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Evaluation of plant derivatives of Meliaceae family as a source of nitrogen for trees

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Abstract

Soil application of fresh organic matter is a way to increase soil organic matter and provide nutrients to trees. The effect of application of organic matter depends on the interaction among soil, root and microbial biomass. The aim of this research was to evaluate the potential release of N for hybrid GF677 (P. persica x P. dulcis) uptake, of 6 neemcakes available on the Italian market compared with fresh leaves of *Melia azedarach*, an ornamental tree that grows in the area of investigation. The release of N, and consequently root uptake was related to C:N ratio, the lower the ratio the higher the N concentration in plant tissues and plant growth. Using the ^{15}N isotope technique, we found that up to 30% of the N applied with fresh *Melia* leaves, was accumulated in the tree, however the mineral N concentration in soil and plant and plant growth was not affected by the application of plant derivatives.

Key words: Azadiracta indica, C:N ratio, ^{15}N, soil respiration, microbial N

Introduction

Soil application of organic fertilizers is a way to increase soil organic matter (OM) and provide nutrients to trees. Organic matter represents an important source of C and energy for soil microbial biomass, hence sustaining a microbial population (Sparling, 1992) with consequent considerable amounts of N immobilized. For this reason, plant roots compete with microorganisms in soil for the same available N, and may reduce N immobilization, thus limiting the supply of mineral N to the microflora (Wang and Bakken, 1989 and 1997). Among the large availability of plant derivatives, neemcake represents a byproduct of neem oil production. Neemcake maintains a valuable fertilization potential which has been recently investigated (Marcolini et al., 2016), that is the result of its chemical and biochemical composition. In particular, the interaction between neemcake and soil promotes a release of

mineral N that may be up taken by roots and used by trees or metabolized by soil microbial biomass (Marcolini et al., 2016).

The potential effect of OM as a source of nutrients for plants depends on soil characteristics, root uptake efficiency, microbial community, environment, etc. The use of isotopic methods can provide useful agronomic information on the amount of N mineralized from the residue, on the root uptake rate and on the fraction used for plant metabolism (Hood et al., 2000). Organic fertilizer derived from plant residues uniformly labelled with ^{15}N (Ladd et al., 1981) can be employed for this purpose. In Northern latitudes, melia (*Melia azedarach*) is often used as an ornamental tree for its resistance to pest and diseases. The release of nutrients from residues of melia, showed a behavior similar to neemcake (Marcolini et al., 2016; Toselli et al., 2010), consequently, considering its adaptability to grow in temperate climate, we used plants of melia to obtain uniformly labelled fresh materials (named leaves enriched with ^{15}N isotope).

The aims of the present study were to 1) evaluate the effectiveness of plant derivatives obtained from neemcake and fresh leaves of melia tree as fertilizer-N for peach and 2) estimate the fraction of ^{15}N released by the mineralization of fresh melia leaves and absorbed by peach tree. The two aims were pursued in two separate experiments, experiment A: Comparison of derivatives as tree fertilizers and experiment B: Estimation of the uptake rate of labelled N.

Material and Methods

Experiment A: Comparison of derivatives as tree fertilizers

The following plant material, commercialized in Italy and described in Marcolini et al., (2016) were used: 1) Green neem 1, from India (green neem 1), 2) Neem pelleted, origin unknown (pelleted neem), 3) Neem, origin unknown (neem cake 1), 4) Deoiled neemcake, from India (deoiled neem), 5) Oiled neem cake, from India (oiled neem), 6) Green neem 2, from India (green neem 2), 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 leaves).

The chemical composition of plant derivatives is listed in Table 1. The derivatives were oven-dried at 65 °C and ball milled. Total C and N concentration were determined with a CHN elemental analyzer (Thermo Fisher, mod. EA 1110, Bremen, Germany). Solid samples were weighed in tin cups and dropped in a tube where in the presence of external oxygen

flash combustion occurred at a temperature of 1800 °C. The gaseous combustion products, N_2, NO_x, CO_2, were carried by the helium through a column filled with copper oxide and from there to a Cu-column where NO_x was reduced to elementary N. With a programmed temperature raise in the column the gases were released separately. They flew along a thermal conductivity detector which produced an electrical signal proportional to the concentration of N and C. 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 g of dry weight (DW) material with 8 mL of nitric acid (65%) and 2 mL of hydrogen peroxide (30%) at 180 °C in an Ethos TC microwave lab station (Milestone, Bergamo, Italy). Phosphorus (P) concentration was spectrophotometrically quantified at 700 nm (Saunders and Williams, 1955) on the same mineralized samples used for metal determinations. Liquid samples 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 color.

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). Plant derivatives were incorporated into a clay loam Bathicalci Eutric Cambisols soil (FAO, 1990) (Table 2), at the application rate of 8 g fresh weight (FW) kg^{-1} soil for neemcakes and 16 g FW kg^{-1} for melia leaves. Micro-propagated plants of GF677 (*P. persica* x *P. dulcis*) rootstocks were placed into 1.5 L pots filled with 1 kg of soil previously mixed with plant derivatives and 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, shoots (leaves and shoot axis). The length of the new shoots was recorded and leaf green color, 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 shoots macro- and micronutrient concentrations were determined as previously described for the derivative characterization, in particular C and N by CHN elemental analyzer (Thermo Fisher, mod. EA 1110, Bremen, Germany), metals by atomic absorption spectrophotometry

(SpectrAA-200, Varian, Mulgrave, Australia) and P by spectrophotometry (Saunders and Williams, 1955).

For each sampling date, data were statistically analyzed 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 (SNK) test.

Experiment B: Estimation of the uptake rate of labelled N

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, Delta Plus Thermo Fisher, Bermen, Germany). Isotope-ratio of N ($^{15}N/^{14}N$) was measured after conversion of the molecule to a gas-phase ion, the resultant flux of electrically charged ions was converted into a proportional electrical current. This allowed to measure total N concentration that was 1.87% and ^{15}N enrichment that was 1.876 atom%.

Experimental design

The experiment started in April 2011 at the experimental station of the University of Bologna, in Cadriano. The same clay loam soil (FAO, 1990) as described in table 2 was collected from the field, mixed with sand at a soil:sand ratio of 3:1 and sieved to 4 mm. Labelled melia leaves were ground and sieved to 2 mm 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, 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, 5 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 color, 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 analyzed for total N and ^{15}N content with the elemental analyzer coupled with an isotope ratio mass

spectrometer 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 (0.3663 atm %).

Then, the amount of N derived from labelled melia leaves (NDFML) in 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 analyzed for total N with a CHN elemental analyzer. Microbial biomass C and N were determined by the fumigation-extraction method (Vance et al, 1987), from 5 g moist soil, 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 moist soil 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 concentrations using a total organic C measuring unit (TOC-Vcpn TNM-1, Shimadzu, Kyoto, Japan). The amount of 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 take into account the incomplete recovery of microbial constituents extracted from soil after fumigation was used, since the values of microbial C and N are used here to reveal differences between derivatives.

For each sampling day, data of plant DW, N concentration and NDFML were statistically analyzed as in a complete randomized design, when analysis of variance showed

statistical significance at P≤0.05, means were separated by SNK test. Data of soil mineral N and microbial biomass C and N were statistically analyzed 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≤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.

Results

Experiment A: Comparison of derivatives as tree fertilizers

Table 1 shows the mineral compositions of the derivatives used in this experiment. With the exception of N, neem cake 1 presented the highest concentration of all the nutrients analyzed. Phosphorus ranged between 11.3 g kg^{-1} in neem cake 1and 2.0 g kg^{-1} in melia leaves. Potassium varied between 24.0 g kg^{-1} in neem cake 1 and 8.1 g kg^{-1} in melia leaves; among the other derivatives, green neem 1, deolid neem and oiled neem presented higher K than green neem 2 and pelleted neem. Calcium and Mg were higher in melia leaves compared to green neem 1, pelleted neem, deoiled neem, oiled neem, and green neem 2. Iron concentration widely varied between 8,447 mg kg^{-1} in neem cake 1 and 160 mg kg^{-1} in melia leaves. Among the others, deoiled neem presented the highest Fe concentration, followed by oiled neem, green neem 1, pelleted neem, and green neem 2. Manganese concentrations ranged between 125 mg kg^{-1} in neem cake 1 and 43 mg kg^{-1} in green neem 2 and melia leaves. Cu concentration was similar in all derivatives, except in neem cake 1, which showed a concentration more than twice that observed in the others derivatives. Zinc concentration ranged between 16 mg kg^{-1} in melia leaves and 70 mg kg^{-1} in neem cake 1.

At the end of the experiment, with the exception of green neem 1, all derivatives, increased leaf chlorophyll content, particularly oiled neem, green neem 2 and melia leaves (Table 3). Shoot length was increased by soil-addition of green neem 1, pelleted neem, neem cake 1, deoiled neem , green neem 2. Pelleted neem, neem cake 1 and green neem 2 increased all organs and, consequently, total plant DW compared to control (Table 3).

At the end of the experiment (day 113), no effect of treatment on organ N concentration was detected (Table 4). Deoiled neem treated-plants showed the highest leaf P concentration, followed by oiled neem and green neem 1, while the lowest P concentration was recorded in green neem 2 treatment (Table 4). No differences between treatments were

found for K concentration that was on average 18.6 mg kg^{-1}. Moreover, leaves of control plants presented the highest Ca concentration, significantly different from that of neem cake 1- and green neem 2-treated-plants. Deoiled neem promoted a higher leaf Mg concentration compared to neem cake 1 and green neem 2. At the end of the experiment, leaf microelement concentrations were not affected by treatments, with the exception of Mn, that was higher in leaves of control, deoiled neem-, oiled neem-, and green neem 2-treated-plants compared to those fertilized with green neem 1 and neem cake 1 (Table 4).

Considering the amount of N in plants as a mean to measure the N removal, 68 days after treatment application, plant fertilized with neem cake 1 and deoiled neem removed a higher amount of N than control (Table 5). At the end of the experiment, in addition to plant treated with neem cake 1 and deoiled neem, those fertilized with green neem 1 and green neem 2 removed a higher amount of N than control (Table 5).

Experiment B: Estimation of the uptake rate of labelled N

Although soil application of ^{15}N-enriched melia leaves increased soil N concentration (1,555 mg kg^{-1}) compared to untreated control (1,266 mg kg^{-1}), soil nitrate- and ammonium-N were not affected by the application of melia leaves. In detail NH_4^+-N ranged between 2.35 and 2.37 mg kg^{-1} while NO_3^--N ranged between 5.1 and 5.6 mg kg^{-1} for treated and untreated soil, respectively. Nitrogen concentration of plant organs was not affected by treatment (data not tabulated) at any of the sampling times.

Plant organ DW resulted similar in control and ^{15}N-melia treated plants and increased with time during the experiment. Total plant DW 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 (data not tabulated).

At day 30, only a little of NDFML were detected in GF677 organs (Figure 1), with a higher amount in roots compared to stem and shoots (Figure 1, inset). Nitrogen derived from melia leaves increased with time and on day 90, shoot showed a higher amount of NDFML than root and stem. On day 131, NDFML in shoot and root was higher than in stem; on day 173, no organ effect was found (Figure 1). At day 131, 9.02 mg of NDFML were found in shoot (Figure 1), corresponding to a NDFML recovery of 4.55% of total N applied with plant derivatives (Table 6). At that day, stem and roots presented a NDFML of 24% and 29.6%, respectively (Table 6). At day 173, NDFML decreased in the different organs investigated, in term of amount (Figure 1) and percentage (Table 6).

Soil application of labelled melia leaves immediately increased microbial biomass C (Figure 2), the highest value was found at day 1, and then it decreased with time to reach values similar to the control soil at the end of the experiment. In addition, microbial N increased after the amendment application, in this case, the highest value was found at day 131, and then it decreased (Figure 3). The microbial C/N ratio decreased from day 1 to day 173 in melia leaf treated plants, compared with unamended control that presented a steady C/N ratio ranging from 4 to 6 throughout the investigation period of time.

Discussion

The *Meliaceae* derivatives showed a different mineral composition in response to their different origin, species, organ, production processes, year of harvest, etc. (Marcolini et al., 2016). The mineral composition of neem cake 1 deserves some comments. In fact, neem cake1 presented, for most of the nutrients (i.e. P, K, Ca, Mg, Fe, Mn, Cu and Zn), concentrations that were at least twice higher than the other neemcakes here used and reported in earlier study (Toselli et al., 2010). Patra et al. (2011) reported a N, P, and K neemcake concentration of 49.3 g kg^{-1}, 10.3 g kg^{-1} and 13.2 g kg^{-1}, respectively, that were similar for P, higher for N and lower for K compared to the neem cake 1used in this experiment.

The mineral composition of melia leaves resulted different from that reported in other studies. For example, melia leaves tested by Toselli et al. (2010) presented a concentration of K, Ca, Cu and Zn two times higher compared to that found here and a Mg concentration almost 20 times lower. In addition, the chemical composition of melia 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 fresh leaves, respectively.

This different chemical composition was probably the main reason of the different effect of the incorporation of *Meliaceae* derivatives on plant growth that was stimulated by green neem 2, pelleted neem and neem cake 1. These cakes had the highest N concentration and the lowest C:N ratio, that made the N added (37, 44, and 31 mg kg^{-1} N for neem cake 1, green neem 2, and pelleted neem, respectively) promptly available for root uptake (Marcolini et al., 2016). However, the higher availability of N did not promote a significant increase of leaf N, this because of the high growth rate induced by the neemcakes that diluted the N absorbed by root. In fact, the amount of N removed by the plants treated with pelleted neem, neem cake 1 and green neem 2 was double the amount removed by control plants; at the same time leaf N concentration was not different.

The effect of the derivatives on leaf P concentration is not easy to explain, since plant treated with deoiled neem, oiled neem and green neem 1 showed the highest leaf P concentration, although the highest P concentration was found in neem cake 1 and green neem 2 compounds. Probably, also in this case, there was a dilution effect induced by the application of pelleted neem, neem cake 1 and green neem 2 that stimulated plant growth and prevented the increase of concentration of P in the tissues.

At the end of the experiment, green neem 2- and neem cake 1-treated-plants presented lower leaf Ca concentration compared to the control ones. This can be only partly explained by the high concentration of K and Mg in neem cake 1 and green neem 2, respectively. In fact, the possible antagonistic effect of K and Mg on Ca uptake (Marschner, 1995) was not observed in deoiled neem- and green neem 1-treated-plants, despite of their high concentration of K. A similar behavior was found on one-year-old peaches fertilized with organic amendments (Baldi et al., 2010), where leaf K was increased, while Ca and Mg concentration were depressed by soil application of compost.

Although the high concentration of Fe in all the neemcakes, leaf Fe concentration was not increased by treatments, this means that Fe was not available for root uptake. However, the root system of GF677 hybrid is considered one of the most efficient in absorbing Fe (Tagliavini and Rombolà, 2001), and this ability may have reduced the effect of Fe soil supply.

Soil-applied melia leaves was less effective than neemcakes in increasing nutrient status in peach tree. In fact, none of the nutrient leaf concentration was increased and plant growth was not stimulated by fresh melia leaves compared to the untreated control. Only leaf chlorophyll obtained benefit from soil incorporation of leaf of melia, this can be the first appearance of a positive effect that may involve also the nutritional status later. This response was probably related to the high C:N ratio of melia leaves (17.5%) that limited the mineralization of N and delayed the fertilization effect (Marcolini et al., 2016). Previous research on wheat reported a grain yield and a nutrient content (N, P, K) increase after the application of leaf litter of poplar (*Populus deltoides*), eucalypt (*Eucalyptus* hybrid) and dek (*Melia azedarach*), with or without the inoculation of cellulolytic fungus culture of *Aspergillus awamori* to accelerate the decomposition rate of litters (Singh and Sharma, 2007). In addition, yield of sorghum increased significantly with increasing levels of leaf litter application. Nevertheless, in their experiment, Singh and Sharma (2007), applied N, P and K

at a rate of 50, 11, 10 mg kg^{-1} soil, respectively that could have enhanced the positive effect of plant litter compared to our study.

The N mineralization behavior of fresh melia leaves was studied in detail in experiment B where, 131 day after application, the amount of N from mineralization of fresh organic material tilled into the soil accounted for up to 39% of total N present in plant. In particular, a little amount of ^{15}N released by melia leaves was available one month after the amendment and was found mainly in shoots. Thereafter, the amounts of NDFML increased with time in all organs, with shoot and leaves representing the strongest sink organs for melia leaf-derived-N. Also, at the end of the experiment (September) shoot and leaves presented a higher amount of melia-derived N, compared to stem, but it was lower than the previous sampling days, indicating a possible root uptake of soil-native-N that diluted the ^{15}N fraction within the plant. The availability and uptake of ^{15}N did not increase plant N concentration, so that treated and untreated trees showed the same N concentration throughout the season. In addition, the mineral N concentration of both control and treated soil was similar during all the experiment. This means that the sum of fractions of labelled N from melia leaves and unlabeled N from soil-native-N was not modified, if one increased, the other decreased and vice versa.

Microbial biomass was significantly increased by application of melia leaves, as testified by the increase of both microbial C and N. In particular, microbial N ranged between 5 and 10 mg kg^{-1} and between 10 and 20 mg kg^{-1} in control and amended soil, respectively. This difference can be explained considering an increase of N mineralization in the amended compared to the unamended soil, followed by an immediate microbial immobilization, and a consequent competition for this N between microbial biomass and plants. However, the exact origin of this extra amount of N mineralized cannot be defined; it can derive from both soil-native-N or melia leaf.

At the same time a fast increase of microbial biomass C:N ratio (12) was observed at day 1, followed by a decrease to 3 (at day 173), as a consequence of incorporation of melia leaves into the soil. Considering that in control soil the C:N ratio was steady and ranged from 4 to 6, this response may indicate a variation in C and N availability throughout the experiment, with C available at the beginning, and N later. The different C and N flux in the soil was related to melia leaf C:N ratio of 17.5 that pushed microbial biomass to immobilize N. This explanation is indirectly supported by Li et al. (2016) who found a significant, negative correlation of annual rice yield with soil microbial biomass C:N. In fact, in our

experiment the high microbial biomass C:N induced a N immobilization, which reduced the flux of mineral N for peach root. In fact, although soil total N increased after melia leaf application, mineral N remained stable, meaning that the release of N by melia leaf mineralization, and detected as ^{15}N was equalized by soil-native-N microbial immobilization.

In conclusion the effectiveness of neemcakes as N fertilizer is variable, and in part predictable from the C:N ratio, in fact the cakes with highest fertilization aptitude showed a C:N ratio lower than 17. In this short-term (6 months) experiment, fresh melia leaves showed a low effectiveness in term of N fertilization, because the rate of N released was compensated by the same rate of N immobilization by microbial biomass. However, plants showed a beneficial effect in term of increase of leaf chlorophyll not justified by the increase of leaf N. Other investigations can possibly explain the physiological reason of this positive response.

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Table 1. Dry weight (DW) and mineral composition of plant derivatives used in experiment A. In brackets standard deviation (n=2).

Derivative	DW (g kg^{-1})	C (g kg^{-1})	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (%)	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
Melia leaves	290	420	24	1.9 (±0.02)	8.1 (±0.05)	13.6 (±0.01)	3.8 (±0.04)	160 (±7.92)	43 (±0.56)	8 (±0.71)	16 (±0.35)
Green neem	890	470	22	3.5 (±0.03)	15.3 (±0.02)	6.2 (±0.007)	1.9 (±0.02)	1324 (±241)	56 (±0.35)	11 (±2.05)	22 (±1.2)
Pelleted neem	940	500	31	3.0 (±0.08)	9.7 (±0.03)	3.0 (±0.007)	1.7 (±0.007)	1096 (±0.21)	26 (±1.70)	7 (±0.92)	30 (-)
Neem cake 1	920	350	37	11.3 (±0.20)	24.0 (±0.09)	14.6 (±0.01)	6.7 (±0.007)	8447 (±1.06)	125 (±0.35)	50 (±11.88)	70 (±0.84)
Deoiled neem	960	410	17	2.1 (±0.02)	15.2 (±0.05)	4.9 (±0.02)	2.0 (±0.01)	3035 (±158)	72 (±2.26)	19 (±3.53)	23 (±3.11)
Oiled neem	910	440	16	2.0 (±0.01)	16.3 (±0.03)	6.1 (±0.06)	2.1 (±0.01)	2414 (±54.7)	61 (±0.28)	10 (±1.70)	19 (±0.56)
Green neem 2	940	490	44	6.5 (±0.02)	12.5 (±0.04)	5.0 (±0.007)	3.5 (±0.007)	620 (±59.2)	43 (±1.27)	10 (-)	54 (±2.12)

Table 2. Selected characteristics of the soil used in the 2 studies

Characteristic	Value	Analytical method
Sand (g kg^{-1})	250	
Silt (g kg^{-1})	500	
Clay (g kg^{-1})	250	
pH	7.5	Water
Calcium Carbonate (g kg^{-1})	30	HCl/De Astis
Organic Matter (g kg^{-1})	14	$K_2Cr_2O_7$ Oxidation
Total N (g kg^{-1})	11	Kjeldahl
Exchangeable K (mg kg^{-1})	219	Barium chloride
Exchangeable P (mg kg^{-1})	12	Olsen
Cation Exchange Capacity (meq 100 g^{-1})	16.1	Barium chloride
Electric Conductivity μScm^{-1})	220	

Table 3. Effect of soil-applied *Meliaceae* derivatives on leaf chlorophyll, shoot length and plant organ dry weight (DW) after 113 days from treatment application (experiment A)

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

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

Table 4. Effect of soil-applied *Meliaceae* derivatives on N, P, K, Ca, Mg, Mn, Fe, Cu and Zn leaf concentrations at the end of the experiment A.

Treatment	**N**	**P**	**K**	**Ca**	**Mg**	**Mn**	**Fe**	**Cu**	**Zn**
	(g kg^{-1})					(mg kg^{-1})			
Control	13.7	4.3bc	15.8	11.1a	3.1ab	31a	122	5.27	22
Melia	14.6	4.1bc	18.6	10.5ab	3.1ab	29ab	97	6.50	22
Green neem 1	15.8	5.8ab	20.1	9.6ab	3.3ab	24b	99	6.2	27
Pelleted neem	13.4	5.5abc	17.1	9.1abc	3.0ab	29ab	100	6.02	24
Neem cake 1	13.4	5.0bc	19.4	8.7bc	2.5b	24b	92	5.53	22
Deoiled neem	16.2	7.1a	20.9	10.3ab	3.8a	33a	79	6.92	23
Oiled neem	15.8	6.0ab	18.9	9.7ab	3.1ab	31a	91	5.84	27
Green neem 2	14.5	3.6c	17.7	7.7c	2.5b	31a	90	5.50	23
Significance	*ns*	***	ns	***	*	*	ns	ns	ns

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

Table 5. Effect of soil-applied *Meliaceae* derivatives on total N removed by plant at day 68 and 113. Experiment A.

Treatment	**N (mg)**	
	Day	
	68	113
Control	20.8b	29.2c
Melia	38.3ab	49.9bc
Green neem 1	45.0ab	55.7b
Pelleted neem	42.4ab	52.7b
Neem cake 1	49.8a	60.7ab
Deoiled neem	39.7ab	45.2bc
Oiled neem	37.8ab	45.5bc
Green neem 2	51.2a	75.9a
Significance	*	***

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

Table 6. Mean ±standard deviation (n =4) of the percentage of N derived from labelled melia leaves (NDFML) and correspondent recovery (%) of ^{15}N in plant organs 30, 90, 131 and 173 day after soil application of ^{15}N-enriched melia leaves (experiment B).

Treatment	**NDFML (%)**				**^{15}N Recovery (%)**			
	day				**day**			
	30	**90**	**131**	**173**	**30**	**90**	**131**	**173**
Shoot and leaves	7.31(±11.2)	38.6(±9.1)	39.1(±7.2)	30.2(±2.7)	0.008(±0.01)	3.57(±1.9)	4.55(±1.9)	2.40(±2.47)
Stem	0.14(±0.02)	15.7(±3.5)	24.0(±4.3)	21.1(±2.6)	0.003(±0.02)	0.29(±0.13)	0.56(±0.19)	0.69(±0.43)
Roots	1.09(±0.37)	26.2(±4.7)	29.6(±7.8)	22.4(±2.9)	0.04(±0.02)	1.43(±0.79)	2.66(±1.15)	2.47(±2.29)

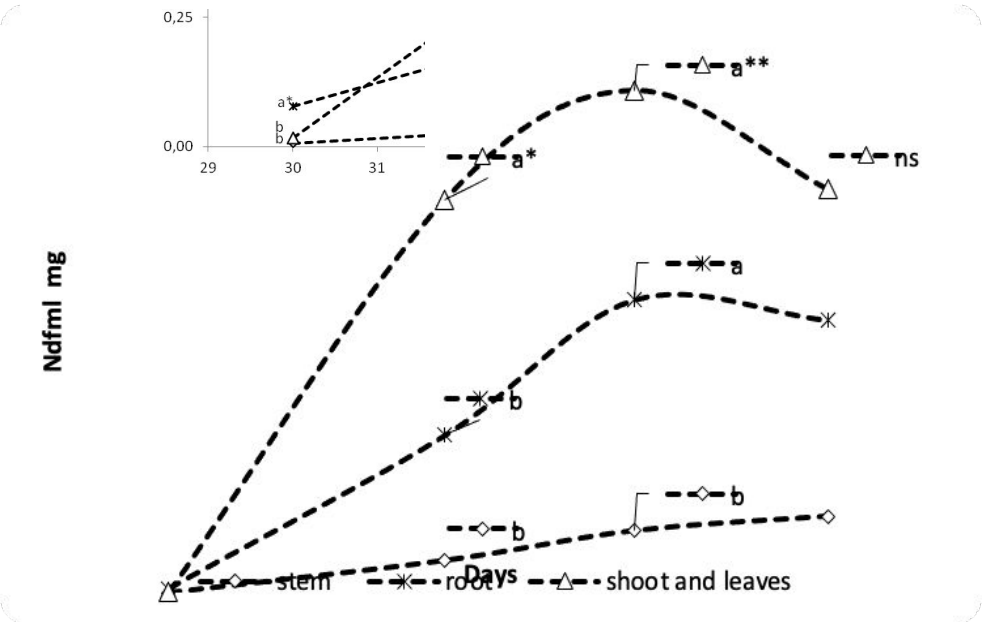

Figure 1. Effect of soil-applied ^{15}N-enriched melia leaves on N derived from labelled leaves (NDFML) in roots, stem, and shoot+leaves. At each sampling date: ns, *, ** = effect of organ not significant, significant at $P \leq 0.05$, and $P \leq 0.01$, respectively. Within the same date, values followed by the same letter are not significantly different. In box, the detailed trend at the first sampling time (day 30) is reported.

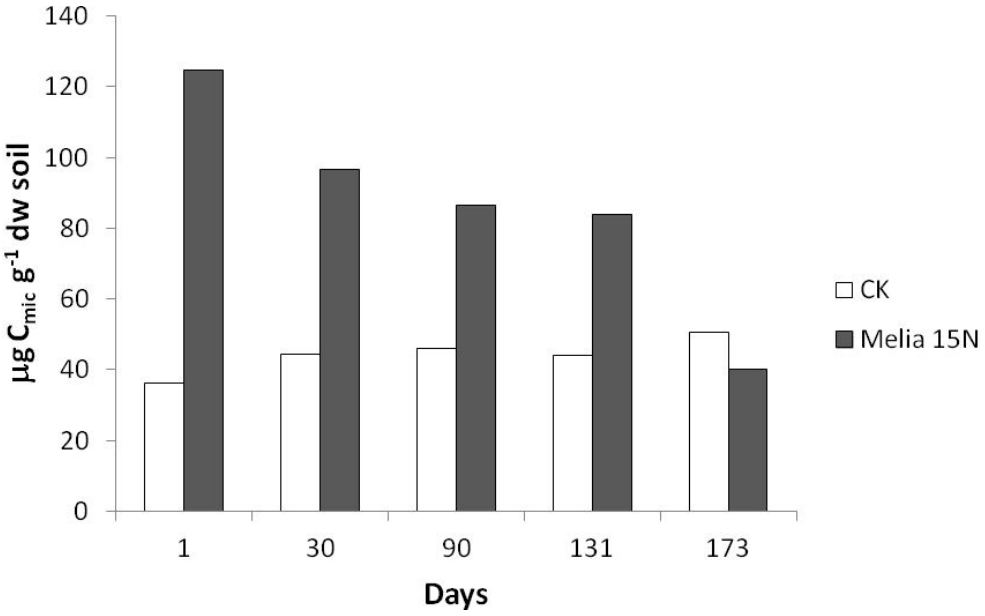

Figure 2 Effect of soil-applied ^{15}N-enriched melia leaves on soil microbial biomass C. Interaction time*treatment significant at P≤0.001. Two standard error of means (SEM) = 16 is minimum difference between two statistically different values.

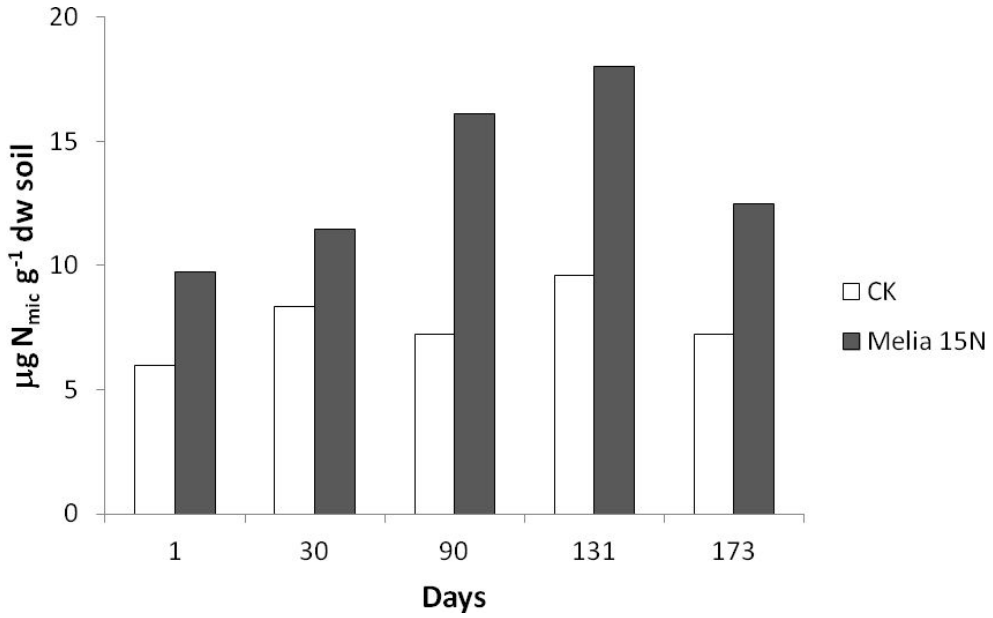

Figure 3. Effect of soil-applied ^{15}N-enriched melia leaves on soil microbial biomass N. Interaction time*treatment not significant. Two standard error of means (SEM) = 3.3 is minimum difference between two statistically different values.