application of 2 t ha^{-1} commercial fertilizer in comparison to GM and CF1. The accumulation of K in potato was not significantly different between experimental treatments. Compost application at the rate of 20 and 40 t ha^{-1} before potato crop plantation increased N accumulation by lettuce compared to the other treatments. Phosphorus, K, Ca and Mg accumulation in lettuce also increased for C40 compared to GM and CF1 (table 3.6).

Table 3.6 Potato (tubers and shoots) and lettuce (shoots) nutrient accumulation (kg ha^{-1}) on the 1st year of crop rotation in response to green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

Crops	Nutrient	T0	GM	C20	C40	CF1	CF2
Potato	N	77.9 b	70.5 b	81.1 b	93.8 ab	98.1 ab	129.9 a
	P	19.0 ab	17.1 b	21.2 ab	23.0 ab	17.2 b	24.5 a
	K	61.6 a	66.4 a	75.1 a	81.4 a	57.7 a	89.1 a
	Ca	23.5 b	24.1 b	19.4 b	26.0 ab	27.8 b	45.5 a
	Mg	8.5 b	8.2 b	7.9 b	9.6 ab	8.3 b	11.6 a
Lettuce	N	17.4 b	16.1 b	29.8 a	30.3 a	12.8 b	17.4 b
	P	2.3 ab	1.8 b	3.2 ab	3.9 a	1.7 b	2.0 ab
	K	12.5 abc	7.4 c	15.7 ab	16.9 a	8.4 bc.	10.8 abc
	Ca	4.4 bc	3.5 c	6.8 ab	7.5 a	3.4 c	5.1 abc
	Mg	1.5 b	1.2 b	2.3 a	2.4 a	1.2 b	1.8 ab

Mean values followed by different letters within the same parameter are statistically different ($P<0.05$).

Nitrogen, Ca and Mg accumulation in Swiss chard, in the 2nd year of crop rotation, increased for CF2 compared to the other remaining treatments. However, P and K accumulation in chard was similar for C40 and CF2. Soil supplied with 40 t ha^{-1} compost before chard increased P and K accumulation in turnip compared to commercial fertilizer application, but differences in N, Ca and Mg were not significant between compost and commercial fertilizer application (table 3.7).

In the 3rd year of crop rotation, N accumulation in cabbage was not significantly different between CF2 and C40 treatments and significant differences were also not found for P accumulation with compost or 2 t ha^{-1} commercial fertilizer application. On the other hand, N and P accumulation increased for C20, C40 and CF2 in comparison to T0 and GM treatments. Potassium accumulation increased for C40 compared to all the other treatments. The accumulation of Ca and Mg in cabbage was similar for compost or commercial fertilizer application (table 3.8).

The application of 40 t ha^{-1} compost before cabbage plantation increased N, P and K accumulation in carrot compared to all other treatments except for C20. Nutrient accumulation of Ca and Mg in carrot were not significantly different with compost or commercial fertilizer application, but was enhanced for C20, C40, CF1 and CF2 compared to T0 and GM treatments (table 3.8).

Table 3.7 Swiss chard (shoots) and turnip (shoots and roots) nutrient accumulation (kg ha^{-1}) on the 2nd year of crop rotation in response to green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

Crops	Nutrient	T0	GM	C20	C40	CF1	CF2
S. chard	N	23.4 c	22.6 c	42.7 b	37.6 bc	29.2 c	91.3 a
	P	3.6 bc	2.8 c	6.1 ab	7.1 a	2.6 c	8.5 a
	K	90.1 cd	75.6 d	131.8 bc.	186.0 a	91.3 cd	162.0 ab
	Ca	12.9 c	15.2 c	14.3 c	19.2 bc	25.0 b	39.9 a
	Mg	8.7 d	10.4 d	11.3 cd	16.1 b	15.2 b	24.1 a
Turnip	N	107.2 b	104.0 b	136.8 ab	171.4 a	128.9 ab	136.9 ab
	P	13.3 b	13.6 b	16.4 ab	20.0 a	13.3 b	13.2 b
	K	139.9 b	148.4 b	179.9 b	235.5 a	142.5 b	138.9 b
	Ca	23.9 b	43.5 ab	45.3 ab	47.0 a	55.0 a	55.8 a
	Mg	4.3 b	7.2 ab	8.6 a	8.7 a	7.8 ab	8.7 a

Mean values followed by different letters within the same parameter are statistically different ($P<0.05$).

Table 3.8 Portuguese cabbage (shoots) and carrot (roots and shoots) nutrient accumulation (kg ha^{-1}) on the 3rd year of crop rotation in response to green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

Crops	Nutrient	T0	GM	C20	C40	CF1	CF2
P. cabbage	N	37.2 cd	30.0 d	66.4 b	89.6 ab	63.2 c	96.9 a
	P	5.8 c	3.5 c	10.5 ab	14.3 a	7.3 bc	11.7 a
	K	57.8 c	42.1 c	105.8 b	143.4 a	71.0 bc	101.8 b
	Ca	36.5 bc	23.9 c	61.8 ab	65.8 ab	59.7 ab	87.1 a
	Mg	3.5 bc	2.9 c	7.2 ab	8.1 a	5.7 abc	8.6 a
Carrot	N	62.6 bc	54.5 c	79.0 ab	98.8 a	66.0 bc	65.8 bc
	P	12.2 b	10.9 b	15.7 ab	19.8 a	12.8 b	13.3 b
	K	108.9 bc	95.9 c	194.8 ab	260.1 a	111.1 bc	120.7 bc
	Ca	26.8 b	27.6 b	32.8 ab	41.3 a	30.0 ab	29.1 ab
	Mg	6.3 b	5.8 b	7.4 ab	9.9 a	6.5 ab	6.7 ab

Mean values followed by different letters within the same parameter are statistically different ($P<0.05$).

3.1.4 Nutrient recovery

At the 1st year of the crop rotation, differences on N, P, K, Ca and Mg recovery between compost and commercial fertilizer for the first crop of the season (potato) were not significant (table 3.9). However, in the 2nd year of crop rotation N, P, Ca and Mg recovery from 2 t ha^{-1} commercial fertilizer increased compared to 40 t ha^{-1} compost for the first crop of the season (S. chard). Although K recovery by Swiss chard from 2 t ha^{-1} commercial fertilizer numerically increased compared to 40 t ha^{-1} compost, significant differences were not attained (table 3.10). In the 3rd year of crop rotation, significant differences on N, P and Ca recovery between compost and commercial fertilizer were not found for the first crop of the season (Portuguese cabbage), but K and Mg recovery for P. cabbage increased for compost compared to commercial fertilizer (table 3.11).

Table 3.9 Apparent nutrient recovery (%) during the 1st year of crop rotation in potato and lettuce from green manure, 20 or 40 t ha^{-1} of compost and 1 or 2 t ha^{-1} of commercial fertilizer.

Crops	Nutrient	Green manure	Compost		Commercial fertilizer	
			20 t ha^{-1}	40 t ha^{-1}	1 t ha^{-1}	2 t ha^{-1}
Potato	N	0.0 b	10.4 ab	11.4 ab	30.3 ab	32.5 a
	P	0.0 b	12.0 ab	8.7 ab	0.8 ab	44.0 a
	K	4.7 a	3.8 a	3.3 a	0.0 a	29.5 a
	Ca	3.2 a	0.0 a	1.7 a	43.8 a	125.2 a
	Mg	0.0 a	0.0 a	1.7 a	0.6 a	23.2 a
Lettuce	N	0.0 a	13.3 a	6.9 a	0.0 a	0.7 a
	P	0.0 a	4.3 a	3.2 a	0.0 a	1.6 a
	K	0.0 a	3.6 a	2.1 a	2.4 a	4.3 a
	Ca	0.0 b	5.8 ab	3.5 ab	0.0 b	9.5 a
	Mg	0.0 a	2.6 a	1.4 a	0.2 a	4.2 a

Mean values followed by different letters within the same parameter are statistically different ($P<0.05$). Nutrient recovery = "0" means that there was no recovery.

For the second crop of the season (lettuce, turnip and carrot), there were no significant differences on nutrient recovery between treatments, except for occasional differences. Although, N recovery by lettuce, turnip and carrot apparently increased with compost compared to commercial fertilizer, no significant differences were found (table 3.9, 3.10 and 3.11).

Table 3.10 Apparent nutrient recovery (%) during the 2nd year of crop rotation in S chard and turnip from green manure, 20 or 40 t ha^{-1} of compost and 1 or 2 t ha^{-1} of commercial fertilizer.

Crops	Nutrient	Green manure	Compost		Commercial fertilizer	
			20 t ha^{-1}	40 t ha^{-1}	1 t ha^{-1}	2 t ha^{-1}
S. chard	N	0.0 c	18.7 b	7.0 bc	7.4 bc	38.4 a
	P	0.0 c	12.8 ab	8.3 bc	0.0 c	24.3 a
	K	0.0 b	49.1 ab	48.3 ab	61.0 ab	167.4 a
	Ca	6.7 b	0.0 b	2.0 b	32.6 a	41.2 a
	Mg	28.1 c	2.4 c	8.0 c	169.1 b	239.5 a
Turnip	N	0.0 a	40.7 a	41.9 a	37.2 a	24.6 a
	P	1.4 a	15.7 a	16.6 a	0.0 a	0.0 a
	K	15.0 a	46.7 a	50.8 a	0.0 a	0.0 a
	Ca	77.3 a	28.4 a	2.3 a	50.8 a	27.2 a
	Mg	62.2 a	16.4 a	2.9 a	31.3 a	37.4 a

Mean values followed by different letters within the same parameter are statistically different ($P<0.05$). Nutrient recovery = "0" means that there was no recovery.

Table 3.11 Apparent nutrient recovery (%) during the 3rd year of crop rotation in P. cabbage and carrot from green manure, 20 or 40 t ha^{-1} of compost and 1 or 2 t ha^{-1} of commercial fertilizer.

Crops	Nutrient	Green manure	Compost		Commercial fertilizer	
			20 t ha^{-1}	40 t ha^{-1}	1 t ha^{-1}	2 t ha^{-1}
P. cabbage	N	0.0 b	29.0 a	23.7 ab	36.8 a	37.1 a
	P	0.0 b	24.1 a	18.7 ab	32.4 a	35.5 a
	K	0.0 b	43.9 b	34.9 b	123.8 a	127.9 a
	Ca	0.0 c	38.9 abc	21.5 bc	120.4 a	106.4 ab
	Mg	0.0 b	17.2 b	10.4 b	93.1 a	95.2 a
Carrot	N	0.0 b	19.5 a	17.6 a	12.7 ab	6.2 ab
	P	0.0 a	16.6 a	15.4 a	16.7 a	10.2 a
	K	0.0 a	68.1 a	56.5 a	65.5 a	53.3 a
	Ca	2.3 a	5.4 a	7.1 a	8.3 a	2.5 a
	Mg	0.0 a	6.5 a	8.1 a	22.2 a	15.3 a

Mean values followed by different letters within the same parameter are statistically different ($P<0.05$). Nutrient recovery = "0" means that there was no recovery.

3.1.5 Nitrogen mineralization

Nitrogen mineralization, determined by field incubation, increased in fertilized treatments both with compost and with the commercial organic fertilizer compared with GM and T0. Mineralized N increased numerically for CF2 in comparison with all other treatments during the first crop of the season (potato, chard and cabbage), although mineralized N only increased significantly for CF2 compared to GM and T0 during potato and P. cabbage growth, respectively. The amount of mineralized N for C40 during the second crop of the season for lettuce (84 kg ha^{-1}), turnip (63 kg ha^{-1}) and carrot (166 kg ha^{-1}) was numerically above that found for treatment CF2 (60 kg ha^{-1}, 33 kg ha^{-1} and 149 kg ha^{-1} respectively). However, mineralized N just increased significantly for C40 compared to GM and C20 compared to T0 during the periods of lettuce and carrot growth, respectively. The mineralized N numerically decreased with GM compared to the unfertilized plots (T0) during the first two years of the crop rotation, except for Swiss chard, but the same was not true for the last year although significant differences were not detected (table 3.12).

Table 3.12 Accumulated mineralized N (kg ha^{-1}) during each crop growing season for green manure (GM), GM with 20 and 40 t ha^{-1} compost (C20 and C40) and GM with 1 or 2 t ha^{-1} organic fertilizer (CF1 and CF2) and for the control without fertilization (T0).

Year	crops	N mineralization (kg ha^{-1})											
		T0		GM		C20		C40		CF1		CF2	
1st year	Potato	104	ab	88	b	152	ab	160	ab	148	ab	201	a
	Lettuce	59	ab	49	b	62	ab	84	a	59	ab	60	ab
2nd year	S. chard	67	b	67	b	96	b	99	b	123	b	199	a
	Turnip	61	a	47	a	55	a	63	a	42	a	33	a
3rd year	P. cabbage	71	b	78	ab	112	ab	160	ab	142	ab	187	a
	Carrot	112	b	123	ab	179	a	166	ab	144	ab	149	ab

Mean values followed by different letters within the same parameter are statistically different ($P<0.05$). The mineralized N (kg ha^{-1}) was calculated based on a soil depth of 15 cm

The commercial organic fertilizer was rapidly mineralized as opposed to compost that was mineralized at a slower rate throughout the crop rotation. The daily N mineralization of commercial organic fertilizer in 2012, 2013 and 2014 is shown in the figures 3.6, 3.7 and 3.8, respectively.

In 2012, N mineralization of the commercial fertilizer in the first 7 days of incubation, for 1 and 2 t ha^{-1}, respectively, was 23% and 49% of the total N mineralized during the growth period of potato and lettuce. Consequently, mean daily N mineralization, during this period was 1.7 and 6.6 mg N kg^{-1} day^{-1} for CF1 and CF2, respectively. The application of 40 t ha^{-1} of compost caused an initial N immobilization during the potato growing season, promoting N remineralization for the last period of potato growth and during the lettuce growing period. Although, N immobilization occurred with the application of 40 t ha^{-1} compost, mean daily N mineralization during potato and lettuce growth was greater with 40 t ha^{-1} compost (0.41 mg N kg^{-1} day^{-1}) compared to the incorporation of 20 t ha^{-1} of compost (0.30 mg N kg^{-1} day^{-1}). On the contrary, the incorporation of green manure without other fertilizers promoted N immobilization (0.11 mg N kg^{-1} day^{-1}). During this year, a peak of N mineralization occurred between 77 and 91 days after incorporation of 20 and 40 t ha^{-1} compost and 1 and 2 t ha^{-1} commercial fertilizer (1.1; 1.8; 1.1 and 1.6 mg N kg^{-1} day^{-1}, respectively). Nitrogen immobilization also increased with green manure application (1.1 mg N kg^{-1} day^{-1}) during this period of incubation (figure 3.6).

In 2013, during the growth period of Swiss chard and turnip, N mineralization for 1 and 2 t ha^{-1} of the commercial fertilizer in the first week of incubation was 60% and 44%, respectively of the total N mineralized. Thus, mean daily N mineralization, during this period, was 3.3 and 5.7 mg N kg^{-1} day^{-1} for 1 and 2 t ha^{-1}, respectively. The application of 40 t ha^{-1} of compost caused an initial N immobilization during the Swiss chard growing season, likewise in the preceding year during the period of potato growth. Afterwards, N remineralization was promoted during the last period of Swiss chard and turnip growth. The mean daily N mineralization during the growth period of Swiss chard and turnip with the incorporation of 40 t ha^{-1} compost (0.32 mg N kg^{-1} day^{-1}) was also greater compared to the incorporation of 20 t ha^{-1} compost (0.24 mg N kg^{-1} day^{-1}) as it occurred in 2012. On this year, the incorporation of green manure promoted a lower N immobilization (0.02 mg N kg^{-1} day^{-1}) compared to the preceding year (figure 3.7).

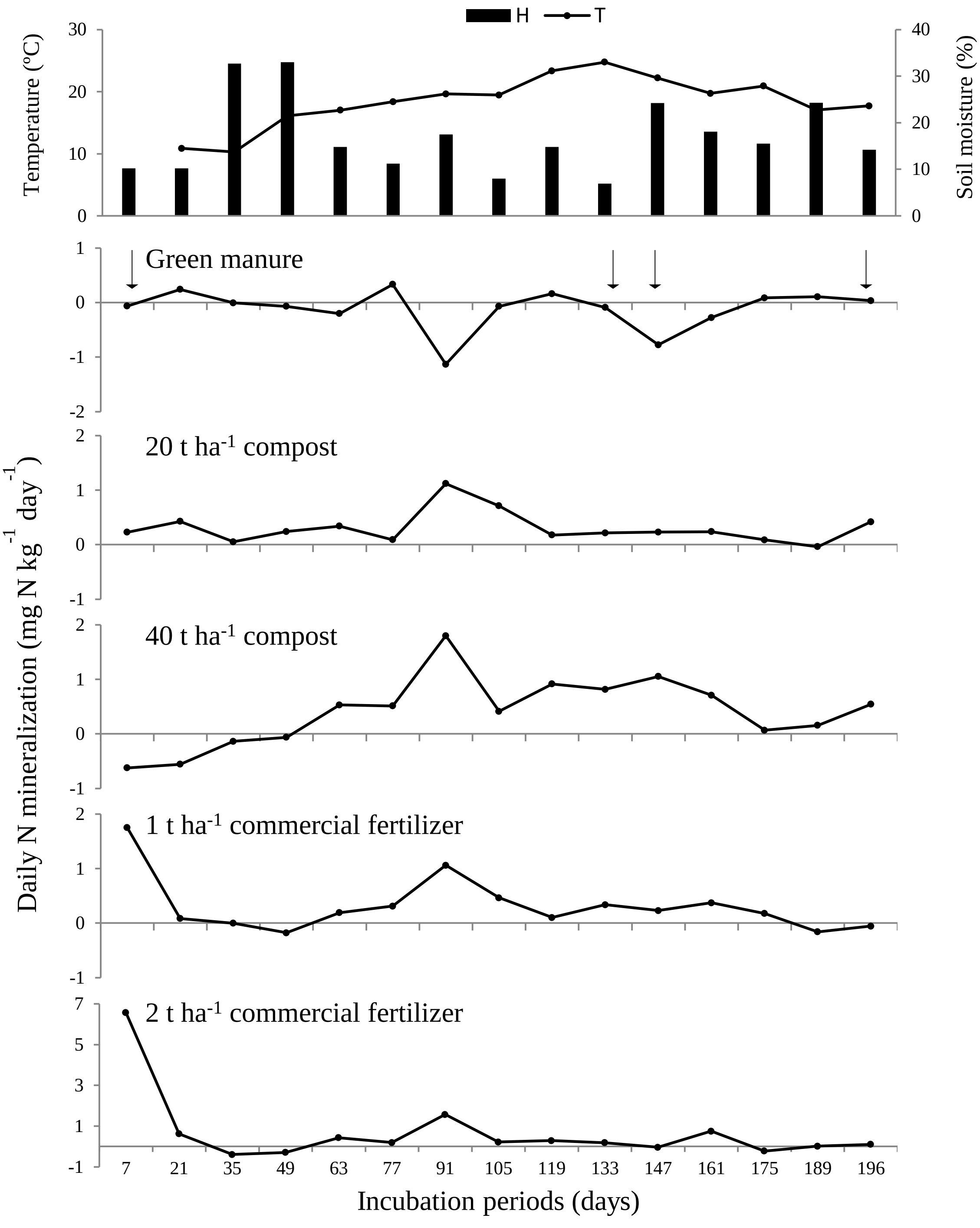

Figure 3.6 Mean temperature (ºC), soil moisture (%) and mean daily N mineralization (mg N kg^{-1} day^{-1}) during the growing seasons of potato and lettuce (from 28th March to 10th October 2012). The arrows indicate the beginning and the end of each crop growing season.

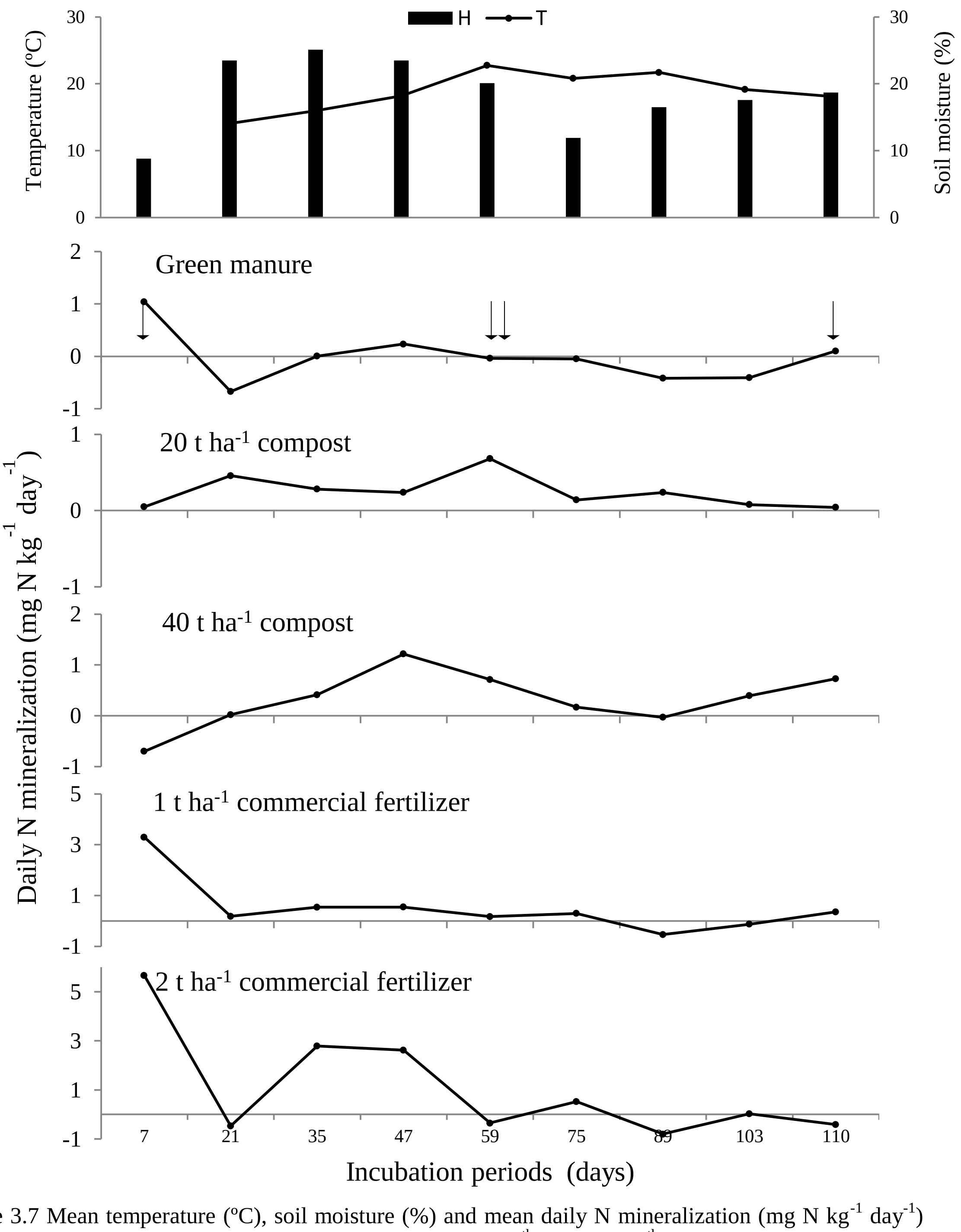

Figure 3.7 Mean temperature (ºC), soil moisture (%) and mean daily N mineralization (mg N kg^{-1} day^{-1}) during the growing seasons of S. chard and turnip (from 7th May to 25th August 2013). The arrows indicate the beginning and the end of each crop growing season.

In 2014, N mineralization for 1 and 2 t ha^{-1} of the commercial fertilizer, respectively, in the first 7 days of incubation was 30% and 44% during the growth period of Portuguese cabbage and carrot. Consequently, mean daily N mineralization, during this period was 2.7 and 6.4 mg N kg^{-1} day^{-1} for 1 and 2 t ha^{-1}, respectively. During this year, the event of N immobilization with the application of 40 t ha^{-1} compost did not occur and mean daily N mineralization increased to 0.58 mg N kg^{-1} day^{-1}. Nitrogen mineralization increased with the application of 20 t ha^{-1} compost (0.41 mg N kg^{-1} day^{-1}) during this year compared to the first and second years of crop rotation (0.30 and 0.24 mg N kg^{-1} day^{-1} respectively). The incorporation of green manure caused an initial low N immobilization but net N mineralization increased during Portuguese cabbage and carrot growth (0.05 mg N kg^{-1} day^{-1}). A peak of N mineralization occurred about 4 months after incorporation of 20 and 40 t ha^{-1} compost and 1 and 2 t ha^{-1} commercial fertilizer (1.1, 0.45, 0.33 and 0.83 mg N kg^{-1} day^{-1} respectively). N immobilization also increased with green manure application (0.68 mg N kg^{-1} day^{-1}) during this period of incubation (figure 3.8).

A nitrogen mineralization model was fitted to the results in order to better understand N mineralization throughout crop rotation (figure 3.9; table 3.13). The Pearson test was performed to find the correlation between the N mineralization values measured in the laboratory and the values calculated by the models. The correlation was always positive with a probability level of $P<0.01$.

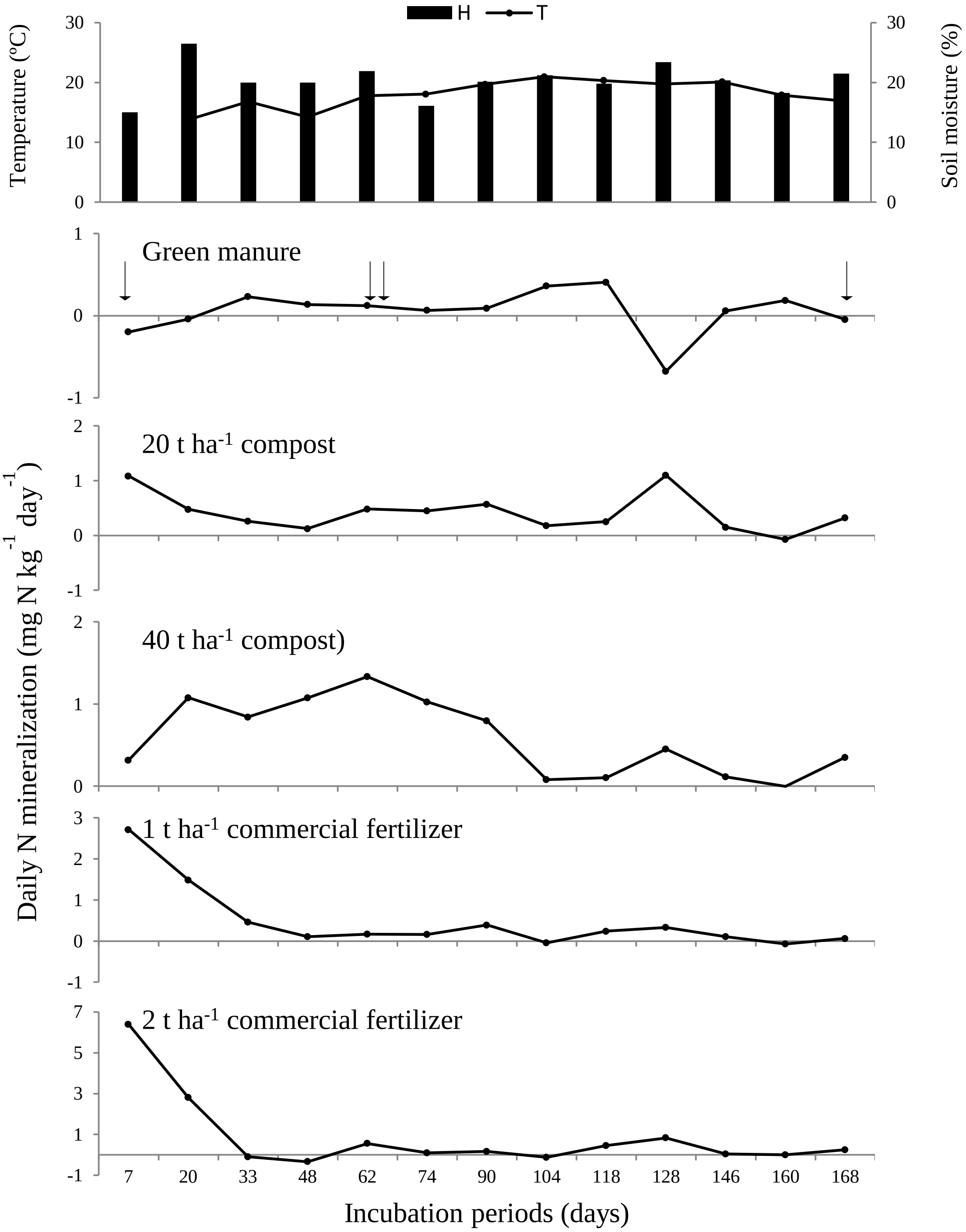

Figure 3.8 Mean temperature (ºC), soil moisture (%) and mean daily N mineralization (mg N kg^{-1} day^{-1}) during the growing seasons of P. cabbage and carrot (from 16th April to 1st October 2014). The arrows indicate the beginning and the end of each crop growing season.

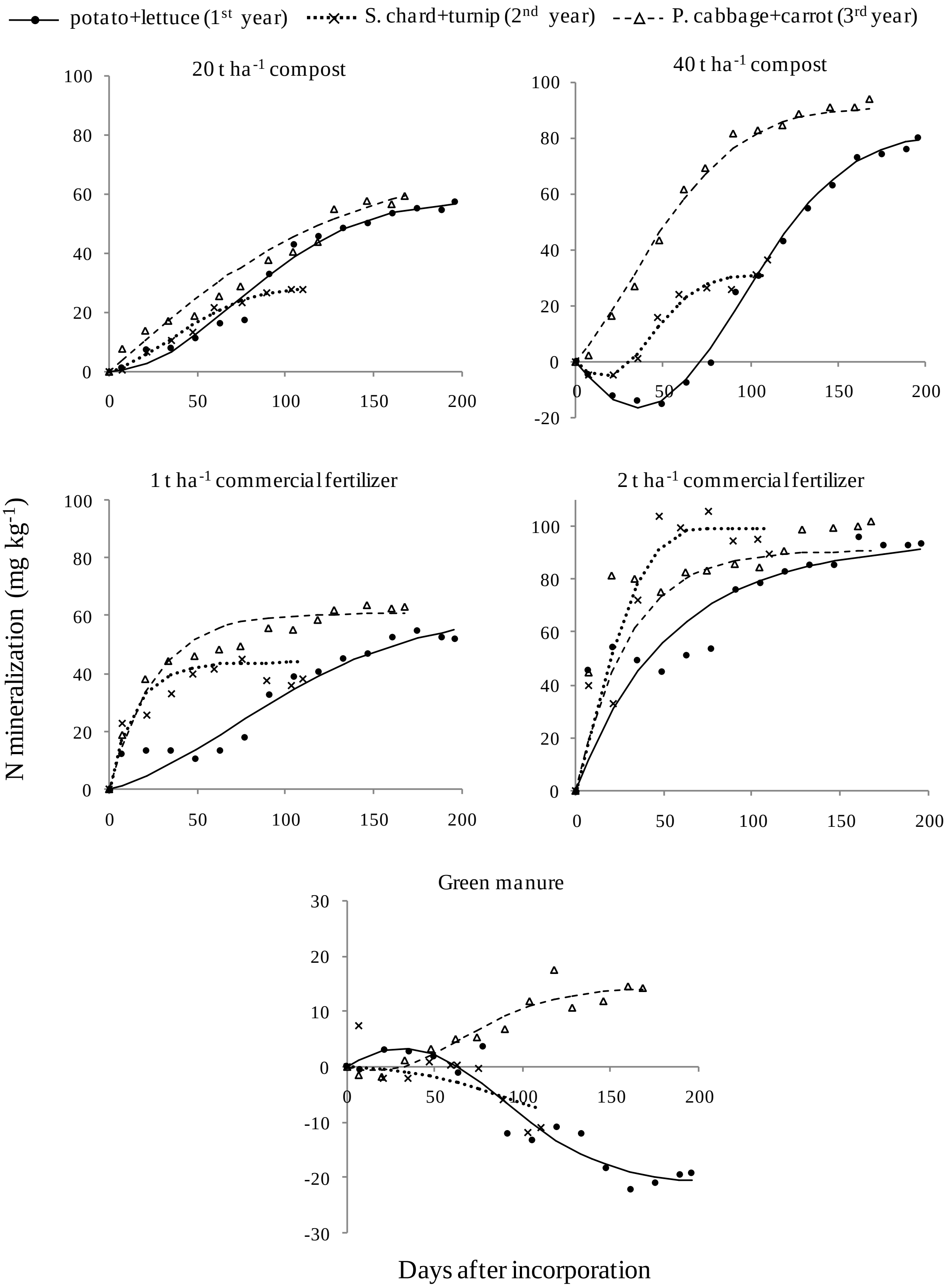

Figure 3.9 Accumulated apparent mineralized N (mg kg^{-1}) from compost, commercial fertilizer, and green manure during the incubation periods for the three years of crop rotation. N mineralization equations are described on table 3.13.

Table 3.13 Apparent N mineralization equations during the growing seasons, for compost, commercial fertilizer and green manure.

Year	Organic fertilizers	Model equation	R^2	P
1st year	20 t ha^{-1} compost	N_m=58 [1-exp(-0.0001t-0.0001t^2)]	0.98	P<0.001
	40 t ha^{-1} compost	N_m=80 [1-exp(0.01t-0.00014t^2)]	0.99	P<0.001
	1 t ha^{-1} fertilizer	N_m=60 [1-exp(-0.003t- 0.00005t^2)]	0.94	P<0.001
	2 t ha^{-1} fertilizer	N_m=101 [1-exp(-0.018t-0.000031t^2)]	0.82	P<0.001
	Green manure	N_m=-21 [1-exp(-0.0091t-0.00015t^2)]	0.91	P<0.001
2nd year	20 t ha^{-1} compost	N_m=30 [1-exp(-0.005t-0.00025t^2)]	0.99	P<0.001
	40 t ha^{-1} compost	N_m=36 [1-exp(0.022t-0.0007t^2)]	0.97	P<0.001
	1 t ha^{-1} fertilizer	N_m=44 [1-exp(-0.068t-0.000006t^2)]	0.97	P<0.001
	2 t ha^{-1} fertilizer	N_m=99 [1-exp(-0.023t-0.0006 t^2)]	0.96	P<0.001
	Green manure	N_m=-20 [1-exp(-0.00001t-0.00004 t^2)]	0.70	P<0.01
3rd year	20 t ha^{-1} compost	N_m=70 [1-exp(-0.008t-0.00002t^2)]	0.97	P<0.001
	40 t ha^{-1} compost	N_m=91 [1-exp(-0.0085t-0.00013t^2)]	0.99	P<0.001
	1 t ha^{-1} fertilizer	N_m=61 [1-exp(-0.04t-0.000003t^2)]	0.97	P<0.001
	2 t ha^{-1} fertilizer	N_m=91 [1-exp(-0.034t-000006t^2)]	0.94	P<0.001
	Green manure	N_m=14 [1-exp(0.0064t-0.0002 t^2)]	0.91	P<0.001

N mineralization from compost and green manure was calculated by one mineralization pool equation $N_m = N_0$ [1-exp(-k_1t-k_2t^2)], where N_m (mg kg^{-1}) represents the accumulated mineralized N; k_1 and k_2 are mineralization constant rates; and N_0 (mg kg^{-1}) represents the amount of potentially mineralizable N.

In the 1st year of crop rotation the application of 40 t ha^{-1} of compost caused an initial N immobilization for 71 days (-k_1/k_2) during the potato crop growing season, but the accumulated mineralized N (N_m) (80 mg kg^{-1}) increased compared to the application of 20 t ha^{-1} compost (57 mg kg^{-1}) during the growth period of potato and lettuce (196 days). In the 2nd year the application of 40 t ha^{-1} compost caused N immobilization for 31 days (-k_1/k_2) during the growth period of S. chard, but Nm (31 mg kg^{-1}) increased compared to the application of 20 t ha^{-1} compost (28 mg kg^{-1}) during the growth period of S. chard and turnip (110 days). In the 3rd year of the crop rotation N immobilization did not occur and N_m during the growth period of cabbage and carrot (168 days) with the incorporation of 20 and 40 t ha^{-1} compost was 60 and 90 mg kg^{-1} respectively (figure 3.9; table 3.14).

The amount of N_m for the first crop in each season (potato, chard and cabbage) was largest with the addition of 2 t ha^{-1} of commercial fertilizer, whereas the amount of N_m

for the second crop of the season (lettuce, turnip and carrot) was largest with the addition of 40 t ha^{-1} of compost (table 3.14). The amount of N_m for potato (85 mg kg^{-1}), chard (97 mg kg^{-1}) and cabbage (80 mg kg^{-1}) was 842 g kg^{-1}, 976 g kg^{-1} and 881 g kg^{-1} respectively, of the potentially mineralizable N (N_0) with the application of 2 t ha^{-1} of organic fertilizer. The amount of N_m for lettuce (23 mg kg^{-1}), turnip (10 mg kg^{-1}) and carrot (32 mg kg^{-1}) with the application of 40 t ha^{-1} of compost was 278 g kg^{-1}, 318 g kg^{-1} and 352 g kg^{-1} of the N_0, respectively. Whereas, the amount of N_m for lettuce (6 mg kg^{-1}), turnip (2 mg kg^{-1}) and carrot (11 mg kg^{-1}) with the application of 2 t ha^{-1} of commercial fertilizer was 61 g kg^{-1}, 24 g kg^{-1} and 116 g kg^{-1} of the N_0, respectively. Importantly, the amount of potentially mineralizable N was always greater for the first crop in each season compared to the second crop. However, the amount of potentially mineralizable N was always greater with compost compared to commercial fertilizer in the second crop of each season, except for lettuce in the first year crop rotation.

The model for green manure (figure 3.9) shows that in the 1st and 2nd years of crop rotation, green manure incorporation into the soil caused N immobilization. The amount of N_m during the growth period of potato and lettuce (196 days) was -20.6 mg N kg^{-1} and during the growth period of Swiss chard and turnip (110 days) was -7.7 mg N kg^{-1}. On the contrary, in the 3rd year of crop rotation, the green manure, after an initial short period of N immobilization of 32 days ($-k_1/k_2$) after soil application, increased net N mineralization. The amount of N_m during the growth period of P. cabbage and carrot (168 days) was 13.9 mg N kg^{-1}.

At the end of each year of the crop rotation the potentially mineralizable N (N_0) of both compost and commercial organic fertilizer was residual because the mineralized N (N_m) was greater than 852 g kg^{-1} of N_0 (table 3.14).

The N mineralization rate was always larger for commercial organic fertilizer (between 705 and 953 g kg^{-1} initial N) in comparison to compost (between 220 and 750 mg kg^{-1} initial N). Nitrogen mineralization rate with compost application was smaller during the chard and turnip growth period of 110 days (354 g kg^{-1} initial N and 195 g kg^{-1} initial N for 20 and 40 t ha^{-1} compost, respectively) in comparison to N mineralization rates during the 1st and 3nd years with longer crop growth periods, 196 and 168 days, respectively. The proportion of mineralized N decreased with the increasing amount of compost application rate. The mineralized organic N decreased with the incorporation of 40 t ha^{-1} compared to 20 t ha^{-1} compost application from 750 to 526 g kg^{-1} initial N, 354 to 195 g kg^{-1} initial N and 674 to 503 g kg^{-1} initial N, for the 1st, 2nd, 3rd year of crop rotation, respectively (table 3.14).

Table 3.14 Accumulated mineralized N (mg kg^{-1}), proportion of potentially mineralizable N that was mineralized, organic N applied (mg kg^{-1}) and N mineralization rate (g kg^{-1} initial N) during the growing seasons for compost and commercial fertilizer.

Organic fertilizers	Accumulated mineralized N (mg kg^{-1})		Proportion of potentially mineralizable N* (g kg^{-1})		N_{org} applied (mg kg^{-1})	N mineralization rate** (g kg^{-1} initial N)
1st year	Potato	Lettuce	Potato	Lettuce	Potato+Lettuce	
20 t ha^{-1} compost	48	9	832	147	76	750
40 t ha^{-1} compost	57	23	692	278	152	526
1 t ha^{-1} c. fertilizer	43	12	721	196	64	859
2 t ha^{-1} c. fertilizer	85	6	842	61	128	711
2nd year	Chard	Turnip	Chard	Turnip	Chard+Turnip	
20 t ha^{-1} compost	20	8	685	220	79	354
40 t ha^{-1} compost	21	10	680	318	159	195
1 t ha^{-1} c. fertilizer	43	1	982	17	55	800
2 t ha^{-1} c. fertilizer	97	2	976	24	110	900
3rd year	Cabbage	Carrot	Cabbage	Carrot	Cabbage+Carrot	
20 t ha^{-1} compost	31	29	436	416	89	674
40 t ha^{-1} compost	58	32	642	352	179	503
1 t ha^{-1} c. fertilizer	56	5	917	81	64	953
2 t ha^{-1} c. fertilizer	80	11	881	116	129	705

* The proportion of potentially mineralizable N (N_0) that was mineralized (g kg^{-1}) during the growth period of each crop is calculated by the rate between accumulated mineralized N (Nm) from compost or commercial fertilizer and potentially mineralizable N (N_0) calculated by the model during the N mineralization period (in MM: 2.8 Statistical analysis and kinetic models). ** N mineralization rate (g kg^{-1} initial N) is calculated by the quotient between the accumulated mineralized N (Nm) from compost or commercial fertilizer during the growth period of each crop and the total amount of N_{org} applied during the N mineralization period (in MM: 2.4 Field incubation).

3.1.6 Soil characteristics

At the end of the crop rotation, there were no significant differences between treatments among the amounts of total soil C and N. The amount of extractable P and K increased in the treatment C40 compared to all the other treatments (P<0.05). The EC was not different between C40 (0.038 dS m^{-1}) and CF2 (0.036 dS m^{-1}). However, the EC increased for treatment C40 compared with T0, GM, C20 and CF1.

Differences between the beginning and the end of the crop rotation for C40 were not significant for C, total N or extractable P and K contents. There were also not significant differences in EC between the beginning (0.032 dS m^{-1}) and the end of the crop rotation (0.038 dS m^{-1}) after three years of 40 t ha^{-1} compost and green manure application. The pH value increased from 4.9 to 6.1, three years after dolomite lime application at the rate of 5 t ha^{-1} in the beginning of the crop rotation (table 3.15).

Table 3.15 Soil chemical characteristics at the beginning and the end of the crop rotation for the control treatment (T0) without soil amendments and in response to green manure (GM), GM and 20 or 40 t ha^{-1} of compost (C20 and C40), GM and 1 or 2 t ha^{-1} of commercial organic fertilizer (CF1 and CF2).

		pH (H_2O)	EC	C	N	Extractable phosphorus (P_2O_5)*	Extractable potassium (K_2O)*
			(dS m^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
Beginning		4.9	0.032	29.0	1.9	208	215
End	T0	6.2 a	0.032 b	29.1 a	1.4 a	133 c	124 c
	GM	6.1 a	0.030 b	29.6 a	1.4 a	135 c	131 c
	C20	6.1 a	0.032 b	31.8 a	1.5 a	152 b	184 b
	C40	6.2 a	0.038 a	32.4 a	1.6 a	173 a	272 a
	CF1	6.1 a	0.030 b	31.7 a	1.5 a	144 bc	133 c
	CF2	6.1 a	0.036 a	31.8 a	1.4 a	129 c	134 c

*Egner-Riehm method. Mean values followed by different letters within the same parameter are statistically different ($P<0.05$).

The average soil mineral N content increased for the first season crops, potato (48.9 kg ha^{-1}), S. chard (85.7 kg ha^{-1}) and P. cabbage (74.5 kg ha^{-1}) in the CF2 treatment compared to all other treatments (16.4 to 33.9 kg ha^{-1}, 23.4 to 61.4 kg ha^{-1} and 21.3 to 43.4 kg ha^{-1} respectively) (figure 3.10). In contrast, the mean soil mineral N content was not significantly different between treatments during the second season crops, lettuce (20.5 to 28.0 kg ha^{-1}) and turnip (33.8 to 50.8 kg ha^{-1}) but increased in C40 for carrot (37.2 kg ha^{-1}) in comparison to the remaining treatments (21.1 to 28.1 kg ha^{-1}).

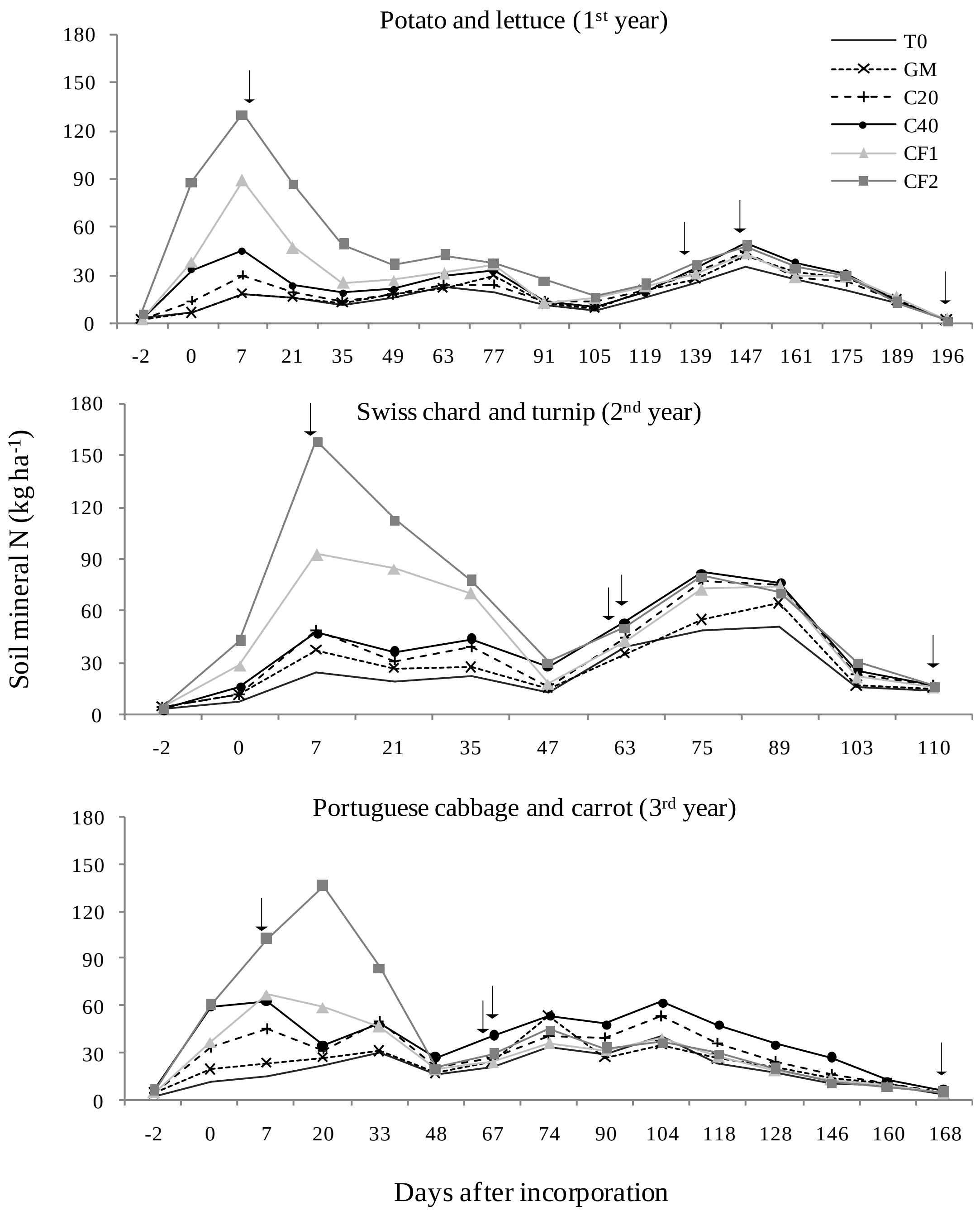

Figure 3.10 Soil mineral N (kg ha^{-1}) content during the horticultural crop rotation for the green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), GM with 1 or 2 t ha^{-1} of commercial organic fertilizer (CF1 and CF2), and the unfertilized control treatment (T0). The arrows represent the beginning and the end of crop growing seasons.

Soil mineral N increased fast in the first 7 days in CF1 and CF2 treatments for potato and S. chard. Soil mineral N also increased fast in the first 7 days in CF1, and in the first 20 days in CF2 for Portuguese cabbage. Then, soil mineral N content decreased in CF1 and CF2 for potato between 7 and 35 days after commercial organic fertilizer incorporation (from 89.2 to 25.1 kg ha^{-1} for CF1 and from 130.8 to 49.2 kg ha^{-1} for

CF2), for Swiss chard between 7 and 47 days after organic fertilizer incorporation (from 92.5 to 17.7 kg ha^{-1} for CF1 and from 158.1 to 30.4 kg ha^{-1} for CF2) and during the cabbage between 20 and 48 days after organic fertilizer incorporation (from 59.0 to 19.7 kg ha^{-1} for CF1and from 136.0 to 20.5 kg ha^{-1}, respectively for CF2).

3.2 Pot experience

3.2.1 Soil chemical characteristics at the beginning of the pot experience

At the beginning of the pot experience there were no significant differences between treatments for pH, EC, total C and N. However, the amount of extractable P and K increased for treatments C20 and C40 in comparison to the other treatments.

Table 3.16 Soil chemical characteristics at the beginning of the pot experience for the control treatment (T0) without soil amendments and in response to green manure (GM), GM and 20 or 40 t ha^{-1} of compost (C20 and C40), GM and 1 or 2 t ha^{-1} of commercial organic fertilizer (CF1 and CF2).

	pH (H_2O)	EC	C	N	Extractable phosphorus (P_2O_5)*	Extractable potassium (K_2O)*
		(dS m^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
T0	6.3 a	0.032 a	29.5 a	2.2 a	197 b	164 c
GM	6.0 a	0.033 a	29.2 a	2.3 a	203 b	171 c
C20	6.2 a	0.031 a	29.0 a	2.2 a	259 b	255 b
C40	6.3 a	0.043 a	28.9 a	2.1 a	254 a	366 a
CF1	6.1 a	0.034 a	30.4 a	2.3 a	203 b	163 c
CF2	6.3 a	0.037 a	30.5 a	2.3 a	206 b	177 c

*Egner-Riehm method. Mean values followed by different letters within the same parameter are statistically different (P<0.05).

3.2.2 Lettuce fresh weight

Lettuce fresh weight 19 days after plantation was increased (P<0.05) with compost compared to the remaining treatments, including those fertilized with green manure and commercial fertilizer. Although these growth differences become less clear 30 days after planting, final lettuce growth was significantly enhanced with compost application compared to the unfertilized treatment or lettuce grown only with green manure (figure 3.11). Lettuce yield with the organic fertilizer was not significantly lower compared to lettuce yield with compost application, but it was also not significantly enhanced compared to unfertilized lettuce or lettuce grown with green manure.

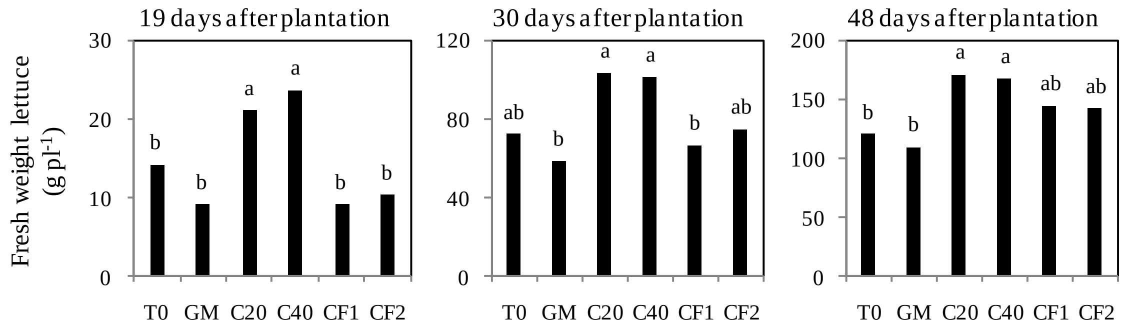

Figure 3.11 Fresh weight (g pl^{-1}) in organic lettuce for the control treatment (T0) and for the treatments with green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

The DM content of lettuce was not significantly different between treatments 19, 30 and 48 days after plantation. However, dry matter content increased lightly along the growth period of lettuce from 5% to 6% 19 days after plantation to 6% to 8% 48 days after plantation (table 3.17)

Table 3.17 Dry weight (DW) and dry matter content (DM) in organic lettuce for the control treatment (T0) and for the treatments with green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

Treatments	19 days after plantation		30 days after plantation		48 days after plantation	
	DW (g pl^{-1})	DM (%)	DW (g pl^{-1})	DM (%)	DW (g pl^{-1})	DM (%)
0	0.9 b	6,1 a	4,9 ab	6,6 a	7,7 b	6,4 a
GM	0.5 b	5,9 a	4,2 b	6,8 a	6,8 b	6,1 a
C20	1.2 a	5,9 a	7,6 a	7,2 a	12,7 a	7,5 a
C40	1,4 a	5,8 a	5,9 a	5,8 a	11,2 a	6,7 a
CF1	0,4 b	4,8 a	4,3 b	6,4 a	10,2 ab	7,1 a
CF2	0,6 b	5,2 a	4,6 ab	6,2 a	10,3 ab	7,5 a

Mean values followed by different letters within the same parameter are statistically different ($P<0.05$).

3.2.3 Nutrient content

Nutrient content in lettuce shoots was not significantly different between experimental treatments, except for occasional differences on N content between T0, GM and CF2. As expected, N and K contents in lettuce shoots were considerably higher compared to the other macronutrients (table 3.18).

Table 3.18 Nutrient content (g kg^{-1}) of organic lettuce for the control treatment (T0) and for the treatments with green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

Nutrient	T0	GM	C20	C40	CF1	CF2
N	15.5 b	22.5 a	18.9 ab	17.7 ab	18.2 ab	22.1 a
P	3.3 a	2.7 a	3.3 a	3.3 a	3.0 a	3.1 a
K	15.9 a	17.1 a	17.5 a	13.6 a	18.1 a	16.6 a
Ca	9.1 a	8.8 a	9.2 a	8.2 a	8.8 a	8.8 a
Mg	2.4 a	3.6 a	2.7 a	2.2 a	2.5 a	2.9 a

Mean values followed by different letters within the same parameter are statistically different ($P<0.05$).

3.2.4 Nutrient accumulation

Nitrogen accumulation was not significantly different with compost or commercial fertilizer application, but N accumulation increased with CF2 and C20 in comparison to T0 or GM. There were also no significant differences for P accumulation with compost or 2 t ha^{-1} commercial fertilizer, but P accumulation increased with compost compared to the unfertilized treatment or green manure application. Potassium accumulation was not significantly different with 20 t ha^{-1} compost or commercial fertilizer, but increased for C20 compared to T0 and GM. Calcium and Mg accumulation was not significantly different with compost or commercial fertilizer application. However, Ca accumulation increased with compost and commercial fertilizer application compared to green manure application and Mg accumulation was enhanced by compost and commercial fertilizer application compared the control treatment (table 3.19).

Table 3.19 Nutrientaccumulation (mg pl^{-1}) in organic lettuce for the control treatment (T0) and for the treatments with green manure (GM), GM with 20 or 40 t ha^{-1} of compost (C20 and C40), and GM with 1 or 2 t ha^{-1} of commercial fertilizer (CF1 and CF2).

Nutrient	T0	GM	C20	C40	CF1	CF2
N	117.0 b	145.8 b	234.0 a	197.5 ab	185.8 ab	232.9 a
P	25.3 cd	17.4 d	40.6 a	36.1 ab	30.8 bc	32.4 abc
K	122.3 b	115.9 b	228.1 a	151.8 b	182.6 ab	171.3 ab
Ca	69.0 bc	59.1 c	114.3 a	90.9 ab	88.3 ab	90.2 ab
Mg	17.9 b	21.1 ab	33.5 a	23.7 ab	25.0 ab	30.8 ab

Mean values followed by different letters within the same parameter are statistically different ($P<0.05$).

3.3 Laboratory incubation

3.3.1 Carbon dynamics

The use of compost, commercial fertilizer and green manure increased the rate of CO_2 emission from the soil. The mean mineralization rate of CO_2 for the control treatment (T0), and for the treatments with compost (C) (applied at the rate of 7.5 g kg^{-1}), commercial fertilizer (CF) (applied at the rate of 1.4 g kg^{-1}), green manure (GM) (applied at the rate of 3.9 g kg^{-1}), farmyard manure compost with green manure (CGM) and commercial organic fertilizer with green manure (CFGM) were 8.9, 10.3, 12.8, 29.4, 32.1 and 34.6 mg CO_2-C kg^{-1} day^{-1}, respectively in 2013 and 9.0, 10.5, 14.7, 26.7, 29.7 and 33.6 mg CO_2-C kg^{-1} day^{-1}, respectively in 2014. For all treatments, the maximum release of CO_2 evolved occurred in the first day of incubation (figures 3.12 and 3.13). The flush of CO_2 was extremely high with green manure treatments compared to the other treatments. In 2013, the amount of CO_2 release in the first day of incubation was 221 mg CO_2-C kg^{-1} day^{-1} for GM, 238 mg CO_2-C kg^{-1} day^{-1} for CGM and, 283 mg CO_2-C kg^{-1} day^{-1} for CFGM. In contrast, the amount of CO_2 release in the first day of incubation was 24, 41 and 61 mg CO_2-C kg^{-1} day^{-1} for T0, C and CF, respectively (figure 3.12).

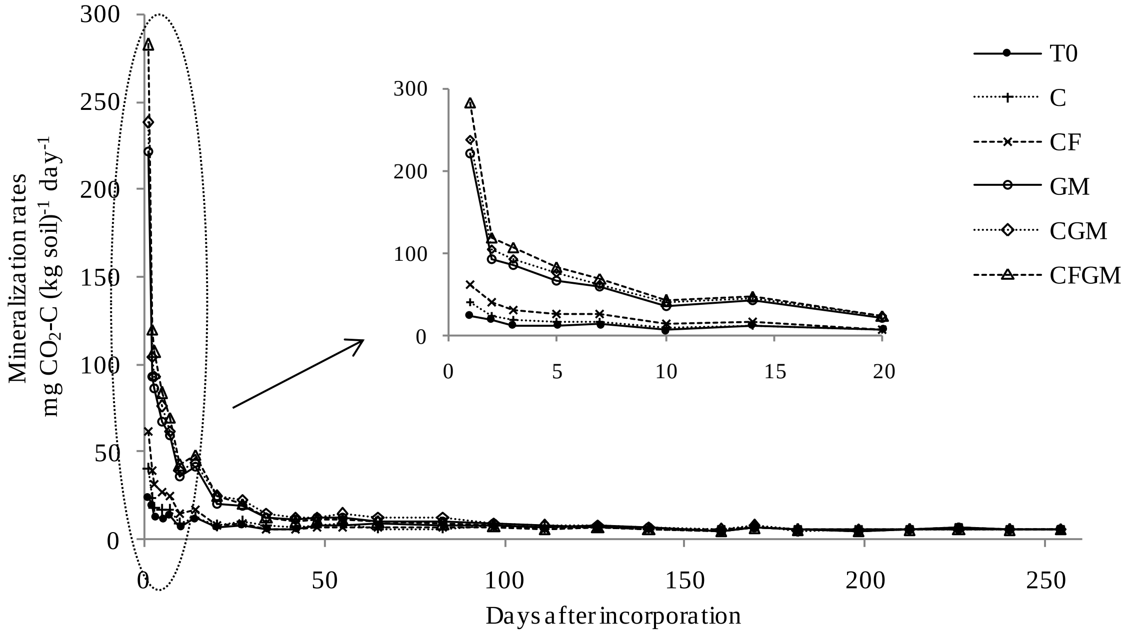

Figure 3.12 C mineralization rates (mg CO_2-C (kg soil)$^{-1}$ day^{-1}) for the control treatment (T0) and for the treatments with compost (C), commercial fertilizer (CF), green manure (GM), compost with green manure (CGM) and commercial organic fertilizer with green manure (CFGM) incorporated into the soil during the 2nd year crop rotation (2013).

In 2014, the flush of CO_2 in the first day of incubation increased slightly compared to the flush of CO_2 in the first day of incubation in 2013. The fast release of CO_2 in the first day of incubation was 231, 253 and 292 mg CO_2-C kg^{-1} day^{-1} for GM, CGM and CFGM, respectively. Similarly to 2013, the CO_2 released from the control treatment and composts greatly decreased compared to the CO_2 released from green manure treatments. The flush of CO_2 in the first day of incubation was 27, 39 and 71 mg CO_2-C kg^{-1} day^{-1} for T0, C and CF, respectively (figure 3.13).

After the initially high mineralization rate, CO_2-C mineralization rate decreased in all treatments before it became fairly constant (approximately 7 mg CO_2-C (kg soil)$^{-1}$ day-^{1}). The CO_2-C mineralization rate became constant earlier with compost or commercial fertilizer compared to GM. CO_2 release became fairly constant approximately 20 days after the beginning of the incubation for T0, C and CF (aproximatelly below 7 mg CO_2-C (kg soil)$^{-1}$ day-^{1}) and about 80 days for GM, CGM and CFGM (aproximatelly below 9 mg CO_2-C (kg soil)$^{-1}$ day-^{1})

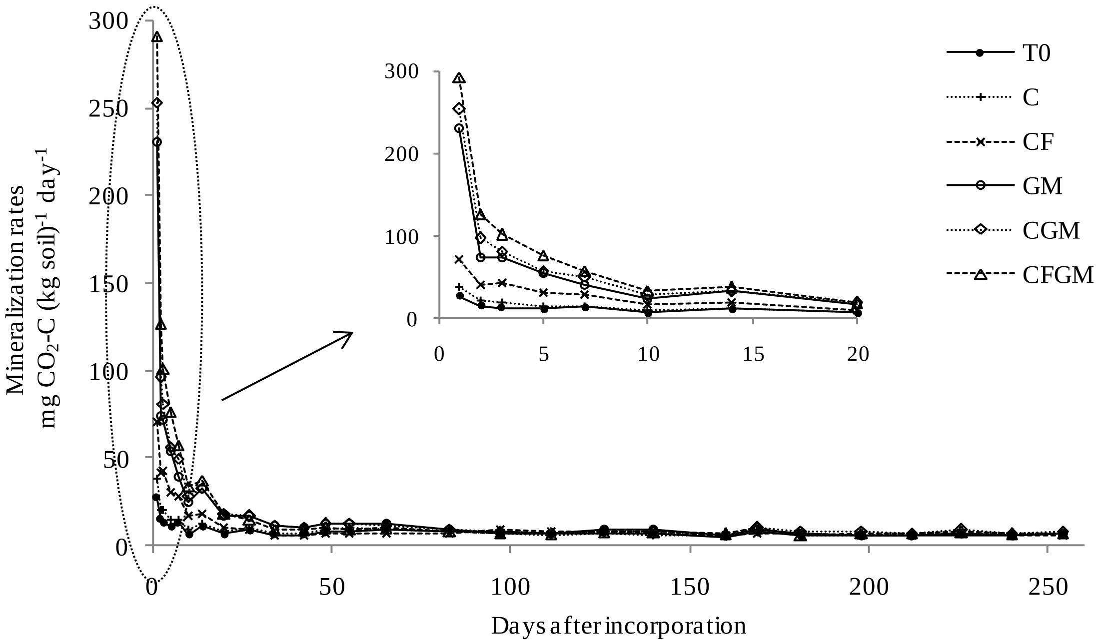

Figure 3.13 C mineralization rates (mg CO_2-C (kg soil)$^{-1}$ day^{-1} for the reference treatment (T0) and for the treatments with compost (C), commercial fertilizer (CF), green manure (GM), compost with green manure (CGM) and commercial organic fertilizer with green manure (CFGM) incorporated into the soil during the 3rd year crop rotation (2014).

The CO_2-C evolution was well described by one mineralization pool first-order kinetic model (figures 3.14 and 3.15; table 3.20). The Pearson test was performed to find the correlation between the C mineralization values measured in the laboratory and the values calculated by the models. The correlation was always positive with a probability level of P<0.001. The R^2 values were between 0.87 and 0.99 (P<0.001) except for the commercial fertilizer in 2013 which R^2 value was 0.65 (P<0.001) because of an unexpected drop on CO_2-C evolved on samples between 48 and 148 days after the beginning of incubation (figure 3.14a; table 3.20).

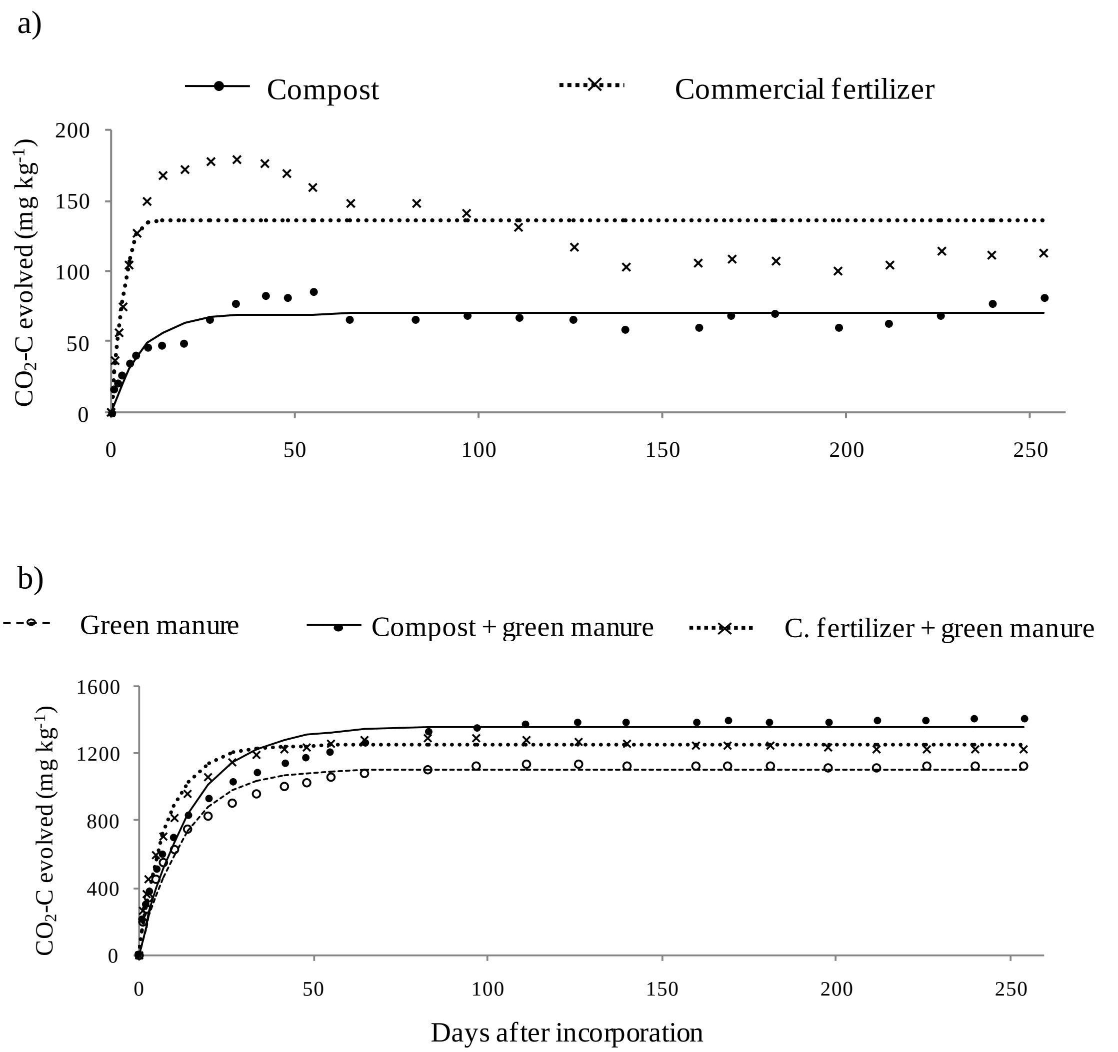

Figure 3.14 The apparent accumulated CO_2-C (mg (kg soil)$^{-1}$) evolved from (a) compost and commercial fertilizer and evolved from (b) green manure, compost with green manure and commercial fertilizer with green manure incorporated into the soil during the 2nd year crop rotation (2013). N mineralization equations are described on table 3.18.

The rates of apparent CO_2 evolution decreased with time in all organic fertilizers and practically ceased with time. Therefore, the accumulated CO_2-C mineralized (Cm) until the end of the incubation was equal to the potentially mineralizable carbon of the pool C_0. In 2013 the highest accumulation of CO_2-C was always found for treatments with green manure. The accumulated CO_2-C evolved increased for green manure (1105 mg kg^{-1}),compost with green manure (1360 mg kg^{-1}) and commercial fertilizer with green manure (1248 mg kg^{-1}) compared to compost (70 mg kg^{-1}) or commercial fertilizer (136 mg kg^{-1}) (figure 3.14; table 3.21). CO_2 evolution for compost and commercial fertilizer almost stopped after 55 and 20 days of incubation, respectively. CO_2 evolution from green manure decomposition increased at a slower rate compared to CO_2 evolution from compost or commercial fertilizer. The accumulated CO_2-C from OM mineralization was identical to the potentially mineralizable C for commercial fertilizer with green manure, green manure, and compost with green manure approximately 65, 97 and 126 days, respectively after the beginning of the incubation.

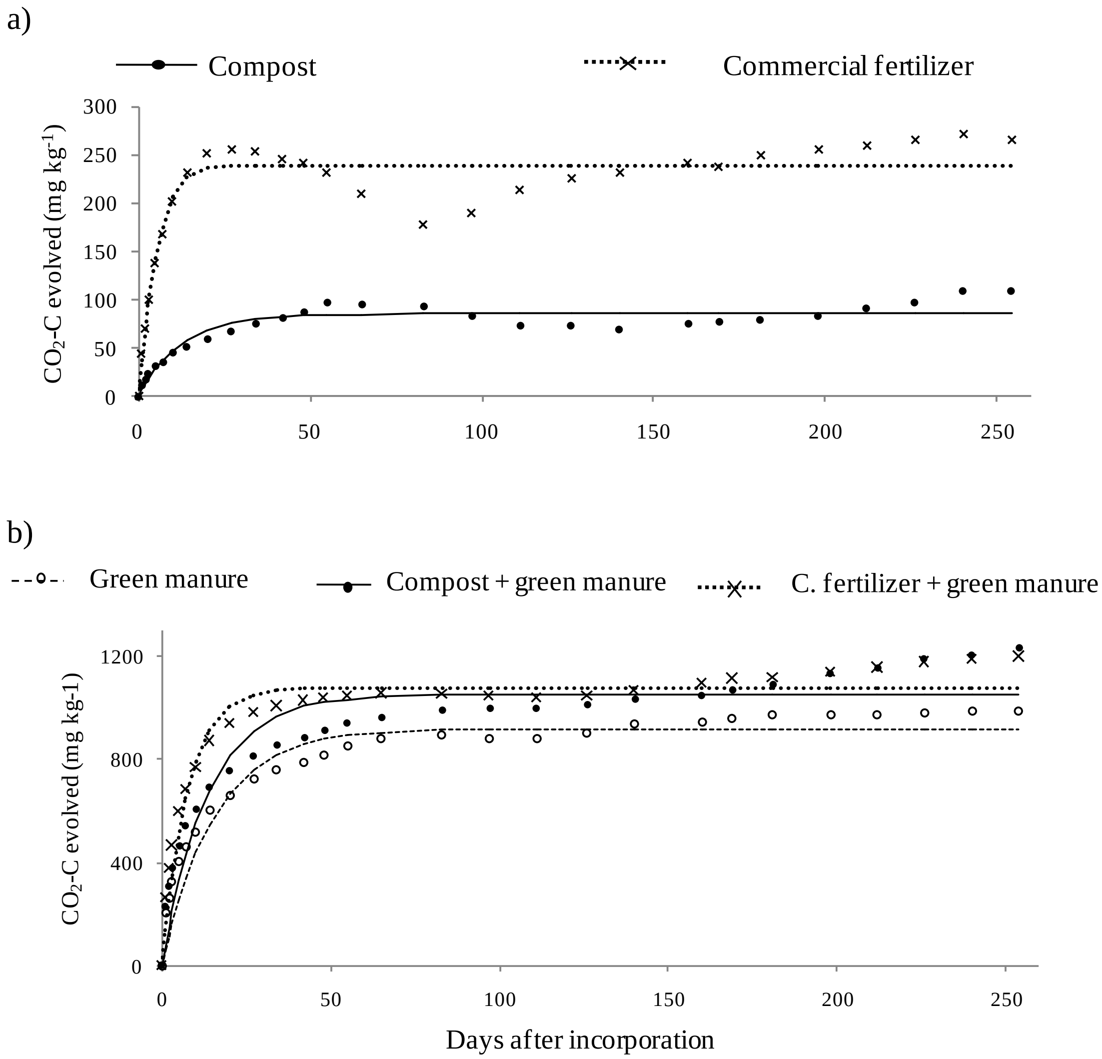

Figure 3.15 The apparent accumulated CO_2-C (mg (kg soil)$^{-1}$) evolved from (a) compost and commercial fertilizer and evolved from (b) green manure, compost with green manure and commercial fertilizer with green manure incorporated into the soil during the 3rd year crop rotation (2014). N mineralization equations are described on table 3.18.

In the year of 2014 the accumulated CO_2-C also increased for green manure (918 mg kg^{-1}), compost with green manure (1050 mg kg^{-1}) and commercial fertilizer with green manure (1080 mg kg^{-1}) compared to compost (85 mg kg^{-1}) or commercial fertilizer (238 mg kg^{-1}) without green manure. The CO_2 evolution rate from compost also decreased compared to that of the commercial fertilizer (figure 3.15; table 3.21). The CO_2 evolution for compost and commercial fertilizer almost stopped after 111 and 34 days of incubation, respectively. There were no further increases for CO_2-C evolution with commercial fertilizer with green manure, compost with green manure and green manure alone after 83, 140 and 160 days of incubation, respectively.

Table 3.20 Apparent CO_2-C evolved equations for compost, commercial fertilizer, green manure, compost with green manure and commercial fertilizer with green manure.

Year	Organic fertilizers	Model equation	R^2	P
2013	Compost	Cm= 70 [1-exp(-0.12t-0.0001t^2)]	0.87	P<0.001
	C. fertilizer	Cm= 136 [1-exp(-0.23t-0.02t^2)]	0.65	P<0.001
	Green manure	Cm= 1105 [1-exp(-0.077t-0.0001t^2)]	0.98	P<0.001
	Compost+GM	Cm= 1360 [1-exp(-0.068t-0.00001t^2)]	0.97	P<0.001
	C. fertilizer+GM	Cm= 1248 [1-exp(-0.123t-0.0001t^2)]	0.99	P<0.001
2014	Compost	Cm= 85 [1-exp(-0.08t-0.00001t^2)]	0.88	P<0.001
	C. fertilizer	Cm= 238 [1-exp(-0.161t-0.004t^2)]	0.91	P<0.001
	Green manure	Cm= 918 [1-exp(-0.065t-0.00001t^2)]	0.95	P<0.001
	Compost+GM	Cm= 1050 [1-exp(-0.075t-0.00001t^2)]	0.92	P<0.001
	C. fertilizer+GM	Cm= 1080 [1-exp(-0.0131t-0.0001t^2)]	0.96	P<0.001

C mineralization was calculated by one mineralization pool equation $C_m = C_0 [1-exp(-k_1t-k_2t^2)]$. C_m (mg kg^{-1}) represents the accumulated mineralized C; k_1 and k_2 are mineralization constant rates; C_0 (mg kg^{-1}) represents the amount of potentially mineralizable C.

Compost C mineralization rate was greater in 2014 (36 g kg^{-1} initial C, respectively) compared to 2013 (29 g kg^{-1} initial C, respectively). On the contrary, green manure C mineralization rate was greater in 2013 (717 g kg^{-1} initial C) compared to 2014 (586 g kg^{-1} initial C). Compost showed the lowest loss of organic C. The amount of C mineralized from compost (between 29 to 36 g kg^{-1} initial C) was much lower compared to C mineralized from commercial fertilizer (between 242 and 412 g kg^{-1} initial C) or C mineralized from green manure (586 to 717 g kg^{-1} initial C) (table 3.21).

Table 3.21 Accumulated CO_2-C evolved (mg (kg soil)$^{-1}$), C applied (mg (kg soil)$^{-1}$) and C mineralization rate (g kg^{-1} initial C) from compost, commercial fertilizer, green manure, compost with green manure and commercial fertilizer with green manure.

Year	Organic fertilizers	Accumulated CO_2-C evolved (mg kg^{-1})	C applied (mg kg^{-1})	C mineralization rate* (g kg^{-1}initial C)
2013	Compost	70	2434	29
	Commercial fertilizer	136	560	242
	Green manure	1105	1540	717
	Compost+green manure	1360	3974	342
	C. fertilizer+green manure	1248	2100	594
2014	Compost	85	2335	36
	Commercial fertilizer	238	578	412
	Green manure	918	1566	586
	Compost+green manure	1050	3901	269
	C. fertilizer+green manure	1080	2144	504

* C mineralization rate (g kg^{-1} initial C) was calculated by the quotient between the accumulated CO_2-C evolved (mg kg^{-1}) from compost, commercial fertilizer or green manure during the incubation period and the total amount of C applied (mg kg^{-1}).

3.3.2 Nitrogen mineralization

Nitrogen mineralization was well described by a first-order kinetic model with one mineralization pool (figure 3.16; table 3.22). The correlation between the N mineralization values measured in the laboratory and the values calculated by the models was always positive with a probability level of $P<0.001$ except for green manure in 2013 and compost, commercial fertilizer and green manure in 2014 with a probability level of $P<0.01$. The R^2 values ranged from 0.69 to 0.97.

The commercial organic fertilizer was rapidly mineralized (figure 3.16). N mineralization of the commercial fertilizer in the first 7 days of incubation was 35% of the total N mineralized in 2013 and in 2014. The accumulated mineralized N (N_m) from commercial fertilizer (103 and 88 mg kg^{-1}, in 2013 and 2014, respectively) was higher than the accumulated mineralized N in compost (28 and 32 mg kg^{-1}, in 2013 and 2014, respectively).

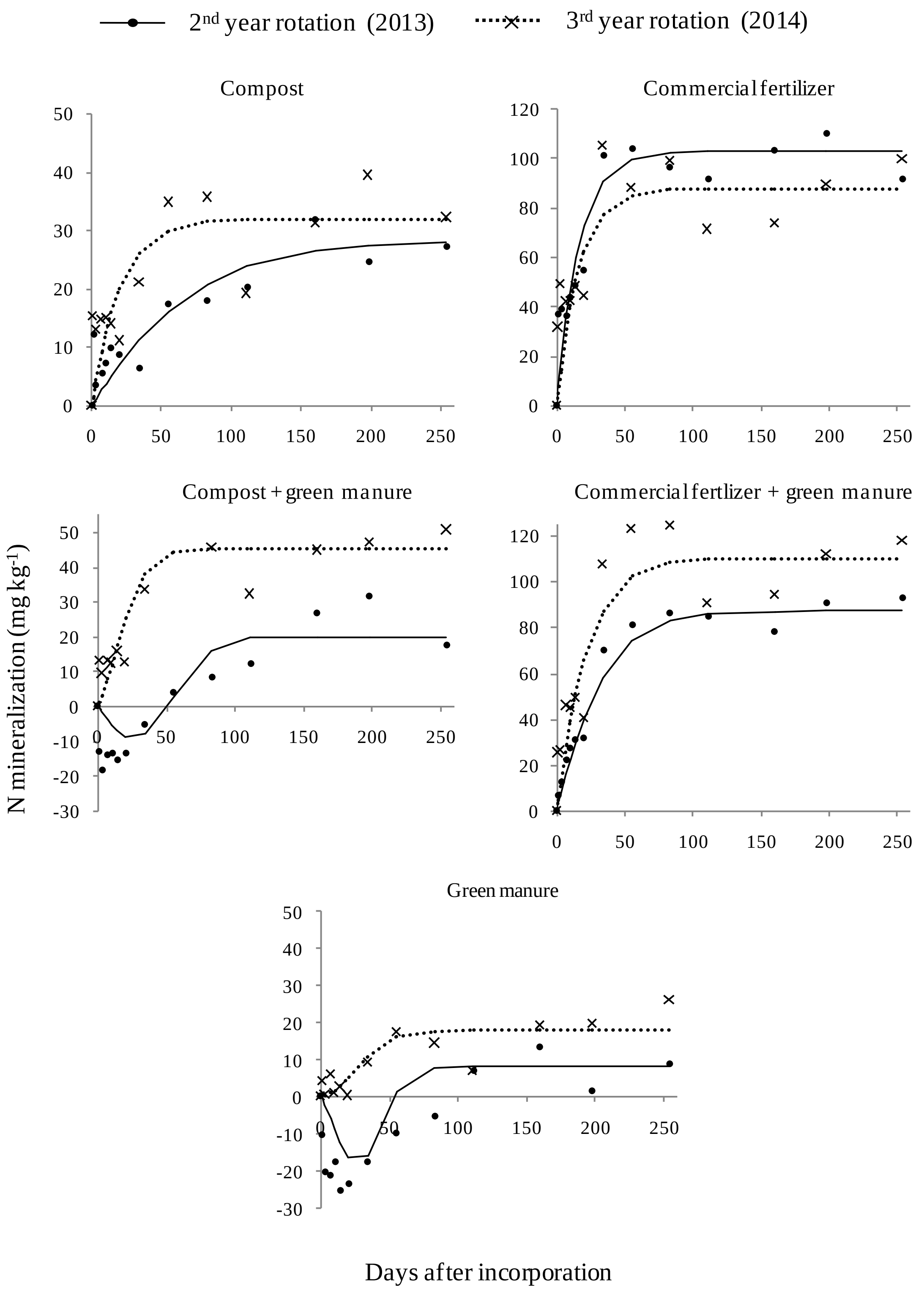

Figure 3.16 Accumulated mineralized N (mg kg^{-1}) from compost, commercial fertilizer, green manure, compost with green manure and commercial fertilizer with green manure incorporated into soil during the 2^{nd} and 3^{rd} year of crop rotation. N mineralization equations are described on table 3.20.

The compost N mineralized faster in 2014 compared to 2013 (figure 3.16). Moreover, compost was mineralized at a slower rate compared to commercial fertilizer. The chemical characteristics of compost and commercial fertilizer of both years are shown at table 2.3 and 2.4, respectively. After 20 days of incubation, compost N mineralization was 25% and 63% of the potentially mineralizable N, in 2013 and 2014, respectively. In contrast, after 20 days of incubation N mineralization of commercial fertilizer was 71% of potentially mineralizable N in both years. The accumulated mineralized N increased with compost application in 2014 (32 mg kg^{-1}) compared to 2013 (28 mg kg^{-1}), as well as the N mineralization rate that increased from 166 g kg^{-1} initial N in 2013 to 178 g kg^{-1} initial N in 2014 (table 3.23).

Table 3.22 Apparent N mineralization equations for compost, commercial fertilizer, green manure, compost with green manure and commercial fertilizer with green manure.

Year	Organic fertilizers	Model equation	R^2	P
2013	Compost	Nm= 28 [1-exp(-0.014t-0.00003t^2)]	0.84	P<0.001
	Commercial fertilizer	Nm= 103 [1-exp(-0.062t-0.00001t^2)]	0.89	P<0.001
	Green manure	Nm=8 [1-exp(0.09t-0.0017t^2)]	0.72	P<0.01
	Compost+GM	Nm=20[1-exp(0.055t-0.001t^2)]	0.82	P<0.001
	C. fertilizer+GM	Nm=87[1-exp(-0.029t-0.0001t^2)]	0.97	P<0.001
2014	Compost	Nm=32 [1-exp(-0.049t-0.00003t^2)]	0.69	P<0.01
	Commercial fertilizer	Nm=88 [1-exp (-0.061t-0.00006t^2)]	0.77	P<0.01
	Green manure	Nm=19 [1-exp(-0.003t-0.0007t^2)]	0.73	P<0.01
	Compost+GM.	Nm=45 [1-exp(-0.021t-0.001t^2)]	0.85	P<0.001
	C. fertilizer+GM	Nm=110[1-exp(-0.043t-0.0001t^2)]	0.88	P<0.001

N mineralization is calculated by one mineralization pool equation $N_m = N_0 [1-exp(-k_1t-k_2t^2)]$. N_m (mg kg^{-1}) represents the accumulated mineralized N; k_1 and k_2 are mineralization constant rates; N_0 (mg kg^{-1} soil) represents the amount of potentially mineralizable N.

Green manure incorporation caused an initial N immobilization (53 days) in 2013, promoting N remineralization afterwards (Nm = 8 mg kg^{-1}). However, N immobilization did not occur in 2014 and Nm increased to 19 mg kg^{-1}. The potentially mineralizable N was fully mineralized after 111 days of incubation in 2013 and 2014 (figure 3.16; table 3.23).

Green manure addition to compost in 2013 caused an initial N immobilization of 50 days. Afterwards, N mineralization increased and accumulated mineralized N reached 20 mg kg^{-1} after 111 days of incubation. On the contrary, N immobilization did not occur with the addition of green manure to compost in 2014, so the accumulated mineralized N was enhanced to 45 mg kg^{-1} in 83 days (figure 3.16; table 3.23).

Green manure addition with commercial fertilizer increased accumulated mineralized N in 2014 (110 mg kg^{-1}) compared to 2013 (87 mg kg^{-1}) (table 3.23). In addition, N mineralization from commercial fertilizer with green manure was faster in 2014 compared to 2013. After 34 days of incubation, N mineralization of commercial fertilizer with green manure increased from 67% of the potentially mineralizable N in 2013 to 79% in 2014 (figure 3.16).

The accumulated mineralized N from compost with green manure in both years was lower than the sum of the accumulated mineralized from compost and green manure. In 2013, the accumulated mineralized N from compost with green manure (20 mg kg^{-1}) was lower compared to accumulated mineralized N from compost (28 mg kg^{-1}). In 2014, the accumulated mineralized N from compost with green manure (45 mg kg^{-1}) increased in comparison to compost (32 mg kg^{-1}) (table 3.23).

Table 3.23 Accumulated N mineralized (mg kg^{-1}), N_{org} applied (mg kg^{-1}) and N mineralization rate (g kg^{-1} initial N) from compost, commercial fertilizer, green manure, compost with green manure and commercial fertilizer with green manure.

Year	Organic fertilizers	Accumulated mineralized N (mg kg^{-1})	N_{org} applied (mg kg^{-1})	N mineralization rate* (g kg^{-1} initial N)
2013	Compost	28	169	166
	Commercial fertilizer	103	110	936
	Green manure	8	52	154
	Compost+g. manure	20	222	90
	C. fertilizer+g.manure	87	162	537
2014	Compost	32	179	178
	Commercial fertilizer	88	129	682
	Green manure	19	81	235
	Compost+g. manure	45	260	173
	C. fertilizer+g.manure	110	210	524

* N mineralization rate (g kg^{-1} initial N) was calculated by the quotient between the accumulated mineralized N (mg kg^{-1}) from compost, commercial fertilizer, green manure, during the incubation period and the total amount of N_{org} applied (mg kg^{-1}).

In 2013, the accumulated mineralized N from commercial fertilizer with green manure (87 mg kg^{-1}) also decreased compared to the accumulated mineralized N from commercial fertilizer (103 mg kg^{-1}). On the contrary, in 2014, the addition of green manure with commercial fertilizer increased the accumulated mineralized N (110 mg kg^{-1}), being approximately the sum of the accumulated mineralized N from commercial fertilizer (88 mg kg^{-1}) and green manure (19 mg kg^{-1}).

The ammonia (NH_3) evolved from the organic fertilizers was residual in all treatments in 2013 and 2014 (between 0 and 0.07 mg kg^{-1}).

4. DISCUSSION

4.1 Nutrient content of vegetable crops

Nitrogen, P and K contents of the vegetable crops were not significantly different between treatments, in the 1st year of the crop rotation, except for occasional differences found for lettuce, specifically a lower N content for CF1 and increased K content for T0 compared to other treatments (table 3.2). Potato tuber N (8.1-8.9 g kg^{-1}) and K (5.4-8.2 g kg^{-1}) contents were below the values reported by Srek et al. (2012) of respectively 12.0 g kg^{-1} for N and 11.0 g kg^{-1} for K with cattle slurry fertilization, whereas the P, Ca and Mg content were similar. Potato tuber N and K content were also lower than potato tuber N and K content (9.8 g kg^{-1} and 14.3 g kg^{-1} respectively) observed by Palmer et al. (2013) with dairy cattle manure application. On the contrary, Alvarez et al. (2006) found tuber N content values with sheep manure compost and farmyard manure similar to those found in the present experiment. This may be explained because differences in nutrient content may depend on the cultivar (Belanger et al. 2001) and on soil and climatic conditions. Srek et al. (2012) found that there were no differences either for N or P contents of the tubers between potatoes grown with mineral or organic fertilization. However, K content of the tubers was reduced significantly with organic fertilization. Potato tuber N content were also similar between crops grown with composted organic pig manure and conventional mineral fertilizers (Mourão et al. 2008). In contrast, Palmer et al. (2013) found higher potato tuber N concentrations in conventional (14.0 g kg^{-1}) compared to organic fertilization (9.8 g kg^{-1}). The contradiction between these results may be attributed to the farming system, soil type, cultivar and the amounts of organic and mineral fertilizers used (El-Sayed et al., 2015).

Lettuce N, P, Ca and Mg contents were close to the values found by Brito et al. (2012b) with farmyard manure compost, whereas K content (15.4-20.7 g kg^{-1}) was lower compared to values found by these authors (33.6-47.0 g kg^{-1}) and those detected by Lairon et al. (1984) which vary between 48.1 and 50.4 g kg^{-1} with organic fertilization. These last authors did also not found significant differences on lettuce nutrient content under organic or mineral fertilization (table 3.2).

Swiss chard N (9.9-27.7 g kg^{-1}), P (1.1-3.1 g kg^{-1}) and K (39.7-70.0 g kg^{-1}) contents were below the values determined for N, P and K by Dzida and Pitura (2008) with mineral fertilization (33.9-59.1 g N kg^{-1}, 5.3-11.9 g P kg^{-1} and 63.9-92-3 g K kg^{-1}). On the other hand, Ca and Mg content (7.2-12.2 and 6.1-7.3 g kg^{-1}, respectively) were similar to the contents determined by these authors. The lower content of N, P and K in Swiss chard found here, with organic fertilizers, was probably due to the fact that Swiss chard is a very demanding crop (Pockluda and Kuben, 2002) and mineral fertilizers are soluble and immediately available for uptake (table 3.3).

Turnip N (23.7-30.4 g kg^{-1}), P (3.8-4.2 g kg^{-1}), K (33.6-44.8 g kg^{-1}), Ca (1.4-3.4 g kg^{-1}) and Mg (0.7-1.3 g kg^{-1}) contents (table 3.3) were slightly lower than those reported by Furlani et al. (1978), which were 31.3, 7.4, 53.3, 4.7 and 3.3 g kg^{-1} for N, P, K, Ca and Mg, respectively. The lower nutrient content values found here, compared to those reported by Furlani et al. (1978), maybe explained by lower nutrient availability with organic fertilization compared to conventional fertilization. On the other hand, Termine et al. (1997) compared conventional with organic fertilization and found that turnip yields were equivalent with mineral fertilizers, manure compost and blood meal. In this experiment organic fertilization with manure compost maintained low nitrate contents in turnips, most likely resulting from a progressive N availability.

Mean N content of Portuguese cabbage varied between 39.6 g kg^{-1} (with commercial organic fertilizer) to 27.1 g kg^{-1} (control treatment). These values were above those observed by Rodrigues (2009) that varied between 18.9 g kg^{-1} (organic fertilization with Dix) and 23.6 g kg^{-1} (recommended mineral fertilization) (table 3.4).

Carrot N (10.1-13.0 g kg^{-1}), P (3.1-3.7 g kg^{-1}) and K (22.9-38.6) contents were similar to the values previously reported for organic and mineral fertilization by Warman and Harvard (1997), but Ca (1.7-1.8 g kg^{-1}) and Mg (0.9-1.0 g kg^{-1}) contents were below the values of Ca (3.1-3.9 g kg^{-1}) and Mg (1.0-1.6 g kg^{-1}) contents observed by these authors (table 3.4).

It is interesting here to take into account that increased K content by Swiss chard, turnip, Portuguese cabbage and carrot with compost results from plant demand, but also from compost K availability (tables 3.3 and 3.4). This is because K does not depend on OM mineralization, being available when the microorganisms die (Varennes, 2003). K has been found to be as available as it is in mineral fertilizers by Wen et al. (1997) for sludge and manure composts.

4.2 Crop yields and N accumulation

4.2.1 Green manure yield and N accumulation

During the three years of the crop rotation, the rye/vetch mixture yield ranged between 17 and 28 t ha^{-1} (figure 3.3) and was higher compared to rye/vetch mixture yields found by Teasdale et al. (2008) between 9 and 11 t ha^{-1}. Although the longer growth period of the vetch-rye mixture in Teasdale et al. (2008) experiment (254, 238 and 233 days in 2003, 2004 and 2005, respectively) in comparison to this study (158, 194 and 189 days in 2012, 2013 and 2014, respectively), the lower sowing rate in Teasdale et al. (2008) experiment (rye and vetch were sown at the rate of 45 kg ha^{-1}) compared to this experiment (between 75 and 120 kg ha^{-1} for rye; between 60 and 150 kg ha^{-1} for vetch) during the three years of the crop rotation accounted for the higher green manure yield in this experiment.

The amount of green manure N accumulation on the above ground biomass was between 65 and 127 kg ha^{-1} for the different treatments (table 3.5). These values are included between those observed by Rannels et al. (1996) for vetch (154 kg ha^{-1}) and for rye (41 kg ha^{-1}).

Green manure N accumulation in the first year of the crop rotation (65.5 to 80.1 kg ha^{-1}) was lower compared to the following years because green manure was chopped earlier (158 days after sowing compared to 194 and 189 days in the following years) (table 3.5) and also because the sowing rate of vetch/rye increased in the following years. The green manure was chopped earlier because potato had to be planted in April. Probably, green manure should have been sowed earlier in September, in order to increase its growing period. The shorter growing period for the cover crop growth in 2012, compared to the other years, might also explain the lower GM yield (17.4 t ha^{-1}) found in 2012 compared to 2013 and 2014 (28.1 and 22.1 t ha^{-1}, respectively) (figures 3.1 and 3.2). The sowing rate of 120 kg ha^{-1} rye and 60 kg ha^{-1} vetch in 2012 originated a C/N ratio of 38 that caused N immobilization and consequently the following crop yield (potato) did not increase with green manure incorporation. Hence, in October 2012 the sowing proportion of vetch was increased compared to rye (100 kg ha^{-1} rye and 100 kg ha^{-1} vetch) in order to decrease the C/N ratio and promote the N availability to the following crop.

Nitrogen accumulation by the cover crop in the year of 2013 (100.2-127.0 kg ha^{-1}) increased in comparison to the year 2012 (65.5-80.1 kg ha^{-1}). This was due to the increased growth period as it was reported before and also because the sowing proportion of rye/vetch decreased from 2/1 in 2012 to 1/1 in 2013, decreasing also the C/N ratio from 38 to 30. Kuo and Sainju (1998) mixed rye with vetch in the proportion of 100/0, 80/20, 60/40, 40/60, 20/80 and 0/100 and found that as the ratio of rye/vetch decreased total N increased from 15.8 to 34.5 g kg^{-1} and the C/N ratio decreased from 23 to 10. The values of C/N ratio were lower than the values found in our study because rye and vetch were collected in the vegetative stage by Kuo and Sainju (1998). Although the increased of N accumulation found for the second year compared to the first year, the crop yield of the following crop (S. chard) did not increase with green manure incorporation because N immobilization persisted due to the high green manure C/N ratio (30). In addition, the laboratory incubation showed a high flush of CO_2 with green manure incorporation which concurs to increase N immobilization. An appropriate proportion of rye consociated with vetch incorporated into the soil should be found in order to increase yields in the succeeding crop. Hence, the sowing proportion of vetch compared to rye was increased again in October 2013 (75 kg ha^{-1} rye and 150 kg ha^{-1} vetch) in order to decrease the C/N ratio and to increase N availability for the following crop.

Green manure N accumulated in 2014 (97.4-117.5 kg ha^{-1}) and 2013 (100.2-127.0 kg ha^{-1}) were similar, although green manure yield decreased between 28.1 t ha^{-1} in 2014 to 22.1 t ha^{-1} in 2013. Green manure yield decreased because the sowing proportion of rye/vetch decreased from 1/1 in 2013 to 1/2 in 2014 and rye produces increased biomass compared to vetch. Nair and Nhouajio (2012) showed that rye yield increased in comparison to rye/vetch mixture yield from 2 to 6 t ha^{-1} average in three years, probably due to the higher seeding rate of rye. In 2014 the C/N ratio decreased to 20 and N immobilization did not occur, but the following crop yield (cabbage) did not increase with green manure application. Probably, green manure should be sown earlier (in September) in order to increase the growth period and consequently enhance N accumulation and promote N uptake by the following crop after N mineralization of green manure residues.

4.2.2 Crop vegetable yields and N accumulation

Crop N uptake and accumulation of horticultural crops depended mostly on the accumulation of dry weight rather than on differences on nutrient contents between treatments.

Potato N accumulation was not significantly increased with compost application compared to the control treatment or the treatment with only green manure application probably because the release of nutrients from compost occurred slowly (Hartl and Erhart, 2005) (table 3.6). However, the increase of potato N accumulated from 77.9 kg ha^{-1} in the control treatment to 129.9 kg ha^{-1} for CF2 was significant and was associated to an increase of potato yield of approximately 40%. This was probably due to increased availability of mineral N and easily mineralizable organic N in the organic fertilizer compared to the compost which increased the N supply to organic potatoes. Previous studies about the effects of organic fertilizers with low C/N ratio on potato yield also showed yield increases, for example, the application of cattle slurry (138 kg N ha^{-1}) increased potato yield by 27% (Srek et al., 2012) and whereas 7 t ha^{-1} of poultry manure with 4% total N (MS) enhanced potato yield by 23% (Ninh et al, 2014).

The long period needed for potato growth (124 days) in the present study allowed N recovery from compost to some extent. Therefore, there were no significant differences for CF1 (98.1 kg ha^{-1}) CF2 (129.9 kg ha^{-1}) and C40 (93.8 kg ha^{-1}). However, potato N accumulation apparently improved for CF2 in comparison to CF1 and C40 (CF2 was 40% higher than C40).

Potato yield (36 t ha^{-1}) with 40 t ha^{-1} of compost and GM was similar to that reported by Ninh et al. (2014) with the application of dehydrated and pelletized poultry manure. These authors found that the application of 3.4, 6.8, 14 and 27 t ha^{-1} of poultry manure increased potato yield by 17% (33 t ha^{-1}), 23% (35 t ha^{-1}), 23% (35 t ha^{-1}) and 36% (39 t ha^{-1}) compared to the control without any amendments. The highest rates of poultry manure enhanced potato yields, soil enzyme activities and microbial biomass (Ninh et al., 2014). However, this organic fertilizer may have negative impacts in the environment due to the high amount of NH_4^+ (15 g kg^{-1} MS) (Cooperband et al., 2002) and fast release of mineral N (Sanchez and Mylavarapu, 2009) leading to potential nitrate leaching (Sallade and Sims, 1993) or ammonia volatilization (Brinson et al., 1994). Although potato yield with the application of 40 t ha^{-1} of farmyard manure compost and GM found here and, with the application of 6.8 t/ha^{-1} of poultry manure (Ninh et al., 2014) were similar, the application of farmyard manure compost and GM resulted in increased physiological efficient because the amount of N applied with poultry manure (300 kg N ha^{-1}) was greater than the amount of N applied with farmyard manure compost (202 kg ha^{-1}) and green manure (42 kg ha^{-1}). In addition, poultry manure releases N faster (69% of added N in 60 days reported by Sanchez and Mylavarapu, 2009) in comparison to 36% of added N released from 40 t ha^{-1} of compost during the growth period of potato (124 days) in this experiment. Hence the residual effect to the following crop of farmyard manure compost may increase compared to poultry manure.

Lettuce N accumulation increased with compost application in comparison to the control treatment from 17 to 30 kg ha^{-1} (table 3.6). Manojlovik et al. (2009) also reported increases in lettuce N uptake with organic fertilizers such as guano (66.6 kg ha^{-1}) or farmyard manure compost (63.8 kg ha^{-1}) but to values much above those found here because lettuce yield reflected the fertilizer application whereas here lettuce N uptake reveals only the residual effect of compost application to the previous potato crop. Lettuce yield increased with the compost compared to the commercial fertilizer. These yield differences are consistent with the increase of lettuce N accumulation and N recovery from compost compared to the commercial fertilizer (explained by increase residual N effect of compost compared to the organic fertilizer) because this last fertilizer released most of N to the previous crop. Nevertheless, the lettuce N uptake by compost was not sufficient to achieve an acceptable lettuce yield for this variety. Lettuce yield (19 t ha^{-1}) with the application of 40 t ha^{-1} compost was below that reported by Brito et al. (2012b) for the same variety (33 t ha^{-1}) with the application of 40 t ha^{-1} farmyard manure compost before lettuce plantation.

Swiss chard N accumulation increased with the application of 2 t ha^{-1} commercial fertilizer (91.3 kg ha^{-1}) in comparison to 20 and 40 t ha^{-1} of compost (42.7 and 37.6 kg ha^{-1} respectively) (table 3.7). Accordingly, S. chard yield also increased with 2 t ha^{-1} commercial fertilizer (46 t ha^{-1}) compared to 20 and 40 t ha^{-1} compost application (26 and 36 t ha^{-1} respectively). Probably, the short period for S. chard growth (54 days) was not long enough to allow substantial N release from compost. Nevertheless, Swiss chard yield with the application of 40 t ha^{-1} compost and GM (36 t ha^{-1}) was above Swiss chard yield grown with 40 t ha^{-1} compost and GM (25 t ha^{-1}) for similar environmental conditions reported by Brito et al. (2012b). Moreover, the Swiss chard yield with compost application was in the range of values reported by Pockluda and Kuben (2012) during a study with several varieties of this crop (18 to 52 t ha^{-1}).

Turnip, the second crop of the season, also took advantage of the slow N release from compost to increase N uptake and N accumulation. Although the difference was not significant, apparently N uptake increased with compost compared to the commercial fertilizer enough to enhance turnip yield with 40 t ha^{-1} compost (38 t ha^{-1}) in comparison to 1 and 2 t ha^{-1} of commercial fertilizer (21 and 18 t ha^{-1} respectively) (table 3.7; figure 3.5). The amount of available N for turnip with the addition of 40 t ha^{-1} of compost was sufficient to achieve average yields for Portuguese conditions reported by Almeida (2006) of 33 t ha^{-1}. This may be explained because turnip was planted after a short-cycle crop, Swiss chard (54 days) that allowed N uptake to increase during the growth period of the following crop as opposed to lettuce that was planted after a long-cycle crop, potato that was highly demanding for available N.

At the third year of the crop rotation, Portuguese cabbage N accumulation was similar with 40 t ha^{-1} compost and 2 t ha^{-1} of commercial fertilizer (table 3.8). Consequently, yield differences between the incorporation of 2 t ha^{-1} of commercial fertilizer and 40 t ha^{-1} compost for the first crop of the season were also not significant although the growth period of P. cabbage (56 days) was similar to Swiss chard (figures 3.4 and 3.5). The N accumulation in cabbage with 40 t ha^{-1} compost and green manure (140%) increased significantly in comparison to the control treatment. However for S. chard N accumulation increased only 61%. Indeed, for the first crop of the season (S. chard and P. cabbage) with approximately the same growth period, cabbage in the 3rd year of crop rotation showed increased N accumulated compared to Swiss chard during the 2nd year of the crop rotation. In addition, N recovery from 20 and 40 t ha^{-1} of compost during the growth period of cabbage (29-24%, respectively) increased compared to the growth period of S. chard (19-7%, respectively) in the previous year. The stronger effect of compost on cabbage yield compared to Swiss chard maybe explained by continuous compost application (Erhart et al., 2005) and the increase of the amount of green manure biomass incorporated into the soil amended with 40 t ha^{-1} of compost (figures 3.4 and 3.5). The field and laboratory incubations also show that in the 3rd year of the crop rotation N immobilization was not detected and net N mineralization increased, increasing cabbage yield.

Carrot N accumulated also increased with compost application in comparison to the other treatments similarly to the other 2nd season crops (lettuce and turnip) and was above the average carrot yield for Portuguese conditions reported by Almeida (2006) of 30 t ha^{-1} (table 3.8).

Across all vegetable crops, yield increases with the application of green manure were not significant, as there were no significant differences on N uptake between the control treatment and the treatment with green manure. In addition, N recovery from green manure was also nil for every vegetable crop. Dabney et al. (2001) reported that cover crops improve biological, chemical and physical properties of the soil, including soil organic carbon content and aggregate stability. However, no vegetable crops yield neither soil organic C content increases were detected during the three years of crop rotation. Indeed, rye may increase soil organic carbon or reduce their rate of depletion (Kuo et al., 1997), but this is a long-term process. These authors reported that the cover crop effect on soil organic matter depended on the amount of C input. More than 4 t ha^{-1} of rye may increase soil organic matter content after 6 years of a maize cover crop rotation, whereas vetch was not suited for building soil organic matter during this period. Here, the crop rotation included two spring/summer vegetable crops planted after the incorporation of 22.5 t ha^{-1} $year^{-1}$ average rye/vetch and two tillage operations. Hence, the amount of C mineralized would increase in comparison to the experiment reported before because of the increased of tillage intensity (Rouw et al., 2010) and consequently the process of soil C sequestration decreases. The amount of rye incorporated into the soil counteract with the effect of tillage. The sowing proportion of rye/vetch in the third year of the crop rotation (75 kg ha^{-1} rye and 150 kg ha^{-1} vetch) promoted N mineralization as opposed to the previous years because of the increased amount of vetch in comparison to rye (Drinkwater et al., 2000). Probably, it would be advantageous to increase the amount of vetch in comparison to rye throughout all the crop rotation in order to avoid N immobilization during the growth period of the crops and increase yields. However, soil C sequestration would decrease.

4.3 Nitrogen mineralization in field incubation

4.3.1 Nitrogen mineralization from the organic fertilizers

Organic fertilizer composition had a great effect on organic N mineralization (table 3.12). The rapid N mineralization from the commercial fertilizer that occurred in the first seven days of crop rotation was the result of its high content of total N (97-100 g kg^{-1}), low C/N ratio (4.2-4.6) and high content of soluble C and N (43-52 g $kg^{-1}$and 21-37 g kg^{-1}, respectively) (table 2.4). These characteristics have been reported to increase the soil microbial activity with the release of high amounts of mineral N in a short period of time (Antil et al., 2011; Kautz et al., 2004; Stadler et al., 2006). The raise of N mineralization between the day 21 and the day 59 after incorporation of 2 t ha^{-1} commercial fertilizer in the second year of the crop rotation can probably be explained by the high NH_4^+ content of the commercial fertilizer (17.6 mg kg^{-1}) that accounted for short-term N availability. The ion ammonium was highly available to plant uptake because the microorganisms did not need to immobilize it, as high amounts of soluble C and N were available in the commercial fertilizer (Gutser et al., 2005).

Nitrogen mineralization from the commercial organic fertilizer in 2012 was not well fitted to the mineralization model (figure 3.9). This was probably due to the soil moisture content that caused N immobilization and impaired the process of nitrification under anaerobic conditions (Mikha et al., 2005; Xiang et al., 2008). In this year the soil moisture content during the core incubation between the day 21 and 49 was above 128% of the water holding capacity (PF 2.7).

The incorporation of 40 t ha^{-1} of compost in the 1st and 2nd years of the crop rotation (C/N = 17 and 14 and, N = 20 and 23 g kg^{-1}, respectively) caused an initial N immobilization for 71 and 31 days, respectively, due to increased available C (Reddy et al., 2008) (figure 3.9). This initial soil N immobilization was followed by net N remineralization. Probert et al. (2005) during a study performed with 9 farmyard manure composts also reported N immobilization periods greater than 8 weeks for farmyard manure composts with C/N ratio lower than 20. Moreover, Antil et al. (2011) reported N immobilization during the first three weeks after hen-cattle manure compost incorporation (C/N = 9.2) and Mubarak et al. (2010) observed N immobilization in the first four weeks of incubation after farmyard manure incorporation (C/N = 19). Here, the period of N immobilization decreased between the first and second years probably due to the reduction of C/N ratio from 17 to 14 since the higher C/N ratio stimulates microbial activity and increases N immobilization (Abera et al., 2013).

On the third year of crop rotation, N immobilization was not detected and net mineralized N (0.58 mg N kg^{-1} day^{-1}) increased with the application of 40 t ha^{-1} compost compared to the first and second years of the crop rotation (0.41 mg N kg^{-1} day^{-1} and 0.32 mg N kg^{-1} day^{-1} respectively) (figure 3.10). This can be explained by the lower compost C/N ratio (C/N = 12) and higher total compost N content (25 g kg^{-1}) compared to the C/N ratio and total N of composts applied in the first and second year of the crop rotation and by the effect of continuous application of compost, increasing soil organic N content and consequently net N mineralization and crop yields in the long-term (Kuo and Jellum, 2000; Sorensen and Amato, 2002). D'Hose et al. (2012) found that crop yields of beet, maize, potato and Brussels sprouts increased after the fourth year of farm compost application with a mean C/N ratio of 18.2. Bonanomi et al. (2014) also reported that crop yields of lettuce, melon and pepper increased after the second year of municipal solid waste compost application with C/N ratios of 15 and 25. Unlike the commercial fertilizer, the application of farmyard manure compost promoted N mineralization throughout all crop rotation after the N immobilization period.

The application of green manure into the soil promoted N immobilization on the 1st and 2nd years of the crop rotation (0.11 and 0.02 mg N kg^{-1} day^{-1} respectively) probably due to the high C/N ratio (38 and 30, respectively) of the green manure (figure 3.10). Although an optimal C/N ratio is difficult to define, Rosecrance et al. (2000) suggested that net N mineralization would be expected with rye and vetch used as green manure, whereas other authors reported that N immobilization happened with rye and vetch with C/N ratio > 10 (Kuo and Sainju, 1998). The N immobilization is also consequence of the high GM content of soluble C on the 1st and 2nd year (62.4 and 78.3 g kg^{-1} respectively) in comparison to the green manure soluble N (3.2 and 4.4 g kg^{-1} respectively) which stimulated the microorganisms to immobilize N from soil to decompose the soluble C of the green manure (Kuo and Sainju, 1998). In the third year of the crop rotation, the C/N ratio (C/N = 20), as well as the total N content (21 g kg^{-1}) and the soluble C to soluble N ratio (12) were expected to stimulate N immobilization, as suggested by Kuo and Sainju (1998). These authors reported total N content < 32 g kg^{-1} and the soluble C to soluble N ratio > 9 as indicative of possible N immobilization, after green manure application to soil. Here, an initial small N immobilization (for 32 days) was observed in this third year, but net N mineralization (0.05 mg N kg^{-1} day^{-1}) increased for the overall period of incubation probably because of continuous green manure incorporation over the three years of the crop rotation (Loes et al., 2011).

The fluctuations in N mineralization along the crop rotation also depend on the interaction between temperature and soil moisture (Wang et al., 2006). In 2012 the peak of N mineralization between the day 77 and the day 91 after compost, commercial fertilizer and green manure application maybe linked to the increase of soil moisture from 11.2% to 17.5% at a mean temperature of 20 ºC (figure 3.6). For example, Wang et al. (2006) reported that at temperatures above 15 ºC, N mineralization increased 50% when soil moisture increased from 15% to 25%.

4.3.2 The effect of N mineralization on crop yields

Field incubation is a simple method and a good indicator of soil N mineralization dynamics when utilized to compare different treatments (Monaco et al., 2010). The main disadvantages of this technique are the disturbance of soil aeration during sampling and incubation, and, isolation of the soil from natural drying-wetting cycles (Hatch et al., 1990). In addition, field incubation may underestimate N mineralization in soils because of the oxygen depletion due to microbial activity, and, the increased microbial activity due to the release of organic N through root exudation (Hatch et al., 1990). Nevertheless, it has been observed a good correlation between N accumulated in the crops and N mineralized during the incubation periods (Carsky et al., 1990; Monaco et al., 2010). In our experiment N mineralization determined by field incubation increased in fertilized treatments both with compost and with the commercial organic fertilizer, and it was found a positive correlation between the sum of mineralized N and crop yield during the growth period of: potato ($R^2 = 0.90$, $P<0.01$), Swiss chard ($R^2 = 0.84$, $P<0.01$) and Portuguese cabbage ($R^2 = 0.79$, $P<0.05$), showing that field incubation may be a good indicator to predict fertilization requirements for crop growth (Yan et al., 2006). During the growth period of the second season crop (lettuce, turnip and carrot) this correlation was not so strong probably due to the fact that a certain amount of mineralized N over the growth period of the first crop of the season may have been used by the second crop of the season. Another reason may result from the amount of N mineralized between the first and the second crop of the season (figure 4.1).

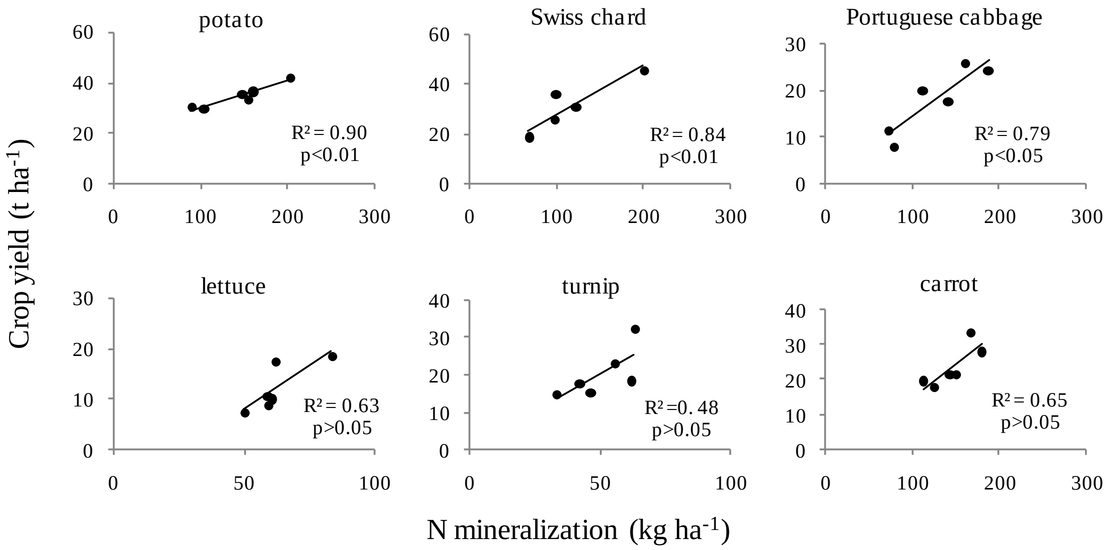

Figure 4.1 Correlation between N mineralization (kg ha^{-1}) and crop yield (t ha^{-1}) during the growth period of potato, S. chard, P.cabbage, lettuce, turnip and carrot.

Lettuce, turnip and carrot yields increased with compost application compared to the commercial fertilizer because compost seems to promoted N mineralization throughout crop rotation whereas commercial fertilizer was mostly mineralized during the previous crop (figures 3.3, 3.4 and 3.5). Therefore, the largest content of mineral N released after harvesting the first crop of each year occurred with the addition of 2 t ha^{-1} commercial fertilizer, whereas the largest content of mineral N released during the second season crop occurred with the application of 40 t ha^{-1} compost (table 3.14). However, the potentially mineralizable N available during the period of lettuce growth was not enough for an acceptable lettuce yield for this variety. The largest content of mineral N released during the lettuce crop was 22 mg kg^{-1} DM (equivalent to about 29 kg ha^{-1}) with the addition of 40 t ha^{-1} of compost. A mid-cycle potato (90-120 days) instead of the long-cycle potato (120-150 days) would probably allow increased lettuce yield because lettuce could be planted earlier when a higher content of mineralizable organic N was available and the higher temperatures would probably increase N mineralization and consequently N availability for lettuce growth.

Although the compost released N slowly throughout all crop rotation, increasing yield in both crops of the season as opposed to commercial fertilizer that released N mostly on the first crop of the season, the N efficiency of commercial organic fertilizer was greater than compost throughout all crop rotation. The N efficiency of 40 t ha^{-1} compost incorporation (34%) decreased compared to the N efficiency of 2 t ha^{-1} commercial fertilizer (49%) application during the three years of crop rotation.

The amount of mineralizable N during the period of turnip (10 mg kg^{-1}) and carrot (32 mg kg^{-1}) growth with the addition of 40 t ha^{-1} of compost was sufficient to achieve suitable yields, probably because both crops were planted after two short-cycle crops, Swiss chard (54 days) and Portuguese cabbage (56 days). The plantation of short-cycle crops instead of long-cycle crops allowed turnip and carrot to be sown earlier than lettuce (9th july, 22nd june and 20th August, respectively), increasing the amount of available N for the second season crop of the year. In addition, the amount of mineralized N during the period of chard growth (20 and 21 mg kg^{-1} with 20 and 40 t ha^{-1} compost, respectively) and during the period of cabbage growth (31 mg kg^{-1} with 20 t ha^{-1} compost) was lower compared to the period of potato growth (48 and 57 mg kg^{-1} with 20 and 40 t ha^{-1} compost, respectively). Hence, the amount of N available increased for the second crop season.

There were no significant differences on potato yield between the application of 2 t ha^{-1} of commercial fertilizer and the application of 40 t ha^{-1} of compost (figure 3.4). This can be explained by the long period for potato growth (124 days) which allowed N mineralization to increase with the application of 40 t ha^{-1} compost and GM (57 mg kg^{-1}), since mineral N was released slowly along the growth period of potato as observed in the field incubation. On the contrary, the short period for Swiss chard growth (54 days) was not long enough to allow substantial compost N mineralization (21 mg kg^{-1}) (table 3.14). Crop species with a long period to uptake N, such as potatoes, can make better use of the slow and prolonged release of available N (Berry et al., 2002). However**,** for short period crops, such as Swiss chard, the yield decreased with the application of 40 t ha^{-1} of compost and GM compared to the application of 2 t ha^{-1} of commercial fertilizer and GM. The time between compost incorporation and chard plantation was 5 days whereas N remineralization took place 26 days after planting Swiss chard. Hence, it would be advantageous to increase the interval between compost incorporation and S. chard plantation to increase the synchronization between net N mineralization and N demand.

In the third year of the crop rotation, mineralized N with the application of 40 t ha^{-1} compost increased for the growing period of cabbage (58 mg kg^{-1}) compared to Swiss chard (21 mg kg^{-1}), although the length of the growing period for cabbage (56 days) was similar to the growing period for Swiss chard (54 days) (table 3.14). This is explained because N immobilization did not occur during the cabbage growth period. For this reason, there were no significant differences on cabbage yield between CF2 and C40.

Crop yield increases with green manure application without additional fertilizers compared with the control treatment were not significant (figures 3.3, 3.4 and 3.5). Several studies reported yield reductions when non-leguminous green manures (Kumar and Goh, 2002) or even leguminous green manures (Salmeron et al., 2011) were incorporated into the soil. This was explained by the lack of synchrony between N mineralization and N crop demand (Sainju et al., 2006), which depends mainly on the balance between N mineralization and immobilization largely determined by the C/N ratio (Tejada et al., 2008). However, previous studies about crop rotation showed that soil incorporation of green manure might increase yields in later years (Kumar and Goh, 2002). For example, cereal yields increased close to 30% after 4 years of rye and clover application (Loes et al., 2011). The net N mineralization in the third year, with a sowing rate of 75 kg ha^{-1} rye and 150 kg ha^{-1} vetch suggests that this green manure seeding density is probably advantageous to promote the yield increase of the following crop. Crop yield increases in the third year of the crop rotation with the application of green manure were not significant. This is probably explained by the timing of green manure incorporation into the soil (Baggs et al., 2000). Since the time between green manure incorporation and Portuguese cabbage transplantation was only 6 days, whereas N remineralization took place 19 days after cabbage plantation (figure 3.9). Under these conditions, it may be advantageous to increase the period of time between the green manure incorporation into the soil and the plantation of Portuguese cabbage and so, to match the cabbage N demand with the net N remineralization from green manure.

4.4 Carbon mineralization in laboratory incubation

The patterns and the amount of total C mineralized in the soil varied considerably depending on the type of organic fertilizer used. However, all organic fertilizers showed a rapid increase in mineralization during the initial stage of incubation followed by a slower and almost linear release, since remaining C after the bio-oxidative phase is relatively resistant to microbial degradation (Bernal et al., 1998a). Carbon mineralization became fairly constant earlier with compost compared to commercial fertilizer and green manure (figures 3.12 and 3.13). Bernal et al. (1998c) also found that C mineralization became constant earlier with matured composts after a period of approximately 2 months in comparison to not decomposed samples. The maximum release of CO_2 evolution for all treatments occurred in the first day of incubation (figures 3.12 and 3.13). Similarly, Bernal et al. (1998c) found that the maximum C mineralization rate also occurred between day 1 and 3 of incubation for composted and uncomposted samples. This was because of the presence of high percentage of easily degradable organic carbon that leads to a flush of CO_2 production immediately after their addition to soil. Here, the greatest amount of CO_2-C evolved in the first day of incubation occurred with green manure incorporation (221 and 292 mg kg^{-1} day^{-1}), followed by commercial fertilizer (61 and 71 mg kg^{-1} day^{-1}). This can be explained by the high amount of green manure soluble C in 2013 and 2014 (78.4 and 70.4 g kg^{-1}, respectively) and commercial fertilizer soluble C in 2013 and 2014 (50.4 and 52.2 g kg^{-1}, respectively) (tables 2.4 and 2.5). Morvan and Nicolardot (2009) also explained that the initial flush of C mineralization was due to the rapid mineralization of low molecular weights compounds contained in the soluble fractions.

The accumulated CO_2 evolution increased with the soluble organic C content of the organic fertilizers. The accumulated CO_2-C increased for compost (70 to 85 mg kg^{-1}) followed by commercial fertilizer (136 to 238 mg kg^{-1}) and green manure treatments (918 to 1105 mg kg^{-1}) as the amount of soluble C increased for compost (7.8 to 9.2 g kg^{-1}) followed by commercial fertilizer (50.4 to 52.2 g kg^{-1}) and green manure (70.4 to 78.4 g kg^{-1}). These results are similar to Antil et al. (2011). This author reported that the accumulated CO_2 evolution after 168 days of incubation increased with hen cattle manure compost compared to cattle manure compost probably due to increased amounts of soluble C in hen cattle manure compost (36.9 g kg^{-1} C in cold water) compared to cattle manure (3.6 g kg^{-1} C in cold water).

The increased amount of total organic C mineralized in the commercial fertilizer was also probably correlated to the greater amount of N content in the commercial fertilizer in 2013 and 2014 (97 and 99 g kg^{-1}, respectively) compared to compost (23 and 25 g kg^{-1}, respectively). These results were similar to the previous data published by Ajwa and Tabatabai (1994) reporting an increase in total C mineralized from alfalfa (2.8% N), sorghum (2.2% N), soybean (1.2% N) and corn (0.7% N) with the higher total N content. The accumulated CO_2 from commercial fertilizer with green manure together in 2013 (1248 mg (kg soil)$^{-1}$) and 2014 (1080 mg (kg soil)$^{-1}$) was similar or decreased in comparison to the amount of CO_2 accumulated from the sum of accumulated CO_2 individually from commercial fertilizer and from green manure in 2013 and 2014 (1241 and 1156 mg kg^{-1}, respectively). On the contrary, the accumulated CO_2 from compost together with green manure in 2013 (1360 mg kg^{-1}) and 2014 (1050 mg kg^{-1}) was similar or increased compared to the amount of CO_2 acumulated from the sum of accumulated CO_2 individually from compost and from green manure in 2013 and 2014 (1203 and 1099 mg kg^{-1}, respectively). Hence, there was probably a synergistic effect on C mineralization when compost was applied with green manure, as opposed to commercial fertilizer with green manure.

In the years of 2013 and 2014, CO_2 evolution was faster in commercial fertilizer compared to compost during the first 42 days of incubation because in both years the C/N ratio was substantially lower for the commercial fertilizer (4.2 and 4.3) compared to compost C/N ratio (14 and 12) and the soluble C also increased for the commercial fertilizer (50.4 and 52.2 g kg^{-1}) compared to compost (9.2 and 7.8g kg^{-1}) (figures 3.14 and 3.15). These results are in agreement with Antil et al. (2011), that showed a positive correlation between C mineralization rate and soluble C during the first 42 days of incubation. Morvan and Nicolardot (2009) also found faster C decomposition with farmyard manure (C/N = 13) compared to composted farmyard manure (C/N = 29) during 107 days of incubation. In 2013, CO_2-C evolution was faster for green manure associated with commercial fertilizer in comparison to green manure and green manure associated with compost for similar reasons, namely, the C/N ratio of commercial fertilizer was lower than that of the compost. Instead, CO_2-C evolution in 2014 was faster with green manure associated with compost compared to green manure alone. This probably is partly linked to the decrease of compost C/N ratio from 14 in 2013 to 12 in 2014.

Although the amount of total C added through the compost in 2013 and 2014 (2434 and 2335 mg kg^{-1}, respectively) was greater than the amount of C added by the organic fertilizer in 2013 and 2014 (560 and 578 mg kg^{-1}, respectively) (table 3.21), the compost C mineralization rate (29 and 36 g kg^{-1} initial C, respectively) was lower than that of the commercial fertilizer (242 and 412 g kg^{-1} initial C, respectively). This can be explained by the presence of easily decomposable substances in the commercial fertilizer as opposed to the decreased amount of easily degradable C in compost, being the availability of C to microorganisms by recalcitrant fractions of compost slow (Bernal et al., 2009).

Green manure C mineralization rate in 2013 and 2014 (717 and 586 g kg^{-1} initial C, respectively) were the highest compared to the other treatments. The stronger effect of green manure in CO_2 evolution might be due to the presence of more easily decomposable substances (Tian et al., 2011) compared to the other fertilizers. Bernal et al. (1998c) always found the highest proportion of C mineralized in untransformed samples. These authors found that the proportion of C mineralized ranged between 623 g kg^{-1} and 932 g kg^{-1} of initial C with untransformed mixtures of sewage sludge, poultry manure, pig slurry and city refuse. Ajwa and Tabatabai (1994) studied C mineralization from plant residues (alfalfa, corn, sorghum and soybean) and found that the proportion of C mineralized was lower in clay soils (270 and 580 g kg^{-1} initial C). These later values are below those found here for rye and vetch, probably because carbon C mineralization decreased in clay soils compared to sandy soils (Antil et al., 2011). The amount of clay in the soil (between 23% and 29%) was higher compared to the amount of clay in the soil used in the present experiment (5%) whereas the amount of sand (between 4.6% to 4.7%) was lower compared to the amount of sand in the soil used here (78%). Moreover, the incubation time was much shorter (30 days) compared to the incubation time in this experiment (252 days).

The lowest C mineralization rates were always found for mature composts (29 and 36 g kg^{-1} initial C in 2013 and 2014, respectively) compared to commercial fertilizer and green manure (table 3.21). These values were lower compared to the C mineralization rate found in mature composts of a mixture of sewage sludge and cotton waste by Bernal et al. (1998a) (90 to 379 g kg^{-1} initial C) probably because these composts reached a lower degree of maturity and stability compared to the compost used in the present experiment.

The accumulated CO_2-C and C mineralization rate in compost were higher in 2014 (85 mg kg^{-1} of soil and 36 g kg^{-1} initial C, respectively) compared to 2013 (70 mg kg^{-1} and 29 g kg^{-1} initial C, respectively). This may be partly explained by the increase of N from 23.1 to 25.4 g kg^{-1} and the decrease of C/N ratio from 14 to 12 for the composts used in 2013 and 2014, respectively because N content and the C/N ratio are important factors to determine C decomposition (Wang et al., 2010). As opposed to compost, the accumulated CO_2-C and C mineralization rate in green manure were lower in 2014 (918 mg kg^{-1} and 586 g kg^{-1} initial C, respectively) compared to 2013 (1105 mg kg^{-1} and 717 g kg^{-1} initial C, respectively). Green manure N content increased in 2014 (20.6 g kg^{-1}) compared to 2013 (13.3 g kg^{-1}) and consequently, C/N rate decreased in 2014 (20) compared to 2013 (30) due to increased amount of vetch compared to rye. As the proportion of rye decreased in comparison to vetch in 2014 compared to 2013, the amount of soluble C also decreased from 78389 (mg kg soil) $^{-1}$ to 70370 mg (kg soil)$^{-1}$ (Kuo and Sainju, 1998). Probably for this reason, the accumulated CO_2-C decreased in 2014 compared to 2013 and the rate of C mineralization also decreased because the amount of C applied was similar.

A major aim of organic agriculture is to build up soil OM content. However, if this mechanism is used to supply nutrients to the crops it implies that a certain amount of OM is lost, being the carbon converted to CO_2 (Fuleky and Benedek, 2010). This loss can only be accepted to a certain limit imposed by soil structure, water holding capacity and erosion resistant. For instance, green manure released a large amount of CO_2-C in 2013 and 2014 (1105 and 918 mg (kg soil)$^{-1}$, respectively) indicating an increase in available soil organic C (Zhou et al., 2012). On the other hand, composts released a small amount of CO_2-C in 2013 and 2014 (70 and 85 mg kg^{-1}, respectively) representing an additional reserve of more stable organic carbon for the cultivated soil (Atallah et al., 1995).

4.5 Nitrogen mineralization in laboratory incubation

The accumulated mineralized N from commercial fertilizer decreased in 2014 compared to 2013 from 103 mg kg^{-1} to 88 mg kg^{-1}. Apparently, there is no reason for this to happen since N content (97 and 100 g kg^{-1} in 2013 and 2014, respectively) and C/N ratio (4.2 and 4.3 in 2013 and 2014, respectively) were similar in both years. This was probably due to the increased amount of NH_4^+ in 2013 (17557 mg kg^{-1}) compared to 2014 (5309 mg kg^{-1}). However, accumulated mineralized N and N mineralization rate from compost were enhanced in 2014 (32 mg kg^{-1} and 178 g kg^{-1} initial N, respectively) compared to 2013 (28 mg kg^{-1} and 166 g kg^{-1} initial N, respectively) (figure 3.17; table 3.21). This can be explained by the higher N content in 2014 (25.4 g kg^{-1}) compared to

2013 (23.1 g kg^{-1}) and lower C/N ratio in 2014 (12) compared to 2013 (14) (Morvan et al., 2006).

The characteristics that enable faster N release from commercial fertilizers compared to compost are higher total N and mineral N contents, low C/N rate and high amount of soluble C and soluble N (Stadler et al., 2006). The compost N mineralization rate varied between 166 and 178 g kg^{-1} of initial N. These values were higher than those observed by Hadas and Portnoy (1997), Morvan and Nicolardot (2009) and Antil et al. (2011) between 100 and 150 g kg^{-1} for farmyard manure compost, although they were lower than the mean N mineralization rate of 320 g $kg^{-1}$of initial N found by (Amlinger et al., 2003) for maize during 5 years of compost incorporation. The commercial fertilizer N mineralization rate was 682 g $kg^{-1}$of initial N in 2014. This value was similar to that found by Stadler et al. (2006) from industrial fertilizers with a N content from 4.7% and 8.5% and a C/N ratio from 5.0 to 8.6 which varied between 600 and 700 g kg^{-1} of initial N. The commercial fertilizer N mineralization rate was higher in 2013 (936 g kg^{-1} initial N) than in 2014 because the amount of organic N applied decreased in 2013 due to the high NH_4^+ content (17557 mg kg^{-1}).

The green manure from a sowing rye/vetch rate of 1:1 (C/N = 30 and total N = 13 g kg^{-1}) incorporated into the soil in 2013 caused an initial N immobilization of 53 days promoting N remineralization afterwards (figure 3.17). In 2014, the sowing rye/vetch rate decreased to 1:2, subsequently C/N rate decreased to 20, total N increased to 21 g kg^{-1} and N immobilization was not detected. The time of N immobilization depends on the value of the C/N rate and total N. Kuo and Sainju (1998) studied N mineralization with different rates of rye and vetch and found that the immobilization period of time was 30 weeks with rye (C/N = 23 and N = 16 g kg^{-1}). When the proportion of vetch/rye increased from 20:80 to 40:60, the C/N ratio was reduced from 17 to 15 and N content was enhanced from 22 to 26 g kg^{-1}. Consequently, the immobilization time decreased from 15 to 10 weeks. At a vetch/ rate of 80:20, the C/N rate decreased to 10 and N immobilization was not detected. In this study, N immobilization periods were lower than the ones found by Kuo and Sainju (1998) for equivalent values of C/N and total N. However, the N immobilization periods were similar to those found by Kumar and Goh (2003). These authors found continuous net N release with oat (C/N = 18) and N immobilization periods between 0 and 35 days for cover crops with C/N ratio between 21 and 30. It is generally reported in the literature that the cut-off point with respect to

N mineralization/immobilization is around 25 (Clark et al., 1997; Kumar et al., 2000). Nevertheless, according to Tian et al. (1995), the effect of C/N ratio, lignin and polyphenols needs to be integrated to have better predictions of N release.

The sum of mineralized N accumulated individually from compost and from green manure was higher than the accumulated mineralized N from the treatment with compost together with green manure. On the contrary, Sanchez et al. (2001) found that there was a synergistic effect when compost was applied with clover as green manure, being the sum of mineralized N from compost with clover higher than their individual N mineralization. During 2013, the sum of accumulated mineralized N from compost with green manure and commercial fertilizer with green manure was below the mineralized N from compost and commercial fertilizer, respectively because of the initial green manure immobilization. During 2014, in contrast, when N immobilization was not observed for green manure, the sum of accumulated mineralized N from compost with green manure and commercial fertilizer with green manure was higher than N mineralization from compost and commercial fertilizer, respectively (table 3.21). These results are in agreement with Carrera et al. (2007) that found an increment in tomato yield with 10 t ha^{-1} compost poultry manure and vetch in comparison to compost alone.

4.6 Ammonia evolution in laboratory incubation

In 2013 and 2014, the ammonia (NH_3) volatilization from organic fertilizers was residual for all the treatments. This can be explained because of the low NH_4^+ content of composts and green manure (Brinson et al., 1994). However, the high amount of NH_4^+ in commercial organic fertilizers would suggest that NH_4^+ could be transformed into ammonia and consequently increase N losses. Probably, this did not occur because ammonium was incorporated into the soil and nitrified. In fact, the amount of ion ammonium decreased after 7 days of incubation while the amount of ion nitrate raised to approximately 100 mg kg^{-1} in 34 days (figure 4.2).

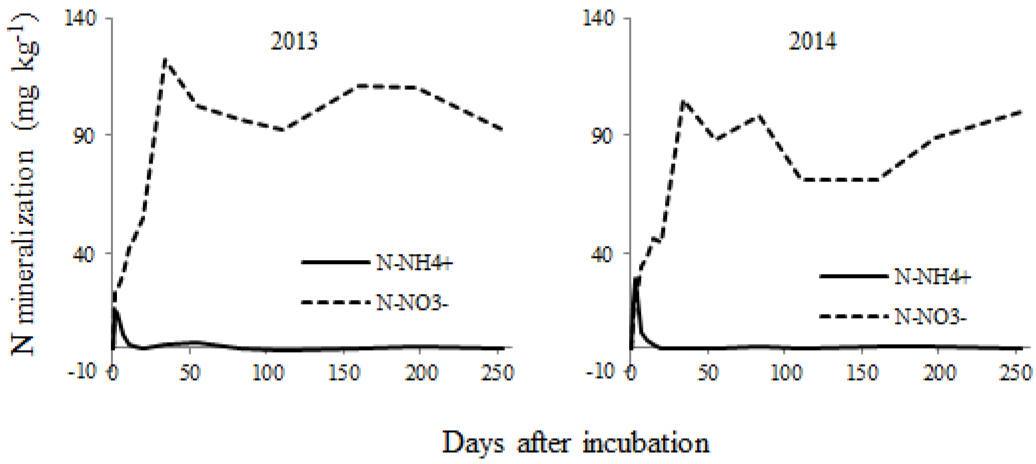

Figure 4.2 Acumulated ammonium ion N (NH_4^+-N) and nitrate ion N (NO_3^--N) from commercial organic fertilizer during the 2nd and 3rd year crop rotation in laboratory incubation.

4.7 Differences in N mineralization between incubations

The differences in N mineralization in laboratory and field incubation were determined with the incorporation of 40 t ha^{-1} compost and 2 t ha^{-1} commercial fertilizer in field incubation and the equivalent to 40 t ha^{-1} compost and 2 t ha^{-1} commercial fertilizer incorporated at the top 15 cm of the layer in laboratory incubation. In 2013, the N mineralization rate from compost was 195 g kg^{-1} of the initial compost N (31 mg kg^{-1} of accumulated mineral N in the soil) during 110 days of field incubation. This value decreased to 144 g kg^{-1} initial N (24 mg kg^{-1} accumulated N) during 111 days in laboratory incubation. In addition, N immobilization was not detected in laboratory incubation. In 2014, N mineralization rate from compost was 503 g kg^{-1} initial N (90 mg $kg^{-1}$of accumulated mineral N) during 168 days in field incubation. In the laboratory incubation N mineralization rate from compost decreased to 178 g kg^{-1} initial N (32 mg kg^{-1}accumulated N) in 160 days (table 3.14 and 3.21). Therefore, N mineralization in compost increased approximately 35% and 182% in 2013 and 2014, respectively, in field incubation compared to laboratory incubation. The difference between N mineralization in laboratory and field incubation was higher in 2014 compared to 2013 because the period of N immobilization in field incubation did not allow N mineralization to increase during the period of Swiss chard growth (figure 3.9).

Several studies have reported that N mineralization was similar or even greater in laboratory compared to field incubations. For example, N mineralization estimated in buried bags in the field with soil amended with poultry manure and yard waste compost was similar to laboratory measured values determined by Hanselman et al. (2004). In this experiment the amendments were air-dried and passed through a 4-mm sieve before mixed with soil inside the plastic bags and the average temperature in laboratory incubation was nearly the same as the average field soil temperature. Knoepp and Swank (1995) reported that N mineralization values for forest soils in laboratory incubations were greater than field incubation because laboratory incubation was performed under optimal conditions of temperature and soil moisture content. Here, the average daily air temperature in the field during the growth period of horticultural crops was approximately 15 ºC while the temperature in the laboratory incubation was 25 ºC. Hence, it was supposed that N mineralization would increase in the laboratory incubation since Agehara and Warncke (2005) found that N mineralization was enhanced 13% in 12 weeks when temperature increased from 15 to 25 ºC.

However, N mineralization from compost increased in field incubation compared to laboratory incubation probably due to the effect of continuous compost incorporation in the field. It also can be hypothesized that this increase may also be related to a significant change and decrease of microbial biomass in laboratory incubation. For instance, measurements of N transformation rates in tropical forest soils showed approximately 150% lower N mineralization in laboratory incubation after a period of cold storage at 5ºC than in-situ incubation (Arnold et al., 2008). Wallenius et al. (2010) found a decrease between 20% and 30% of enzyme activity in compost after freezing. Moreover, frozen storage reduced accumulated CO_2 evolution in composts by 110 to 277% indicating a deep decrease of microbial activity (Wu and Ma, 2001).

Jost et al. (2013) reported that microbial characteristics of cow faeces revealed higher impacts on N mineralization in soil than soil microbial properties. Here, the microbial characteristics of the compost may have been improved in the field due to the changes of microbiology in frozen compost samples in the laboratory incubation and promote greater N mineralization in the field. Moreover, materials derived from root exudates that may increase N mineralization are excluded in laboratory incubation (Yamasaky et al., 2011).

The addition of fresh C from cover crops into the soil can promote the degradation of resistant soil organic matter which is known as priming effect, increasing N mineralization in field incubation (Zhou et al., 2012). Indeed, the priming effect is usually induced by fresh OM with a high C/N ratio (Blagodatskaya and Kuzyakov, 2008). Several studies indicate that the incorporation of green manure may cause a flush of decomposition from the native soil organic matter (Fu et al., 2000; Pascault et al., 2013). The incorporation of compost may also induce decomposition of SOM, increasing soil mineral N content (Nendel and Reuter, 2007). These authors found that the incorporation of mature biowaste compost in vineyards induced a priming effect leading to mineral N contents in soil in between 87 and 440 kg N ha^{-1}.

To sum up, the increased rate of N mineralization in field incubation compared to laboratory incubation may be due to the effect of continuous compost application in the field, to increased microbial activity in the field compared to laboratory incubation and, eventually, to the effect of compost and green manure in the decomposition of native SOM (priming effect).

Nitrogen mineralization rate from commercial fertilizer was similar in laboratory (936 and 682 g kg^{-1} initial N in 2013 and 2014, respectively) and in field incubation (900 and 705 g kg^{-1} initial N in 2013 and 2014, respectively), indicating the impact of its high total N and mineral N content, low C/N rate, and high amount of available C and N to increase N mineralization (Martin-Olmedo and Rees, 1999; Stadler et al., 2006; Antil et al., 2011) (table 3.14 and 3.21). In this case, the colonization by the soil microorganisms is so rapid that N mineralization increases fast (Bending and Turner, 1999). For example, the particle size of the substrate influenced the activity of microbial population due to the protection to C and N afforded by fine particles. However, the decomposition of an organic fertilizer with high N and low C/N rate mineralized so quickly that the protection from fine particles was limited. So, the process of sieving may probably decrease N mineralization from compost but not influence N mineralization from commercial fertilizer.

In 2014, compost N mineralization was faster in laboratory incubation (k_1 = 0.049) compared to field incubation (k_1 = 0.0085) (tables 3.13 and 3.20). Moreover, in 2013 and 2014, commercial fertilizer N mineralization was also faster in laboratory incubation (k_1 = 0.062 and k_1 = 0.061, respectively) compared to field incubation (k_1 = 0.023 and k_1 = 0.034, respectively). Similarly, Sistani et al (2008) also found that N mineralization from litter broiler was faster in laboratory incubation than in field incubation. This tendency to release mineral N for a shorter period of time in laboratory incubation than in field incubation may be explained by the fact that when organic fertilizers were added to the soils samples under favourable conditions, the microbes quickly utilized the easily decomposable organic C and N. The N immobilization from compost in field incubation compared to no N immobilization in laboratory incubation in 2013 and the faster N remineralization from green manure in laboratory incubation compared to N immobilization in field incubation in the same year may also be linked to the favourable conditions in laboratory incubations. For example, the soil moisture content from 7 to 59 days after the beginning of field incubation in 2013 (approximately 100% water holding capacity) was higher compared to soil moisture content in laboratory incubation (70% water holding capacity). Probably, this increase in soil moisture content may increase the period of N immobilization. The N immobilization rate was lower at moisture content equivalent to pF 2.5 than at pF 3.9, irrespectively of organic sources and soil type (Abera et al., 2012). Furthermore, Sistani et al. (2008) reported that the environmental conditions such as temperature and precipitation may result in increased immobilization in field incubation compared to laboratory incubation.

The net nitrification in commercial fertilizer also increased fast in field incubation because the amount of ion ammonium decreased in 20 days as the amount of ion nitrate increased (figure 4.3). Even though, nitrification was faster in laboratory incubation (figure 4.2) because the ammonium ion content declined in 10 days as the the amount of nitrate ion increased. Moreover, the ammonia volatilization was also probably residual in field incubation because nitrification occurred after the incorporation of commercial fertilizer into the soil.

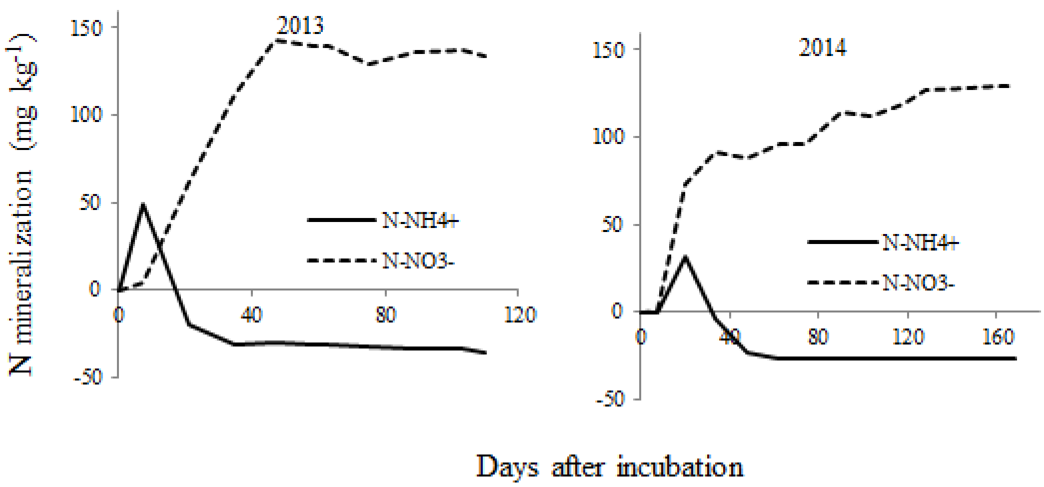

Figure 4.3 Acumulated ammonium ion N (NH_4^+-N) and nitrate ion N (NO_3^--N) from commercial organic fertilizer during the 2nd and 3rd year crop rotation in field incubation.

The present study suggests that the potential N mineralization in unstable commercial organic fertilizers that mineralize quickly, were similar under field and controlled laboratory incubations. On the contrary, the rates of N mineralization and the pattern of N release which occur in the field from matured farmyard manure composts that mineralize slowly are different in field incubation and under controlled laboratory conditions. Further research is needed to better understand the behavior of N mineralization in field and laboratory incubation.

4.8 Differences in N accumulation and lettuce yield during the pot and field experiment

The weight of lettuce grown in the pots were lower compared to lettuces grown in the field, which is explained by the smaller volume of soil in the pots. Lettuce crop yield decreased 57% from 269.6 g pl^{-1} in field experiment to 171.1 g pl^{-1} in pot experiment for C20 and decreased 70% from 284.5 g pl^{-1} in field experiment to 167.6 g pl^{-1} in pot experiment for C40 (figure 24). Lettuce yields were similar for CF1 and CF2 between field and pot experiment, since commercial fertilizer was mostly mineralized during the previous crop. Therefore, there were no significant differences with compost and commercial fertilizer application in the pot experiment, whereas in the field experiment lettuce yield increased with compost application compared to all the other treatments. Plant N content was higher in the field compared to pot experiment. Lettuce N content decreased from 28.8 to 15.5 g kg^{-1}, from 32.4 to 22.5 g kg^{-1}, from 32.3 to 18.9, from 28.9 to 17.7, from 23.8 to 18.2 and from 28.1 to 22.1 g kg^{-1} in field experiment compared to pot experiment for T0, GM, C20, C40, CF1 and CF2, respectively. This can be explained by the increase on soil exploration by the roots in the field compared to the pot experiment. Norby (1994) reported that small pots may alter root growth and morphology. Pot experiments can be accomplished in smaller space in green houses and growth rooms. Then, it would be useful if the results found in pot experiments were similar to the ones found in the field because it would save time and costs. However, pot experiments may be useful to compare different treatments but are not accurate enough to predict organic fertilization as opposed to long-term experiences that are a valuable resource to determine soil's mineral N balance in order to decide on fertilization practices (Fuleky and Benedek, 2010).

4.9 Changes in soil characteristics during the crop rotation

The amounts of extractable P and K decreased in soil after 3 years of crop rotation with incorporation of green manure, 1 and 2 t ha^{-1} commercial fertilizer with green manure and 20 t ha^{-1} compost with green manure. In contrast, the amounts of extractable P and K were similar in the beginning and the end of the crop rotation for 40 t ha^{-1} compost and green manure because the amounts of P and K added to soil with 40 t ha^{-1} compost were greater compared to P and K applied with the all other treatments. The P added to soil with green manure (17 kg ha^{-1} $year^{-1}$), 1 and 2 t ha^{-1} commercial fertilizer (10 and 20 kg ha^{-1} $year^{-1}$, respectively) and 20 t ha^{-1} compost (30 kg ha^{-1} $year^{-1}$) was lower compared to the incorporation of 40 t ha^{-1} compost (60 kg ha^{-1} $year^{-1}$). Similarly, K added to soil with green manure (94 kg ha^{-1} $year^{-1}$), 1 and 2 t ha^{-1} commercial fertilizer (29 and 58 kg ha^{-1} $year^{-1}$, respectively) and 20 t ha^{-1} compost (161 kg ha^{-1} $year^{-1}$) was lower compared to the incorporation of 40 t ha^{-1} compost (322 kg ha^{-1} $year^{-1}$).

Edmeades et al. (2003) reported that the use of manures can result in soils becoming excessively enriched in P and K because the ratio of nutrients in manures is different from the ratio of nutrients removed by the crops. Here, the amount of extractable P and K in the soil was similar after 3 years of 40 t ha^{-1} compost and green manure incorporation. The highly K uptake from turnip (314 kg ha^{-1}) and carrot (260 kg ha^{-1}) probably did not allow extractable K in soil to increase too much. Hence, it is important to set up a crop rotation accordingly to the release of nutrients from the organic fertilizers, including K crop demands, since organic fertilizers usually have a large anmount of this nutrient.

It is generally accepted that soil pH (H_2O) should be maintained near 6.5 (Fuleky and Benedek, 2010). The pH increased in the first year from 4.9 to 6.7 and gradually declined to values of 6.1 in the end of experiment (table 3.15). These results are in agreement with Cifu et al. (2004) that found an enhancement of pH from 5.0 to 7.2 in the first year after 7.5 t ha^{-1} liming amendment, decreasing to pH values of 6.8 three years later.

Salinity is well known for its negative effects on crop yields. EC increased for treatment C40 compared with T0, GM, C20 and CF1, probably because composts EC values (between 3.6 to 5.2 dS m^{-1}) were above the recommended values for composts used as soil amendments (3 dSm^{-1}) (Soumaré et al., 2002). EC increased due to compost application has been previously reported (Chang et al., 2007).

Soil mineral N increased in the first seven days after commercial organic fertilizer incorporation, but decreased in the next few days (figure 3.11). This mineral N decrease was not caused only by N uptake from the crops once it occurred before potato plantation and 2 days after the plantation of S. chard in 2012 and 2013 respectively. These mineral N losses from the system occurred probably as a result of N leaching because rainfall is responsible for high N losses in the presence of high soil mineral N content (Huang et al., 2011; Zhu et al., 2005). Xin-Qiang et al. (2011) suggested that higher potential leaching would be engendered when the rainfall intensity was over 5.9 mm day^{-1}. Here, between the day 7 and the day 42 after commercial organic fertilizer application the daily rainfall intensity was over 5.9 mm day^{-1} for 14 and 9 days in 2012 and 2013 respectively (data obtained from the nearest weather station in Braga). In 2012 the moisture content in the incubation cores between the day 21 and 49 was above 128% of the water holding capacity (PF 2.7). In 2013, the moisture content in the incubation cores between the day 21 and 47 was above 92% of the water holding capacity. Probably, between the day 7 and day 21 in both years, there was no synchrony between soil moisture inside and outside the incubation cores, being the soil moisture greater in the field. This is one disadvantage of the field incubation method (Hatch et al., 1990). Mineral N might also have been leached to deeper layers (below 15 cm) where it was still available for crops. These mineral N losses with commercial fertilizer incorporation might also be due to denitrification induced by oxygen depletion under anaerobic conditions and high mineral N content (Rosecrance et al., 2000; Calderon et al., 2005). Aulakh et al. (2000) reported that nearly saturated soils supported greater nitrification of applied ammonia fertilizer, resulting in higher rates of denitrification. Moreover, mineral N decreases may also partly be due to N immobilization by microorganisms, since N immobilization was observed after green manure incorporation (GM).

More than 83% of the potential mineralizable N (N_0) was mineralized during spring/summer crops. Therefore, the risk of leaching during autumn and winter was small. In addition, the sowing of a combination of rye and vetch in October is advantageous to minimize N losses (Rosecrance et al., 2000). The risk of leaching was even smaller during autumn and winter after the short and the long cycle crop compared to two short cycle crops, since the amount of soil mineral N after the short and long cycle crop (1.9 to 6.0 kg ha^{-1}) was lower than the soil mineral N content after the two short cycle crops (14.1 to 16.6 kg ha^{-1}).

Soil quality depends on the amount of both soil organic C and N content (D'Hose et al., 2014) for building a self-sustainable soil nutrient cycle that provides a wide range of nutrients for crop growth (Hepperly et al., 2009). Here, there were no significant differences in total organic C for all treatments after the three years of the crop rotation. Total N content in soil was similar with the incorporation of 40 t ha^{-1} of compost and green manure in the end of the experience compared to the beginning of the experience, whereas total N decreased after the three years of crop rotation for green manure, green manure with commercial organic fertilizer and green manure with 20 t ha^{-1} of compost (table 3.15). Zhou et al. (2012) also found no significant differences in soil total C content 3 years after soil incorporation of oat and wheat. Here, compost was supposed to represent an additional reserve of more stable organic carbon for the cultivated soil due to the small amount of CO_2-C released in 2013 and 2014 (72 and 96 mg kg^{-1}, respectively) (Atallah et al., 1995). On the other hand, green manure released a large amount of CO_2-C in 2013 and 2014 (1120 and 970 mg kg^{-1}, respectively) indicating an increase in available soil organic C (Zhou et al., 2012). The total organic C and total N did not increase after 3 years of accumulated incorporation of organic fertilizers, probably due to the increase in OM mineralization. In the present experiment the rate of N mineralization in soil without organic fertilizers application in the 1^{st}, 2^{nd} and 3^{rd} year of the crop rotation was 163, 158 and 183 kg N ha^{-1}, respectively. These values are higher than the estimated N mineralization (50 to 150 kg N ha^{-1}) when the OM content of the soil is approximately 5% (Soltner, 1996). This fact was probably partly due to tillage opperations that were performed twice each year of the crop rotation (Kristensen et al., 2003).

Other studies suggested that total C and N increased after three years of compost incorporation (Bonanomi et al., 2014; Chang et al., 2007; Evanylo et al., 2008; Reider et al., 2000). This is probably explained by the higher compost rates added to the soil (between 17 to 60 t DM ha^{-1} $year^{-1}$) by those authors compared to the incorporation of about 10 t DM ha^{-1} $year^{-1}$ of compost (C40) for the present experiment, since the C/N ratios and N contents of the composts used by those authors were similar to the composts used in this study.

Although, after 3 years of accumulated application of green manure, compost and commercial fertilizer there were no significant differences in total C between treatments, Fraga et al. (2015a), for the same samples detected significant differences between treatments with the permanganate oxidizable carbon method. This is an alternative method that is supposed to quantify biologically active soil carbon and seems to be a sensitive indicator to evaluate the evolution of soil C sequestration under alternative management practices (Weil et al., 2003). The addition of compost and commercial organic fertilizer over green manure increased soil permanganate oxidizable carbon content in comparison to green manure application. Fraga et al. (2015a) reported that the application of 40 t ha^{-1} of compost and green manure increased soil permanganate oxidizable carbon compared to all other treatments, having the most pronounced effect on soil C sequestration.

The application of compost, commercial fertilizer and green manure after 3 years crop rotation contributed to the increased biological quality of the soil. Soil from the control treatment showed significantly lower values for the activities of *ß-glucosidae, ß-glucosaminodase* and *acid phosphomonoesterases* compared to the fertilized plots (Fraga et al., 2015b). The application of compost and commercial fertilizer with green manure also revealed significantly higher activity of these three enzymes in comparison to green manuring alone. However, the highest activity of these three enzymes was also detected with the application of 40 t ha^{-1} of compost and green manure. (Fraga et al., 2015b). Hence, the application of 40 t ha^{-1} farmyard manure compost enhanced crop yields throughout the crop rotation contributing to increase the biological quality and C sequestration of the soil.

5. CONCLUSIONS

During the three years of organic crop rotation N availability from the organic fertilizers incorporated into the soil could explain crop yield and N accumulation differences between treatments. Increases of crop yield and N accumulation were found with both rates of either compost or organic fertilizer, compared to the control and to the green manuring alone. Nitrogen mineralization determined by field incubation predicted soil N availability and may be a good indicator to predict fertilization requirements for crop growth during an organic horticultural crop rotation.

The commercial organic fertilizer was rapidly mineralized, increasing the risk of an early spring leaching and/or denitrification as opposed to the farmyard manure compost which released mineral N throughout the crop rotation. Thus, the unstable commercial organic fertilizer (with a mean C/N ratio = 4.4 and total N = 99 g kg^{-1}) increased crop yields only for the first crop of each year whereas a matured compost of farmyard manure (with a mean C/N ratio = 14 and a total N = 23 g kg^{-1}) enhanced the second crop yield of the year.

Crop yields did not increase with green manure application for the first and second year of crop rotation (C/N = 38 and 30 respectively). On the third year the increased sowing proportion of vetch/rye (150 kg ha^{-1} and 75 kg ha^{-1}, respectively), decreased C/N ratio (20) and net N mineralization was detected but was not enough to increase crop yields. Probably, green manure should be sown earlier in September in order to increase the growth period and consequently enhance N accumulation. However, the application rate of 40 t ha^{-1} $year^{-1}$ of farmyard manure compost (223 kg N $ha^{-1}year^{-1}$) with 22.5 t ha^{-1} $year^{-1}$ of rye with vetch as green manure (74 kg N ha^{-1} $year^{-1}$) showed the potential to increase the 1^{st} and 2^{nd} season crop yields during the three years of the organic horticultural rotation. The potato, lettuce, S. chard, turnip, P. cabbage and carrot yields increased 20%, 74%, 94%, 79%, 124% and 74%, respectively compared to the control.

The selection of crops and cultivars and the timing of plantation during a horticultural crop rotation should be carried out accordingly to the release of nutrients from the composts and green manure. The lettuce yield in the first year of the crop rotation was small for this variety, probably because the preceding crop, a long-cycle potato did not allow N mineralization to be extended during the growth period of lettuce. Therefore, a mid-cycle potato would be advantageous, since lettuce could be planted earlier when a higher content of mineralizable organic N would increase N availability and lettuce yield.

A long-cycle crop such as potato should be planted immediately after 40 t ha^{-1} compost because the long period for potato growth (124 days) allowed N mineralization to increase after the period of N immobilization. In contrast, a short period crop such as Swiss chard should be planted later after the application of the compost, since the period of N immobilization avoided the synchronization between N uptake and N mineralization and S. chard yield decreased.

The amount of total N, extractable P and extractable K contents in the soil were similar after 3 years crop rotation with the incorporation of 40 t ha^{-1} compost and green manure whereas the amount of these nutrients decreased for all the other treatments from the beginning till the end of the crop rotation. The pH remained at 6.1 in the end of crop rotation after the incorporation 5 t ha^{-1} of dolomite lime in the beginning of crop rotation to increase the initial pH (4.9).

In the end of each year of the crop rotation the amount of soil mineral was residual (1.9 to 16.6 kg ha^{-1}) and more than 85% of the potential mineralizable N was mineralized during spring/summer crops, so the risk of leaching during autumn and winter was reduced.

Total C did not increase between treatments after 3 years of green manure, commercial fertilizer and compost incorporation. Probably, total C was not the ideal parameter to assess C sequestration during short periods of time.

Field experiment is more accurate than pot experiment to predict organic fertilization because of the increased soil exploration by roots. Here, letuce yields and N uptake were much higher in the field compared to the pot experiment.

Nitrogen mineralization rate from compost in the field incubation (503 g kg^{-1} initial N), in the third year of the crop rotation increased compared to laboratory incubation (178 g kg^{-1} initial N) during approximately 160 days. On the contrary, N mineralization from commercial fertilizer in the third year of the crop rotation in field incubation (900 g kg^{-1}initial N) was similar to laboratory incubation (936 g kg^{-1} initial N) during 168 days. This was explained by the biochemical characteristics of this fertilizer (low C/N ratio; high total N content, soluble N and soluble C) that encouraged a fast colonization by soil microorganisms.

Briefly, (i) nitrogen mineralization in field incubations predicted soil N availability; (ii) commercial organic fertilizer increased crop yields only for the first crop of each year, increasing the risk of N losses; (iii) compost of farmyard manure released mineral N over all crop growing seasons (iv) the application rate of 40 t ha^{-1} $year^{-1}$ compost and 22.5 t ha^{-1} $year^{-1}$ rye and vetch as green manure is suitable to fertilize a three year organic horticultural rotation (v) the plantation of a short-cycle crop after the incorporation of 40 t ha^{-1} and green manure should be delayed in order to match N uptake with N mineralization (vi) a long-cycle crop should be planted immediately after the application of 40 t ha^{-1} and green manure; (vii) total C and N were similar between treatments after three years of organic fertilizers application.

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