Alma Mater Studiorum - Università di Bologna

DOTTORATO DI RICERCA IN

Colture Arboree ed Agrosistemi Forestali Ornamentali e Paesaggistici

Ciclo XXV

Settore Concorsuale di Afferenza: 07/B2
Settore Scientifico Disciplinare: AGR/03

Soil application of *Meliaceae* derivatives: effect on carbon and nitrogen dynamics in the soil-plant system

Presentata da **Graziella Marcolini**

Coordinatore Dottorato
Prof. Luca Corelli Grappadelli

Relatore
Dr. Moreno Toselli

Esame Finale Anno 2013

Index

Chapter 1

General introduction

1.1 Soil Organic Matter

1.1.1 Definition, composition, significance

The soil organic matter (SOM) is a heterogeneous mixture of materials ranging from fresh plant and microbial residues to relatively inert compounds (Haynes, 2008). Although it includes a continuum of materials ranging from highly decomposable to very recalcitrant, it is generally considered as constituted by two major pools: a labile and a stabilized fraction. Labile pools of organic matter (OM) are more sensitive to changes with soil management or environmental conditions than total SOM. In fact, due to the generally high background levels and also natural soil variability on total SOM it is often difficult to detect changes in total OM in response to soil management (Haynes and Beare, 1996). Changes in labile fractions provide an indication of changes in total SOM content that may become evident in the long-term (Haynes, 2008).

The labile fraction of SOM consists mainly on materials in transition between fresh plant residues and stabilized OM and is characterized by a short turnover time (less than ten years). Within the labile pool of SOM, different fractions have been identified, including: particulate OM, soluble C, extractable C, microbial biomass C, potentially mineralizable-C, and enzymes present in soluble and sorbed forms. (Haynes, 2008). Nature, significance and typical quantities of these labile fractions are listed in Table 1.1.

Stabilized OM consists mainly on humic substances (humic and fulvic acids, humine) which are complex systems of high molecular weight organic molecules made up of phenolic polymers produced from the products of biological degradation of

Table 1.1. Typical quantities, nature and significance of organic matter pools present in soil (modified from Haynes, 2008)

Organic fraction	**Typical quantities**	**Nature and significance**
Total organic C and N	Organic C = 7-60 g kg^{-1} Organic N = 0.6-5 g kg^{-1}	Sum of organic materials (both living and dead) present in soil excluding living plant material. Single most important factor involved in soil productivity. It has mass effects on chemical, physical, and biological properties and processes in soils.
Particulate organic matter	LF=2-18% of organic C, 1-16% of total N SSF=20-45% of organic C 13 - 40% of total N	Partially decomposed plant litter isolated by density fractionation (LF) or sieving (SSF). Substrate and center for soil microbial activity, short term reservoir for nutrients, food source for earthworms for formation of water stable aggregates
Microbial biomass	1-5 % of organic C 1-6 % of total N	Organic matter associated with cells of living soil microorganisms. Agent for transformation and cycling of organic matter and nutrients, formation and decay of humic materials, dynamic source or sink of plant nutrients, and an agent involved in formation and stabilization of aggregates.
Soluble organic matter	About 0.05-0.40 % organic C and total N	Water soluble organic compounds present in soil solution, including simple compounds of plant and microbial origin as well as humic material. Available substrate for microbial activity, primary source of mineralizable N, S and P, its leaching greatly influences nutrient and organic matter status and pH of groundwater.
Extractable organic C and N	Variable amount of organic C (1-40 %) depending on the extractant	Organic C and N solubilized /hydrolyzed/oxidized by various chemical reagents.
Potentially mineralizable C and N	About 1-5 % of organic C and total N	Organic C and N released by indigenous soil microflora during a laboratory incubation. Values are the result of an integration of physical, chemical and microbiological properties of the soil. Indicator of the N fertility of soils and their ability to supply N to crops

plant and animal residues and the synthetic activity of microorganisms (Stevenson, 1994; Baldock and Nelson, 2000). They are long lasting critical components of natural soil systems, persisting for hundred of even thousands of years (Mayhew, 2004). These materials make up 70-80 % of the SOM content of most soils, they are highly resistant to microbial decomposition because of their chemical structure and/or their association with soil minerals.

Soil organic matter is considered an extremely important attribute of soil quality (Haynes, 2008) that affects productivity and physical well-being of soils (Lal, 2006; Komatsuzaki and Ohata, 2007) and is now recognized as a key factor in the evaluation of the sustainability of soil management practices (Gregorich et al., 1994, 1997). Indeed, SOM profoundly affects the physical, chemical and biological properties and processes of soil.

The main physical properties influenced by SOM are: aggregate formation and stabilization, water retention, resistance and reliance to compaction and thermal properties.

The main chemical properties affected by SOM are charge characteristics, cation exchange capacity, buffering capacity, formation of soluble and insoluble complexes with metals and interactions with xenobiotics such as pesticides.

The most important biological properties of OM deals with its role as a reservoir of metabolizable energy for microbial and faunal activity, its effect in stabilizing enzyme activity and its value as a source of plant-available N, S and P via mineralization.

In European semi-arid Mediterranean regions, the decrease in OM content of many soils is becoming a major process of soil degradation (Diacono and Montemurro, 2010). Addition of exogenous organic materials to soil can be an excellent means to replace SOM, thus improving soil physical, chemical and biological conditions (Powlson et al., 2011) and it is almost always desirable (Johnston et al., 2009).

Different kind of exogenous organic material can be used in this sense, including: crop residues and organic wastes such as by-product of farming, animal manures, compost, food processing wastes and municipal biosolids, waste from agri-food industries. The application of these organic materials provides a means of recycling nutrients via their mineralization.

1.1.2 Soil organic matter mineralization

The term mineralization indicates the conversion of an element from an organic to an inorganic form (Alexander, 1977; Paul and Clark, 1989; White, 1997) available to plants. Applied specifically to C, mineralization may be defined as the release of carbon dioxide (CO_2) from metabolizing organisms (Zibilske, 1994); this concept is comparable to soil microbial respiration which is the most popular method to estimate microbial activity and substrate decomposition in soils.

In the specific case of nitrogen (N), the process of mineralization can be defined as the conversion of organic N into inorganic form of N as ammonium-N (NH_4^+) and is performed exclusively by heterotrophic microorganisms, able to operate in both aerobic and anaerobic conditions and to produce a wide variety of extracellular enzymes capable to degrade proteinaceous (proteinases, peptidases) and nonproteinaceous substrate (chitanase, kinases) into NH_4^+ (Pierzynski et al., 2000).

Mineralization is driven by the soil microbial biomass which can be defined as the part of SOM that constitutes living organisms smaller than 5-10 μm^3 (Alef and Nannipieri, 1995). It includes mainly bacteria and fungi, but also actinomycetes and microalgae and excludes soil animals and plant roots. Although the soil microbial biomass represents only 1-5% of total organic C and 1-6% of total N (Sparling, 1997) it performs critical functions in the soil system as it contributes to its structure and, above all, regulates energy and nutrient cycling that take place in the soil (Haynes, 2008; Murphy et al., 2007).

During mineralization, the soil microbial biomass assimilates complex organic substrates for energy and biomass C with excess inorganic nutrients being released to the soil solution. The fate of C utilized by these decomposer microorganisms is to be either assimilated into their tissues, released as metabolic products, or respired as carbon dioxide (CO_2) (Diacono and Montemurro, 2010). The inorganic ions of N (NH_4^+), P (PO_4^{3-}), S (SO_4^{2-}), and metals produced during this process can be taken up by plants, adsorbed (to colloidal surfaces), precipitated (e.g. as insoluble salts like Ca, Fe, Al-phosphate) or leached from the system (Figure 1.1) (Smith, 1994). In most cultivated, well aerated soils, NH_4^+ is rapidly converted into NO_3^- (nitrification) mainly through the activity of two groups of autotrophic aerobic bacteria *Nitrosomonas* (responsable of the conversion of NH_4^+ to NO_2^-) and *Nitrobacter* (responsable of the conversion of NO_2^- into NO_3^-). In this conditions NO_3^- represents the predominant available mineral form of the element. On the

other hand, if low O_2 concentration is present (i.e. waterlogged conditions), nitrification cannot take place and inorganic N accumulates as NH_4^+.

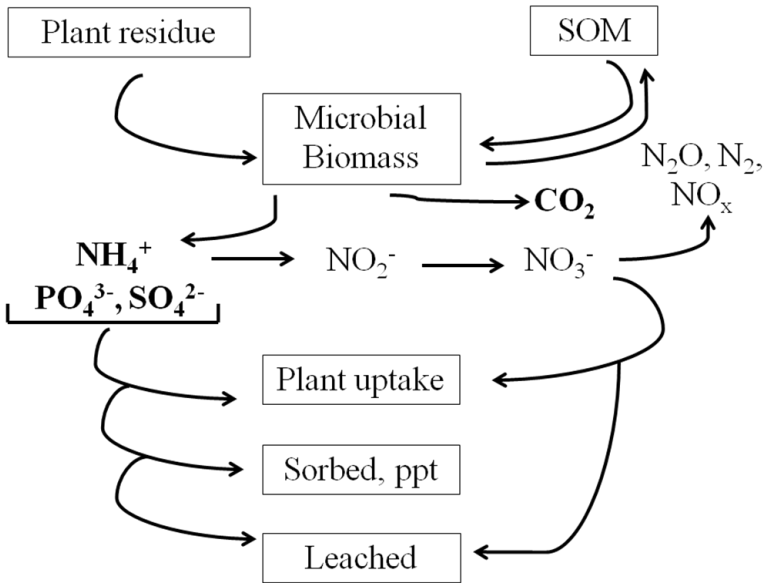

Figure 1.1. Schematical representation of production and fate of inorganic ions released by microbial mineralization of plant residue and SOM. Modified from Smith, 1994.

Nearly always, the mineralization process is accompanied by the immobilization of N, due to activities of the soil living biomass and, since the two processes of mineralization and immobilization take place simultaneously, the increase of mineral N concentration at a defined time indicates the net mineralization (Canali and Benedetti, 2006 and literature cited).

In the absence of plant assimilation, leaching, gaseous losses, net rates of mineralization are determined from the change in the soil inorganic-N pool size over time (t):

Net N mineralization = $\Delta NH_4^+ + \Delta NO_3^- = (NH_4^+\text{-N} + NO_3^-\text{-N})_{t+1} - (NH_4^+\text{-N} + NO_3^-\text{-N})_t$

A negative value indicates net immobilization.

The determination of net N mineralization is considered a way to evaluate the capacity of a soil to supply N for crop growth and potential risk of water and atmospheric pollution. Thus, N mineralization is often included in minimum data sets, arranged to evaluate the capacity of a soil to operate within the boundaries of the ecosystem: promote

biological productivity, maintain environmental quality and safeguard the health of plants and animals (Canali and Benedetti, 2006).

All environmental factors affecting the microbial activity can affect the mineralization rate of SOM. Among these factors, the most important are soil temperature, moisture, oxygen concentrations, pH and nutrient availability (Canali and Benedetti, 2006; Pierzynski et al., 2000). Because of a wide variety of microorganisms participate in the process of mineralization, the optimum condition for it can be of large extent. For example, the optimum conditions for mineralization-immobilization are a temperature range of 40-60 °C, with a Q_{10} (change in reaction rate when temperature increases 10 °C) of about 2.0. The optimum soil moisture content varies with soil texture, but generally ranges between 50-70 % of soil water-holding capacity. Moreover, aerobic conditions promote mineralization, but this process can proceed also under anaerobic conditions.

Actually, other factors such as tillage, clay content, cycles of drying and rewetting can also influence the mineralization of SOM. Moreover, the addition of exogenous organic materials to the soil can affect the mineralization rate of the native soil organic matter, a phenomen called: priming effect (Kuzyakov, 2010) (§ Paragraph 1.2).

In addition, the biochemical characteristics and physical nature, named ‘quality’ (Swift et al., 1979), of the exogenous OM largely affects its rate of mineralization. Numerous studies have attempted to relate biochemical quality to decomposition and mineralization, and a wide range of components have been found to control these processes (Bending and Turner, 1999). In the ’20 some studies demonstrated that the carbon to nitrogen ratio (C/N) of the organic materials can predict their mineralization in soils (Waksman, 1924; Jensen, 1929) and thereafter numerous experiments conducted to the same conclusions (Giller and Cadisch, 1997; Nicolardot et al., 2001). It is generally proven that the addition of OM with differing C:N ratios to the soils can cause significant, but reasonably predictable, changes in the amount of plant-available inorganic soil N (Pierzinsky et al., 2000). A C:N ratio of ~25:1 is commonly used as the ratio where mineralization and immobilization are in balance. Adding materials with high (>30:1) C:N ratios can cause a rapid increase in microbial biomass and a depletion of available soil N to the point where N deficiency can occur in many plants. Conversely, some organic amendments with very low C:N ratios can produce large excess of soluble N and must be managed carefully to avoid N losses to sensitive parts of the environment. The C:N ratio of added organic material does not remain constant during the decomposition process as C from microbial respiration is evolved from the soil as CO_2. With time, therefore, the C:N

ratio will decrease into the range where mineralization, not immobilization, predominates and the soil once again provides some available N for plant uptake. However, the use of C:N ratio to predict N mineralization has been later criticised as this parameter do not take into consideration the C and N availability for decomposer microorganisms, often essential to describe the decomposition kinetics (Recous et al., 1995).

Another biochemical characteristics of exogenous OM, generally plant residues, that in many studies, has been found to be related to the dynamic of mineralization is the initial N content (Frankenberger and Abidelmagid, 1985; Tian et al., 1992, 1995; Vigil and Kissel, 1991). This finding has later been attributed to the experimental condition of limited N availability for decomposer; when the N requirements of the soil decomposers are not fulfilled by the soil N content (N availability is a limiting factor of decomposition), the kinetic of decomposition or C mineralization observed does not allow the effect of biochemical quality to be assessed or distinguished from the effects of N availability on C decomposition (Trinsoutrot et al., 2000).

Actually, other quality factors that have been found to be correlated with N release are: lignin content (De Neve et al., 1994; Müller et al., 1988), lignin-to-N (Vigil and Kissel, 1991), polyphenol-to-N (Palm and Sanchez, 1991). Moreover, Bending et al. (1998) found that the quality components controlling net N mineralization changed during decomposition, with water-soluble phenolic content significantly correlated with net N mineralization at early stages, and water-soluble N, followed by cellulose at later stages. They also found that C-to-N and total N were correlated with net N mineralization towards the end of the incubation only.

Hence, a general relationship between biochemical composition and dynamic of C and N mineralization that can be validated for a wide range of residues is still missing.

1.2 The Priming effect

1.2.1 Definition

The definition most frequently used for priming effect (PE) is ‘strong short-term changes in the turnover of SOM caused by comparatively moderate treatments of the soil’ given by Kuzyakov et al. (2000) in his review.

But the first definition is attributed to Bingeman and co-workers which, in 1953, defined PE as 'the extra decomposition of native SOM in a soil receiving an organic amendment'.

This phenomenon was discovered first by Löhnis, in 1926, who found intensified mineralization of native N by the addition of fresh residues (green manure of legume plants) in soil. Although Löhnis outlined the central role of PE in the long-term SOM dynamics, in the following twenty years the phenomenon was not considered. Then, in the middle of '40-'50, PEs were studied again thanks to isotopic facilities (Kuzyakov et al., 2010). In the meantime, some synonyms of PE were used in the literature, for example 'priming action' and 'added nitrogen interaction' (ANI), introduced by Jenkinson et al. in 1985.

In their review, Kuzyakov et al. (2000) classified PEs in positive and negative. A positive PE occurs when the added substrate cause an acceleration of SOM decomposition, with an extra release of CO_2 or mineral N (or other nutrients) from the native soil compared to the unamended soil. On the contrary, a negative priming effect occurs when the addition of the substance to the soil causes a retardation of native SOM decomposition (Figure 1.2).

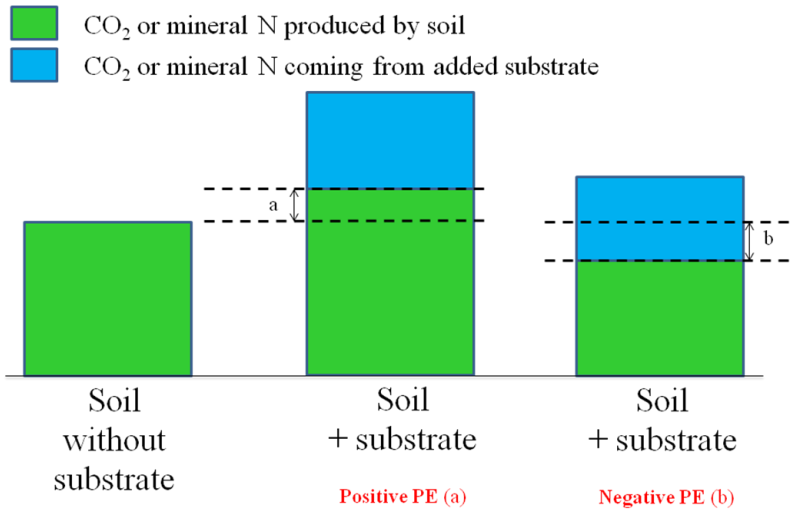

Figure 1.2 Schematic representation of positive and negative priming effect (PE). Modified from Kuzyakov et al., 2000.

The ‘treatments’ of the soil that can induce PEs are:

- addition of mineral fertilizers to the soil (Kuzyakov et al., 2000 and literature cited),
- addition of organic substances as carbohydrate, aminoacids, root extracts etc. (De Nobili et al., 2001; Dilly and Zyakun, 2008; Fontaine et al., 2004a; Fontaine et al., 2004b; Hamer and Marschner 2002; Hamer and Marschner, 2005; Kuzyakov and Bol, 2006; Mary et al., 1993; Nottingham et al., 2009; Shen and Bartha, 1997),
- incorporation of plant residues (Azam et al., 1993; Bending and Turner, 2009; Bending et al., 1998; Breland and Hansen, 1998; Paré and Gregorich, 1999),
- the presence of earthworms (Postma-Blaauw et al., 2006)
- the presence of roots (rhizosphere PE) (Bader and Cheng, 2007; Cheng, 1996, 2009; Cheng et al., 2003; Cheng and Kuzyakov, 2005; Dijkstra and Cheng, 2007; Dijkstra et al., 2006, 2009, 2010; Fu and Cheng, 2002; Fu et al., 2002; Kuzyakov, 2002; Kuzyakov and Cheng, 2001; Kuzyakov and Cheng, 2004; Kuzyakov et al., 2001).

Priming effects are usually quantified by comparing the native soil element/nutrient release in amended and unamended soil, the most studied elements being C and N, although nutrient as sulphur (S) and phosphorous (P) can be monitored too.

Research in which PE induced by the incorporation of plant materials were studied in term of N release are limited. In this case the first step in the quantification of PE is the separation, in the amended soil, of the amount of N released by the residue from that released by soil and this can be done with isotope techniques. Then the native soil mineral-N released by the amended soil is compared to that of the unamended soil to find the amount of primed N (Figure 1.3).

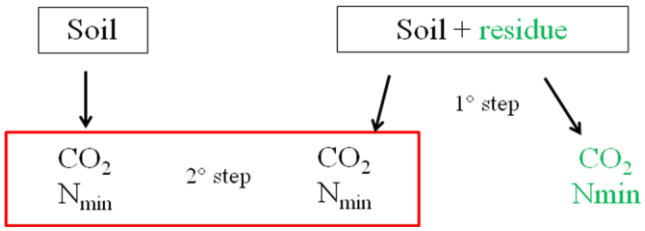

Figure 1.3. Schematic representation of the steps for the quantification of primed-C and -N.

Using this approach, Azam and co-workers (1993) studied the N mineralization of N from ^{15}N-labelled soybean (*Glycine max* L.) tops, vetch (*Vicia villosa* Roth.) tops and corn (*Zea mays* L.) stover in six Mollisols from Illinois and they found that from 13 to 41% of the applied N was mineralized during the 4-week incubation (25°C), the proportion decreasing in the order, vetch › corn › soybean. They also found that the three plant materials had different effects on the mineralization of soil N, depending on soil type and resulting in either a positive or negative added N interaction that ranged between -31.8 mg N kg^{-1} observed after the application of 15 g kg^{-1} of soybean tops in the Flanagan soil and +25.3 mg N kg^{-1} after the application of 15 g kg^{-1} of vetch in the Drummer soil.

In another laboratory incubation experiment, three different plant residues (mais, soybean and alfalfa) were added to 3 different types of soil (clay, loam, sandy), with the result that alfalfa residue stimulated native soil N mineralization in the sandy soil, whereas soybean and maize residue led to negative ANI in all soils (Paré and Gregorich, 1999).

Even if the use of isotopes is necessary to have precise quantification of PE, in some cases the 'difference method' has permitted to identify PE (Bending et al., 1998). For example, the incorporation of 5 g of fresh different plant materials as mature leaves and petioles of brussels sprouts (*Brassica oleracea* cv. Peer Gynt), ryegrass (*Lolium perenne* L. cv. Parcour), sugar beet (*Beta vulgaris* L. cv. Saxon), french bean (*Phaseolus vulgaris* L. cv. Double White) and patato (*Solanum tuberosum* L. cv. Wilja) into 100 g of a sandy –loam soil induced a fast mineral N release following the incorporation of the shoot materials. In addition, within the first 14-84 days, for all shoot residues, net N mineralization exceeded the quantity of residue-N added, demonstrating that additional N was mineralized from SOM. In particular, the mineral-N pool in excess of N added ranged from above 90 μg g^{-1} dw soil for sugar beet and patato to 50 μg g^{-1} dw soil for the other shoot materials.

1.2.2 Duration of PE

Many early studies on PE stated, as also suggested by the definition of Kuzyakov et al. (2000), that PEs arise immediately after substrate addition, actually, it has now been accepted that real PE may be delayed for days or even weeks, and that it can last at high levels, even after exhaustion of the initial priming substrate (Kuzyakov et al., 2010 and literature cited).

1.2.3 Real and apparent PE

Priming effect have also been divided into real and apparent. A real PE occurs when the extra CO_2 released by priming is originated by the extra-mineralization of native SOM, whereas an apparent PE occurs when the extra CO_2 released is originated from increased microbial biomass turnover, thus becoming an artefact (Blagodatskaya and Kuzyakov, 2008; Fontaine et al., 2004b; Kuzyakov, 2010; Nottingham et al., 2009).

According to Kuzyakov (2010), the extra CO_2 release occurring in the first 0-3 days is mainly due to accelerated turnover or pool substitution in microbial biomass (apparent PE) and, at that time, real PE is small. However, real PE increases with time and completely replace apparent PE after several days (Kuzyakov et al., 2010).

Moreover, Fontaine et al. (2011) suggested that the developing of real, instead of apparent, PE is determined by the nature of incorporated fresh C (soluble or polymerized) which determine the type of substrate utilization by microbes (microbial turnover or production of SOM degrading enzymes). These authors support the hypothesis that apparent PE is generally observed with the supply of soluble C (sugars) with mineral nutrients. In this situation, in fact, soluble C and nutrients diffuse in the soil so that many dormant soil microbes may be activated, with a release of unlabeled C of their own biomass as CO_2, leading to the apparent PE. In contrast, the addition of polymerized fresh C induces local activation and growth of microbes around the pieces of organic matter limiting in space, the apparent PE; moreover these polymerised organic compounds stimulate slow-growing K-strategists microbes that co-mineralize fresh C and recalcitrant SOM (Fontaine et al., 2003, 2011).

1.2.4 Mechanisms behind PE

The mechanisms behind PEs are still not fully understood. Priming effects were first attributed to the following two main mechanisms: (i) increased microbial activity or enzyme production of the whole microbial community following the addition of energy to the soil and (ii) increased microbial biomass induced by the input of fresh OM to soil, the newly formed microbes being able to decompose the SOM especially when the fresh organic matter (FOM) is exhausted (Fontaine et al, 2004a and literature cited).

However, the findings of Wu et al., (1993) who observed PE after soil addition of ryegrass but not after soil addition of glucose, which highly stimulates growth and activity

of microbial biomass led to the conclusion that neither increased microbial biomass or activity were involved in PE.

Fontaine et al. (2003) formulated the following two hypothesis to describe the mechanisms responsible of PE: given that in soil, even if a huge range of microorganisms are present, only a few of them are adapted to the dominant soil organic source, the other being dormant. After the supply of fresh organic matter (FOM) to soils, many dormant microorganisms are triggeredd into activity; these FOM specialized microorganisms, commonly classified as r-strategists, are adapted to intervals of rapid growth, depending on availability of their substrate. In contrast, SOM feeding microorganisms, classified as K-strategists, are continuously active because they use the almost inexaustible SOM. They grow slowly and dominate only in the last stages of FOM decomposition. When FOM is added to a soil r-strategists microorganisms produce enzymes in order to decompose this FOM. These enzymes are supposed to be partly efficient for degrading SOM (Mechanism 1) (Figure 1.4).

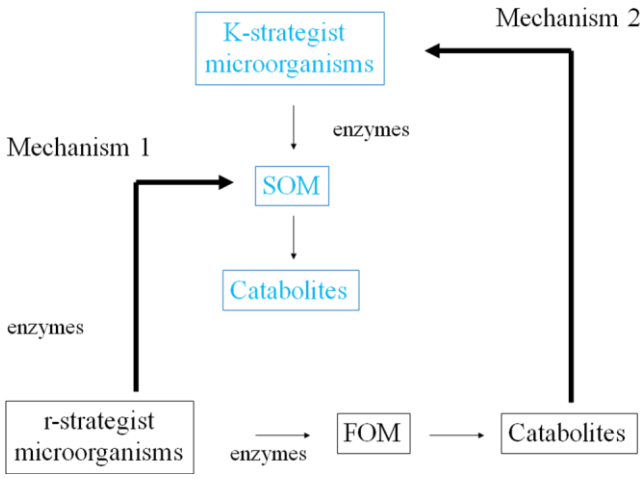

Figure 1.4. Mechanisms behind priming effect (PE). Modified from Fontaine et al., 2003.

This mechanism depends on biochemical similarities between FOM and SOM. The second mechanism deals with the FOM availability for r-strategists (Fontaine et al., 2003). Some types of r-strategist grow very quickly on simple and soluble substrates, which are exausted in some hours. In this condition, supplies of soluble and quickly assimilable carbon generally have no effect on the activities of K-strategists. In fact, most available

substrates are taken up by r-strategists before the slow K-strategists can increase their populations. In contrast, polymerised compounds of plant litter persist in soils longer than simple substrate (for example cellulose decomposition takes several weeks), and K-strategists mineralizing SOM, with slow growth rates, may benefit from these polymerised substrates which have a long residence time in soil. In these conditions, K-strategists populations increase the amount of SOM decomposing enzymes released in soil, since these microorganisms apparently do not adjust their enzyme production according to exogenous substrate at individual level. The sur-production of SOM-decomposing enzymes leads to the priming effect (Mechanism 2). The intensity of the mechanism relies on the competition for FOM between r- and K-strategists (Figure 1.4).

Later, Fontaine et al. (2004b) quantified the PE in a Savannah soil amended with ^{13}C-cellulose at a rate of 495 mg C kg^{-1}. Cellulase was applied to the soil at a rate of 30000 units kg^{-1} soil to quantify the contribution of cellulase to the PE of cellulose. The rate of soil C decomposition increased by 55% with cellulose addition leading to PE of 234 mg C kg^{-1}. Cellulase released 32 mg C-glucose from soil cellulose, representing only 14% of the PE. According to the authors this indicated that the decomposition of soil C required the production of specific enzymes, and that the PE resulted from the stimulation of microbes able to provide soil C decomposing enzymes. This results also showed that cellulose stimulated at least two types of microbes: soil C decomposing microbes that may also use cellulose and cellulose specialized microbes that exclusively decompose cellulose. So they concluded that PE depends on microbial competition (Mechanism 2).

The same authors (Fontaine et al., 2011) validated the ‘bank’ mechanism, based on the assumption that microbial degradation of recalcitrant SOM (PE) is modulated by the concentration of nutrients in soil solution (Fontaine et al., 2003, 2004b) (Figure 1.5). According to these mechanism, in conditions of low nutrient availability, for example when high nutrient uptake by plants occurs, the microbial mining of SOM could be intense and eventually exceed the formation of new SOM trough humification of fresh C, causing net destruction of SOM and release of mineral nutrients.

On the contrary, with high nutrient availability, microbial immobilization of N increases while mining of SOM decreases, leading to a greater sequestration of mineral nutrients (Fontaine et al., 2011).

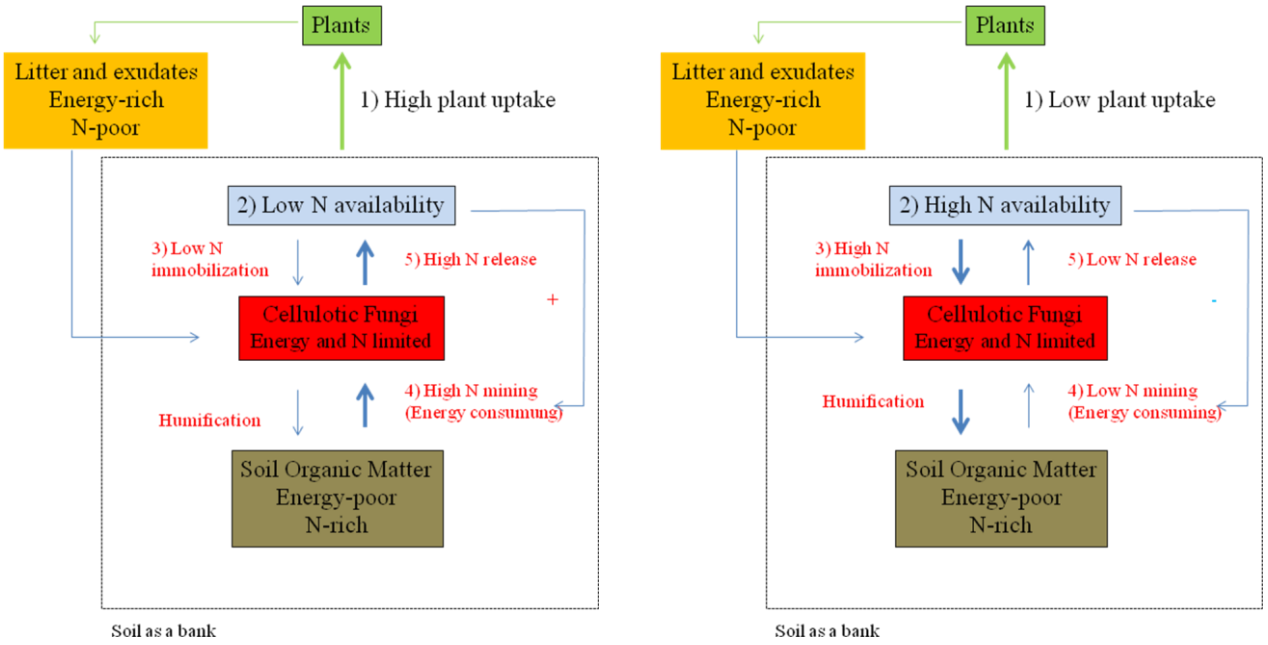

Figure 1.5. The bank mechanism. Modified from Fontaine et al., 2011.

1.2.5 Microorganisms responsible of PE

No clear information on the microorganisms responsible of PE is available yet. According to Fontaine et al. (2011), fungi are the main actors of real PE occurring after addition of cellulose, but when more complex substances than cellulose are used (i.e. plant litter), microbial population controlling the PE could be different. For example, Nottingham et al. (2009) found by ^{13}C-PLFA, that Gram-negative bacteria may also contribute to real PE. Moreover the implication of microbial groups as actinomycetes, protozoa and archea have not yet been investigated in priming experiments. As a consequence, little is known on the microbial groups involved in the different stages of PE (Kuzyakov, 2010).

1.2.6 Important implications of PE

In the course of priming, large amounts of C, N and other nutrients can be released in soil very quickly (Blagodatsky et al., 2010 and literature cited). If, into one hand this means a higher availability of nutrients, for example for plants, on the other hand, this

implies a decrease of soil C content (Fontaine et al., 2004a and 2011) thus inducing a negative carbon balance.

The importance of a better understanding of mechanisms involved in PE is lined out by very recent works in wich it is emphatized that models describing SOM dynamics, generally based on first-order kinetics, should be re-elaborate considering PE and thus the interactions between microbial biomass and SOM (Blagodatsky et al., 2010; Neill and Gignoux, 2006; Neill and Guenet, 2010).

1.3 *Meliaceae* derivatives

Plant derivatives of trees of some species of the familily of *Meliaceae* (ord. *Sapindales*) have been studied because of their insecticide and antimicrobial properties, thus attracting the interest of entomologists and phytochemists all over the world (Schmuetterer, 1990). *Azadiractha indica* A. Juss and *Melia azedarach* L. are the two plants belonging to this family that have been most studied in the last years. The term *Meliaceae* derivatives will be hereafter used to designate all products originated from different organs of plant belonging to this family.

1.3.1 *Azadiractha indica* A. Juss

The tree *Azadiractha indica* A. Juss (syn. *Antelaea azadirachta, Melia azedirachta*), also named neem tree, morgosa tree or Indian lilac, has its origin in southern and southeastern Asia, but grows also in tropical and subtropical areas of Africa, America and Australia (Schmuetterer, 1990). It is an evergreen, fast-growing tree which may reach a height of 25 meters. The fruits are oval, 1.4-2.4 cm long and have, when ripe, a yellowish sweet pulp that encloses a brown seed kernel, embedded in a hard white shell. They are produced in drooping panicles and are considered the most important source of compounds with insecticidal effects. The leaves, unpaired pinnate, that may reach a length of 30 cm, may also be used to produce extracts for pest control.

The main active component present in neem seed kernel is azadirachtin (AZ), a steroid-like tetranortriterpenoid (limonoid), but also other active compounds as salannin, salannol, salannolacetate, 3-deacetylsalannin, azadiradon, 14-epoxyazaradion, gedunin, nimbinen and deacetylnimbinen have been isolated from neem seed kernels (Schmuetterer, 1990 and literature cited). Some of these compounds have also been isolated from other parts of the plant, such as leaves, flowers, bark and roots (Mordue and Nisbet, 2000). These compounds, particularly AZ, are effective in controlling many economically important insects (Amadioha, 2000; Charleston et al., 2005, 2006) such as *Aphis gossypii*, *Bemisia tabaci*, *Plutella xylostella* and nematodes (Akhtar, 1999; Gopal et al., 2007 and literatue cited).

The most important product derived from neem trees is neem oil (Isman, 2006), generally obtained by cold-pressing seeds eventually followed by extraction with organic solvents (Nicoletti et al., 2012). Neem oil is mainly used for medical devices, production of cosmetics and soap, and as insecticide (Nicoletti et al., 2012). Quality and composition of neem oil can vary according to the origin of the raw material and the different oil extraction processes. For example, the presence of limonoids in neem oil change with the geographical area and the season in which seed are harvested (Nicoletti et al., 2012 and literature cited).

Neem cake is the final by-product of oil extractions from neem kernels. As for neem oil, its composition changes with the raw material used for the oil extraction. It is a product of low cost and widely available on the world market, already used as fertilizer and livestock feed supplement (Nicoletti et al., 2012 and literature cited). India produces, alone, 80,000 tons of oil and 330,000 tons of neem cake from 14 million of naturally growing trees (Nicoletti et al., 2012).

Besides their effect on insects and nematodes, neem extracts have been found to have antimicrobial activity (Méndez-Bautista et al., 2009) with notable effects on some fungal phytopathogens (Coventry and Allan, 2001).

In addition, neem cake, neem bitter and seed kernel powder have been reported to inhibits nitrification (Mohanty et al., 2008; Patra et al., 2002 and literature cited), although this effect has not been always confirmed (Toselli et al., 2010).

1.3.2 *Melia azedarach* L.

Melia azedarach L., also known as chinaberry, Persian lilac tree or melia, is a deciduous tree, very similar to neem in habitat and gross morphology (Safaei-Ghomi et al., 2010). It is native of India and China, but grows in different warm regions of America, Australia, Europe (Spain, Greece, Italy and southern France), where it is used as ornamental tree (Abou-Fakhr Hammat et al., 2001; Banfi and Consolino, 1996).

Similar active component to those found in neem tree have been reported for *M. azedarch* (Carpinella et al., 2002; Isman, 2006) and numerous studies have shown toxic effects of melia extracts for a variety of insects, mainly *Lepidoptera*, *Coleoptera*, *Diptera* and *Heteroptera* (Banchio et al., 2003; Defagò et al., 2011 and literature cited).

Moreover, leaves and seeds of *M. azedarach* contain meliacarpins, triterpenoids that are similar to the azadirachtins, with insecticidal and growth disrupting activities (Bohnenstengel et al., 1999; Isman, 2006). Conversely, development of melia commercial insecticides has not paralleled that of neem insecticides. The main reason is the presence in chinaberry seeds of additional triterpenoids, the meliatoxins that have demonstrated toxicity to mammals. However the chemistry of chinaberry varies considerably across its natural and introduced range, so that meliatoxins are not always present in melia seeds (Isman, 2006).

The interest for melia extracts is not limited to their effect on insects. A number of studies investigated the antimicrobial properties of melia extracts. For example, water and organic solvent extracts of *M. azedarach* leaves have been found to exhibit antifungal activity against *Ascochyta rabiei*, responsible for chickpea blight (Jabeen and Javaid, 2008; Jabeen et al., 2011). An *in vitro* study on 'MRS 2/5' shoot cultures, showed a bactericidal effect of melia leaf extracts against *Sphingomonas paucimobilis* and *Bacillus circulans* (Marino et al., 2009). In contrast, Spyrou et al. (2009) found, in a laboratory study, that soil application of 30 g kg^{-1} of pulverized melia fruit stimulates the soil microbial community, probably as a consequence of the increased availability of organic substrates and nutrients after the amendment application.

Finally, soil incorporation of *M. azedarach* derivatives has also been studied as a tool to increase soil fertility. In particular, fresh leaf litter of melia have been used as plant material containing high amounts of ash alkalinity to increase the pH of acid soils (Marschner and Noble, 2000). The addition of 5 g kg^{-1} of melia ground leaves improved acclimatization of micro propagated 'MRS 2/5' plantlets by improving their growth and

mineral uptake (Marino et al., 2009a e b). Moreover, soil application of 20 g kg^{-1} and 40 g kg^{-1} of fresh ground melia fruits increased N uptake of peach trees (Baldi et al., 2009; Toselli et al., 2010), leaf green colour and plant biomass compared to control plants (Toselli et al., 2010).

1.4 Aim of the research

Soil addition of organic materials can be useful to replace organic matter losses, hence improving soil fertility. However, some aspects of this agricultural practice still need to be assessed, in particular: i) the dynamic of nutrient release, and ii) the effect on the mineralization rate of native SOM, the so called 'priming effect'.

The aim of the research was to evaluate the effect of soil incorporation of some *Meliaceae* derivatives (6 commercial neem cakes and fresh melia leaves) on C and N dynamics, and nutrient availability in the soil-plant system. For this purpose 4 experiments were conducted.

In the first experiment (described in Chapter 2) the C and N mineralization process of the seven derivatives was compared in controlled conditions, in the absence of plant.

In the second experiment, we evaluate the effect of *Meliaceae* derivative-soil addition on nutrient uptake by micropropagated plants of GF677. In addition, to distinguish the source of N uptaken by plants (soil- or derivative-derived), the ^{15}N isotope technique was used in a third experiment in which GF677 one-year old plants were supplied with uniformly labelled melia leaves. Second and third experiment are described in Chapter 3.

Finally, in a laboratory incubation experiment, a natural ^{13}C abundance method was used to quantify the PE induced by the addition of 3 of the *Meliaceae* derivatives that, in the previous experiment, showed different N mineralization kinetics. This experiment is described in Chapter 4.

The conclusions of the research are reported in Chapter 5.

1.5 References

Abou-Fakhr Hammad E.M., Zournajian H., Talhouk S., 2001. Efficacy of extracts of *Melia azedarach* L. callus, leaves and friuts against adults of the sweetpotato whitefly *Bemisia tabaci* (Hom., Aleyrodidae). J. Appl. Ent., 125: 483-488.

Akhtar M., 1999. Biological control of plant parasitic nematodes in pigeon pea field crops using neem-based products and manurial treatments. Appl. Soil Ecol., 12: 191-195.

Alef K., Nannipieri P., 1995. Microbial biomass. In: Methods in applied Soil Microbiology and Biochemistry. Alef K., Nannipieri P (Eds), Academic Press, p.375.

Alexander M., 1997. Introduction to soil microbiology. 2nd ed. Jhon Wiley and Sons, New York.

Amadihoa A.C., 2000. Controlling rice blast *in vitro* and *in vivo* with extracts of *Azadiractha indica*. Crop. Prot., 19, 287-290.

Azam F., Simmons F.W., Mulvaney R.L., 1993. Mineralization of N from plant residues and its interaction with native soil N. Soil Biology and Biochemistry, 25 (12) 1787-1792.

Bader N.E., Cheng W., 2007. Rhizosphere priming effect of *Populus fremontii* obscures the temperature sensitivity of soil organic carbon respiration. Soil Biology and Biochemistry, 39: 600-606.

Baldi E., Toselli M., Sorrenti G., Quartieri M., Marangono B., 2010. Evaluationof the effectiveness of soil-applied melia (Melia azedarach L.) derivatives on nitrogen availability to peach trees. Acta Hort. 868: 129-134.

Baldock J.A., Nelson P.N., 2000. Soil Organic matter. In: Handbook of soil science. Summer M.E. (Ed.), CRC, Boca Raton, ppB25-B28.

Banchio E., Valladares G., Defagó M., Palacios S., Carpinella C., 2003. Effects of *Melia azedarach* (Meliaceae) fruit extracts on the leafminer *Liriomyza huidobrensis* (Diptera,

Agromyzidae): Assessment in laboratory and field experiments. Ann. Appl. Biol., 143: 187-193.

Banfi E., Consolino F., 1996. Alberi. Conoscere e riconoscere tuttele specie più diffuse di alberi spontanei e ornamentali. Istituto Geografico De Agostini, Novara, p272.

Bending G.D., Turner M.K., 1999. Interaction of biochemical quality and particle size of crop residues and its effect on the microbial biomass and nitrogen dynamics following incorporation into soil. Biol Fertil Soils, 29: 319-327.

Bending G.D., Turner M.K., 2009. Incorporation of nitrogen from crop residues into light-fraction organic matter in soils with contrasting management histories. Biol. Fertil. Soils, 45: 281-287.

Bending G.D., Turner M.K., Burns I.G., 1998. Fate of nitrogen from crop residues as affected by biochemical quality and the microbial biomass. Soil Biology and Biochemistry, 30(14): 2055-2065.

Bingeman C.W., Varner J.E., Martin W.P., 1953. The Effect of the Addition of Organic Materials on the Decomposition of an Organic Soil. Soil Science Society of America Proceedings, 17: 34-38.

Blagodatskaya E.V, Kuzyakov Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and Fertility of Soils, 45: 115-131.

Blagodatsky S., Blagodatskaya E., Yuyukina T., Kuzyakov Y., 2010. Model of apparent and real priming effects: Linking microbial activity with soil organic matter decomposition. Soil Biology and Biochemistry, 42: 1275-1283.

Bohn H.L., McNeal B.L., O'Connor G.A., 1985. Soil Chemistry. New York, Wiley-Interscience.

Bohnenstengel F.I., Wray V., Witte L., Srivastava R.P., Proksch P., 1999. Insecticidal meliacarpins (C-*seco* limonoids) from *Melia azedarach.* Phytochemistry, 50: 977-982.

Breland T.A., Hansen S., 1998. Comparison of the difference method and ^{15}N technique for studying the fate of nitrogen from plant residues in soil. Biol Fertil Soils, 26: 164-168.

Canali S., Benedetti S., 2006. Soil Nitrogen Mineralization. In: Microbiological Methods for Assessing Soil Quality. Bloem J., Hopkins D., Benedetti A. (Eds). CABI Publishing, pp 127-135.

Carpinella C., Ferrayoli C., Valladares G., Defago M., Palacios S., 2002. Potent Limonoid Insect Antifeedant from *Melia azedarach*. Biosci. Biotechnol. Biochem., 66: 1731-1736.

Charleston D.S., Kfir R., Dicke M., Vet L.E.M., 2006. Impact of botanical extracts derived from *Melia azedarach* and *Azadirachta indica* on populations of *Plutella xylostella* and its natural enemies: A field test of laboratory findings. Biological Controlo, 39: 105-114.

Charleston D.S., Kfir R., Dicke M., Vet L.E.M., 2005. Impact of botanical pesticides derived from *Melia azedarach* and *Azadirachta indica* on the biology of two parasitoid species of the diamondback moth. Biological Control, 33: 131-142.

Chaves B., Opoku A., De Neve S., Boeckx P., Van Cleemput O., Hofman G., 2006. Influence of DCD and DMPP on soil N dynamics after incorporation of vegetable crop residues. Biol. Fertil Soils, 43: 62-68.

Cheng W., 1996. Measurement of rhizosphere respiration and organic matter decomposition using natural ^{13}C. Plant and Soil, 183: 263-268.

Cheng W., 2009. Rhizosphere priming effects: its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. Soil Biology and Biochemistry, 41: 1795-1801.

Cheng W., Johnson D.W., Fu S., 2003. Rhizosphere effects on decomposition: controls of plant species, phenology and fertilization. Soil Sci. Soc. Am. J., 67: 1418-1427.

Cheng W., Kuzyakov Y., 2005. Root Effects on Soil Organic Matter Decomposition. In: Roots and Soil Management: Interactions between Roots and the Soil. R.W. Zobel and S.F. Wright (Eds). Madison: American Society of Agronomy.

Coventry E., Allan E.J., 2001. Microbiological and Chemical Analysis of Neem (Azadirachta indica) Extracts: New Data on Antimicrobial Activity. Phytoparasitica, 29 (5), 441-450.

Defagó M.T., Dumón A., Avalos D.S., Palacios S.M., Valladares G., 2011. Effects of *Melia azedarach* extract on *Cotesia ayerza*, parasitoid of the alfalfa defoliatot *Colias lesbia*. Biological Control, 57: 75-78.

De Neve S., Pannier J., Hofman G., 1994. Fractionation of vegetable crop residues in relation to in situ N mineralization. European Journal of Agronomy, 3: 267-272.

De Nobili M., Contin M., Mondini C., Brookes P.C., 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate. Soil Biology and Biochemistry, 33:1163-1170.

Diacono M., Montemurro F., 2010. Long-term effects of organic amendments on soil fertility. A review. Agron. Sustain. Dev., 30: 401-422.

Dijkstra F.A., Cheng W., 2007. Moisture modulates rhizosphere effects on C decomposition in two different soil types. Soil Biology and Biochemistry, 39: 2264-2274.

Dijkstra F.A., Bader N.E., Johnson D.W., Cheng W., 2009. Does accelerated soil organic matter decomposition in the presence of plants increase plant N availability? Soil Biology and Biochemistry, 41: 1080-1087.

Dijkstra F.A., Cheng W., Johnson D.W., 2006. Plant biomass influences rhizosphere priming effects on soil organic matter decomposition in two differently managed soils. Soil Biology and Biochemistry, 38: 2519-2526.

Dijkstra F.A., Morgan J.A., Blumenthal D., Follet R.F., 2010. Water limitation and plant inter-specific competition reduce rhizosphere-induced C decomposition and plant N uptake. Soil Biology and Biochemistry, 42: 1073-1082.

Dilly O., Zyakun A., 2008. Priming Effect and Respioratory Quotient in a Forest Soil Amended with Glucose. Geomicrobiology Journal, 25: 425-431.

Fontaine S., Mariotti A., Abbadie L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology and Biochemistry, 35: 837-843.

Fontaine S., Bardoux G., Abbadie L., Mariotti A., 2004a. Carbon input to soil may decrease soil carbon content. Ecology Letters, 7: 314-320.

Fontaine S., Bardoux G., Benest D., Verdier B., Mariotti A., Abbadie L., 2004b. Mechanisms of the Priming Effect in a Savanah Soil Amended with Cellulose. Soil Science Society Of America Journal, 68: 125-131.

Fontaine S., Henault C., Aamor A., Bdioui N., Bloor J. M. G., Maire V., Maron P. A. (2011). Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biology and Biochemistry, 43(1): 86-96.

Frankenberger W.T., Abdelmagid H.M., 1985. Kinetic parameters of nitrogen mineralization rates of leguminous crop incorporated into soil. Plant and Soil, 87: 257-271.

Fu S., Cheng W., 2002. Rhizosphere priming effects on the decomposition of soil organic matter in C_4 and C_3 grassland soils. Plant and Soil, 238:289-294.

Fu S., Cheng W., Susfalk R., 2002. Rhizosphere respiration varies with plant species and phenology: A greenhouse pot experiment. Plant and Soil, 239: 133-140.

Giller K.E., Cadisch G., 1997. Driven by Nature: A sense of arrival or departure? In: Driven by Nature. Plant Litter Quality and Decomposition, ed. G. Cadisch and Giller K.E., pp 393-399. CAB International, Wallingford, UK.

Gioacchini P., Nastri A., Marzadori C., Giovannini C., Vittori Antisari L., 2002. Influence of urease and nitrification inhibitors on N losses from soils fertilized with urea. Biol. Fertil. Soils., 36: 129-135.

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

Gregorich E.G., Drury C.F., Ellert B.H., Liang B.C., 1997. Fertilization effects on physically protected light fraction organic matter. Soil Science Society of America Journal, 61: 482-484.

Hamer U., Marschner B., 2002. Priming effects of sugars, amino acids, organic acids and catechol on the mineralization of lignin and peat. Journal of Plant Nutrition and Soil Science, 165: 261-268.

Hamer U., Marschner B., 2005. Priming effects in soils after combined and repeated substrate additions. Geoderma, 128: 38-51.

Haynes R.J., 2008. Soil Organic Matter Quality and the Size and Activity of the Microbial Biomass: Their Significance to the Quality of Agricultural Soils. In: Soil Mineral-Microbe-Organic Interactions. Q. Huang, P.M. Huang and A. Violante (Eds.), Springer.

Haynes R.J., Beare M.H., 1996. Aggregation and organic matter storage in mesothermal, humid soils. In: Carter M.R., Stewart BA (Ed), Structure and organic matter storage in agricultural soils. CRC Press, Boca Raton, pp213-262.

Isman M.B., 2006. Botanical Insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomolo., 51:45-66.

Jabeen K., Javaid A., 2008. Antifungal activity of aqueous and organic solvent extract of allelopathic trees against *Ascochyta rabiei*. Allelopathy Journal, 22: 231-238.

Jabeen K., Javaid A., Ahmad E., Athar M., 2011. Antifungal compounds from *Melia azedarach* leaves for management of *Ascochyta rabiei*, the cause of chickpea blight. Natural Product Research, 25: 3, 264-276.

Jenkinson D.S., R.H. Fox, Rayner J.H., 1985. Interactions between fertilizer nitrogen and soil nitrogen –the so called 'priming' effect. Journal of Soil Science, 36: 425-444.

Jensen H., 1929. On the influence of carbon:nitrogen ratios of organic material on the mineralization of nitrogen. J. Agr. Sci., 19: 71-82.

Jhonston A.E., Poulton P.R. and Coleman K., 2009. Soil organic matter: its importance in sustainable agriculture and carbon dioxide fluxes. Advances in Agronomy, 101:1-57.

Komatsuzaki M., Ohta H., 2007. Soil management practices for sustainable agro-ecosystems. Sustain. Sci., 2: 103-120.

Kuzyakov Y., 2002. Review: Factors affecting rhizosphere priming effects. J. Plant Nutr. Soil Sci., 165: 382-396.

Kuzyakov Y. and Bol R., 2006. Sources and mechanisms of priming effect induced in two grasslands soils amended with slurry and sugar. Soil Biology and Biochemistry, 38: 747-758.

Kuzyakov Y. and Cheng W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology and Biochemistry, 33: 1915-1925.

Kuzyakov Y. and Cheng W., 2004. Photosynthesis controls of CO_2 efflux from maize rhizosphere. Plant and Soil, 263: 85-99.

Kuzyakov Y., Ehrensberger H. and Stahr K., 2001. Carbon partitioning and below-ground translocation by *Lolium perenne.* Soil Biology and Biochemistry, 33: 61-74.

Kuzyakov Y., Friedel J.K., Stahr K., 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry, 32: 1485-1498.

Lal R., 2006. Enhancing crop yields in the developing countries through restoration of the soil organic carbon pool in agricultural lands. Land Degrade. Dev., 17: 197-209.

Löhnis F., 1926. Nitrogen availability of green manures. Soil Science, 22: 253-290.

Marino G., Gaggia F., Saiano F., Biavati B., Marangoni B., 2009a. Elimination of in vitro bacterial contaminants in shoot cultures od 'MRS 2/5' plum hybrid by the use of *Melia azedarach* extracts. Eur J Plant Pathol, 123: 195-205.

Marino G., Toselli M., Baldi E., Marangoni B., 2009b. Effects of soil-applied ground leaves of *Melia azedarach* L. on acclimatization of bacteria-contaminated rooted plum shoots. Acta Hort. 812: 447-453.

Marschner B., Noble A.D., 2000. Chemical and biological processes leading to the neutralisation of acidity in soil incubated with litter materials. Soil Biology and Biochemistry, 32: 805-813.

Mary B., Fresneau C., Morel J.L. and Mariotti A., 1993. C and N cycling during decomposition of root mucilage, roots and glucose in soil. Soil Biology and Biochemistry, 25 (8): 1005-1014.

Mayhew L., 2004. Humic substances in Biological Agriculture, Acres USA 34.

Méndez-Bautista J., Fernández-Luqueño F., López-Valdez F., Mendoza-Cristino R, Montes-Molina J.A., Gutierrez-Miceli F.A., Dendooven L., 2009. Effect of pest controlling neem (*Azadirachta indica* A. Juss) and mata-raton (*Gliricidia* sepium Jacquin) leaf extracts on emission of green house gases and inorganic-N content in urea-amended soil. Chemosphere, 76: 293-299

Mohanty S., Patra A.K., Chhonkar P.K., 2008. Neem (*Azadirachta indica)* seed kernel powder retards urease and nitrification activities in different soils at contrasting moisture and temperature regimes. Bioresource Technology, 99: 894-899.

Montes-Molina J.A., Luna-Guido M.L., Ceballos-Ramirez J.M., Fernández-Luqueño F., Espinoza-Paz N., Rincon-Rosales R., Dendooven L., Gutierrez-Miceli F.A., 2008. Effect of pest-controlling neem and mata-raton on bean growth, soil N and soil CO2 emissions. Agron. Sustain. Dev., 28:187-194.

Mordue A.J., Nisbet A.J., 2000. Azadirachtin from the neem tree *Azadirachtin indica*: its action against insects. An. Soc. Entomol. Bras., 29: 615-632.

Müller M.M., Sundman V., Soininvaara O., Marilainen A., 1988. Effects of chemical composition on the release of N from agricultural plant material decomposing in soil under field conditions. Biol. Fertil. Soils, 6: 78-83.

Murphy D.V., Stockdale E.A., Brookes P.C., Goulding K.W.T., 2007. Impact of microorganisms on chemical transformation in soil. In: Abbott L. K., Murphy D.V.(Eds), Soil biological fertility – A key to sustainable land use in agriculture, Springer, pp 37-59.

Neill C. and Gignoux J., 2006. Soil organic matter decomposition driven by microbial growth: a simple model for a complex network of interactions. Soil Biology and Biochemistry, 38: 803-811.

Neill C. and Guenet B., 2010. Comparing two mechanistic formalisms for soil organic matter dynamics: A test with in vitro priming effect observations. Soil Biology and Biochemistry, 42: 1212-1221.

Nicolardot B., Recous S., Mary B., 2001. Simulation of C and N mineralization during crop residue decomposition. A simple dynamic model based on the C:N ratio of the residues. Plant and Soil, 228: 83-103.

Nicoletti M., Mariani S., Maccioni O., Cuccioletti T., Murugan K., 2012. Neem cake: chemical composition and larvicidal activity on Asian Tiger mosquito. Parasitol. Res.doi 10.1007/s00436-012-2819-8.

Nottingham A.T., Griffiths H., Chamberlain P.M., Stott A.W. and Tanner E.V.J., 2009. Soil priming by sugar and leaf-litter substrates: A link to microbial groups. Applied Soil Ecology, 42: 183-190.

Palm C.A., Sanchez P.A., 1991. Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. Soil Biology and Biochemistry, 23: 83-88.

Paré T. and Gregorich G., 1999. Soil Textural Effects on Mineralization of Nitrogen from Crop Residues and the Added Nitrogen Interaction. Commun. Soil Sci. Plant Anal., 30 (1&2): 145-157.

Patra D.D., Anwar M., Chand S., Kiran U., Rajput D.K., Kumar S., 2002. Nimin and *Mentha Spicata* oil as nitrification inhibitors for optimum yeld of Japanese mint. Commun. Soil Sci. Plant Anal. 33 (3&4): 451-460.

Paul E.A., Clark F.E., 1989. Soil microbiology and biochemistry. Academic Press, San Diego.

Pierzynski G.M., Sims J.T., Vance G:F., 2000. Soils and Environmental Quality. CRC Press, Boca Raton, pp459.

Postma-Blaauw M.B., Bloem J., Faber J.H., Van Groenigen J.W., de Goede R.G., BrussaardsL., 2006. Earthworm species composition affects the soil bacterial community and net nitrogen mineralization. Pedobiologia, 50: 243-256.

Powlson D.S., Riche A.B., Coleman A.B., Glendining M.J. and Whitmore A.P., 2008. Carbon sequestration in European soils through straw incorporation: limitation and alternatives. Waste management, 28, 741-746.

Powlson D.S., Whitmore A.P. Goulding W.T., 2011. Soil Carbon sequestration to mitigate climate change: a critical re-examination to identify the true and the false. European Journal of Soil Science, 62: 42-45.

Recous S., Robin D., Darwis D., Mary B., 1995. Soil inorganic N availability: effect on maize residue decomposition. Soil Biology and Biochemistry, 27: 1529-1538.

Safaei-Ghomi J., Ahmadi T., Baatoli H., 2010. GC-MS identification of essential oil components and *in vitro* investigation of antioxidant activity of methanol extracts from flower and friut fractions of *Melia azedarach* cultivated in central Iran. Chemistry of Natural Compounds, 46 (5): 816-818.

Schmutterer H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadiractha indica.* Annu. Rev. Entomol., 35: 271-297.

Shen J. and Bartha R., 1997. Priming effect of glucose polymers in soil-based biodegradation tests. Soil Biology and Biochemistry, 29: 1195-1198.

Shumetterer H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu. Rev. Entomol., 35: 271-297.

Smith J.L., 1994. Cycling of Nitrogen through Microbial Activity. In: 'Soil Biology, effects on soil quality. Hatfield S.L., Stewart B.A. (Eds.). Lewis Publishers, CRC Press, pp 91-120.

Sparling G.P, 1997. Soil microbial biomass, activity and nutrient cycling as indicators of soil health. In: Biological indicators of soil health. Pankurst C.E., Doube B.M., Gupta VVSR (Eds), CAB International, Wallingford, pp 97-119.

Spyrou I.M., Karpouzas D.G., Menkissoglu-Spiroudi U., 2009. Do Botanical Pesticides Alter the Structure of the Soil Microbial Community? Microb. Ecol., 58: 715-727.

Stevenson F.J., 1986. Cycles of soil: carbon, nitrogen, phosphorus, sulfur, micronutrients. New York, Wiley-Interscience.

Stevenson F.J.,1994. Humus chemistry: genesis, composition, reactions. John Wiley, New York.

Swift M.J., Heal O.W., Anderson J.M., 1979. Decomposition in terrestrial ecosystems. Blackwell, Oxford.

Tian G., Kang B.T., Brussaard L., 1992. Biological effects of plant residues with contrasting chemical composition under humid tropical conditions: decomposition and nutrient release. Soil Biology and Biochemistry, 24: 1051-1060.

Tian G.L., Brussaard L., Kang B.T., 1995. An index for assessing the quality of plant residues and evaluating their effects on soil and crop in the (sub-)humid tropica. Appl. Soil Ecol., 2:25-32.

Toselli M., Baldi E., Sorrenti G., Quartieri M., Marangoni B., 2010. Evaluation of the effectiveness of soil-applied plant derivatives of *Meliaceae* species on nitrogen availability to peach trees. Scientia Horticulturae 124: 183-188.

Trinsoutrot I., Recous S., Bentz S., Linères M., Chèneby D., Nicolardot B., 2000. Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions. Soil Sci. Soc. Am. J., 64: 918-926.

Vigil M.F., Kissel D.E., 1991. Equations for estimating the amount of nitrogen mineralized from crop residues. Soil Science Society of America Journal, 5: 757-761.

Waksman S., 1924. Influence of microorganisms upon the carbon-nitrogen ratio in the soil. J. Agr. Sci., 14: 555-562.

White R.E., 1997. Principles and practice of soil science: the soil as a natural resource. London: Blackwell Science.

Wu J., Brookes P.C., Jenkinson D.S., 1993. Formation and destruction of microbial biomass during decomposition of glucose and ryegrass in soil. Soil Biology and Biochemistry, 25: 1435-1441.

Xue J.M., Clinton P.W., Sands R., Payn T.W. and Skinner M.F., 2008. Fate of biuret ^{15}N and its effect on net mineralisation of native soil N in forest soils. Australian Journal of Soil Research, 46: 363-644.

Zibilske L.M., 1994. Carbon mineralization. In: Methods of Soil Analysis, Part 2. Microbiological and Biochemical properties.SSSA, Madison, pp 835-863.

Chapter 2

Nitrogen and carbon mineralization of different *Meliaceae* derivatives incorporated into the soil

2.1 Introduction

In the last decades concern about environment preservation increased interest on sustainable agricultural practices such as the use of natural product in pest control or the use of organic matter (OM) in soil fertility and plant nutrition management. In sustainable orchard management, for example, the use of organic materials such as crop residue, animal manure, compost or agro-industrial by-products are encouraged as they can improve soil physical characteristics and replace soil OM losses (Johnston et al., 2009). Moreover, they can represent a source of nutrient for plants (Powlson et al., 2011): their decomposition is responsible for nutrient release and fluxes in agrosystems and this process is being investigated to optimize the induced soil nutrient availability to tree demand (Ha et al., 2008; Jacob et al., 2009; Singh and Sharma, 2007; Tognetti et al., 2008).

The process of decomposition of plant materials is the result of complex microbial activities controlled by numerous factors. Among these, the biochemical composition of the material has been recognised to exert an important influence (Swift et al., 1979) and many studies have been aimed at finding quality components that control the rate of decomposition and mineralisation of nutrients with contrasting results. For example, a wide range of quality factors have been found to be correlated with N release, these include the C to N ratio, N content, lignin content, lignin/N, polyphenol/N and polyphenol plus lignin/N ratios (Bending et al., 1998), soluble C concentrations. Moreover the quality factors controlling mineralization are likely to change over time as the nature of the remaining substrate changes (Killham, 1994).

Meliaceae are a family of plants including the tree *Azadiractha indica* A. Juss that grows wild in tropical and sub-tropical areas and whose derivatives are worldwide used for their antifungal and antibacterial properties. The most commercially important product obtained from *A. indica* is neem oil used as an insecticide. Neem cake, a by-product of oil

extraction of neem fruits, was found to act as a natural inhibitor of nitrification (Rao and Prasad, 1980; Joseph and Prasad, 1993), but was also found to increase soil concentration of nitrate N (NO_3^--N) and ammonium N (NH_4^+-N), and consequently N root uptake of peach trees (Toselli et al., 2010). The quality of the oil, as well as the chemical composition of the cake, strictly depends on the origin and quality of the seeds and on extraction process (Nicoletti et al., 2012).

Melia azedarach L. is taxonomically related to *Azadiractha indica* and it is native to India and China, grows in temperate areas of Europe, America and Australia where it is used as ornamental plant. Similar active components (limonoids) as those reported for *A. indica* were obtained from different organs of *M. azedarach*.

The aim of the research was to compare the carbon (C) and nitrogen (N) mineralization process of different *Meliaceae* derivatives incorporated into the soil and evaluate the possibility to use them in tree fertilizer and agronomical management.

2.2 Materials and Methods

Meliaceae derivatives

The following six commercial neem cakes (Nicoletti et al., 2012) were used in the experiments:

1 - Green neem, from Virdhunagar, India, (neem cake 1),

2 - Neem Italia, from Brescia, Italy, N content reported 3%, pelletted, (neem cake 2)

3 - Neem Italia, from Brescia, Italy, (neem cake 3),

4 - Deoiled neem cake, from Medors Biotech P. Ltd, Delhi, India, (neem cake 4)

5 - Oiled neem cake, from Medors Biotech P. Ltd, Delhi, India, (neem cake 5)

6 - Green neem, from Virudhunagar, India, (neem cake 6)

7 - In addition, plant derivative of *Melia azedarach* L. were used, specifically leaves harvested in June 2010 from seedlings grown in pots and frozen at -20°C prior to use. This treatment is named melia.

The derivatives were oven-dried at 65° and ball-milled prior to analysis. Total C and N concentration were determined with a CHN elementar analyser (Thermo Fisher, mod. EA 1110) on two replicates.

Incubation study

A clay loam Bathicalci Eutric Cambisols soil (FAO, 1990) was collected from the field of the Experimental Station of the University of Bologna, in Cadriano (44°35'N, 11°27'E), mixed with sand at a ratio soil:sand of 3:1, sieved at 2 mm, air-dried, moistened with distilled water to reach a water content of 14% and incubated at 20°C at constant soil humidity for one week prior to use. Neem cakes were pulverized and incorporated into the soil at a rate of 8 g fresh weight (fw) kg^{-1} dry weight (dw) soil. Melia leaves were chopped in little pieces and incorporated at a rate of 16 g fw kg^{-1} dw. A control treatment consisting of unamended soil and a mineral treatment containing 0.5 g of urea kg^{-1} dw were also included.

For each treatment, 50 g soil sub-samples were placed in 250 ml glass jars that were covered with perforated black polyethilene bags to allow aeration and incubated in a growth chamber with a costant temperature of 23°C ± 2 and relative air humidity of 85%. Throughout the incubation period soil moisture was maintained to the initial level by weighing and corrected, if necessary, by adding distilled water.

At days 0 (2 hours after the start of the experiment), 1, 2, 6, 12, 26, 54 and 118, four jars per treatment were destructively sampled for soil total-N, mineral-N and microbial C and N determinations. Total N concentrations were determined by Kjeldhal method (Schuman et al., 1973) by mineralizing 1.5-2 g of ground soil with 18 ml of a 95:5 (v/v) H_2SO_4:H_3PO_3 mixture, at 420°C, for 180 min, distillation with 32 % (v/v) NaOH and titration with 0.2 M HCl. Nitrate- (NO_3^--), nitrite- (NO_2^--), and ammonium– (NH_4^+-) N fractions were extracted by shaking 10 g of soil in 100 ml of a solution of 2 M KCl for 1 h. After sedimentation, soil extracts were stored at -20°C until analysis that were performed by autoanalyzer (Auto Analyzer AA-3; Bran + Luebbe, Norderstadt, Germany). Ammonium-N was determined after reaction of the sample solution with sodium silicate and dichloroisocyanuric acid with sodium nitroprusside as catalyzer, to develop a blue indophenol colour, whose intensity was spectrophotometrically measured at 660 nm. Nitrate-N was first reduced, in an alkaline hydrazine enriched environment, to nitrite to react with sulphanilamide and N(-1-naphthyl)ethylenediammine chlorohydrate and develop a purple colour measured at 520 nm. Because nitrate-N determination includes also the nitrite-N fraction and because, only a minimal presence of nitrite-N, compared to nitrate-N, was expected in most soils, the term NO_3^--Nwill be used hereafter to indicate total amount of nitrate- and nitrite- N.

Microbial C and N were determined by the fumigation extraction method (Vance et al, 1987) as follows: 5 g fw soil were extracted with 20 ml of 0.5 M K_2SO_4 and filtered through filter paper S&S 595 (Ahlstrom, Helsinki, Finland), another sample of 5 g of soil fw was firstly fumigated with chloroform for 24 h and then extracted in the same way. The extracts were frozen at -20°C until analysis for total organic C and total N concentrations using a total organic C and total N measuring unit (TOC-Vcpn TNM-1, Shimadzu, Kyoto, Japan). The total amount of extractable microbial C and N were determined by the difference between K_2SO_4 extractable C and N in fumigated and non-fumigated soils. No correction factor, that is usually applied to correct for the incomplete recovery of microbial constituents extracted from soil after fumigation was used, since the values of microbial C are used here to reveal relatives differences between derivatives. The amount of C extracted with K_2SO_4 0.5 M from non-fumigated soil was considered as extractable organic-C (EOC).

To quantify C mineralization, CO_2 fluxes were periodically measured during all incubation period. Four glass jars per treatment were sealed for approximetively 3 hours, and the CO_2 accumulated in the headspace of the jar was measured with an infrared gas analyzer (EGM-4, PP system; Hitchin, UK). Data of hourly CO_2-C production were used to obtain cumulative CO_2-C fluxes over the incubation period by assuming a linear increase of CO_2 concentration over time during the enclosure period as well as linear changes between subsequent flux measurements.

The rate of C and N applied to soil differed (Table 2.1) according to the derivative C and N concentration. To compare the mineralization of the different *Meliaceae* derivatives, apparent net N mineralization (N derived from the derivative) was calculated as the difference between the mineral N present in the amended soil and that present in the control soil and was expressed as % of added-N. Similarly, apparent C mineralization was calculated as the difference between CO_2-C produced by the amended soil and that produced over the same period by the control soil and was expressed as % of added C. By doing that, it was assumed that the addition of the derivatives did not modify the mineralisation of native soil organic matter (no priming effect occured).

Data were analyzed as in a factorial design with two factors: treatments (9 levels: control, urea, neem 1, neem 2, neem 3, neem 4, neem 5, neem 6, melia leaves) and time (8 levels: day 0, 1, 2, 6, 12, 26, 54, 118). When analysis of variance showed statistical significance at $P \leq 0.05$, means were separated by Student Newman Keuls test. When interaction between factors was significant, 2 standard error of means (SEM) was used as

minimum difference between statistically different values. Pearson correlation coefficient (r) was determined to evaluate the relationship between C %, N % and C:N ratio of the *Meliaceae* derivatives and total C and N mineralized.

2.3 Results

Meliaceae derivatives

The *Meliaceae* derivatives used in the experiments resulted different in term of mineral composition and dry matter content (Table 2.1). Carbon concentration ranged from 35 (neem 3) to 50 % (neem 2) and N concentration from 1.6 % (neem 5) to 4.4 % (neem 6). As a consequence, the C:N ratio of the derivatives ranged between 9 (neem 3) and 27 (neem 5). Melia leaves presented the highest water content (71 %) while in neem cakes water was lower than 11%.

Table 2.1. Chemical characteristics of *Meliaceae* derivatives and amounts of C and N added to soil.

	Derivative characteristics				**Amount of C and N added**	
	Dry matter (%)	**C** (%)	**N** (%)	**C:N**	**C** mg kg^{-1}soil	N mg kg^{-1}soil
Neem cake 1	89	47	2.2	21	3364	159
Neem cake 2	94	50	3.1	16	3770	234
Neem cake 3	92	35	3.7	9	2592	275
Neem cake 4	96	41	1.7	24	3126	128
Neem cake 5	91	44	1.6	27	3192	119
Neem cake 6	94	49	4.4	11	3692	329
Melia leaves	29	42	2.4	17	1982	114

Incubation study

During the incubation period, the average total N concentration (N determined by Kjeldhal method + nitrate-N fraction) of control soils resulted of 957 mg kg^{-1} dw (data not tabulated). In all the treated soils this parameter reflected the amount of N added (Table 2.1) with the fertilizers and resulted of 1167, 1042, 1193, 1217, 1232, 1114, 1031 and 1287 mg N kg^{-1} respectively for urea, melia and neem 1 to 6 treatment (data not tabulated).

Ammonium-N was released immediately after incorporation of urea and neem 3 (Figure 2.1), maximum concentrations resulted of 135 and 96 mg kg^{-1} dw at day 2 and 1, respectively. Also neem 2 and 6 had a peak of release of ammonium-N, but it was delayed of a few days and with maximum values of 22 and 54 mg kg^{-1} dw respectively. In all the other treatments NH_4^+-N was present at very low concentrations and after 26 days, small amounts of ammonium-N were detected in soil for all treatments.

Nitrate-N concentration in control soils ranged between 30 and 70 mg kg^{-1} dw at day 1 and 118, respectively (Figure 2.2). After incorporation of urea, neem 2 and neem 3, NO_3^--N increased with time until day 54, and reached maximum values of 159, 120 and 137 mg kg^{-1} dw, respectively. On the contrary, after incorporation of neem 4 and 5, NO_3^--N decreased and resulted lower compared to the control soil for most of the incubation period. A rapid decrease of nitrate-N was detected also in neem 1 treated soil from day 12 to 52 and in neem 6 at day 6, then, in the latter case, it increased and resulted higher than control soils from day 26 until the end of the incubation. In melia treated soils NO_3^--N concentration was slightly lower compared to control until day 54, but did not resulted significantly different.

The derivates showed a different net apparent N mineralisation (Figure 2.3). It was always positive for neem 3 (C:N=9) which showed the maximum N mineralised (34.5 % added-N) at day 2. The behaviour was similar to urea, which caused a rapid increase of mineral-N, in this case the surplus of mineral-N compared to the control soils represented 60-62 % of added N after 2-6 days, then it decreased and resulted steady around 40 % of added-N from day 26 until the end of the experiment. The incorporation into the soil of all the other derivatives initially caused a net N immobilization although the amount of N immobilized varied with the derivative. In particular, neem 2 and neem 6 (C:N=11 and 16, respectively) caused a short period of immobilization (2 days), after which the amount of N mineralised increased and reached values around 20 % of added-N at day 54. Net N mineralization was always negative (net immobilization) for neem 4 and neem 5 (C:N

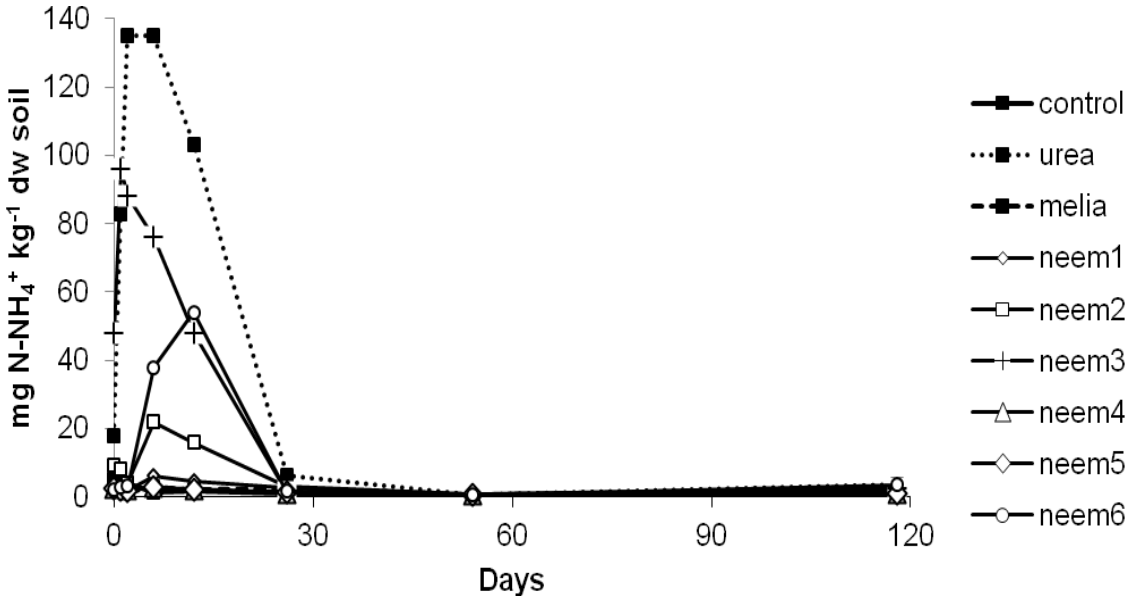

Figure 2.1. Effect of soil addition of plant derivatives on ammonium-N evolution during the incubation. Interaction time*treatment significant at P ≤0.001. Minimum difference between statistically different values (2SEM) = 4 (n=4).

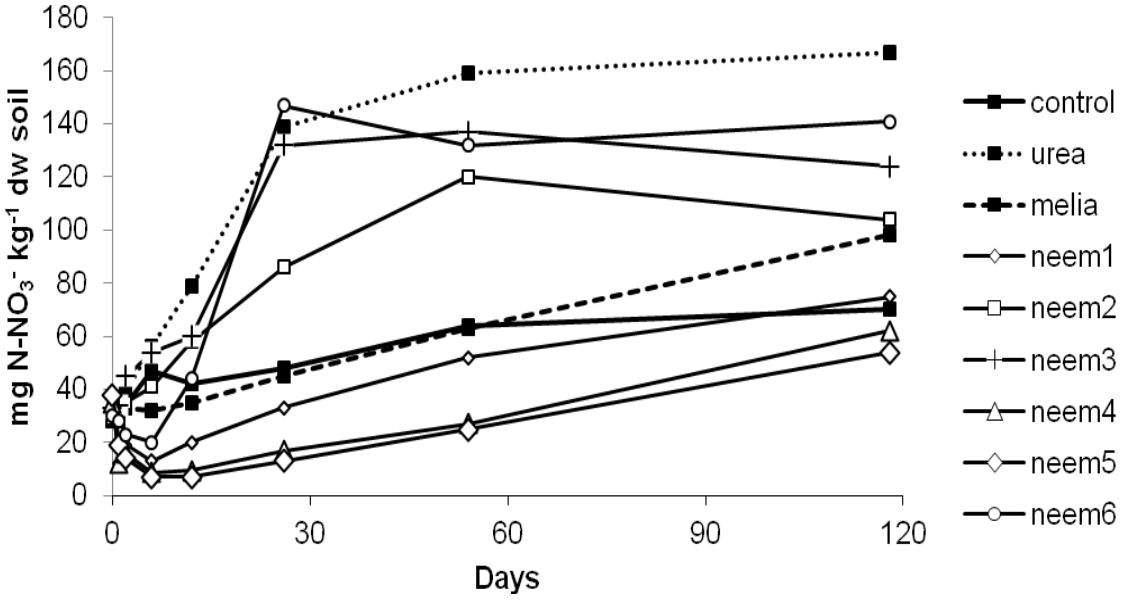

Figure 2.2. Effect of soil addition of *Meliaceae* derivatives and urea on nitrate–N evolution during the incubation. Interaction time*treatment significant at P ≤0.001. Minimum difference between statistically different values (2SEM) = 13 (n=4).

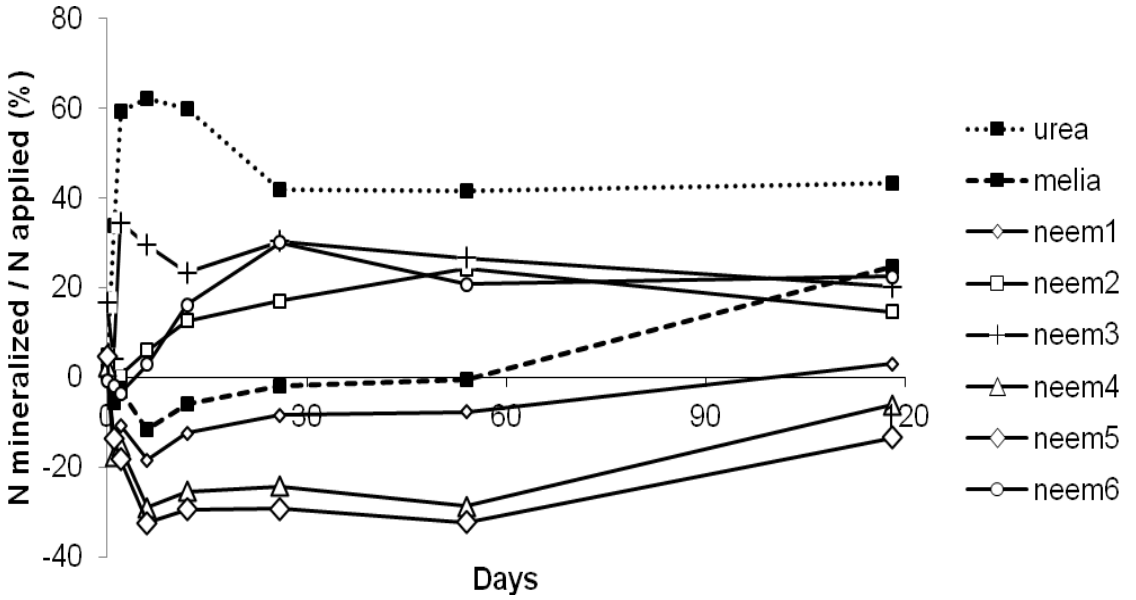

Figure 2.3. Apparent net N mineralization of the *Meliaceae* derivatives. Interaction time*treatment significant at P ≤0.001. Minimum difference between statistically different values (2SEM) = 5.6 (n=4).

>24) which showed a similar dynamic of N mineralization. Melia leaves (C:N =22), and similarly neem 1 (C:N =21) showed a net N immobilization during the first 50 days of incubation, followed by a positive net mineralization

Nitrogen mineralized resulted positively correlated with the N concentration of the derivative and negatively correlated with the C:N ratio of the derivatives after day 1 (r=-0.81, p<0.001) (Table 2.2). It was negatively correlated to C concentration only immediately after the incorporation of the derivatives (t0: r=-0.78, P<0.001).

After 118 days of incubation, the amount of C mineralized in the control soil was 224 mg kg^{-1} dw (Figure 2.4). The addition of the derivatives rapidly increased C mineralization, which resulted higher by 3-5 time at day 118. The amount of C mineralized by the end of the incubation, expressed as a proportion of derivative C, ranged from 25 % for neem 1 and neem 2 to 15 % for neem 3 (Figure 2.5). The observed differences at day 118 resulted mainly from differences in C mineralization during the first 40 days.

The C mineralized was only slightly dependent on the chemical composition of the residue, being positively correlated to the C:N ratio in the first 6 days and to the C concentration of the residue from day 26 (r=0.61, p<0.001) to the end of the experiment and slightly negatively correlated to the derivative N % in the first 6 days (Table 2.2).

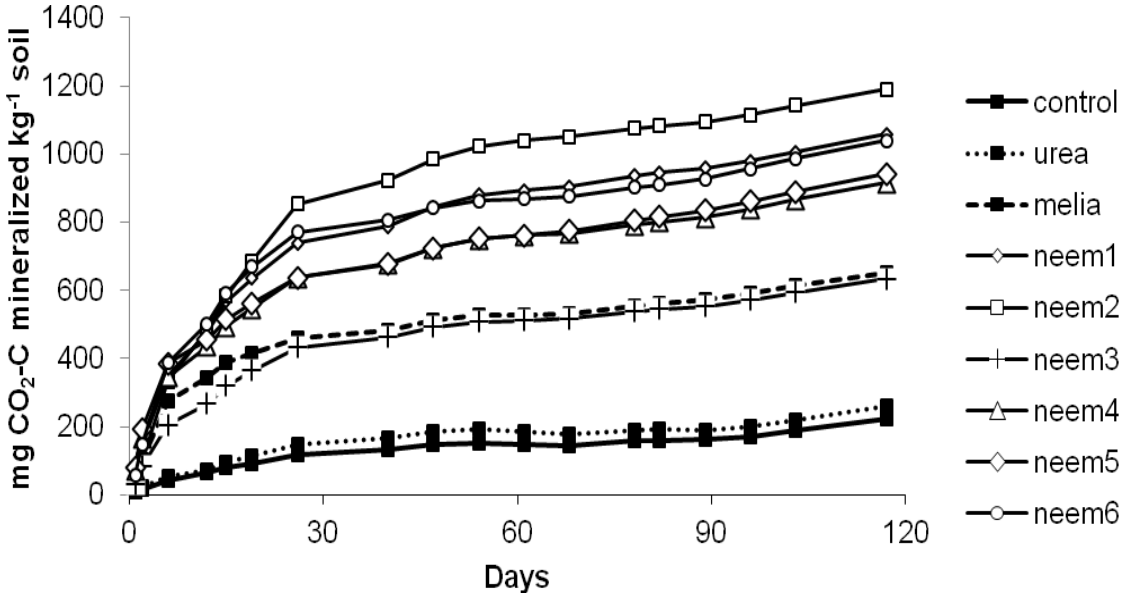

Figure 2.4. Effect of soil addition of *Meliaceae* derivatives and urea on cumulative C mineralized. Interaction time*treatment significant at P≤0.001. Minimum difference between statistically different values (2SEM) = 77 (n=4).

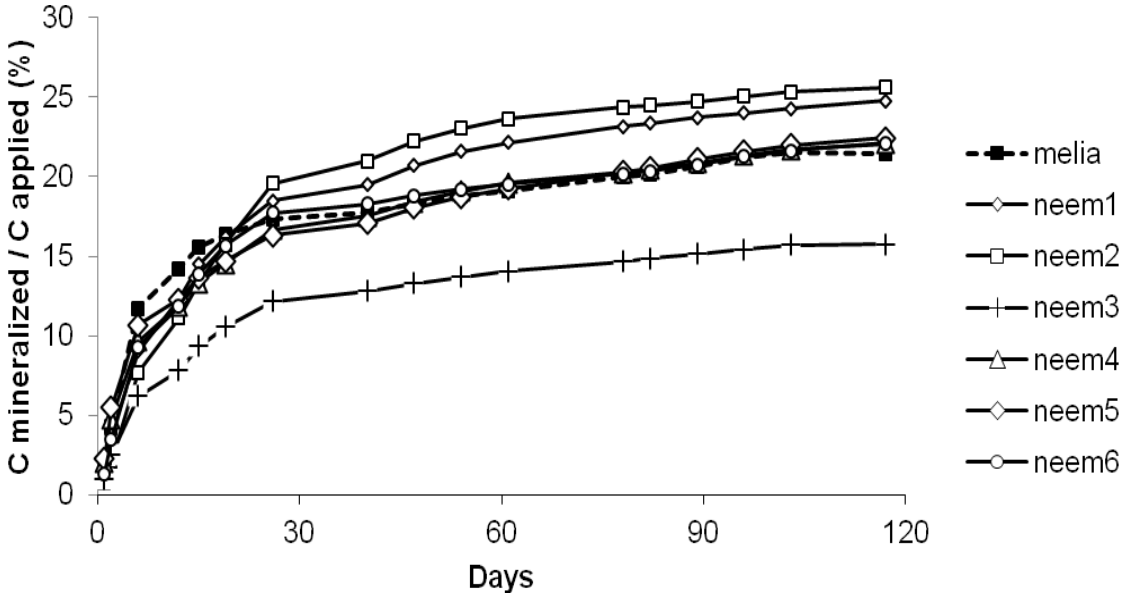

Figure 2.5. Apparent C mineralization of *Meliaceae* derivatives. Interaction time*treatment not significant. Minimum difference between statistically different values (2SEM) =2.62 (n=4).

Table 2.2 Pearson correlation coefficient (r) and significance between N and C mineralized and derivative concentration of C, N and C:N ratio during the incubation period.

	N mineralized (% added-N)							
	Day							
	0	**1**	**2**	**6**	**12**	**26**	**54**	**118**
C %	-0.78 ***	-0.53 *	-0.55 **	ns	ns	ns	ns	ns
N %	ns	0.66 ***	0.64 **	0.83 ***	0.92 ***	0.94 ***	0.87 ***	0.72 ***
C:N	ns	-0.81 ***	-0.82 ***	-0.93 ***	-0.96 ***	-0.96 ***	-0.92 ***	-0.82 ***
	C mineralized (% added-C)							
	Day							
	0	**1**	**2**	**6**	**12**	**26**	**54**	**118**
C%	-	ns	ns	ns	ns	0.61 ***	0.65 ***	0.65 ***
N%	-	-0.43 *	-0.70 ***	-0.46 *	ns	ns	ns	ns
C:N	-	0.44 *	0.77 ***	0.50 ***	ns	ns	ns	ns

ns, *, ** and ***= not significant or significant at P≤0.05, 0.01, 0.001, respectively.

Microbial biomass C peaked during the first 6 days after incorporation of all derivatives (Figure 2.6), with untreated control and urea fertilized soil that showed the lowest amount of microbial C. Then it declined to low amounts for all treatments. As for biomass C, microbial biomass N peaked in the first 6 days of the incubation period except in control soil which showed the lowest microbial N (Figure 2.7).

For almost all the incubation period K_2SO_4-extractable organic C (Figure 2.8) was lower in untreated control and mineral-treated soil compared to the amended soils which showed a peak between day 6 and 12. Among these, melia-treated soil always showed the lowest EOC concentration (maximum 236 mg C kg^{-1} dw) and neem 5 the highest (343 mg C kg^{-1} dw at day 2). After day 12, EOC decreased with time in all treatments.

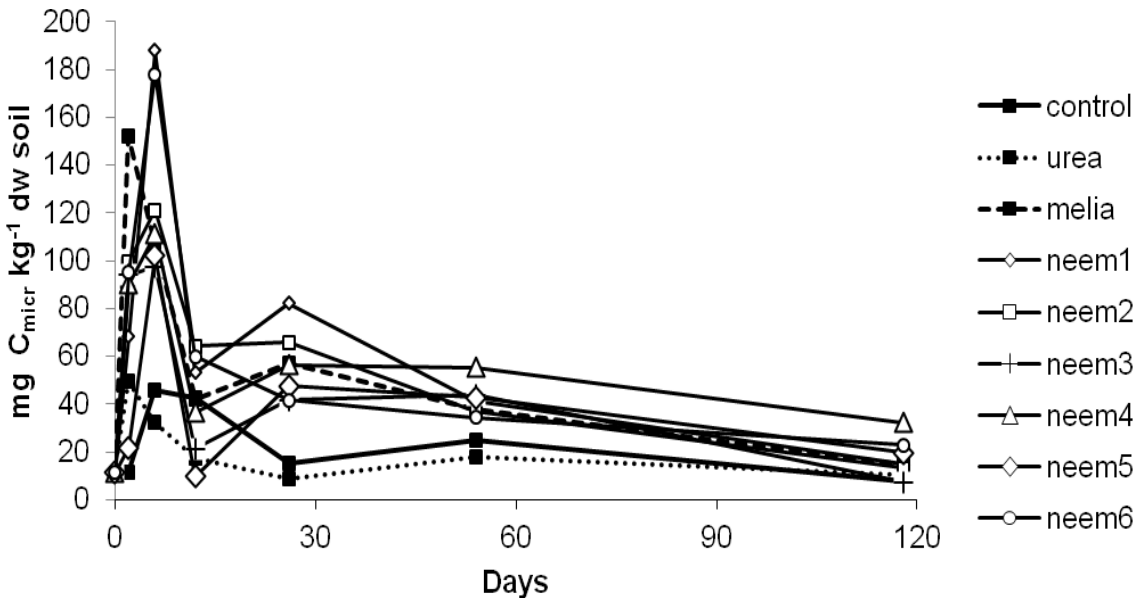

Figure 2.6. Effect of soil addition of *Meliaceae* derivatives and urea on microbial biomass C evolution during the incubation study. Interaction time*treatment significant at P ≤0.001. Minimum difference between statistically different values (2SEM) =29.3(n=4).

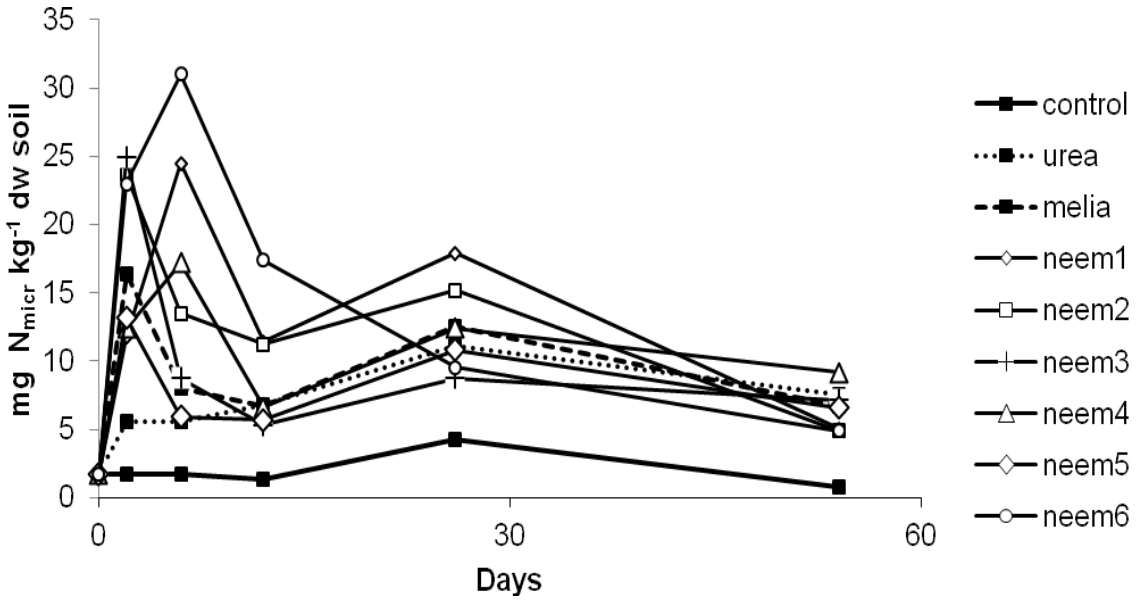

Figure 2.7. Effect of soil addition of *Meliaceae* derivatives and urea on microbial biomass N evolution during the incubation study. Interaction time*treatment significant at P ≤0.05. Minimum difference between statistically different values (2SEM) = 6.45 (n=4).

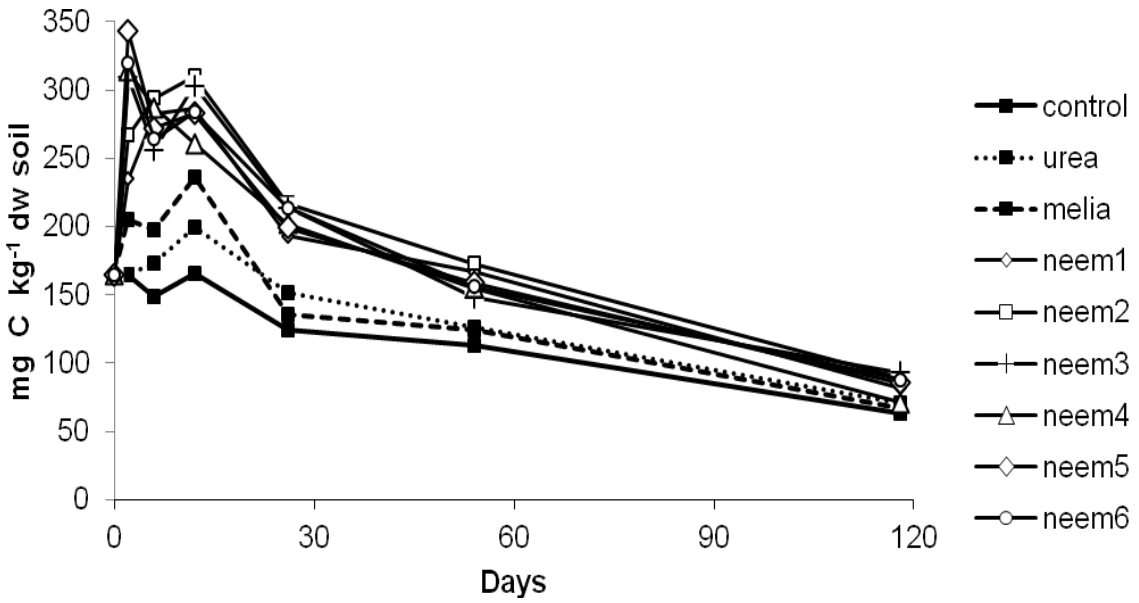

Figure 2.8. Effect of soil addition of *Meliaceae* derivatives and urea on K_2SO_4-extractable organic C evolution during the incubation study. Interaction time*treatment significant at $P \leq 0.05$. Minimum difference between statistically different values (2SEM) = 18 (n=4).

2.4 Discussion

The derivatives had different origin in term of species (i.e. *Azadiractha indica* for neem cake and *Melia azedarch* for melia leaves) and organ (neem kernels for neem cake and leaves for melia). Moreover, the commercial neem cakes employed in the experiment are produced by different companies and are the by-products of different oil extraction processes, resulting in different C and N composition. A similar variability was also found in terms of limonoids composition through complementary use of High Performance Thin Layer Chromatography (HPTLC), Nuclear Magnetic Resonance (NMR) analysis and High Performance Liquid Chromatography (HPLC) (Nicoletti et al., 2012). In particular, the concentration of salannin, the main limonoid, and azadiracthin A changed with the *Meliaceae* derivative.

In our investigation the two variables best related to N release were derivative-N concentration and C:N ratio, which is the criterion most used to predict N mineralization during crop residue decomposition (Vanlauwe et al., 1996). On contrast, C % was not a good indicator of N release of *Meliaceae* derivatives with the exception of the beginning of the essay. The explanation of these results may be the behavior of microbial biomass

that at the beginning of the incubation attacked the new C pool to find energy source; making this, N was immediately immobilized (negative correlation). Later, the presence of C did not control N mineralization as probably the availability of N became the limiting factor of N mineralization.

To define the biochemical quality that best control the dynamics of C and N released by residues (especially those with high C/N ratio), mineral N is usually added at the beginning of the study to prevent decomposition being limited by low mineral N availability (Recous et al., 1995; Trinsoutrot et al., 2000). In this experiment no mineral N was applied because the main purpose was to evaluate the possibility to use the different derivatives in N nutrient management and thus study the effect of incorporation of the different derivatives on N dynamics and its subsequent availability for plant growth without any other N supply. The initial mineral N content of the unamended soil was 35 mg kg^{-1} dw which probably was not sufficient to fulfill the N requirements of the soil decomposers (especially during the decomposition of derivatives with high C:N ratio), but, on the other hand, it is more than sufficient for tree demand.

Neem 3, the derivative with the lowest C:N ratio, had a similar behavior to urea. Both urea hydrolysis and neem 3 apparent mineralization were immediate, then the mineral-N decreased similarly from day 2 to day 12, probably because of N volatilization losses that occurred after urea and neem 3 application. It is known, in fact, that in soil, urea is hydrolyzed by urease to NH_3 and CO_2 with a rise in pH and an accumulation of NH_4^+. Surface application of urea can promote gaseous losses of NH_3 equal to 50 % of the fertilizer N applied (Terman, 1979). Nitrogen losses by NH_3 volatilization are frequent also after surface application of ammoniacal fertilizers or readily decomposable organic wastes, particularly if the soil (or organic waste) is alkaline in nature (Pierzynski et al., 2000). In this experiment the fertilizers were not surface applied but incorporated into the soil, however, the soil was mixed with sand that can have promoted N volatilization losses for both urea and neem 3.

Neem 2 and neem 6 (C:N of 11 and 16, respectively) showed a slower release of mineral-N, but at the end of the incubation, they mineralized in the same proportion as neem 3. Melia leaves seemed to have no effect on mineral-N concentration except at the end of the incubation. Neem 1, but especially neem 4 and 5 (C:N of 24 and 27, respectively) caused a long period of immobilization. Thus, the use of neem cakes as ‘fertilizer’ for improving N management should be defined after their characterization. The use of neem cake 3, 2 and 6 can be suggested when a rapid N supply to plants is

required, on the contrary cakes as neem 4 and neem 5 could be potentially used in N management strategies to temporary reduce soil nitrate concentration, helping to prevent nitrate from leaching, as hypothesized for other organic amendments (Chaves et al., 2008; Jin et al., 2008; Rhan et al., 2003). However, as suggested by Jin et al., (2008), care should be paid to the extrapolation to field conditions because differences in conditions of decomposition may occur.

In previous experiments, neem cake was found responsible for nitrification inhibition (Sahrawat, 1989). Nimin, a tetranortriterpinoid obtained after alcohol extraction of *Azadirachta indica* oil, showed some regulatory effect on urea hydrolysis (Patra et al., 2002). Also neem seed kernel powder was found to have potential to retard the urease activity in acid soil, and nitrification in all the soil tested (Mohanty et al., 2008). Montes-Molina et al. (2008) firstly found that neem leaf extracts strongly inhibited the release of NO_3^- but, in another experiment, Méndez-Bautista et al. (2009) found that the dynamics of NH_4^+, NO_2^- and NO_3^- were not affected by neem leaf extract, when soil was amended with or without urea and incubated at 40% and 100% water holding capacity. The authors explained the contrasting results mentioning that possible effects will depend on characteristics of the soil and/or neem leaf extracts, i.e. the concentration of the active components. In our conditions no indication of this kind of effect was detected for none of the neemcakes as high NH_4^+ concentrations never have been maintained constant by time; rather, peaks of NH_4^+-N were always followed by immediate increase on NO_3^--N concentrations. These findings are partially in agreement with previous studies in which soil-applied commercial neem cake (10 g kg^{-1}) was ineffective in decreasing the level of NO_3^--N after soil application of urea-N, rather it increased soil concentration of NO_3^--N and NH_4^+-N (Toselli et al., 2010).

We used the amount of C extracted with 0.5 M K_2SO_4 as a measure of labile C and defined it as EOC, aware that it may differ significantly from that measured by water extraction, generally used for dissolved organic carbon (DOC) determinations (Haynes, 2005) and considered labile substrate for soil microbial activity. With the incorporation of the derivatives, the EOC immediately increased, then progressively decreased reaching amount similar to control and mineral soils, indicating a relationship between EOC and respired CO_2 that resulted enhanced immediately after incorporation of the derivatives, and similar to control and mineral soils at the end of the incubation. These findings are in agreement with the laboratory study of Spyrou et al. (2009) who found that the application of pulverized melia fruits (PMF) resulted in significant increase in the soil microbial

community and this response was attributed to the release of copious amount of organic C and nutrients in the soil by the PMF; on the contrary, in the same experiment, azadirachtin did not modify the soil microbial community.

2.5 References

Bending G.D., Turner M.K. and Burns I.G., 1998. Fate of nitrogen from crop residues as affected by biochemical quality and the microbial biomass. Soil Biology and Biochemistry, 30: 2055-2065.

Chaves B., De Neve S., Boeckx P., Dupont R., Van Cleemput O., Hofman G., 2008. Manipulating the N release from ^{15}N-labelled celery residues by using straw and vinasses in Flanders (Belgium). Agriculture, Ecosystems & Environment, 123: 151-160.

Food and Agriculture Organization (FAO), 1990. Guidelines for soil profile description, 3rd ed. Soil Resources Management and Conservation Service, Land and Water Development Division, FAO, Rome.

Ha K.V., Marschner P., Bünemann E.K., 2008. Dynamics of C, N, P and microbial community composition in particulate soil organic matter during residue decomposition. Plant Soil, 303: 253-264.

Haynes R.J., 2005. Labile organic matter fractions as central components of the quality of agricultural soils: an Overview. Advances in Agronomy, 85: 221-268.

Jacob M., Weland N., Platner C., Schaefer M., Leuschner C., Thomas F.M., 2009. Nutrient release from decomposing leaf litter of temperate deciduous forest trees along a gradient of increasing tree species diversity. Soil Biology and Biochemistry, 41: 2122-2130.

Jin K., Sleutel S., De Neve S., Gabrield D., Cai D., Jin J., Hofman G., 2008. Nitrogen and carbon mineralization of surface-applied and incorporated winter wheat and peanut residues. Biol Fertil Soils, 44: 661-665.

Johnston A.E., Poulton P.R., Coleman K., 2009. Soil Organic matter: its importance in sustainable agriculture and carbon dioxide fluxes. Advances in Agronomy, 101: 1-57.

Joseph P.A., Prasad R., 1993. The effect of dicyandiamide and need cake on the nitrification of urea-derived ammonium under field conditions. J. Biol. Fertil. Soils, 15: 149-152.

Killhan K, 1994. Soil Ecology. Cambridge, Cambridge University Press.

Montes-Molina J.A., Luna-Guido M.L., Ceballos-Ramirez J.M., Fernández-Luqueño F., Espinoza-Paz N., Rincon-Rosales R., Dendooven L., Gutierrez-Miceli F.A., 2008. Effect of pest-controlling neem and mata-raton on bean growth, soil N and soil CO2 emissions. Agron. Sustain. Dev., 28:187-194.

Méndez-Bautista J., Fernández-Luqueño F., López-Valdez F., Mendoza-Cristino R, Montes-Molina J.A., Gutierrez-Miceli F.A., Dendooven L., 2009. Effect of pest controlling neem (*Azadirachta indica* A. Juss) and mata-raton (*Gliricidia* sepium Jacquin) leaf extracts on emission of green house gases and inorganic-N content in urea-amended soil. Chemosphere, 76: 293-299

Mohanty S., Patra A.K., Chhonkar P.K., 2008. Neem (Azadiractha indica) seed kernel powder retards urease and nitrification activities in different soils at contrasting moisture and temperature regimes. Bioresource Technology., 99: 894-899.

Nicoletti M., Mariani S., Maccioni O., Cuccioletti T., Murugan K., 2012. Neem cake: chemical composition and larvicidal activity on Asian Tiger mosquito. Parasitol. Res.

Patra D.D., Anwar M., Chand S., Kiran U., Rajput D.K., Kumur S., 2002. Nimin and *Mentha spicata* oil as nitrification inhibitors for optimum yield of Japanese mint. Comm. Soil. Sci. Plant. Anal., 33 (3&4): 451-460.

Pierzynski G.M., Sims J.T., Vance G.F., 2000. Soils and Environmental Quality. CRC Press, Boca Raton , 459 pp.

Powlson D.S., Whitmore A.P. and Goulding W.T., 2011. Soil carbon sequestration to mitigate climate change: a critical re-examination to identify the true and the false. European Journal of Soil Science, 62: 42-55.

Rao P., Prasad R., 1980. Nitrogen leaching losses from conventional and new nitrogenous fertilizers in low-land rice culture. Plant and Soil, 57: 383-392.

Recous S., Robin D., Darwis D., Mary B., 1995. Soil inorganic N availability: effect on maize residue decomposition. Soil Biology and Biochemistry, 27: 1529-1538.

Rhan C.R., Bending G.D., Turner M.K., Lillywhite R.D., 2003. Management of N mineralization from crop residues of high N content using amendment materials of varying quality. Soil Use and Management, 19: 193-200.

Saharawat K.L., 1989. Effect of nitrification inhibitors on nitrogen transformation, other than nitrification, in soils. Adv. Agron., 42: 279-309.

Schuman G.E., Stanley A.M., Knudsen D., 1973. Automated total nitrogen analysis of soil and plant samples. Proc. Soil Sci. Soc. Am. 37: 480-481.

Singh B., Sharma K.N., 2007. Nutrition and growth of wheat-sorghum rotation in soils amended with leaf litter of trees before planting of wheat. Agroforest Syst, 71: 25-34.

Spyrou I.M., Karpouzas D.G., Menkissoglu-Spiroudi U., 2009. Do Botanical Pesticides Alter the Structure of the Soil Microbial Community? Microb. Ecol., 58: 715-727.

Swift M.J., Heal O.W., Anderson J.M., 1979. Decomposition in terrestrial ecosystems. In Anderson et al. (Ed), Studies in Ecology, 5. Blackwell Scientific Publ., Oxford, UK.

Terman G.L., 1979. Volatilization losses of nitrogen as ammonia from surface-applied fertilizers, organic amendments and crop residues. Adv.Agronon. 31: 189-223.

Tognetti C., Mazzarino M.J., Laos F., 2008. Compost of municipal organic waste: Effects of different management practices on degradability and nutrient release capacity. Soil Biology & Biochemistry, 40: 2290-2296.

Toselli M., Baldi E., Sorrenti G., Quartieri M., Marangoni B., 2010. Evaluation of the effectiveness of soil-applied plant derivatives of *Meliaceae* species on nitrogen availability to peach trees. Scientia Horticulturae 124: 183-188.

Trinsoutrot I., Recous S., Bentz S., Linères M., Chèneby D., Nicolardot B., 2000. Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions. Soil Sci. Soc. Am. J., 64: 918-926.

Vance E.D., Brookes P.C., Jenkinson D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry, 19: 703-707.

Vanlauwe B., Nwoke O., Sanginga N., Merckx R., 1996. Impact of residues quality on the C and N mineralization of leaf and root residues of three agroforestry species. Plant Soil 183: 221-231.

Chapter 3

Nutrient uptake by GF677 micropropagated plants grown in soil treated with different *Meliaceae* derivatives

3.1 Introduction

In the previous chapter we investigated the dynamic of N release from different *Meliaceae* derivatives in a laboratory incubation experiment, without the presence of plant. However, plant roots play a central role in organic matter (OM) mineralization by two opposite effects (Wang and Bakken, 1989). Roots represent an important source of C and energy for the soil microflora, hence sustaining a substantial microbial population in rhizosphere with consequent considerable amounts of N immobilized. On the other hand, plant roots compete for the same available N with microorganisms in soil by reducing N re-immobilization, thus limiting the supply of mineral N to the microflora (Wang and Bakken, 1989 and 1997).

In addition, as suggested by Dijkstra et al. (2009), 'soil N availability' and 'soil fertility' are relative terms, and N availability cannot be determined by soil properties alone, but it is strongly affected by root-soil interactions. Thus, to assess the possibility to use these organic materials as a source of nutrient for plants, experiments in which plant nutrient uptake is monitored should be performed. The research on plant N uptake from organic residues presents methodological difficulties (Hood et al., 2000). Non isotopic methods used to evaluate the amount of N mineralised from the residue uptaken by root as the difference between N uptake in the presence and in the absence of added residues can provide useful agronomic information on the quantity of N becoming available to a crop. However, with this method it is not possible to trace the flow of residue-derived N through soil pools. On the contrary, if a residue of interest can be uniformly labelled with ^{15}N, this provides an unambiguous method of tracing the fate of N from the residue and measuring the amount taken up by plants (Ladd et al., 1981).

Among the *Meliaceae* derivatives considered, we had the possibility to uniformly label only *Melia azedarach* leaves.

The aims of the present study were to (i) compare *Meliaceae* derivative ability to supply nutrients to plants and (ii) estimate plant uptake of melia derived-N by the use of ^{15}N technique. For the first aim experiment A was performed, while for the second aim, the experiment B was conducted.

3.2 Material and Methods

3.2.1 Experiment A

Meliaceae derivatives

The same *Meliaceae* derivatives as for the experiment described in Chapter 2 were used:

1 - Green neem, from Virdhunagar, India (neem cake 1),

2 - Neem Italia, pelletted, from Brescia, Italy, N content reported 3% (neem cake 2),

3 - Neem Italia, from Brescia, Italy (neem cake 3),

4 - Deoiled neem cake, from Medors Biotech P. Ltd, Delhi, India (neem cake 4),

5 - Oiled neem cake, from Medors Biotech P. Ltd, Delhi, India (neem cake 5),

6 - Green neem, from Virudhunagar, India (neem cake 6),

7 - *Melia azedarach* L. leaves harvested in June 2010 from seedlings grown on pots filled with sand and frozen at -20°C prior to use (melia).

The derivatives were oven dried at 65° C and ball milled prior to analysis. Calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were determined by atomic absorption spectrometry (SpectrAA-200, Varian, Mulgrave, Australia) after wet mineralization according to US EPA Methods 3052 (Kingston, 1988) by treating 0.5 of dw material with 8ml of nitric acid (65%) and 2 ml of hydrogen peroxide (30%) at 180°C in a Ethos TC microwave lab station (Milestone, Bergamo, Italy). Phosphorous (P) concentration was spectrophotometrically quantified at 700 nm (Saunders and Williams, 1955) on the same mineralized samples used for metals determinations, which were neutralized with 5 M NaOH and enriched with 30 ml of a mixture of 0.1 M ascorbic acid, 32 mM ammonium molybdate, 2.5 M sulphuric acid and 3μM potassium antimonyl tartrate to develop a phospho-molybdic blue colour.

Experimental design

The experiment was carried out in 2010 at the Experimental Station of the University of Bologna, in Cadriano (44°35'N, 11°27'E). The *Meliaceae* derivatives were incorporated into the soil that had the same characteristics of that used for experiment 1 (§ Chapter 2) and at the same application rate: 8 g fw kg^{-1} soil for neem cakes and 16 g fw kg^{-1} for *Melia* leaves. Micropropagated plants of GF677 (*P. persica* x *P. amygdalus*) rootstocks were placed into 1.5 l pots filled with 1 kg treated soil. These treatments were compared to an unamended control. On day 68 and 113 after planting, 5 pots per treatment were destructively sampled. Plants were harvested and divided into roots, stem, new growth (leaves and shoot axis). The length of the new shoots was recorded and leaf green colour, as an estimation of leaf chlorophyll, was determined by a portable SPAD 502 (Minolta Co., Ramsey, New Jersey, USA). Leaves were washed with a solution of Tween 20, then, as all the other organs, they were rinsed with tap water and distilled water, oven-dried, weighed and milled. Roots and new growth (leaves and shoot axis) were analyzed for macro- and micronutrient concentrations as described for the derivative characterization. The soil of each pot was collected, uniformly mixed and analysed for mineral N as described in Chapter 2 and for microbial biomass C by the substrate induced respiration method (SIR) (Anderson and Domsch, 1978). Briefly, 50 g fresh soil sub-samples were sieved (2 mm), placed in 500 ml glass jars and incubated at 22°C for at least 48 hours. Then, 200 mg of glucose were added and mixed with the soil, the jars were sealed for two hours and hourly CO_2 production was measured with an infrared gas-analyzer (EGM-4, PP system; Hitchin, UK).

For each sampling date, data were statistically analysed as in a complete randomized design with 5 replicates (pot), and, when analysis of variance showed statistical significance at $P \leq 0.05$, means were separated by Student Newman Keuls test.

3.2.2 Experiment B

Melia leaves

Uniformly ^{15}N labelled melia leaves were obtained by feeding seedlings of *Melia azedarach* grown in sand with a nutrient solution containing ^{15}N-labelled $^{15}NH_4{}^{15}NO_3$ (10 atom% ^{15}N). Leaves were harvested in August 2010 and stored at -20°C prior to use. A subsample was oven-dried, ball-milled and analyzed for N and ^{15}N concentration with an

elemental analyzer coupled with an isotope ratio mass spectrometer (CF-IRMS, mod. Delta Plus Thermo Fisher, Bermen, Germany). Analysis are reported in Table 3.1.

Experimental design

The experiment started in April 2011 at the Experimental Station of the University of Bologna, in Cadriano. A clay loam Bathicalci Eutric Cambisols soil (FAO, 1990) was collected from the field, mixed with sand at a ratio soil:sand of 3:1 and sieved to 4 mm. Labelled melia leaves were chopped in little pieces and incorporated into the soil at the same application rate as for experiment A (16 g fw kg^{-1} soil). One year-old plants of GF677 rootstocks were placed into pots filled with 2 kg fw amended soil. Another set of plants was potted with unamended soil (control treatment). Immediately after potting, plants were pruned and trained to one shoot; pots were first placed in the greenhouse and watered daily. After one month, plants were transferred outdoor, on a bench, under a plastic shelter to protect them from rain. At day 30, 90, 131 and 173 after planting, five control and 4 treated plants were destructively harvested and divided into roots, stem, new growth (leaves and shoot axis). The length of the new shoots was recorded and leaf green colour, as an estimation of leaf chlorophyll, was determined by a portable SPAD 502 (Minolta Co., Ramsey, New Jersey, USA). All organs were carefully washed with tap water and distilled water, oven-dried, weighed, milled and analysed for total N and ^{15}N content with the same elemental analyzer coupled with a CF-IRMS as described previously. The percentage of N derived from *Melia* leaves (% Ndfml) was calculated as (Hauck and Bremner, 1976):

% Ndfml = (atom ^{15}N excess of plant fertilized with labelled melia leaves / atom ^{15}N excess of labelled melia leaves) × 100;

where atom % ^{15}N excess was obtained by subtracting from values measured in the treated pools, the respective natural abundance measured in control pool.

Then, the amount of N derived from labelled melia leaves (Ndfml) in soil and plant organ were calculated as:

$$\text{Ndfml (mg)} = \text{total N (mg)} \times (\%\ \text{Ndfml} / 100)$$

Finally, the amount of N recovered from the labelled melia leaves was calculated as (Hood et al., 2000):

% N recovery from melia leaves = Ndfml (mg) / (N added as melia leaves (mg)) * 100

The soil of each pot was collected, uniformly mixed and analysed for total N and ^{15}N concentrations. Soil mineral-N was determined as described in Chapter 2. Microbial biomass C and N were determined by the fumigation-extraction method (Vance et al, 1987) as described in Chapter 2.

For each sampling day, data of plant dw, N concentration (%) and Ndfml were statistically analysed as in a complete randomized design, when analysis of variance showed statistical significance at $P \leq 0.05$, means were separated by Student Newman Keuls test. Data of soil mineral N and microbial biomass C and N were statistically analysed as in a factorial design with 2 factors: treatment (2 levels: control and ^{15}N melia) and time (4 levels: day 30, 90, 131, 173). When analysis of variance showed statistical significance at $P \leq 0.05$, means were separated by Student Newman Keuls test. When interaction between factors was significant, 2 standard error of means (SEM) was used as minimum difference between statistically different values.

Table 3.1. Total N and ^{15}N concentration of melia leaves used in experiment B and consequent amount of N added to soil.

Treatment	**N**	**^{15}N**	**Amount of N added**
	%	atom %	mg kg^{-1} fw soil
Control	-	-	-
Melia	1.87	1.876	100

3.3 Results

3.3.1 Experiment A

Table 3.2 shows the mineral compositions of the derivatives used in experiment A. Neem cake 3 presented the highest concentrations for all nutrients analysed. Phosphorous

concentrations ranged between 1.13 % in neem cake 3 and 0.20 % in melia leaves. Potassium concentration varied between 2.40 % in neem 3 and 0.81 % in melia leaves; among the other derivatives neem cake 1, 4 and 5 presented higher K concentration than neem 6 and neem 2. Calcium and Mg concentrations were higher in melia leaves compared to neem cake 1, 2, 4, 5, 6. Iron concentrations widely varied between 8447 mg kg^{-1} in neem 3 and 160 mg kg^{-1} in melia leaves. Among the other neem cakes, neem 4 presented the highest Fe concentration, followed by neem 5, neem 1 and 2, and neem cake 6. Manganese concentrations ranged between 125 mg kg^{-1} in neem 3 and 43 mg kg^{-1} in neem 6 and melia leaves. Finally, Cu concentrations were similar for all derivatives except for neem 3 which showed concentrations more than twice higher, while Zn concentration ranged between 16 mg kg^{-1} in melia leaves and 70 mg kg^{-1} in neem 3.

Sixty-eight days after the start of the experiment, soil application of all *Meliaceae* derivatives increased leaf chlorophyll content which resulted the highest in neem 6 and melia leaves treated plants (Table 3.3).

Shoot length was not significantly affected by the derivatives in the first sampling date (Table 3.3). At that time, the application of neem cake 3 increased new shoot and leaves dry weight along with total plant dry weight compared to untreated control. Also neem cake 1 increased total plant dw (Table 3.3).

At the end of the experiment, all derivatives, except neem 1, increased leaf chlorophyll content, particularly neem 5, neem 6 and melia leaves (Table 3.4). Shoot length was increased by soil addition of neem 1, 2, 3, 4, 6. In addition, neem cake 2, 3 and 6 increased all organ dry weight compared to control, consequently, total plant dry weight of these treatments resulted the highest (Table 3.4).

At day 68, N concentration of new tissues and roots of neem 6 treated plants (Table 3.5) resulted higher than those of untreated plants. In addition, also soil application of melia leaves increased organ N concentration, however, this effect was statistically significant only for roots. In contrast, at the end of the experiment, no effect of treatment in organ N concentration was detected (Table 3.5).

Soil application of neem 3 and 6 significantly increased total amount of N found in shoot and leaves at day 68, while no significant differences were found for roots (Table 3.6). At the end of the experiment, neem 2, neem 3 and particularly neem 6, increased the amount of N in leaves, that resulted more than twice higher than that found in control plants. Neem 6, neem 3 and neem 1 increased the total amount of N in shoot, while all the derivatives increased N in roots. As a consequence, at the end of the experiment, the

Table 3.2. Mineral composition of the *Meliaceae* derivatives used in experiment A.

Derivative	P (%)	K (%)	Ca (%)	Mg (%)	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
Neem cake 1	0.35 (±0.03)	1.53 (±0.02)	0.62 (±0.007)	0.19 (±0.02)	1324 (±241)	56 (±0.35)	11 (±2.05)	22 (±1.2)
Neem cake 2	0.30 (±0.08)	0.97 (±0.03)	0.30 (±0.007)	0.17 (±0.007)	1096 (±0.21)	26 (±1.70)	7 (±0.92)	30 (-)
Neem cake 3	1.13 (±0.20)	2.40 (±0.09)	1.46 (±0.01)	0.67 (±0.007)	8447 (±1.06)	125 (±0.35)	50 (±11.88)	70 (±0.84)
Neem cake 4	0.21 (±0.02)	1.52 (±0.05)	0.49 (±0.02)	0.20 (±0.01)	3035 (±158)	72 (±2.26)	19 (±3.53)	23 (±3.11)
Neem cake 5	0.20 (±0.01)	1.63 (±0.03)	0.61 (±0.06)	0.21 (±0.01)	2414 (±54.7)	61 (±0.28)	10 (±1.70)	19 (±0.56)
Neem cake 6	0.65 (±0.02)	1.25 (±0.04)	0.50 (±0.007)	0.35 (±0.007)	620 (±59.2)	43 (±1.27)	10 (-)	54 (±2.12)
Melia leaves	0.19 (±0.02)	0.81 (±0.05)	1.36 (±0.01)	0.38 (±0.04)	160 (±7.92)	43 (±0.56)	8 (±0.71)	16 (±0.35)

In brackets standard deviation (n=2).

Table 3.3. Effect of soil addition of *Meliaceae* derivatives on leaf chlorophyll, shoot length and plant organ dry weight (dw) after 68 days.

Treatment	Leaf chlorophyll	Shoot length	Shoot and leaves dw	Roots dw	Stem dw	Total dw
	(Spad Unit)	(cm)	(g dw plant^{-1})			
Control	34.0 c	35	1.21 b	0.98	0.53	2.72 b
Melia	42.5 a	46	1.88 ab	1.17	0.60	3.66 ab
Neem 1	39.0 b	62	2.55 ab	1.59	0.70	4.83 a
Neem 2	38.6 b	60	2.49 ab	1.45	0.64	4.59 ab
Neem 3	37.9 b	63	3.05 a	1.34	0.75	5.14 a
Neem 4	37.7 b	48	2.01 ab	1.50	0.76	4.27 ab
Neem 5	40.1 b	49	1.87 ab	1.29	0.52	3.68 ab
Neem 6	43.9 a	55	2.19 ab	0.97	0.57	3.73 ab
Significance	***	*ns*	*	*ns*	*ns*	*

ns, * and *** = effect of *Meliaceae* derivatives not significant, significant at P ≤0.05 and 0.001, respectively. Values followed by the same letter are not statistically different.

highest total (stem excluded) amount of N was recorded in neem 6 (75.9 mg), followed by neem 3 treated plants (Table 3.6). Also the application of neem 1 and neem 2 significantly increased the N content of plants compared to control. Although melia, neem 4 and neem 5 treated soil promoted a total tree N recovery on average higher than 50 % of that found in untreated control, these amounts did not result significantly different.

On day 68, the application of neem 2, 3, 4 and 6 increased P concentration in new growth tissues (Table 3.7). At that time, K concentration was increased by all derivatives but in a significantly manner only by neem 3, 4, 5 and 6. On the contrary, no effect of treatment was detected for Ca and Mg concentration that on average were 0.78 % and 0.23 %, respectively (Table 3.7).

At day 68, only neem 6 increased Mn concentration of new growth tissues, while Cu concentration was increased also by neem 5 and 4, reaching values of 10 ppm in neem 6 treated plants. At day 68, mean values of Zn concentration resulted higher in neem 1, 2, 4 and 6 treated plants compared to untreated control (Table 3.7).

Table 3.4. Effect of soil addition of *Meliaceae* derivatives on leaf chlorophyll, shoot length and plant organ dry weight after 113 days (experiment A).

Treatment	**Chlorophyll content**	**Shoot lenght**	**Leaves**	**Shoot**	**Roots**	**Stem**	**Total**
	(Spad Unit)	(cm)	(g dw plant^{-1})				
Control	35 c	32 c	1.0 d	0.69 c	1.78 b	0.76 c	4.64 c
Melia	40 a	46 abc	1.69 bcd	1.20 bc	2.83 ab	1.08 abc	6.80 bc
Neem 1	36 bc	71 a	1.60 bcd	1.70 ab	3.13 a	1.21 ab	7.64 abc
Neem 2	37 b	56 ab	2.22 abc	1.70 ab	3.56 a	1.23 ab	8.72 ab
Neem 3	38 b	62 ab	2.32 ab	1.96 ab	3.08 a	1.40 ab	8.76 ab
Neem 4	37 b	58 ab	1.11 cd	1.31 bc	2.96 a	1.10 abc	6.49 bc
Neem 5	40 a	42 bc	1.44 bcd	1.18 bc	2.49 ab	1.02 bc	6.14 bc
Neem 6	42 a	65 ab	2.92 a	2.26 a	3.50 a	1.51 a	10.2a
Significance	**	***	***	***	**	**	**

** and *** = effect of *Meliaceae* derivatives not significant, significant at P>0.01 and 0.001, respectively. Values followed by the same letter are not statistically different.

At the end of the experiment (day 113), neem 4 treated plants had the highest leaf P concentration, followed by neem 5 and neem 1, while the lowest P concentration was recorded in neem 6 treatment (Table 3.8). No differences between treatments were found for K concentration, that was on average 1.86 %. Moreover, leaves of control plants presented the highest Ca concentration, significantly different from that of neem 3 and 6 treated plants. Neem 4 treated plants presented higher Mg concentration compared to neem 3 and 6. No differences between treatment were found, at the end of the experiment, for leaf microelement concentration, except for Mn, that was higher in leaves of neem 4 treated plants compared to those of neem 3 and neem 6 treatments (Table 3.8).

In the first sampling date, soil NO_3^--N concentration was similar in all treatments, in particular it resulted of 5.70, 6.34, 6.28, 7.26, 6.05, 4.52, 5.90 and 6.03 mg kg^{-1} in control, melia and neem 1 to 6 treated soil, respectively (data not shown), while NH_4^+-N was not found. At the end of the experiment no mineral-N was detected in any treatment (data not tabulated).

Table 3.5. Effect of soil addition of *Meliaceae* derivatives on shoot, leaf and root N concentrations at day 68 and 113 (experiment A).

Treatment	**N (%)**				
	Day				
	68		**113**		
	shoot and leaves	**roots**	**leaves**	**shoot**	**roots**
Control	1.16 b	0.76 b	1.37	0.60	0.58
Melia	1.60 ab	1.02 a	1.46	0.64	0.66
Neem 1	1.24 b	0.84 ab	1.58	0.58	0.68
Neem 2	1.21 b	0.85ab	1.34	0.51	0.64
Neem 3	1.33 b	0.85 ab	1.34	0.52	0.64
Neem 4	1.36 b	0.86 ab	1.62	0.60	0.67
Neem 5	1.44 ab	0.92 ab	1.58	0.60	0.69
Neem 6	1.86 a	1.07 a	1.45	0.45	0.69
Significance	**	**	*ns*	*ns*	*ns*

ns, ** = effect of *Meliaceae* derivatives not significant, significant at $P \leq 0.01$, respectively. Values followed by the same letter are not statistically different.

No differences among treatments were observed, at day 68, for microbial biomass C that on average was 221 μg C g^{-1} dw soil (Table 3.9), while at the end of the experiment, neem 4 soil presented a higher value of microbial C compared to untreated soil (Table 3.9).

Table 3.6. Effect of soil addition of *Meliaceae* derivatives on amount of N present in shoot, leaves and root at day 68 and 113 (experiment A).

Treatment	**N (mg)**						
	Day						
	68			**113**			
	shoot and leaves	**roots**	**total**	**leaves**	**shoot**	**roots**	**total**
Control	13.3 b	7.5	20.8 b	14.5 c	3.94 b	10.2 b	29.2 c
Melia	26.9 ab	11.4	38.3 ab	24.0 bc	7.08 ab	18.8 a	49.9 bc
Neem 1	31.6 ab	13.3	45.0 ab	24.6 bc	9.85 a	21.2 a	55.7 b
Neem 2	30.1 ab	12.3	42.4 ab	29.8 b	7.19 ab	22.6 a	52.7 b
Neem 3	38.3 a	11.5	49.8 a	30.8 b	10.1 a	19.7 a	60.7 ab
Neem 4	27.1 ab	12.5	39.7 ab	17.4 bc	7.87 ab	19.9 a	45.2 bc
Neem 5	26.2 ab	11.6	37.8 ab	22.2 bc	6.51 ab	16.8 a	45.5 bc
Neem 6	40.9 a	10.3	51.2 a	41.9 a	10.1 a	23.9 a	75.9 a
Significance	**	*ns*	*	***	***	***	***

ns,*, **,*** = effect of *Meliaceae* derivatives not significant, significant at $P \leq 0.05$, 0.01, 0.001, respectively. Values followed by the same letter are not statistically different.

Table 3.7. Effect of soil addition of *Meliaceae* derivatives on P, K, Ca, Mg, Mn, Fe, Cu and Zn concentrations of new growth tissues at day 68 (experiment A).

Treatment	**P**	**K**	**Ca**	**Mg**	**Mn**	**Fe**	**Cu**	**Zn**
	(%)				(mg kg^{-1})			
Control	0.23 c	1.43 b	0.82	0.23	27 b	97	6.2 c	23 c
Melia	0.26 bc	1.66 ab	0.77	0.25	27 b	52	6.7 bc	25 bc
Neem 1	0.29 abc	1.61 ab	0.74	0.19	22 b	86	7.4 abc	32 ab
Neem 2	0.39 a	1.73 ab	0.77	0.23	27 b	73	7.8 abc	31 ab
Neem 3	0.36 ab	2.00 a	0.76	0.24	25 b	59	8.1 abc	26 abc
Neem 4	0.38 a	1.98 a	0.82	0.25	27 b	65	9.4 ab	34 a
Neem 5	0.32 abc	1.88 a	0.77	0.23	31 ab	89	9.2 ab	30 abc
Neem 6	0.36 ab	2.01 a	0.79	0.22	35 a	61	10.0 a	32 ab
Significance	**	***	ns	ns	***	ns	*	**

ns,*, **,*** = effect of *Meliaceae* derivatives not significant, significant at $P \leq 0.05$, 0.01, 0.001, respectively. Values followed by the same letter are not statistically different.

Table 3.8. Effect of soil addition of *Meliaceae* derivatives on P, K, Ca, Mg, Mn, Fe, Cu and Zn concentrations of leaves at the end of the experiment A.

Treatment	**P**	**K**	**Ca**	**Mg**	**Mn**	**Fe**	**Cu**	**Zn**
	(%)				(mg kg^{-1})			
Control	0.43bc	1.58	1.11 a	0.31 ab	31 a	122	5.27	22
Melia	0.41bc	1.86	1.05 ab	0.31 ab	29 ab	97	6.50	22
Neem 1	0.58ab	2.01	0.96 ab	0.33 ab	24 b	99	6.2	27
Neem 2	0.55abc	1.71	0.91 abc	0.30 ab	29 ab	100	6.02	24
Neem 3	0.50bc	1.94	0.87 bc	0.25 b	24 b	92	5.53	22
Neem 4	0.71a	2.09	1.03 ab	0.38 a	33 a	79	6.92	23
Neem 5	0.60ab	1.89	0.97 ab	0.31 ab	31 a	91	5.84	27
Neem 6	0.36c	1.77	0.77 c	0.25 b	31 a	90	5.50	23
Significance	***	ns	***	*	*	ns	ns	ns

ns,*, *** = effect of *Meliaceae* derivatives not significant, significant at $P \leq 0.05$, 0.001, respectively. Values followed by the same letter are not statistically different.

Table 3.9. Effect of soil addition of *Meliaceae* derivatives on soil microbial biomass C at day 68 and 113 (experiment A).

Treatment	**Microbial C** (μg g^{-1} dw soil)	
	day	
	68	**113**
Control	195	105 b
Melia	235	160 ab
Neem 1	236	159 ab
Neem 2	244	161 ab
Neem 3	196	152 ab
Neem 4	252	194 a
Neem 5	255	165 ab
Neem 6	157	169 ab
Significance	ns	*

ns,*= effect of *Meliaceae* derivatives not significant and significant at $P \leq 0.08$, respectively. Values followed by the same letter are not statistically different.

3.3.2 Experiment B

Plant organ dry weight resulted similar in control and ^{15}N-melia treated plants (Table 3.10) and increased with time during the experiment. Total plant dry weight in untreated plant ranged between 1.47 g (at day 30) and 6.07 g (at day 173) and between 1.12 g (at day 30) and 4.58 g (at day 173) in ^{15}N-melia treatment (Table 3.10).

Soil application of ^{15}N-melia leaves had no effect on shoot length which, at the end of the experiment, had a mean value of 26 cm (Table 3.11). On the contrary, SPAD values were increased by the application of melia leaves, but only at day 90 (Table 3.11).

Nitrogen concentration of plant organs was not affected by treatment (Table 3.10) at any of the sampling time. Maximum N concentration in shoot and leaves and in roots was found at day 30, then it decrease with time until day 90.

Soil addition of ^{15}N-melia leaves did not modify the amount of N in plant organs (Figure 3.1). Thus, plant total amount of N was similar in control and in amended pots (Figure 3.1).

At day 30, only little amounts of N derived from labelled melia leaves were observed in GF677 organs (Figure 3.2) and the highest amount were found in roots (Figure 3.2, inset). Nitrogen derived from melia leaves increased with time until day 131, and, unlike day 30, the highest amounts were found in shoot and leaves, although values were not significantly different from roots (Figure 3.2). In these organs, 9.02 mg of Ndfml were found at day 131, corresponding to 39 % of total N (Table 3.12). At that day, stem and roots presented a Ndfml % of 24 % and 30 %, respectively (Table 3.12). At day 173 Ndfml was not different in the different organ investigated.

Soil application of ^{15}N labelled melia leaves increased total pot N content compared to untreated control during all the experiment (Table 3.13).

Thirty day after melia application, Ndfml was found almost exclusively in the soil (Table 3.14). The amount in plant increased at day 90 and 131, while no increase was found from day 131 to day 173. Consequently, also the percentage of melia-N plant recovery increased from day 30 to day 131 and was almost steady from day 131 to day 173 (Table 3.14). One month after the start of the experiment, 14 % of N derived from melia leaves was not recovered, thereafter the percentage of melia N not accounted increased to approximately 22%.

Table 3.10. Effect of soil application of labelled ^{15}N melia leaves on dry weight and N concentration of GF677 plant organs at day 30, 90, 131 and 173 (experiment B).

Treatment	**Shoot and leaves**							
	Dry weight (g)				**N (%)**			
	day				**day**			
	30	**90**	**131**	**173**	**30**	**90**	**131**	**173**
Control	0.013	1.07	1.82	1.87	5.26	1.53	1.26	1.28
Melia	0.007	1.18	1.74	1.35	4.21	1.47	1.32	2.17
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
	Stem							
	Dry weight (g)				**N (%)**			
	day				**day**			
	30	**90**	**131**	**173**	**30**	**90**	**131**	**173**
Control	0.72	0.81	1.29	1.39	0.58	0.50	0.47	0.58
Melia	0.59	0.72	1.08	1.05	0.68	0.51	0.44	0.61
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
	Roots							
	Dry weight (g)				**N (%)**			
	day				**day**			
	30	**90**	**131**	**173**	**30**	**90**	**131**	**173**
Control	0.73	1.14	2.11	2.81	1.21	0.94	0.92	0.78
Melia	0.53	1.04	1.82	2.18	1.45	0.99	0.99	0.99
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

	Total plant			
	Dry weight (g)			
	day			
	30	**90**	**131**	**173**
Control	1.47	3.02	5.22	6.07
Melia	1.12	2.94	4.65	4.58
Significance	*ns*	*ns*	*ns*	*ns*

ns = effect of melia leaves not significant

Table 3.11. Effect of soil application of labelled ^{15}N melia leaves on shoot length and leaf chlorophyll of GF677 at day 30, 90, 131 and 173 (experiment B).

Treatment	**Shoot length (cm)**				**Leaf chlorophyll**			
	day				**day**			
	30	**90**	**131**	**173**	**30**	**90**	**131**	**173**
Control	-	26	30	31	-	30	33	35
Melia	-	26	34	20	-	36	33	35
Significance		*ns*	*ns*	*ns*		*	*ns*	*ns*

ns, * = effect of melia leaves not significant or significant at $P \leq 0.05$

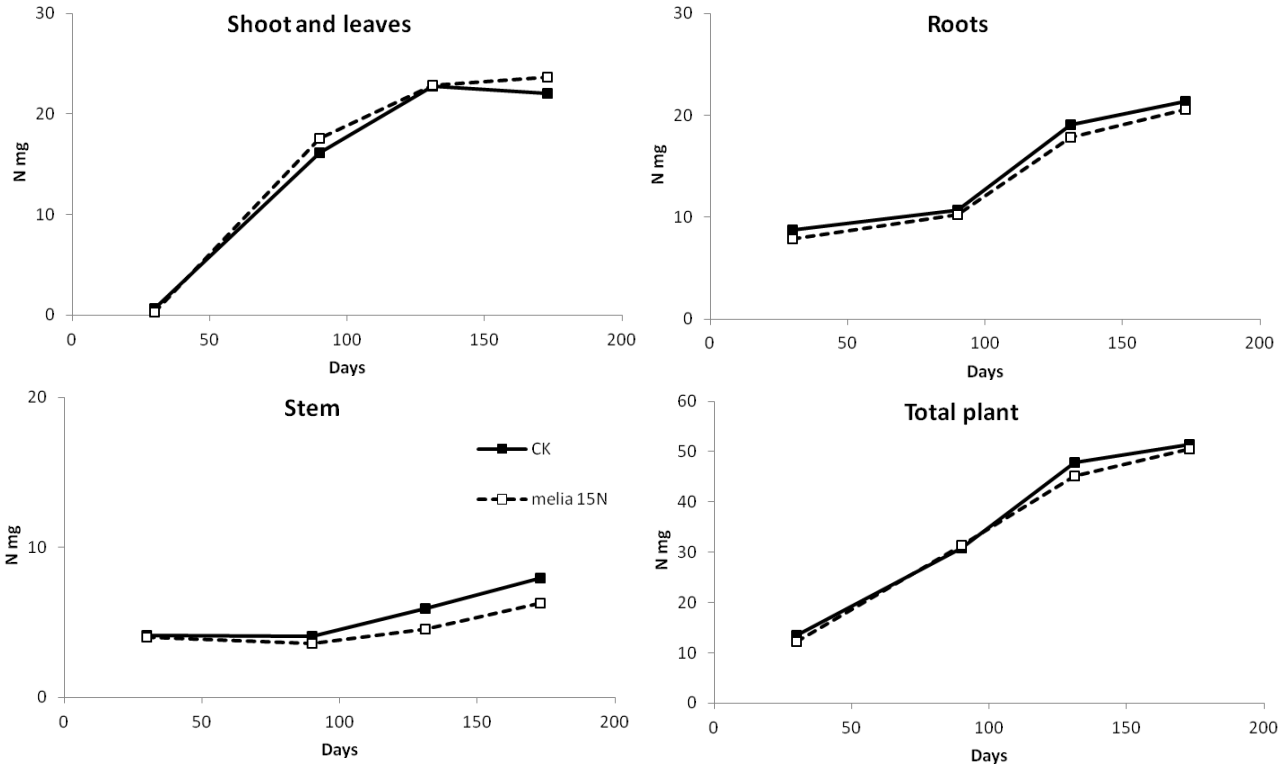

Figure 3.1 Effect of soil application of ^{15}N labelled melia leaves on plant N. At each sampling date, effect of treatment not significant.

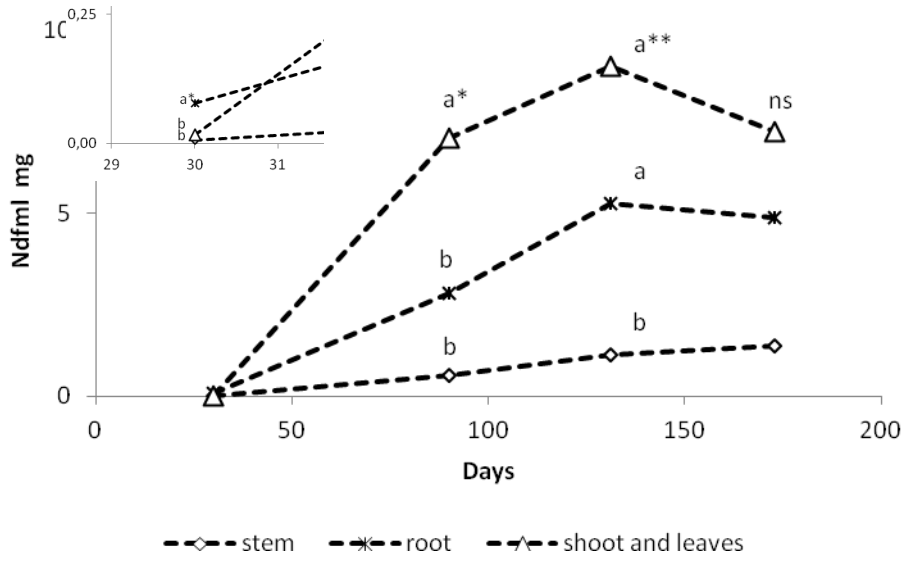

Figure 3.2 Effect of soil application of ^{15}N labelled melia leaves on organ Ndfml. At each sampling date: ns, *, ** = effect of organ not significant, significant at $P \leq 0.05$, 0.01, respectively. Within the same date, values followed by the same letter are not significantly different. In box is reported the detailed trend at the first sampling time (day 30).

Table 3.12. Effect of soil application of labelled ^{15}N melia leaves on percentage of Ndfml and correspondent recovery of melia-N in plant organs at day 30, 90, 131 and 173 (experiment B).

Treatment	**Ndfl** (%)				**Recovery melia-N** (%)			
	day				**day**			
	30	**90**	**131**	**173**	**30**	**90**	**131**	**173**
Shoot and leaves	7.31	38.6	39.1	30.54	0.008	3.57	4.55	3.66
Stem	0.14	15.7	24.0	21.1	0.003	0.29	0.56	0.69
Roots	1.09	26.2	29.6	22.4	0.04	1.43	2.66	2.47

Table 3.13. Effect of soil application of labelled ^{15}N melia leaves on total N present in soil at day 30, 90, 131 and 173 (Experiment B).

Treatment	**Soil N** (mg pot^{-1})			
	day			
	30	**90**	**131**	**173**
Control	1278	1330	1042	1414
Melia	1590	1565	1454	1612
Significance	**	**	**	*

*, ** = effect of treatment significant at $P \leq 0.05$ and 0.01, respectively.

Table 3.14. Amount of Ndfml in plant and soil and correspondent recovery of melia-N 30, 90, 131 and 173 days after the application of melia leaves (Experiment B).

Treatment	**Ndfml** (mg pot^{-1})				**Recovery melia-N** (%)			
	day				**day**			
	30	**90**	**131**	**173**	**30**	**90**	**131**	**173**
Plant	0.09	10.5	15.4	13.5	0.05	5.29	7.77	6.83
Soil	171	143	134	143	86	72	68	72

Soil application of melia leaves did not increase soil mineral-N compared to the control soil (Table 3.15). In both amended and unamended soils, NH_4^+-N concentration increased from day 30 to day 90, when the maximum value of 3.6 mg kg^{-1} was found. Thereafter ammonium concentration decreased in both treatments (Table 3.15). A similar dynamic was found for NO_3^--N that was present at higher concentrations compared to ammonium-N. In particular, nitrate-N increased in both soils until day 90, thereafter it decreased.

Soil application of melia leaves immediately increased microbial biomass C (Figure 3.3), the highest value was found 1 day after treatment, then it decrease with time

Table 3.15. Effect of soil application of labelled ^{15}N melia leaves on ammonium-, nitrate-, and total mineral-N present in soil at day 1, 30, 90, 131 and 173 (Experiment B).

Treatment	NH_4^+-N	NO_3^--N	Nmin
	(mg kg^{-1})		
Control	2.37	5.6	7.97
Melia	2.35	5.1	7.45
Significance	ns	ns	ns
Day			
1	2.12 bc	4.20 c	6.32 c
30	2.08 bc	6.31 b	8.39 b
90	3.63 a	8.19 a	11.82 a
131	2.40 b	4.32 c	6.72 c
173	1.57 c	3.53 c	5.1 c
Significance	***	***	***
*Interaction treatment*day*	ns	ns	ns

ns, ***= not significant and significant at $P \leq 0.001$, respectively

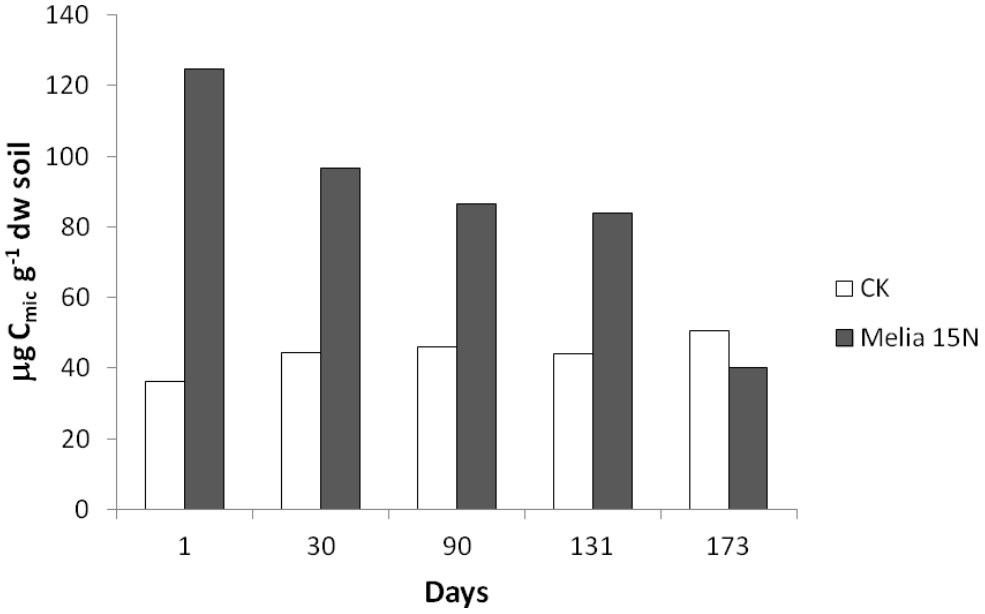

Figure 3.3 Effect of soil application of ^{15}N melia leaves on soil microbial biomass C. Interaction time*treatment significant at $P \leq 0.001$. Minimum difference between statistically different values (2SEM) = 16.

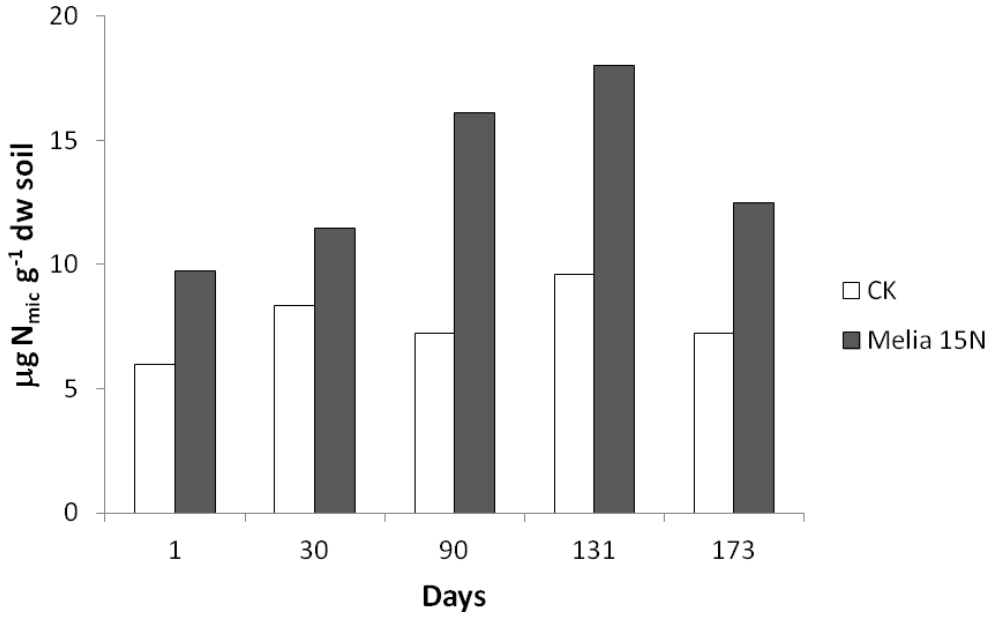

Figure 3.4. Effect of soil application of ^{15}N melia leaves on soil microbial biomass N. Interaction time*treatment not significant. Minimum difference between statistically different values (2SEM) = 3.3.

to reach similar of control soil at the end of the experiment. Also microbial biomass N increased after the amendment, in this case the highest value was found at day 131, then it decreased (Figure 3.4).

3.4 Discussion

The ^{15}N isotope technique provided the evidence of melia-N mineralization as between 0.8 % and 34 % of N present in plants derived from the ^{15}N labeled melia leaves.

In particular, ^{15}N released by melia leaves was available one month after the amendment, when only a low amount of melia-N was uptaken by plants and mostly of it was recovered in roots. Since N is metabolized in root immediately and mobilized to the canopy, the fraction of N found in root at day 30 was probably just absorbed and was going to be converted in aminoacids. Thereafter, the amounts of Ndfml increased with time in all organs until day 131, with shoot and leaves representing the 'sink organs' for melia-N. Also at the end of the experiment (September) shoot and leaves presented the highest amount of melia-N, but it was lower compared to that of the previous

sampling date, indicating a possible remobilization of melia-N within the plant, probably in stem. In fact, between the two last sampling date, the amount of Ndfml in roots was constant, while stem Ndfml increased. The availability and uptake of ^{15}N at the end of the experiment accounted for about 7 %, so we expected an increase of total N % in plant of the same amount. Actually this was not observed, and treated and untreated trees showed the same N content throughout the season. The mineral N concentration of both control and treated soil was similar during all the experiment. However, microbial biomass N increased from day 1 to day 131; at that date, microbial N was 10 mg kg^{-1} and 18 mg kg^{-1} in control and amended soil, respectively. If the correction factor K_N of 0.45, usually applied to correct for the incomplete recovery of microbial constituents extracted from soil after fumigation, is used, the difference in N immobilized in the microbial biomass between the two soil correspond to 18 mg kg^{-1}. This difference can support the idea of an extra N mineralization in the amended compared to the unamended soil, of native soil-N or melia-N, followed by an immediate immobilization, and a competition for this N between microbial biomass and plants, however the exact origin of this extra amount of N mineralized cannot be defined.

At the end of the experiment, 72 % of melia-N was still present in the soil, almost all in the organic form as only 5 mg $kg^{-1}$of mineral N were found in soil, thus representing a potential source of N for the following vegetative growth season.

The *Meliaceae* derivatives showed a different mineral composition. As mentioned in Chapter 2, this can be due to the different origin of the derivative in term of species, organ, production processes. However, the mineral composition of neem cake 3 was unexpected. In fact, neem 3 presented values that were at least twice higher for most of the nutrients (i.e. P, K, Ca, Mg, Fe, Mn, Cu and Zn) than the mean concentration of the other neem cake here used and also reported in earlier study (Toselli et al., 2010).

Actually, also the mineral composition of *Melia* leaves resulted partly different from that reported in other studies. For example, melia leaves tested by Toselli et al. (2010) presented the following chemical composition: P: 0.26 %, K: 1.57 %, Ca: 3.14 %, Mg: 210 mg kg^{-1}, Fe: 287 mg kg^{-1}, Mn: 32 mg kg^{-1}, Cu: 16 mg kg^{-1}, Zn: 33 mg kg^{-1}, thus showing a concentration of K, Ca, Cu and Zn two times higher compared to our derivative and a Mg concentration almost 20 times lower. In addition, the chemical composition of dek (*Melia azedarach*) litter reported by Singh and Sharma (2007) differed from our material, principally for Fe and Zn concentration which resulted about 9 and 3 times higher compared to our.

Despite this different chemical composition, the incorporation of all the *Meliaceae* derivatives generally had a positive effect on plant growth, this was particularly evident for neem 3 and 6 that, by the end of the experiment increased plant dry weight from 4.64 g (control plant) to

10.2 g and 8.76 g respectively. These are also the two derivatives that showed the highest final shoot length and amounts of N in plants. These effects can be related to the higher amounts of N added with the two derivatives that presented the highest N concentration (3.7 % and 4.4 % for neem cake 3 and neem cake 6, respectively), compared to the other (§ Chapter 2).

Also neem 1 (2.2 % N) and neem 2 (3.1 % N), at the end of the experiment, increased the total amount of N present in plants and shoot length compared to control. Altough neem cake 5 and melia leaves decreased N availability as showed in the first experiment (net immobilization) (§ Chapter 2), they did not reduce plant growth neither total amount of plant N, rather they both induced a positive effect on leaf chlorophyll content, and in the case of melia, root N concentration at day 68.

The effect of the derivatives on plant nutrient status was evident in the first sampling date when neem 6 was the derivative with higher positive effect on P, K, Mn, Cu and Zn concentrations of new growth tissues. At day 68, also neem 3 and neem 4 had a positive effect on plant nutrient status as they increased P, K and Zn concentration in shoot and leaves compared to control. However, this improvement did not persist, as, at the end of the experiment, only P concentration induced by neem 4 was higher compared to control plants.

In addition, at the end of the experiment, neem 6 and neem 3 treated plants presented lower leaf Ca concentration compared to control plants. This can be only partly explained by the higher presence of K found in neem 6 and neem 3 treatments in the previous sampling date. In fact, the possible antagonistic effect of K on Ca and Mg root uptake was not observed in neem 4 treated plants despite the high level of K found at day 68. A similar behaviour was found by Baldi et al. (2010) on one-year old peaches fertilized with organic amendments. In their study, they found that compared to control, K leaf resulted higher in plants addressed with compost and that Ca and Mg concentration was reduced in trees fertilized with compost and cow manure.

The increased nutrient composition of treated plants was not related to the mineral composition of the derivatives, this was particularly evident for Fe, whose concentration resulted similar in all plants (control included), despite the great variability between derivative Fe concentrations.

Soil application of melia leaves did not affect mineral composition of plant organs, except for N in roots, that resulted increased compared to control. However, chlorophyll content of melia treated plants resulted higher compared to control, in both experiment A and B. As a result, the positive effect induced by soil addition of melia leaves found in other studies, did not completely emerge in this study. In a previous pot experiment, Singh and Sharma (2007) found that straw, grain yield, and nutrient content (N, P, K) of wheat increased with increasing level of leaf litter of poplar

(*Populus deltoides*), eucalypt (*Eucalyptus* hybrid) and dek (*Melia azedarach*), no matter if inoculated or not with cellulolytic fungus culture of *Aspergillus awamori* to accelerate the decomposition rate of litters. In addition, dry matter yield of sorghum raised on residual fertility, increased significantly with increasing levels of leaf litter application. Nevertheless, in their experiment, Singh and Sharma, applied N, P and K at a dose of 50, 11, 10 mg kg^{-1} soil, respectively that could have enhanced the positive effect of plant litter compared to our experiment.

3.5 References

Anderson J.P.E., Domsch K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry, 10: 215-221.

Baldi E., Toselli M, Marangoni B., 2010. Nutrient partitioning in potted peaches (*Prunus persica* L.) trees supplied with mineral and organic fertilizers. Journal of Plant Nutrition, 33 (14): 2050-2061.

Dijkstra F.A., Bader N.E., Johnson D.W., Cheng W., 2009. Does accelerated soil organic matter decomposition in the presence of plants increase plant N availability? Soil Biology and Biochemistry, 41: 1080-1087.

Food and Agriculture Organization (FAO), 1990. Guidelines for soil profile description, 3rd ed. Soil Resources Management and Conservation Service, Land and Water Development Division, FAO, Rome.

Hauck R.D., Bremner J.M., 1976. Use of tracers for soil and fertiliser nitrogen research. Advances in Agronomy, 28: 219-266.

Hood R., Merckx R., Jensen E.S., Powlson D., Matijevic M., Hardason G., 2000. Estimating crop N uptake from organic residues using a new approach to the ^{15}N isotope dilution technique. Plant and Soil, 223: 33-44.

Ladd J.N., Oades J.M., Amato M., 1981. Microbial biomass from ^{14}C and ^{15}N-labelled plant material decomposing in soils in the field. Soil Biology and Biochemistry, 13: 119-126.

Singh B., Sharma K.N., 2007. Nutrition and growth of wheat-sorghum rotation in soils amended with leaf litter of trees before planting of wheat. Agroforest Syst, 71: 25-34.

Saunders W.M., Williams E.G., 1955. Observations of the determinations of organic phosphorous in soils. Journal of Soil Science, 6: 254-267.

Toselli M., Baldi E., Sorrenti G., Quartieri M., Marangoni B., 2010. Evaluation of the effectiveness of soil-applied plant derivatives of *Meliaceae* species on nitrogen availability to peach trees. Scientia Horticulturae 124: 183-188.

Vance E.D., Brookes P.C., Jenkinson D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry, 19: 703-707.

Wang J.G. and Bakken L.R., 1997. Competition for nitrogen during mineralization of plant residue in soil: microbial response to C and N availability. Soil Biology and Biochemistry, 29 (2): 163-170.

Wang J.G. and Bakken L.R., 1989. Nitrogen mineralization in rhizosphere and non-rhizosphere soil, effect of the spatial distribution of N-rich and N-poor plant residues. In: Nitrogen in Organic Wastes Applied to Soils (J. A. Hansen and K. Henriksen, Eds): pp 81-97. Academic, London.

Chapter 4

Use of a ^{13}C natural abundance method to quantify the priming effect induced by different *Meliaceae* derivatives

4.1 Introduction

In the two first experiments we assumed that the addition of the derivatives did not induce any priming effect (PE). However inputs of fresh organic matter (OM) are known to affect the rate of soil organic matter mineralization (SOM) (§Chapter 1 and literature cited) and can result in both acceleration (positive PE) or retardation (negative PE) of soil organic carbon decomposition. When positive PE occur, large amounts of mineral nutrients can be released in soil solution (Kuzyakov et al., 2010), with a consequent increase in nutrient availability for plants. On the other hand, the mineralization of soil organic C can be intense and eventually exceed the formation of new SOM through humification of fresh C, leading to net destruction of SOM (Fontaine et al., 2011). For example, Fontaine et al. (2004) demonstrated, in controlled conditions, that the supply of fresh C, as cellulose, accelerate the decomposition of soil C particularly in low soil nutrient conditions, inducing a negative C balance.

We tried to quantify the PE induced by soil application of some of the *Meliaceae* derivatives used in the previous experiments in order to evaluate their effect on the C balance of the soil. To evaluate their suitability as N supplier, we studied the dynamic of mineral N considering part of N losses via nitrification and denitrification as N_2O (Velthof et al., 2002). Denitrification is the process by which NO_3^- is reduced to gaseous forms of N (NO, N_2O, N_2) by chemoautotrophic bacteria. The critical factors regulating the rate and duration of denitrification in soils are the availability of NO_3^- (the source) and C (source of energy and electrons) and the absence of O_2 (Pierzynski et al., 2000). Incorporation of fresh plant material into the soil may affect soil N_2O emission as it supplies easily mineralizable N and C (source of N and C for denitrifier) and produce anaerobic zones due to the rapid O_2 consumption by soil microorganisms during decomposition of OM.

To quantify PEs, the native soil C (and/or N release) in amended and unamended soil are usually compared and the identification, in the amended soil, of the amount of C (or/and N) released by soil is done with isotope techniques. We used a 'labelled' soil instead of a 'labelled' amendment introducing a natural ^{13}C method. This methods have been used in many studies of soil C dynamics (Balesdent et al., 1987) and are based on the differences in $\delta^{13}C$ between plants with the C_3 photosynthetic pathway, which have $\delta^{13}C$ values ranging from approximately -32‰ and -22‰, with a mean of -27‰, and plants with C_4 photosynthesis which discriminate less against $^{13}CO_2$ during photosynthesis and, therefore, have larger $\delta^{13}C$, ranging from approximately -17‰ to -9‰ with a mean of -13‰ (Boutton, 1991). Because there is little change in the $\delta^{13}C$ value of plant material as it decomposes, the $\delta^{13}C$ of soil organic carbon strictly reflects the isotopic composition of the plants from which it derives (Boutton, 1991; Cheng, 1996). Indeed, SOM derived from continuous cultivation of C_4 plants has $\delta^{13}C$ value ranging from -12 ‰ to -21 ‰, whereas $\delta^{13}C$ values of SOM derived from C_3 plants generally range from -24 ‰ to -29 ‰ (Cheng, 1996). The changes in $\delta^{13}C$ of emitted CO_2 that follow the addition of a C_3-amendment to the soil of a C_4 ecosystem or vice-versa, allow to distinguish between CO_2 evolved from the microbial mineralization of the amendment and CO_2 evolved from the microbial mineralization of native SOM (Ekblad and Högberg, 2000) hence permitting study on PE.

The aim of this study was to quantify, by the use of a ^{13}C natural method, the PE induced by the *Meliaceae* derivatives and their real C mineralization.

4.2 Materials and Methods

Soil

The soil used in the experiments was collected in January 2012 from the 0-25 cm depth of the continuous corn plot of the Long-Term Experiment of Cadriano (University of Bologna), initiated in 1966 by Prof. Toderi of the Department of Agricultural Sciences. In particular, soil was collected from mineral fertilized plots (300 kg N ha^{-1}). Detailed description of the soil and the management practices are given by Gioacchini et al. (2007) and are here briefly reported. The soil is a Typic Udochrept; pH (in water) 6.9; sand 56%; silt 16%; clay 28%; cation exchange capacity 16.5 $cmol_c$ kg^{-1}. Before the start of the experiment, soil total C and N concentration and C isotope composition were determined on four soil sub-samples previously dried and milled. Analysis was carried out by an elemental analyzer (Thermo Electron, mod. EA 1110) coupled with a continuous flow-isotope ratio mass spectrometer (CF-IRMS, mod. Delta Plus Thermo Fisher, Bermen,

Germany). Soil C and N content resulted of 7.5 g kg^{-1} and 0.98 g kg^{-1}, respectively. The carbon isotope value of soil ($\delta^{13}C$) was -21.1 ‰; where

$$\delta^{13}C\ (‰) = [(R_{sample} / R_{standard})-1]*1000,$$

and R_{sample} and $R_{standard}$ are $^{13}C/^{12}C$ of the sample and the standard, respectively. Delta ^{13}C is relative to the Vienna Pee Dee Belemnite (VPDB).

Meliaceae derivatives

Neem cake 3, neem cake 5, neem cake 6 and melia leaves were first chosen for the experiments because of their different N mineralization dynamic showed in the first experiment (§Chapter 2). Neem 6 and neem 3 were chosen for their relatively rapid net mineralization; melia leaves because of their little effect on mineral-N concentration, and neem 5 because of the long period of N immobilization. These derivatives were reanalyzed for C and N content and for C isotope composition as described for the soil (Table 4.1). Because the $\delta^{13}C$ of neem cake 3 resulted similar to that of the soil, we decided to exclude this derivative from the experiment.

Table 4.1. Chemical characteristics of the *Meliaceae* derivatives used in the incubation experiment (n=3) and amounts of derivative and consequent C and N added to soil.

Derivative	**Characteristics**				**Amount added to soil**		
	C (%)	**N** (%)	**C:N**	**δ ^{13}C** (‰)	**derivative** g fw kg^{-1} dw soil	**C** mg kg^{-1} dw soil	N
Neem cake 3	32.6	3.7	9	-23.15	-	-	-
Neem cake 5	43.3	1.6	27	-26.27	2.00	919	34
Neem cake 6	49.0	4.0	12	-25.89	0.73	386	34
Melia leaves	43.0	1.9	22	-30.07	4.44	677	34

In order to evaluate the chemical structure of the derivatives, thermogravimetric analysis (TG) and differential thermal analysis (DTA) were performed simultaneously using a TG-DTA92 instrument (SETARAM, France) with a heating rate of 10 °C min^{-1} from 30 °C to 800 °C under dynamic air flow (8 l h^{-1}) on dried subsamples of about 3 mg. Details about these procedures have

been previously described by Montecchio et al. (2006). Each sample was analyzed twice. This kind of analysis involves a slow and continuous heating of the sample coupled with a simultaneous measurement of weight loss (TG) and energy change (DTA) (Francioso et al., 2007; Francioso et al., 2009). The latter is generally estimated by measuring the temperature change of the sample compared to a reference (calcined caolinite). The mass loss and the energy changes during heating are strictly related to abundance of more or less labile C of derivatives, and to their structure and chemical composition (Francioso et al., 2007; Francioso et al., 2009).

Incubation study

The C_4 soil was carefully deprived of all maize residues, sieved at 2 mm and air-dried, then moistened with distilled water to reach a water content of 13% and incubated at 20°C at constant moisture for two weeks prior to use. The *Meliaceae* derivatives were pulverised as for experiment 1 (§Chapter 2) and incorporated into the soil in order to supply 34 mg N kg^{-1} soil. The amount of C and N added with the amendment is shown in Table 4.1. A mineral treatment containing 34 mg of ammonium sulphate-N kg^{-1} $(NH_4)_2SO_4$–N soil was also included as well as an unamended control soil to measure mineralization of native SOM. A set of 250 ml glass jars containing 50 g soil sub-samples were used at days 1, 7, 14, 21, 38, 84 and 144 for soil mineral N and microbial C and N determination (see *mineral N and microbial biomass*). A second set of 250 ml glass (4 jars/treatment) containing 100 g of soil was used for CO_2 and N_2O flux measurements (see *flux measurements*) and a third set of 500 ml glass jars containing 100 g of soil was used to trap CO_2 produced during the first two weeks, and at days 43, 113 and 142 by precipitating it as strontium carbonate ($SrCO_3$) in order to analyze the $\delta^{13}C$ of CO_2-C and partition soil derived-C from *Meliaceae* derived-C (see *CO_2 precipitation*). During the experiment, all jars were incubated at constant temperature (23 ±2 °C) and soil moisture, measured by subsequent weight.

Mineral N and microbial biomass. Mineral nitrogen (NO_3^--N, NO_2^--N and NH_4^+-N) was extracted by shaking 10 g of soil in 100 ml of a solution of 2 M KCl for 1 h. After sedimentation, soil extracts were stored at -20°C until analysis that were performed by autoanalyzer (Auto Analyzer AA-3; Bran + Luebbe, Norderstadt, Germany) as described in chapter 1. Microbial biomass C and N was determined by the chloroform extraction method (Vance et al., 1987) (§ Chapter 2), using a solution 0.05 M of K_2SO_4 (Blagodatskaya et al., 2011; Brant et al., 2006). As for the first experiment (Chapter 2), no correction factor was used. The unfumigated samples were also used to quantify extractable organic C (EOC) (Brant et al., 2006).

Flux measurements. Fluxes were measured daily the first week, three times a week for the following 2 weeks, then twice a week and finally weekly. To measure CO_2 and N_2O fluxes, the glass

jars were sealed for approx. 2 hours with lids that allowed the connection to the Innova 1302 photo-acoustic infrared gas analyser (LumaSense Technologies A/S, Ballerup, Danmark) with two Teflon tubes. Once CO_2 measurement was performed, a soda-lime filter was used for N_2O measurements to minimize interferences by CO_2 (Velthof et al., 2002). Four empty jars (blank) were used to correct values for background CO_2 and N_2O concentrations. Values were corrected also for mixing of the gas sample with the previous measurement in the internal volume of the gas analyser. Hourly fluxes were calculated assuming linear increase of CO_2 and N_2O concentrations over time during the enclosure period. Cumulative N_2O and CO_2 emissions were calculated assuming linear changes between subsequent flux measurements. Cumulative N_2O fluxes were calculated only for the first 2 weeks of the experiment as after 14 days N_2O emissions declined to the background level.

Carbon dioxide precipitation. Vials containing 20 ml of sodium hydroxide (NaOH) 0.25 M were placed in the 500 ml glass jars to trap CO_2 and were immediately closed air-tight. During the first two weeks, the vials with NaOH were removed and immediately replaced by vials with new trapping solution. In particular, this was done at day 1, 2, 4, 7, 10, 14. Also at day 43, 113, and 142, other trapping sessions of 96, 120 and 121 hours, respectively, were performed. The duration of the trapping session was increased by time in order to accumulate enough C needed for mass-spectrometry analysis. At each trapping session four empty jars containing only the vial with the trapping solution were used as blank to correct for ambient CO_2 and carbonate-C present in the NaOH solution. The CO_2-C trapped by the NaOH solution was determined with a TOC/TIC analyzer (TOC-Vcpn TNM-1, Shimadzu, Kyoto, Japan). The NaOH solution was then used to precipitate CO_2-C as $SrCO_3$ by adding 10 ml of 1 M $SrCl_2$ aqueous solution. The NaOH solutions containing the $SrCO_3$ precipitated were then centrifuged 4 times at 3600 rpm for 10 minutes and washed in between with CO_2-free distilled water that was obtained by boiling distilled water for 30 minutes and cooling with a soda-lime protection. After washing, the remaining water was removed from the vials and the $SrCO_3$ was dried at 105°C (Blagodatskaya et al., 2011). The $SrCO_3$ was analyzed for $\delta^{13}C$-values with a CF-IRMS (mod. Delta Plus Thermo Fisher, Bermen, Germany). As for gas monitor flux measurements, hourly fluxes were calculated by assuming linear increase of CO_2-C concentrations over time during the enclosure period and cumulative CO_2-C emissions were calculated by assuming linear changes between subsequent trapping sessions.

Partitioning of Meliaceae derivatives derived- and soil-derived-CO_2-C. The following mass balance equation (Balesdent et al., 1988) was used to correct data from the blank (atmospheric CO_2 and carbonates contained in NaOH)

$$C_{trt}\,\delta_{trt} = C_{tot}\,\delta_{tot} - C_{NaOH}\,\delta_{NaOH} \qquad (1)$$

where:

C_{tot} is the total amount of C present in the NaOH vials placed in jars filled with the amended/control soils (= C_{trt} + C_{NaOH}),

δ_{tot} is the $\delta^{13}C$ of $SrCO_3$-C precipitated in the NaOH vials placed in the jar filled with the amended/control soils,

C_{trt} is the total amount of C produced by the amended/control soil (corrected from the blank),

δ_{trt} is the $\delta^{13}C$ of CO_2-C produced by the amended/control soil (corrected from the blank),

C_{NaOH} is the total amount of C present in the NaOH vials placed in the empty jar (blank),

δ_{NaOH} is the $\delta^{13}C$ of $SrCO_3$-C precipitated in the NaOH vials placed in the empty jar (blank).

Once all data were corrected from the blank, to distinguish native soil derived CO_2-C from derivatives derived CO_2-C, the following equation was used:

$$C_{trt}\,\delta_{trt} = C_{soil}\,\delta_{soil} + C_{derivative}\,\delta_{derivative}$$

in the form:

$$C_{derivative} = C_{trt}\,(\delta_{trt} - \delta_{soil})/(\delta_{derivatives} - \delta_{soil}) \qquad (2)$$

(Cerri et al., 1985; Cheng, 1996; Fu and Cheng, 2002)

where:

$C_{derivative}$ is the CO_2-C evolved from the microbial mineralization of the derivative,

$\delta_{derivative}$ is the $\delta^{13}C$ of the CO_2-C evolved from the microbial mineralization of the derivative (we assumed that no fractionation occurred during mineralization of the derivative and we considered this value to be equal to that determined by the CF-IRMS analysis of the derivative (Table 4.1)),

C_{soil} is the CO_2-C evolved from the microbial mineralization of the native soil organic matter.

δ_{soil} is the $\delta^{13}C$ of the CO_2-C evolved from the control soil.

The CO_2-C evolved from the microbial mineralization of the native soil organic matter (C_{soil}) in the amended soil was then determined as:

$$C_{soil} = C_{trt} - C_{derivative}$$

The PE or carbon primed (C_{primed}) induced by the addition of the derivates was calculated by comparing the native soil derived CO_2-C from the amended and unamended (control) soils as:

$$C_{primed} = C_{soil}\,(\text{amended soil}) - C_{soil}\,(\text{control soil})$$

Statistics. Analysis of variance of mineral-N, microbial biomass C and N and EOC data were performed as in a factorial design with two factors: treatments (5 levels: control, mineral, neem 5,

neem 6, melia) and time (7 levels: day 1, 7, 14, 21, 35, 84, 144). When interaction between factors was significant, 2 standard error of means (SEM) was used as minimum difference between statistically different values. Analysis of variance of N_2O and CO_2 hourly fluxes was performed with the repeated measure procedure because measurements were conducted on the same glass jar throughout the incubation experiment. Cumulative amount of CO_2-C, expressed as mg total CO_2-C kg^{-1} or % CO_2-C/C added at the end of the incubation period was analyzed with one-way analysis of variance and the comparison of treatment effects was based on Student-Newman-Keuls test. Pearson correlation coefficient (r^2) was determined to evaluate the relationship between hourly fluxes of CO_2-C determined with NaOH trapping and those determined with the gas monitor in day 1, 2, 4, 7, 14, 42, 106, 142. Pearson correlation coefficient (r^2) was also determined to evaluate the relationship between cumulative amount of CO_2-C mineralized as determined with NaOH trapping and those determined with the gas monitor at day 1, 2, 4, 7, 14, 42, 106, 142. For these last data, Pearson correlation coefficient was also determined plotting data of all sampling day together.

4.3 Results

Meliaceae derivatives

The chemical analysis of the *Meliaceae* derivatives (Table 4.1) reflected the values of the first characterization (§Table 2.1, Chapter 2) with only slightly differences; in particular, melia leaves used in this incubation experiment presented a lower N concentration compared to those used in the first experiment (2.4% vs. 1.9%).

The $\delta^{13}C$ of the derivative ranged between -23.15‰ and -30.07‰, typical values of plants with the C_3 pathway of photosynthesis. Among the derivatives neem cake 3 presented the lowest value of C % and the highest value of $\delta^{13}C$ (Table 4.1).

The TG-DTA curves of the derivative are shown in Figure 4.1. The DTA curves of neem cake 6 and melia leaves were characterized by the presence of two exothermic peaks, while for neem 5 also a third exothermic peak was detected (Figure 4.1). Moreover, only for this latter derivative a first mass loss of 14.7 % was detected at around 193 °C. In synthesis, neem cake 5 showed a first exothermic peak at 340 °C with a mass loss of 37.2 %, a second exothermic peak at 464 °C with a mass loss of 24.8 %, and a third exothermic peak at 601 °C with a little mass loss of 2.1 %. For neem cake 6 and melia the first exothermic peak (EXO 1) was found at around 336 °C and 327 °C, with a mass loss of 50.1 % and 59.7 %, respectively. The second exothermic peak (EXO 2) was found between 487 °C and 518 °C for neem 6, with a mass loss of 32.6 % and at around 475 °C for melia leaves, with a mass loss of 27.1 % (Figure 4.1).

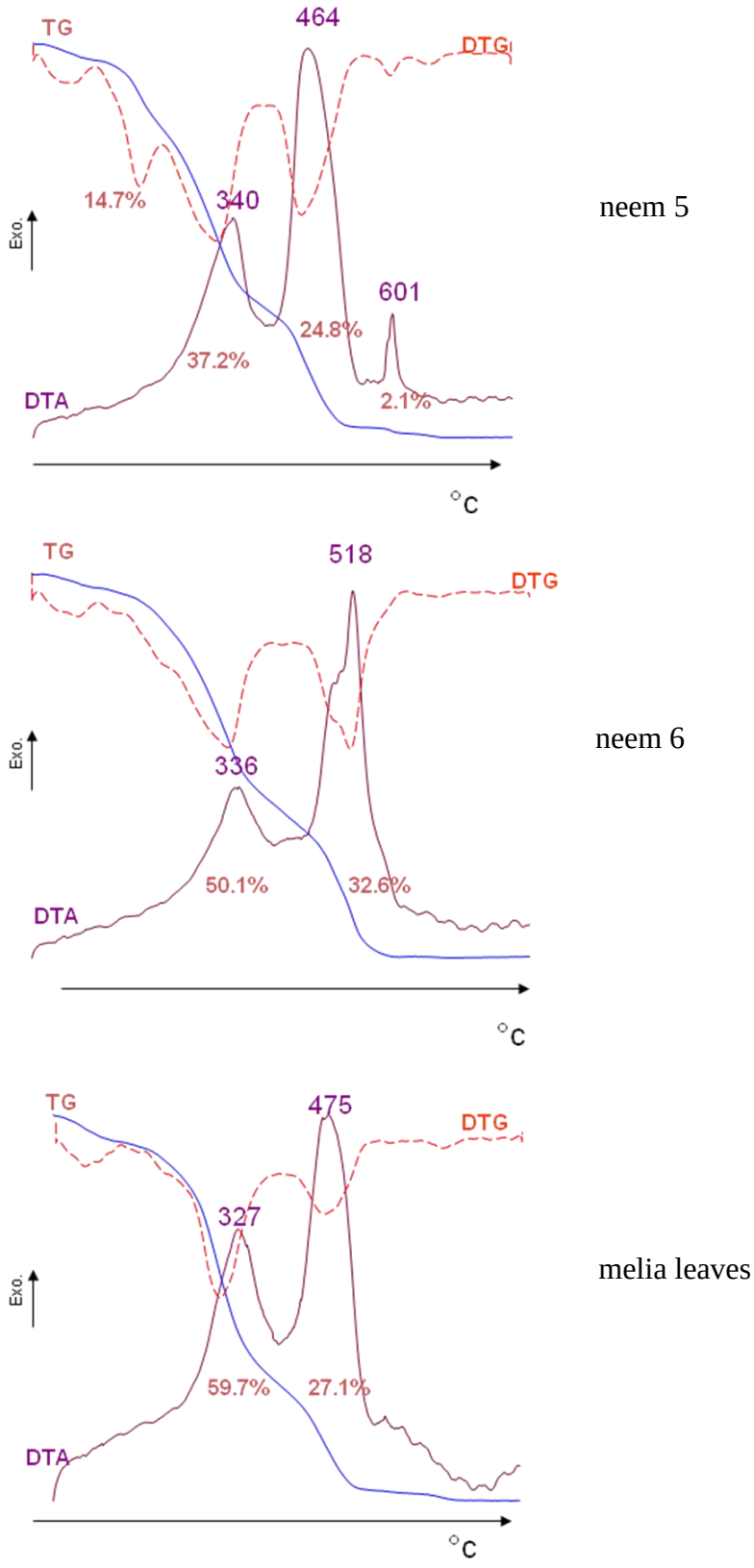

Figure 4.1. TG-DTA curves of neem cake 5, neem cake 6 and melia leaves.

Incubation experiment

As expected, at the beginning of the incubation study (day 1), the soils treated with ammonium sulphate presented the highest value of NH_4^+-N (21 mg N kg^{-1}) which decreased to 8 mg NH_4^+-N kg^{-1} at day 7 (Figure 4.2). The addition of neem cake 6 induced a slight increase of NH_4^+-N release after 7 days (around 4.5 mg N kg^{-1}) which was maintained until day 21. For all the other treatments and until day 35, NH_4^+-N ranged between 2 and 3 mg N kg^{-1}. Thereafter, in all soils, the concentration was lower than 2 mg N/kg^{-1} (Figure 4.2).

In control soils NO_3^-–N ranged from 17 mg N kg^{-1} at day 1, to 23 mg N/kg^{-1} at day 144 (Figure 4.3). As for NH_4^+-N, also nitrate-N concentrations were significantly higher in mineral soils compared to all the others (Figure 4.3), this already after 1 day and until the end of the incubation period, when it resulted of 56 mg NO_3^--N kg^{-1}. The addition of neem cake 6 did not modify NO_3^--N soil concentration at the beginning of the incubation, when values did not result statistically different from control soils, while after day 35, neem 6 increased NO_3^--N compared to all the other treatments except the mineral fertilization. On the contrary, incorporation of melia leaves and especially of neem cake 5 caused a decrease of nitrate-N until day 35, thereafter, they showed higher and lower nitrate-N concentration compared to control soil, respectively, but the differences did not result statistically significant (Figure 4.3).

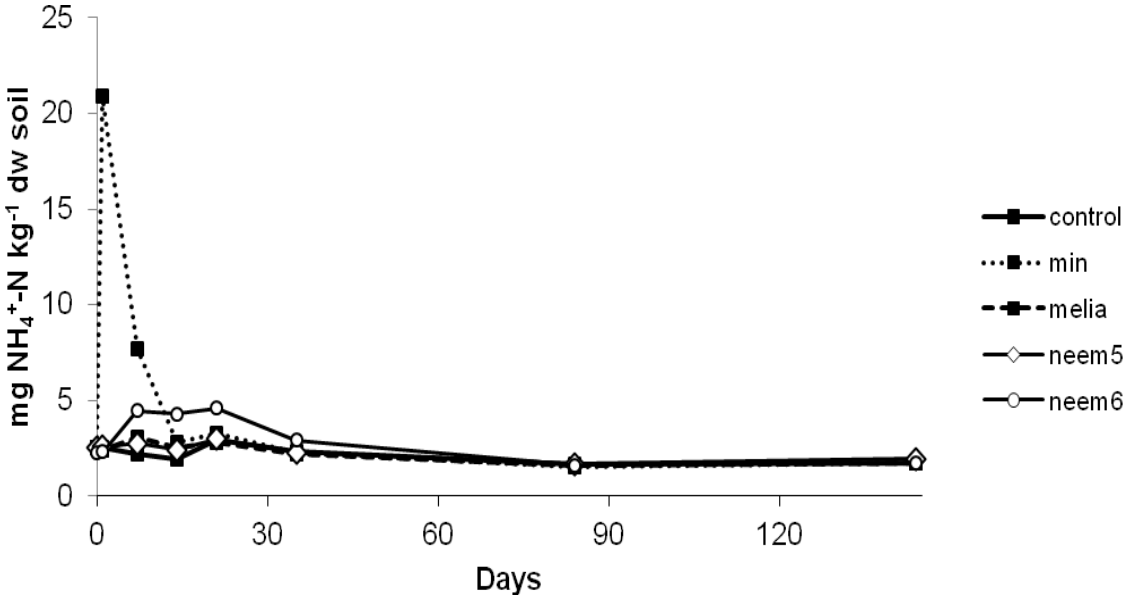

Figure 4.2. Effect of soil addition of *Meliaceae* derivatives and ammonium sulphate on ammonium-N evolution during the incubation. Interaction time*treatment significant at P≤0.001. Minimum difference between statistically different values (2SEM) = 0.36.

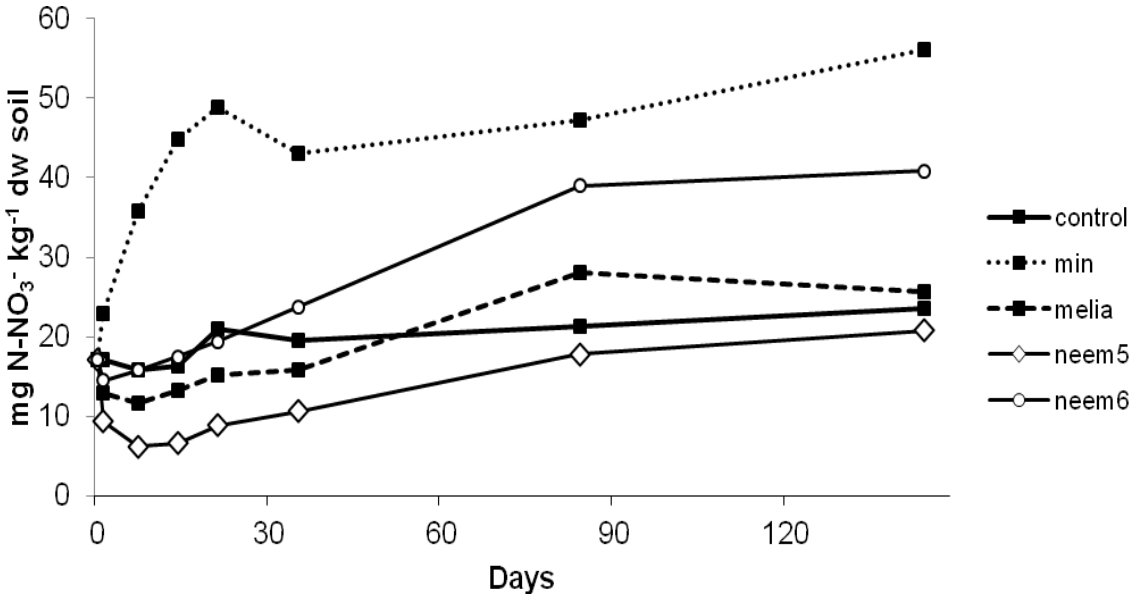

Figure 4.3. Effect of soil addition of *Meliaceae* derivatives and ammonium sulphate on NO_3^--N evolution during the incubation. Interaction time*treatment significant at $P \leq 0.001$. Minimum difference between statistically different values (2SEM) = 3.

At day 1, the three *Meliaceae* derivatives showed a net immobilization (negative apparent N mineralization) (Figure 4.4). From day 7 until the end of the incubation period, neem 6 showed a net mineralization (positive apparent N mineralization) that reached 55% of the applied N at day 144. On the contrary, neem cake 5 always showed a negative apparent N mineralization, whereas melia leaves, after a first period of immobilization, had a little positive apparent N mineralization (Figure 4.4). The recovery of mineral-N in soil treated with ammonium sulphate was of 70 % at day 1 and reached value of 95 % at the end of the incubation period (Figure 4.4).

In the first two days, N_2O emission was highest in the mineral treatment (Figure 4.5), while in the following 10 days melia, leaf-treated soil emitted the highest amount of N_2O-N, followed by neem 6 treated soils. After 14 days, N_2O-N emissions declined to the background level for all treatments. Total amount of N_2O-N emitted during the first 2 weeks of incubation was 35 μg N kg^{-1}, 10 μg N kg^{-1}, 5 μg N kg^{-1}, 2 μg N kg^{-1} and 1 μg N kg^{-1} for melia, neem 6, mineral, neem 5 and control soils, respectively (data not shown).

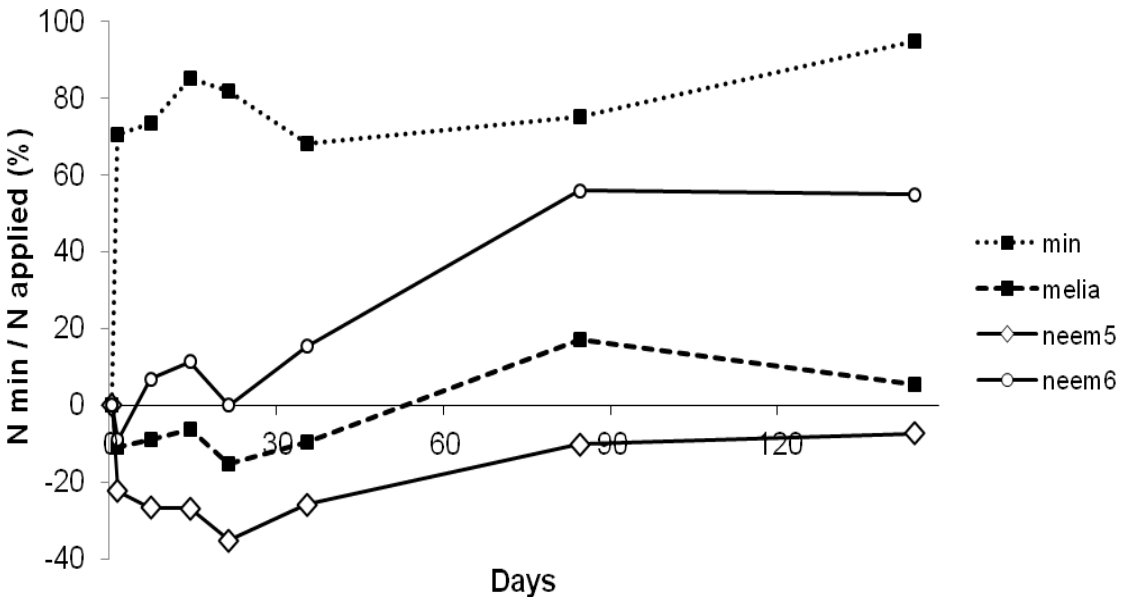

Figure 4.4. Net apparent N mineralization of the *Meliaceae* derivatives during the incubation. Interaction time*treatment significant at P≤0.001. Minimum difference between statistically different values (2SEM) = 9.59.

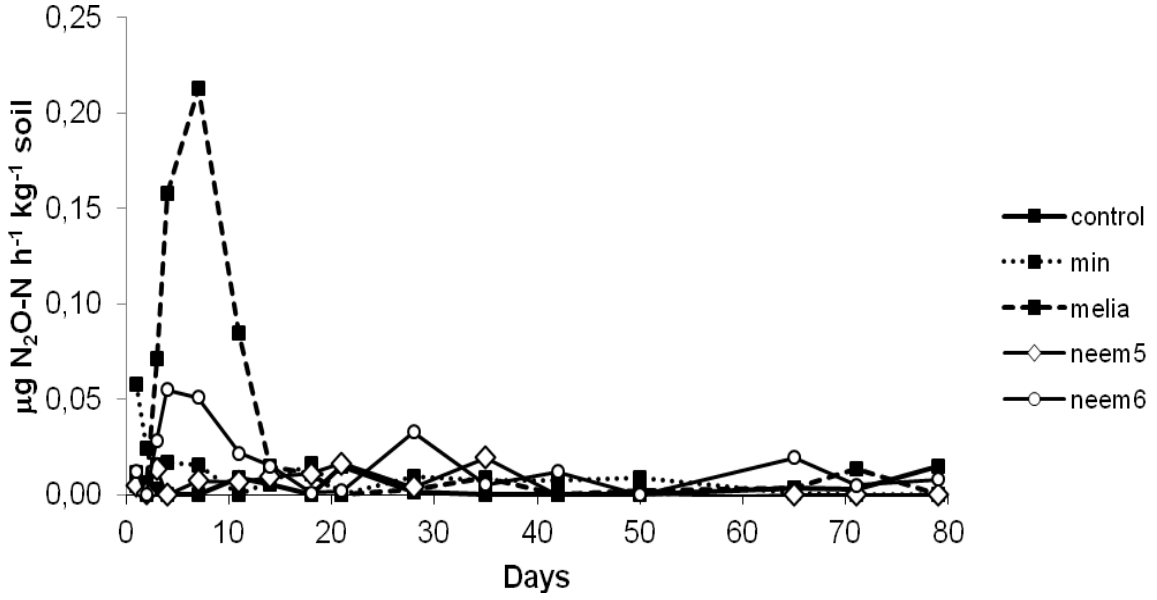

Figure 4.5. Effect of soil addition of *Meliaceae* derivatives and ammonium sulphate on nitrous oxide- nitrogen (N_2O-N) emission during the incubation. Interaction time*treatment significant at P≤0.001. Minimum difference between statistically different values (2SEM) = 0.037.

Total amount of C mineralized

Hourly fluxes of CO_2-C, as determined with the photo-acoustic infrared gas analyser, are reported in Figure 4.6. The incorporation of the *Meliaceae* derivatives caused an immediate release of CO_2-C, that resulted highest in melia and neem 5 treatments, followed by neem 6. After 4 days from the start of the experiment, hourly CO_2-C fluxes decreased in derivatives treated soils, reaching, after day 25, similar values of control and mineral trated soil that generally presented the lowest rate of C mineralization. The same trend of hourly fluxes of CO_2-C was generally found using the NaOH trapping solution (Figure 4.7). Data obtained by both methods were well correlated in the first 43 days, when r ranged between 0.93 of day 1 and 0.83 of day 43 ($P \leq 0.001$) (data not tabulated).

Cumulative amount of mineralized C (mg CO_2-C kg^{-1} soil) determined by the photo-acoustic infrared gas analyser are shown in Figures 4.8 and those by NaOH trapping solution in Figure 4.9. Data determined by the use of the gas-monitor showed that, by the end of the incubation period, control and mineral soils presented the lowest amount of total C mineralized (161 and 144 mg CO_2-C kg^{-1}, respectively), followed by neem 6 (330 mg CO_2-C kg^{-1} soil), while neem 5 and melia leaves induced the highest C mineralization with 620 and 604 mg CO_2-C kg^{-1}, respectively (Figure 4.8). These values were generally confirmed by the NaOH determinations (Figure 4.9), except for neem 5 treatment which showed a total amount of C mineralized of 777 mg CO_2-C kg^{-1}, which resulted, at the end of the incubation period, significantly higher than that measured in melia leaves treatment (Figure 4.8). Despite this discrepancy, data of cumulative amount of mineralized-C obtained by the two methods resulted well correlated in all sampling days ($0.93 < r < 0.96$, $P \leq 0.001$), as also illustrated in Figure 4.10.

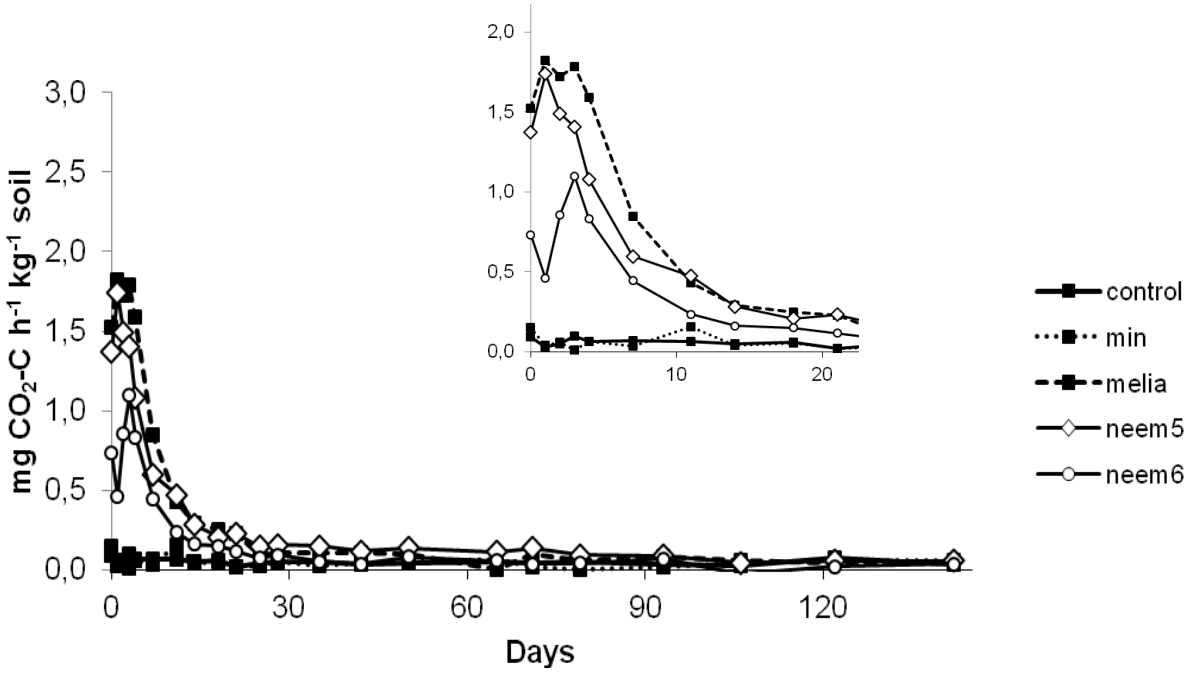

Figure 4.6. Effect of soil addition of *Meliaceae* derivatives and ammonium sulphate on hourly fluxes of C mineralized during the incubation as determined by the gas monitor. Interaction time*treatment significant at P≤0.001. The inset shows the first 20 day trend. Minimum difference between statistically different values (2SEM) = 0.1047.

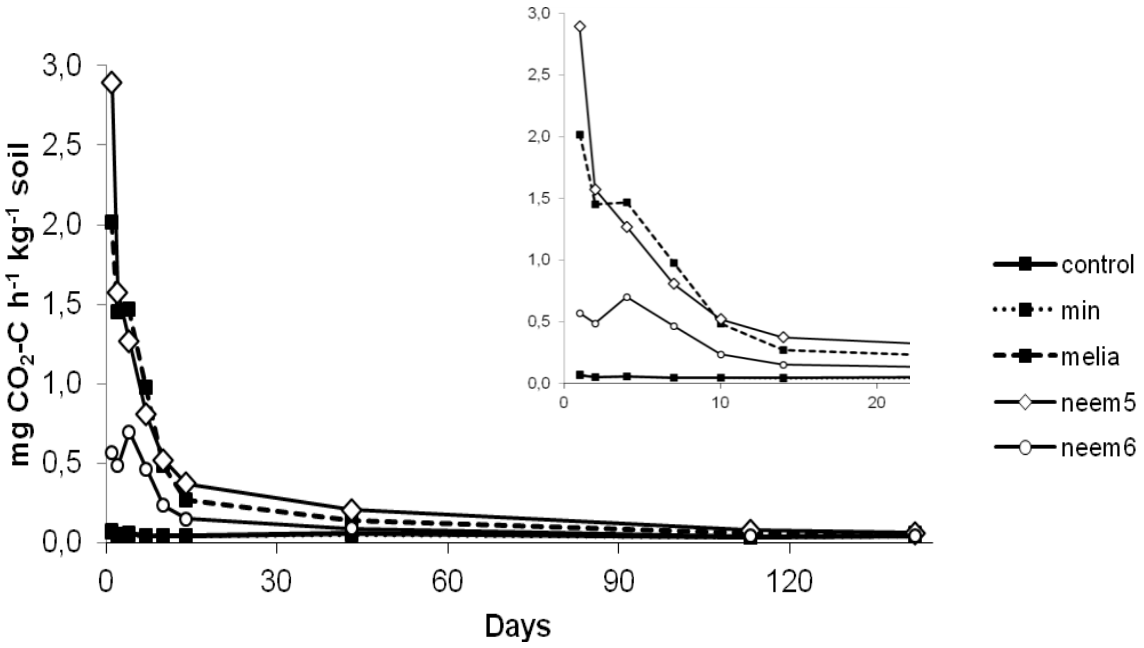

Figure 4.7. Effect of soil addition of *Meliaceae* derivatives and ammonium sulphate on hourly fluxes of C mineralized during the incubation as determined by NaOH trapping. Interaction time*treatment significant at P≤0.001. The inset shows the first 20 day trend. Minimum difference between statistically different values (2SEM) = 0.066.

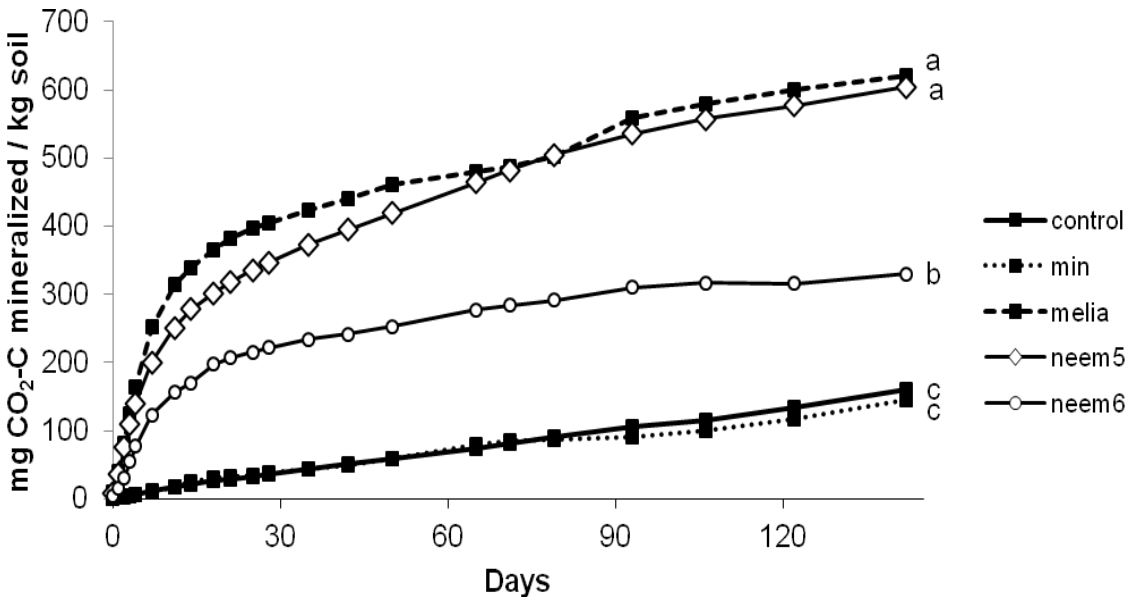

Figure 4.8. Effect of soil addition of *Meliaceae* derivatives and ammonium sulphate on cumulative C mineralized during the incubation as determined by the gas monitor. At day 143, effect of treatment significant at $P \leq 0.001$.

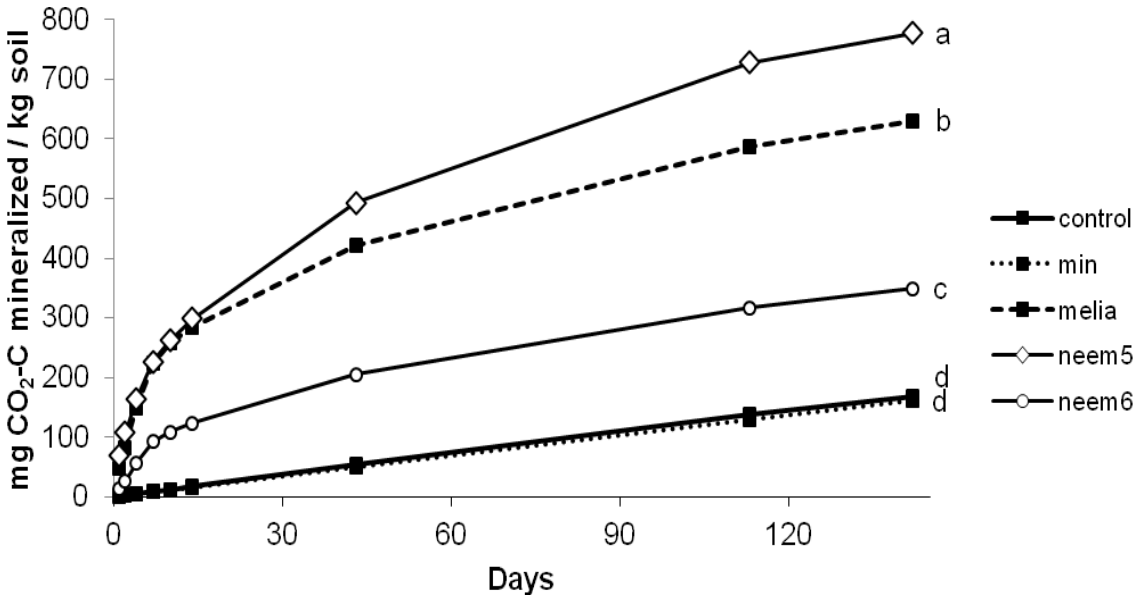

Figure 4.9. Effect of soil addition of *Meliaceae* derivatives and ammonium sulphate on cumulative C mineralized during the incubation, as determined by NaOH trapping. At day 143, effect of treatment significant at $P \leq 0.001$.

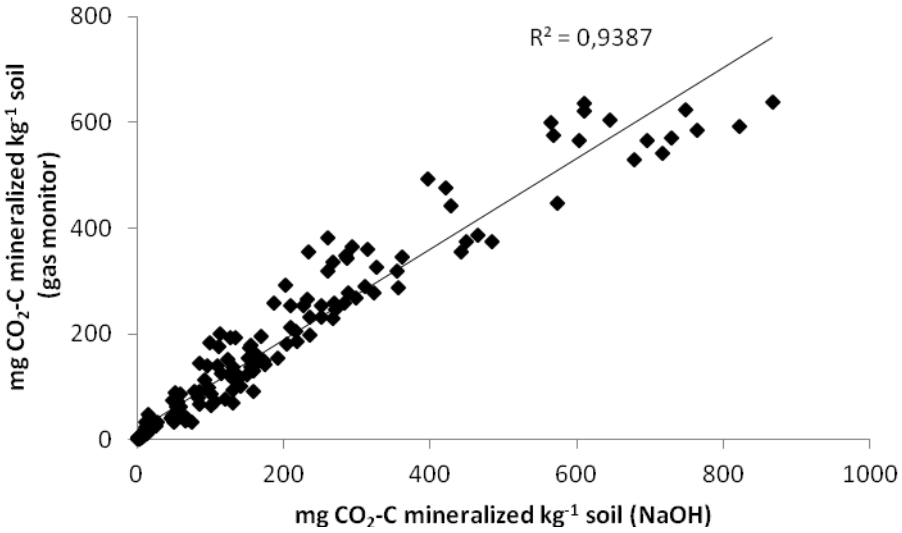

Figure 4.10. Correlation between data of cumulative CO_2-C mineralized obtained with the gas-monitor and those obtained with the NaOH trapping.

Derivative-C mineralization

The real and apparent cumulative C mineralization of *Meliaceae* derivatives was calculated with CO_2-C data obtained with the NaOH trapping solution. In particular, apparent C mineralization was calculated as the difference between total amount of mineralized C in treated soil (C_{trt}) and total amount of mineralized C in control soil ($C_{control\ soil}$), whereas real C mineralization was the real amounts of derivative-C mineralized ($C_{derivative}$) as determined by ^{13}C natural tracing (§equation 2).

The apparent mineralization was greater than real mineralization of derivative-C in all cases (Figure 4.10). By the end of the incubation period, real mineralization of neem 5 and melia leaves were similar, reaching 40 % of added C, while real mineralization of neem 6 was significantly lower (22 % of added C).

However, if considering the first day of the incubation period (Table 4.3), neem 5-C was mineralized faster than melia, and from day 4 until day 14, melia leaf mineralization was higher than neem 5. Neem 6 mineralization was always the lowest.

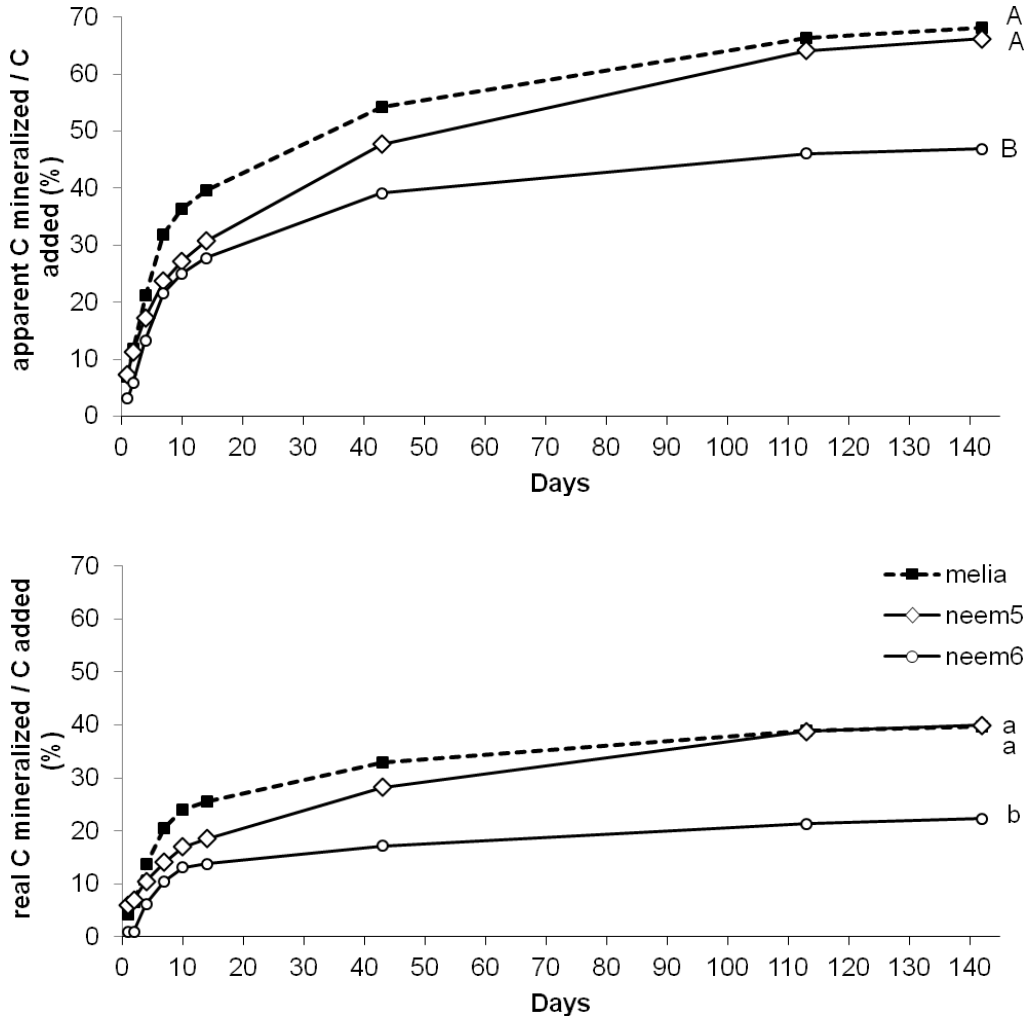

Figure 4.10. Apparent (upper) and real (lower) C mineralization rate of the *Meliaceae* derivatives expressed as percent of added C. Effect of tratment significant at P≤0.001.

Table 4.3. Real mineralization rate of *Meliaceae* derivative-C (% of added C) in the first two weeks of incubation.

Derivative	Mineralized-C (% of added C)				
	Day				
	1	2	4	7	14
Neem 5	6.0 a	7.0 a	10.5 b	14.0 b	18.5 b
Neem 6	1.0 c	1.0 b	6.2 c	10.6 c	13.8 c
Melia	4.2 b	6.6 a	13.7 a	20.6 a	25.6 a
significance	***	***	***	***	***

***: effect of treatment significant at P≤0.001

Native soil-C mineralization and primed C

As shown in Figure 4.11, the incorporation of the *Meliaceae* derivatives always increased mineralization of native SOM (positive PE). The surplus of native soil organic C mineralized compared to unamended control amounted for 193 mg, 241 mg and 45 mg in melia, neem 5 and neem 6 treated soil, respectively.

At at day 144, the amount of C primed as % of added C was similar for all derivatives.

The incorporation of the derivatives had different impact on the carbon balance of the soil. In fact, at the end of the incubation period, the amount of C stored in the system was 142 mg kg^{-1}, 37 mg kg^{-1} and 47 mg kg^{-1} after application of neem cake 5, neem cake 6 and melia leaves and represented 15 %, 10 % and 7 % of added-C, respectively (Figure 4.13).

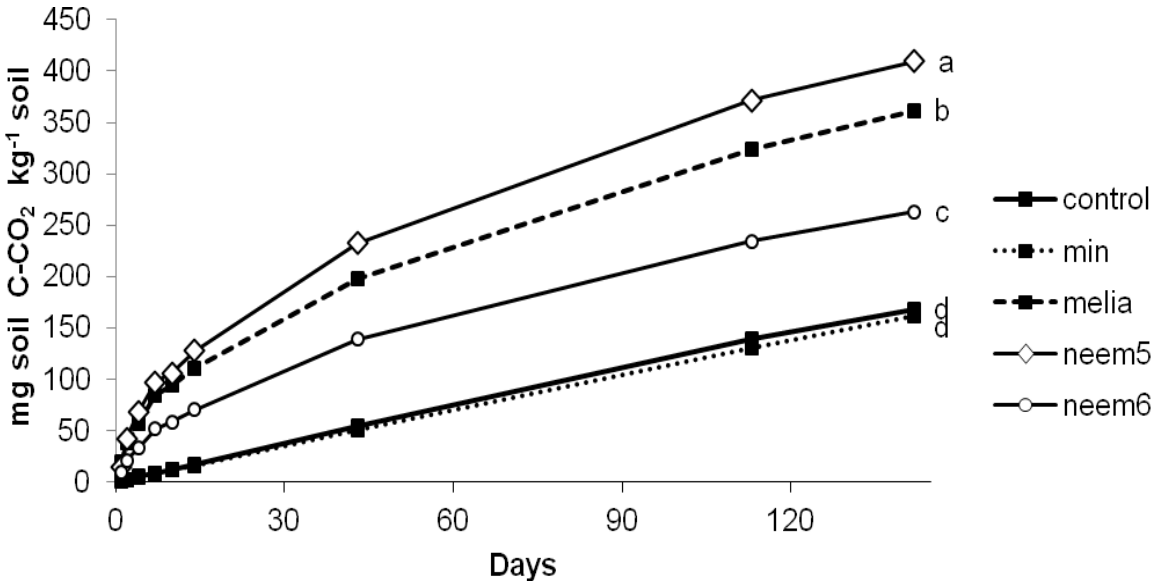

Figure 4.11. Effect of soil addition of *Meliaceae* derivatives on cumulative native soil C mineralized during the incubation period. At day 143, effect of treatment significant at $P \leq 0.001$.

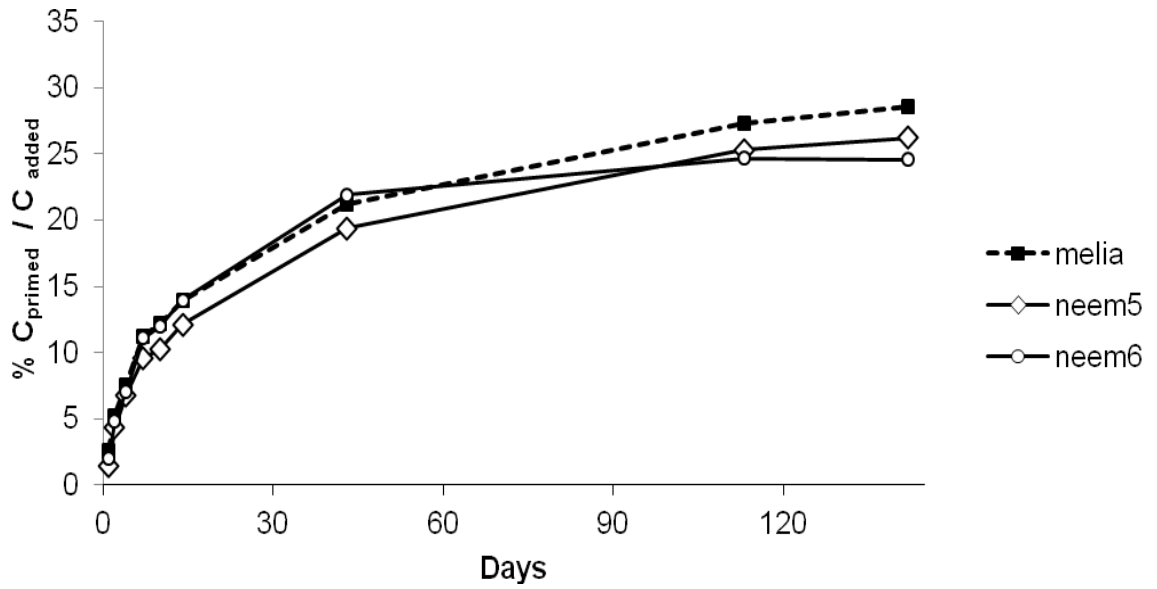

Figure 4.12. Effect of soil addition of *Meliaceae* derivatives on cumulative amount of C primed during the incubation period. At day 143, effect of treatment not significant.

The incorporation of all *Meliaceae* derivatives immediately increased the amount of EOC, which, at day 1, resulted highest in neem 5 treated soil (68 mg C kg^{-1}), followed by melia (47 mg C kg^{-1}), and by neem 6 (39 mg C kg^{-1}) (Figure 4.14). Thereafter, EOC generally decreased with time.

Application of neem 5 and melia leaves caused an immediate increase of microbial biomass C (Figure 4.15) which already at day 1 resulted higher than control soils. After 7 days, all treated soils presented a higher microbial biomass C compared to control soils, in the order: neem 5 (54 mg C kg^{-1} soil) > mineral (42 mg C kg^{-1} soil) > neem 6 (36 mg C kg^{-1} soil) > melia (32 mg C kg^{-1} soil). Successively, the values decreased for all treatments, except in the last sampling day and generally neem 5 presented the highest values.

Incorporation of melia leaves immediatly increased soil microbial biomass N compared to control soils (Figure 4.16) and, at day 7, also neem 5 presented a higher amount of microbial N compared to the three other treatments. The values of microbial N generally decreased with time until day 144 when all treated soils presented a higher amount of microbial N compared to control.

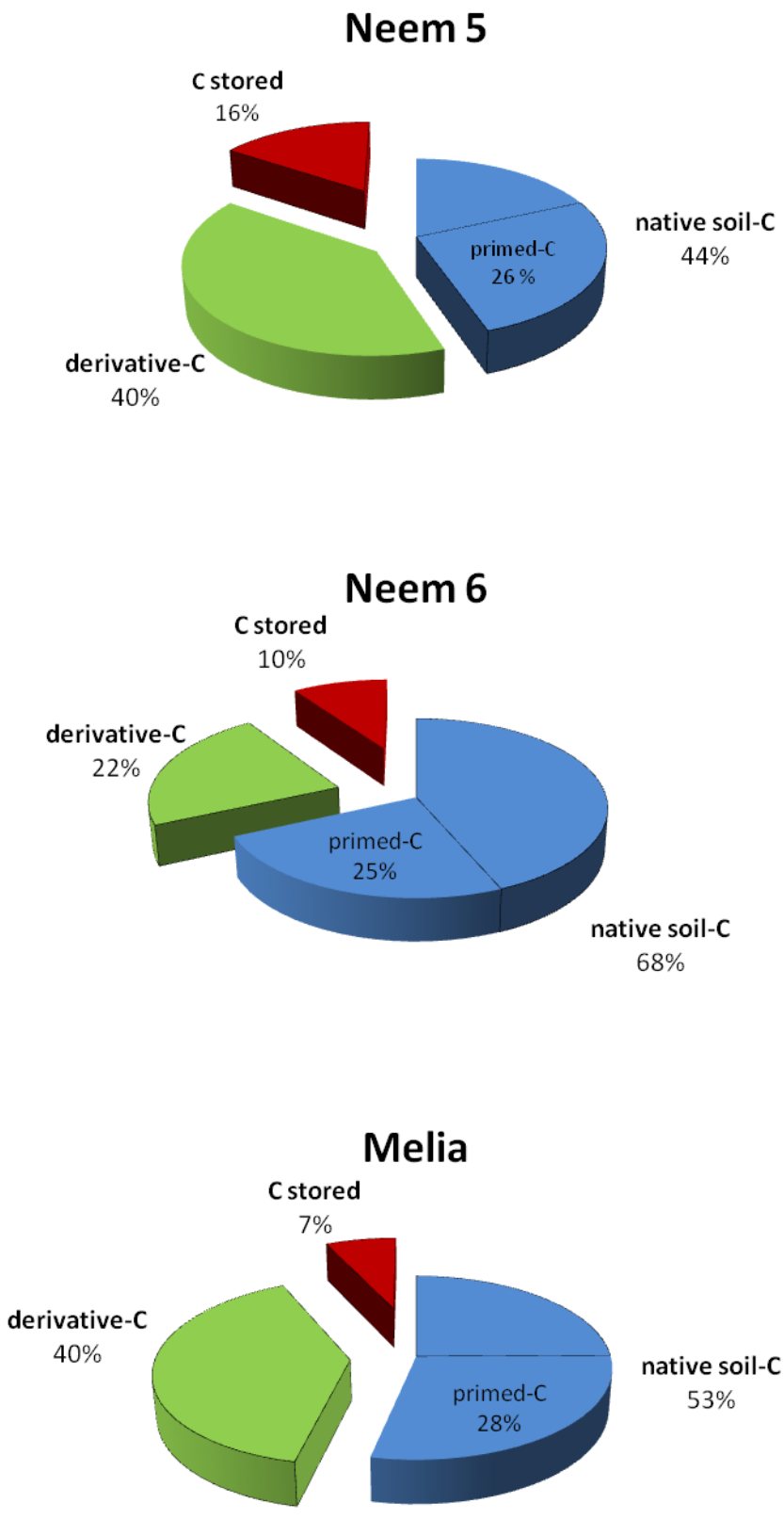

Figure 4.13. Effect of soil addition of *Meliaceae* derivatives on the C balance of soil. Percentage are relative to added-C.

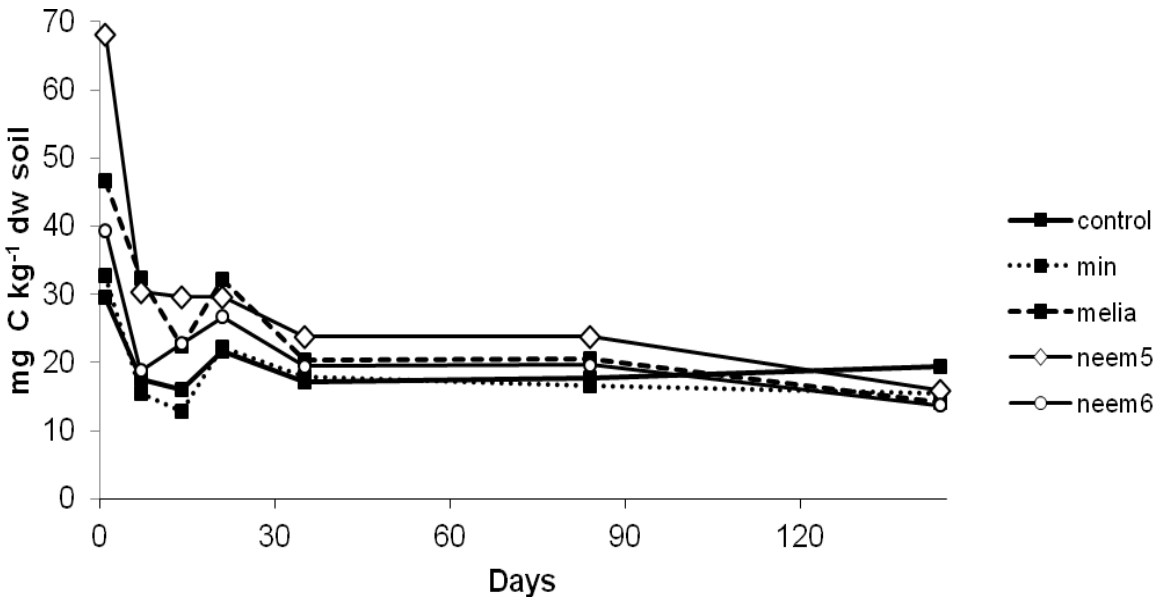

Figure 4.14. Effect of soil addition of *Meliaceae* derivatives and ammonium sulphate on K_2SO_4 extractable organic C during the incubation experiment. Interaction time*treatment significant at P≤0.001. Minimum difference between statistically different values (2SEM) = 3.63.

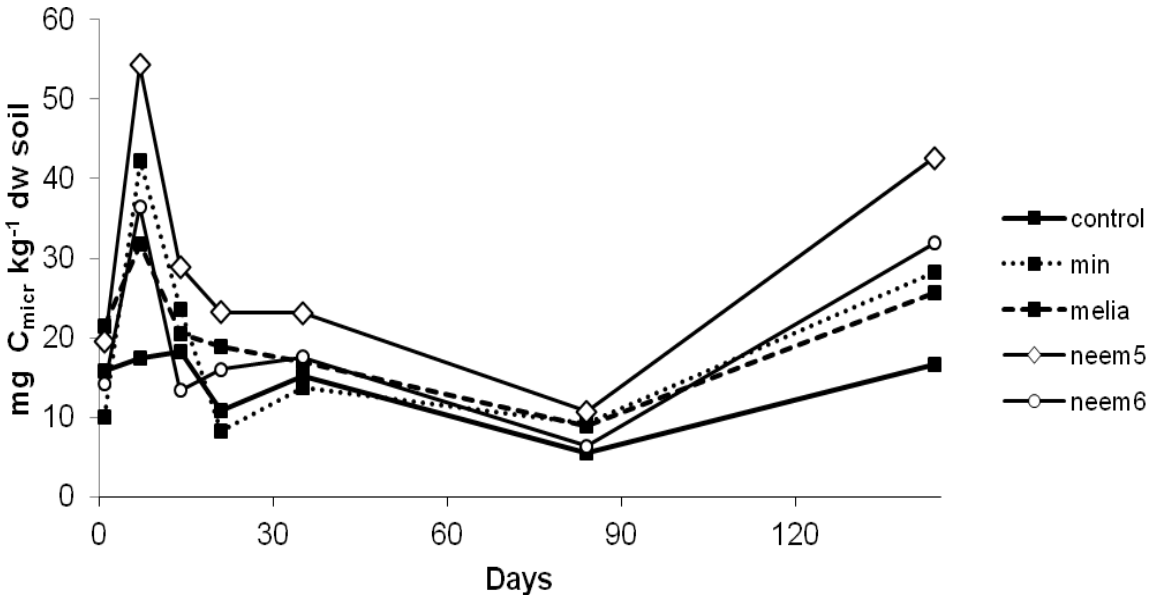

Figure 4.15. Effect of soil addition of *Meliaceae* derivatives and ammonium sulphate on microbial biomass C during the incubation experiment. Interaction time*treatment significant at P≤0.001. Minimum difference between statistically different values (2SEM) = 6.45.

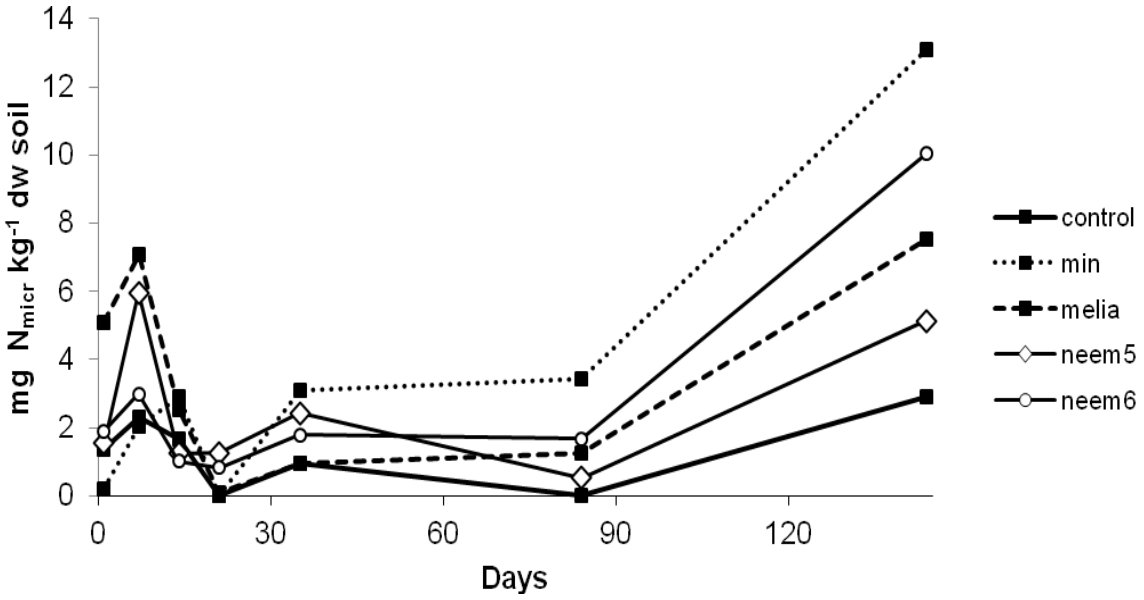

Figure 4.16. Effect of soil addition of *Meliaceae* derivatives and ammonium sulphate on microbial biomass N during the incubation experiment. Interaction time*treatment significant at P≤0.001. Minimum difference between statistically different values (2SEM) = 2.44.

4.4 Discussion

Meliaceae derivatives

The differences in the composition of the derivatives between this experiment and the first incubation study (§Chapter 2), can be explained by the organic nature of the compounds and, especially regarding melia leaves, by the fact that, although leaves were harvested from the same seedlings, this was done in different periods. Neem cake 3, the derivatives that in the first experiment showed the fastest net apparent N mineralization (Figure 2.3, Chapter 2) and, at the same time, the lowest apparent C mineralization (Figure 2.5, Chapter 2), had the lowest C content (%) and also the highest (less negative) value of $\delta^{13}C$, this means that compared to the other derivatives neem 3 is depleted in ^{12}C (or enriched in ^{13}C), this may be the consequence of a loss of C, more precisely a loss of ^{12}C during the process by which this by-product is produced or simply a loss of very labile ^{12}C during a stocking phase. It is known, in fact, that in kinetics reactions the light isotopes usually react faster than respective heavy isotope, and this differential isotope behavior is known as fractionation (Fry, 2006). Anyway, this value of $\delta^{13}C$ did not allow us to use it in combination with the C_4 soil to partition soil derived CO_2-C from derivative derived CO_2-C.

The TG-DTA curves of the three derivatives showed different thermal events indicating a different chemical structure of the derivatives. The first exothermic peak (EXO1), found at around 340 °C, 336 °C and 327 °C for neem 5, neem 6 and melia respectively, is mainly due to the breaking up of labile compounds such as aliphatic and/or alicyclic structures, especially polysaccharides and proteins; therefore, in the case of plant material, water soluble compounds, hemicellulose and cellulose are all included in this exothermic reaction (Reh et al., 1990; Kosheleva and Trofimov, 2008). These compounds are supposed to account for 37 %, 50 % 59 % of neem 5, neem 6 and melia mass, respectively. The second exothermic peak (EXO2), found at around 464 °C, 518 °C and 475 °C for neem 5, neem 6 and melia, respectively, was due to the oxidation of more thermostable compounds such as aromatic rings. In this exothermic reaction lignin is assumed to break up (Flaig et al., 1975; Rovira and Vallejo, 2000). These compounds accounted for 25 %, 33% and 27 % in neem 5, neem 6 and melia mass, respectively.

The mass loss of 14.7 % detected at 193 °C only for neem cake 5 indicates the presence, in this derivative, of very labile and easily degradable compounds. In addition, the final mass loss of 2 % detected at 600 °C is indicative of the presence of thermally resistant organic compounds as hydrocarbons, esters, fatty acids, wax and aromatic and cyclic compounds (Bianchi, 1995).

Nitrogen mineralization

Data on mineral N confirmed the results of the first experiment, in fact neem cake 6 (C:N 12) showed a first short period of immobilization, then a peak of release of ammonium after 7 days as in the first experiment and a net positive apparent N mineralization of 55 % (vs. 22 % in the first experiment). Melia leaves and, in particular, neem cake 5 showed N immobilization as previously found and after 84 days net apparent mineralization resulted of 17 % and -10 %, respectively, percentage comparable to those of the first experiment (20 % and -13 %, respectively, § Chapter 2). The immobilization of N after the addition of melia leaves and neem 5 was confirmed by the values of microbial biomass N which resulted increased at the beginning of the incubation study in these two treated soils compared to the control.

The values of apparent N mineralization were not corrected for N_2O-N losses because these were irrelevant compared to the amount of mineral N found in soil. On the contrary, Velthof et al. (2000), in their experiment on 10 different crop residues added to a sandy and a clay soil, with and without additional nitrate, found that in the sandy soil total N_2O emission from wheat, maize and barley (crop with low mineralizable N content (<10%) and high C:N ratio) were not significantly different from the control, while total N_2O emission from white cabbage, brussels sprouts, mustard, sugar beet residues and broccoli [crop with high amounts of mineralizable N (> 60% of total N) and

small C:N ratios (<20)] ranged from 0.13 to 14.6 % of the amount of N added as residue and were higher with additional NO_3^- than without additional NO_3^-. They found similar effects of crop residue in the clay soil, where less than 1 % of the residue N evolved as N_2O. In our study, only the incorporation of melia leaves and, with a lower extent, neem 6 increased N_2O emissions compared to the control. In the former case, a local depletion of O_2 after the application of 4.4 g kg^{-1} of fw melia leaves may have increased N_2O emission despite the lower presence of mineral N compared to control and neem 6 treated soils. In any case, the total amount of N_2O emitted was very low.

Total carbon mineralization

Data of CO_2-C emissions obtained with the two methods were generally well correlated, in agreement with Alavoine et al. (2008), who compared C decomposition of crop residues and agro-industrial effluent in a highly calcareous ($CaCO_3$ = 65.7 %) and in a loamy soil using three different methods. A static soil incubation method with an alkali trap as the one described in our experiment, and two cylinder methods (CM): a static CM where CO_2 fluxes were measured with an alkali trap and a dynamic CM where CO_2 fluxes were measured with an infrared gas analyser. Carbon dioxide emission fluxes obtained from the three methods were in good agreement and significantly correlated for both soils, nevertheless the static methods tended to overestimate CO_2 emissions for the highly calcareous soil (Alavoine et al., 2008). Also in our experiment CO_2 fluxes resulted higher when measured with the alkali trap, but this contrasts with numerous other studies that highlighted underestimations of CO_2 fluxes with the static method. Alavoine and co-workers (2008) attributed this discrepancy to the displacement of the carbonate-bicarbonate equilibrium of alkaline soil that might have led to excess CO_2 production. However the soil of our experiment was not highly calcareous, moreover the discrepancy between the two methods was found only for neem 5 treatment and not for the others. We believe that in our case, the underestimation of CO_2 fluxes when determined by the photo-acoustic infrared gas analyser was probably due to a saturation of CO_2 in the headspace of the jars during the 2 hour enclosure, especially at the beginning of the incubation study when CO_2 fluxes were high (>2.5 mg C kg^{-1} h^{-1}). In this situation, the amount of CO_2 accumulated inside the jars could have inhibited the production of further CO_2, event that did not happen in the static method where all CO_2 produced was ‘removed’ from the gas headspace by the NaOH trap.

Derivative-, soil-C mineralization and primed-C

The natural ^{13}C abundance method showed differences between the apparent and the real C mineralization of the derivatives. In particular, apparent C mineralization was greater than real mineralization indicating a stimulation of native SOM mineralization.

At the beginning of the incubation period neem 5 mineralized faster than melia leaves and neem 6, indicating the presence of a labile component that rapidly (within 1 day) exhausted, this is in agreement with TG-DTA analysis, as previously reported.

The addition of ammonium sulphate did not modify SOM mineralization, confirming the results of Guenet et al. (2010), who found that adding NH_4NO_3 (85.4 mg kg^{-1}) to the soil did not trigger any change in SOM mineralization compared to the control.

On the contrary, soil incorporation of all the *Meliaceae* derivatives increased mineralization of native SOM. In particular, in neem 5-treated soil, C primed resulted higher than in melia and particularly neem 6-treated soil, where primed C always resulted the lowest. However, the amount of C primed is reported to the amount of C added, no difference among derivatives can be detected. This may indicate that there is a linear relationship between priming effect intensity and the amount of C added, as already reported by Mary et al. (1993) who found a positive PE proportional to C supply (100 and 450 mg C kg^{-1} soil) for glucose, roots and root exudates in a 6-month incubation study. This is apparently in contrast with Guenet et al. (2010) who found that PE intensity did not increase proportionally with the supply of 1.5, 2.2 and 3.2 g straw-C kg^{-1} soil. However, in our case, the C supply by the three derivatives was different both in term of amount and quality, not allowing to define the real relationship between PE intensity and amount of C added.

We are aware that the amounts of primed-C reported in this study probably are overestimated as in our calculations we considered that PEs were always real and not apparent. However, the initial flush of extra CO_2 occurring in the first days can reflect accelerated turnover or pool substitution in microbial biomass (apparent PE) (Kuzyakov, 2010) and should not be accounted as extra-soil OM mineralization (real PE). For a more correct picture of PE, the separation of the real from the apparent PE should be performed. However, according to Fontaine et al. (2011) the nature of incorporated fresh C (soluble or polymerized) seems to determine the type of substrate utilization by microbes (microbial turnover or production of SOM degrading enzymes). These authors support the hypothesis that apparent PE is generally observed with the supply of soluble C (sugars) with mineral nutrients, whereas real PE is observed with the supply of polymerized C like cellulose and ryegrass. The derivatives studied in this experiment can be considered as ‘polymerized’, thus supporting the real nature of the PE observed.

4.5 References

Alavoine G., Houlbert J.C., Nicolardot B., 2008. Comparison of three methods to determine C decomposition of organic materials in soils under controlled conditions. Pedobiologia, 52:61-68.

Balesdent J., Mariotti A., Guillet B., 1987. Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. Soil Biology and Biochemistry, 19: 25-30.

Balesdent J., Wagner G.H., Mariotti A., 1988. Soil Organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. Soil Science Society of America Journal, 52: 118-124.

Bianchi G., 1995. Plant waxes. In: Hamilton R.J. (Ed.), Waxes: chemistry, molecular biology and function. Dundee: the oil press, pp. 175-222.

Blagodatskaya E.V., Blagodatsky S.A., Anderson T.H., Kuzyakov Y., 2007. Priming effects in Cherozem induced by glucose and N in relation to microbial growth strategies. Applied Soil Ecology, 37: 95-105.

Boutton T.W., 1991. Stable Carbon Isotope Ratios of Natural Materials: II. Atmospheric, Terrestrial, Marine, and Freshwater Environments. In: Carbon Isotope Techniques. Edited by Coleman D.C. and Fry B. Academic Press, Inc. San Diego, California.

Brandt J.B., Sulzman E.W., Myrold D.D., 2006. Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. Soil Biology & Biochemistry, 38: 2219-2232.

Cheng w., 1996. Measurement of rhizosphere respiration and organic matter decomposition usinf natural ^{13}C. Plant and Soil, 183: 263-268.

Flaig W., Beutelspacher H., Rietz E., 1975. Chemical composition and physical properties of humic substances. In: Gieseking J.E. (Ed), Soil Component: Organic Components. Springer, Berlin, pp. 1-211.

Fontaine S., Bardoux G., Abbadie L, Mariotti A., 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters, 7: 314-320.
Fontaine S., Barot S., Barré P., Bdioui N., Mary B. & Rumpel C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature, 450: 277-281.

Francioso O., Ferrari E., Saladini M., Montecchio D., Gioacchini P., Ciavatta C., 2007. TG-DTA, DRIFT, and NMR characterization of humic-like fractions from olive wastes and amended soil. Journal of Hazardous Materials, 149: 408-417.

Francioso O., Montecchio D., Gioacchini P., Cavani L., Ciavatta C., Trubetskoj O., Trubetskaya O., 2009. Structural differences of Chernozem soil humic acids SEC-PAGE fractions revealed by thermal (TG-DTA) and spectroscopic (DRIFT) analysis. Geoderma, 152: 264-268.

Fu S., Cheng W., 2002. Rhizosphere priming effects on the decomposition of soil organic matter in C_4 and C_3 grassland soils. Plant and Soil, 238:289-294.

Guenet B., Neill C., Bardoux G., Abbadie L., 2010. Is there a linear relationship between priming effect intensity and the amount of organic matter input? Applied Soil Ecology, 46: 436-442.

Kosheleva Y.P., Trofimov, S.Y., 2008. Characteristics of the biochemical composition of plant litter at different stages of decomposition (according to thermal analysis data). Biology Bulletin, 35: 64-69.

Kuzyakov Y., 2010. Priming effects: Interactions between living and dead organic matter. Soil Biology and Biochemistry, 42: 1363-1371.

Mary B., Fresneau C., Morel J.L. and Mariotti A., 1993. C and N cycling during decomposition of root mucilage, roots and glucose in soil. Soil Biology and Biochemistry, 25: 1005-1014.

Montecchio, D., Francioso, O., Carletti, P., Pizzeghello, D., Chersich, S., Previstali, F., Nardi, S., 2006. Thermal analysis (TG-DTA) and drift spectroscopy applied to investigate the evolution of humic acids in forest soil at different vegetation stages. Journal of Thermal Analysis and Calorimetry 83, 393-399.

Pierzynski G.M., Sims J.T., Vance G.F., 2000. Soils and Environmental Quality. CRC Press, Boca Raton , 459 pp.

Reh U., Kratz W., Kraepelin G., Angern-Bettinazzi C., 1990. Analysis of leaf and needle litter decomposition by differential scanning calorimetry and differential thermogravimetry. Biology and Fertility of Soils, 9: 188-191.

Rovira P., Vallejo V.R., 2000. Evaluating thermal and acid hydrolysis methods as indicators of soil organic matter quality. Communication in Soil Science and Plant Analysis, 31: 81-100.

Vance E.D., Brookes P.C., Jenkinson D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry, 19: 703-707.

Velthof G.L., Kuikman P.J., Oenema O., 2002. Nitrous oxide emission from soils amended with crop residues. Nutrient Cycling in Agroecosystems, 62: 249-261.

Chapter 5

Conclusions

The *Meliaceae* derivatives resulted different in term of mineral composition and chemical structure. They also showed different dynamics of N mineralization. The biochemical quality that best predicted N release was the C:N ratio and, partially, its N concentration. Carbon concentration did not result effective in controlling the N mineralization, except immediately after the amendment application. Generally, the derivatives with a C:N ratio higher than 21 caused net N immobilization, whereas the derivatives with a C:N ratio lower than 16 caused net N mineralization. As a consequence the latter can be used when a fast availability of mineral N is required, while the former are recommended to reduce the risk of nitrate-N leaching.

Soil incorporation of all *Meliaceae* derivatives always increased soil microbial biomass C, thus resulting a source of C for the soil microbial population. However, our results did not provide information on changes in the microbial community composition, topic that certainly deserve further investigations.

Some *Meliaceae* derivatives, in particular those with higher N concentration, had a positive effect on plant growth, probably as a result of a general higher nutrient availability for plants. In these sense, N, P and K resulted the nutrients more available after soil application of derivatives.

Although some *Meliaceae* derivatives caused N immobilization in the incubation studies, they did not reduce plant growth, rather they induced a positive effect on root N, and increased chlorophyll content of GF677 plant leaves, thus confirming that plant-soil interactions should always be included in studies of SOM dynamics and consequent nutrient release.

The ^{15}N isotope technique showed that one month after the fertilization, N derived from melia leaves was already available to plants. At the end of the growing season, about 7% of N added as melia leaves was recovered in plant, while 70% of it was still present in soil, almost all in the organic form, thus representing a potential source of N for the following vegetative seasons. The remaining 20% of melia derived-N resulted lost out of the plant-soil system, probably by leaching and/or gaseous emissions.

Despite its low sensitivity, due to the relatively low difference in $\delta^{13}C$ value between soil CO_2-C and derivative CO_2-C, the natural ^{13}C abundance method allowed the separation, in the amended soil, of the two CO_2-C sources, thus permitting the quantification of the priming effect induced by the *Meliaceae* derivatives and their real C mineralization.

In particular, the real C mineralization differed with the derivatives and ranged between 22 % and 40 % of added-C. All the derivatives studied induced a positive priming effect. The amount of C primed, 144 days after the amendment, corresponded to approximately 26 % of added-C, for all the derivatives. Despite this substantial priming effect, the C balance of the soil, 144 days after the amendment, always resulted positive.

Acknowledgements

I am particularly grateful to my tutor, Dr. Moreno Toselli, for this opportunity to increase my knowledge, its encouragements and its teaching in all these years. I am particularly grateful also to Dr. Paola Gioacchini for the important contribution to this doctorate dissertation, in particular for helping me to conceive and design the priming effect experiment, and for all the advices concerning isotope techniques. A special thanks to Dr Elena Baldi, Maurizio Quartieri and Giovambattista Sorrenti for their useful assistance before, during and after the experiments. I express my gratitude to Dr. G. Marino and Prof. B. Marangoni for their precious and encouraging advices. I am also grateful to Dr. D. Montecchio for the TG-DTA analysis and her valuable comments on the last experiment, to Prof. M. Nicoletti (Università La Sapienza, Rome) and Dr. S. Mariani (ENEA, Rome) for providing the starting material for the research: the neem cakes, and to Prof. G. Baldoni, G. Giordani and M. Bortolotti for providing the valuable C_4 soil used in the last experiment. I also thanks Dr. E. Muzzi for the statistical advices and the team of the Experimental Station of Cadriano for the technical assistance.

The Soil Biology and Biological Soil Quality Group of Wageningen University, The Netherlands, is acknowledged for having accepted my stay. In particular, I thank Prof J.W. van Groenigen and Dr. I. Lubbers, for their teaching in dealing with isotopes and gaseous flux measurements.

Tutor and colleagues reviewed this PhD dissertation, I thank them for this, and any mistakes you found are due to myself, not to the meticulous attention of the reviewers.