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Nitrogen and carbon mineralisation of different Meliaceae derivatives

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ABSTRACT

Among Meliaceae derivatives, neem cake is usually used as a fertilizer; however its origin and industrial processing are often unknown, so that its effect on soil fertility is not predictable. In this study, the effect of soil incorporation of 6 commercial neem cakes and leaves of *Melia azedarach* L. on nitrogen (N) and carbon (C) dynamics was investigated in a 118-day laboratory incubation experiment. Neem cake at a rate of 8 g/kg of soil and melia leaves at 16 g/kg were incorporated into the soil and their net N and C mineralisation were evaluated 2 h after application and at day 1, 2, 6, 12, 26, 54 and 118, by analysing a 50-g soil sample placed in 250 glass jars. The apparent net N mineralisation was well predicted by N concentration and C/N ratio of derivatives. The derivatives with a C/N ratio < 24 caused a net N mineralisation, whereas those with a C/N ratio ≥ 24 caused net N immobilisation. C mineralisation ranged between 15% and 25% and was not related to chemical composition of the derivative. Neem cake with a C/N ratio < 24 can be used to add N, while neem cake with a C/N ratio > 24 can be used to reduce soil mineral N.

Keywords: ammonium-N; *Azadirachta indica*; CO_2; extractable organic C; nitrate-N

In sustainable orchard management, the use of organic materials such as crop residues, animal manure, compost or agro-industrial by-products represent a source of nutrients for plants since their decomposition is responsible for nutrient release and fluxes in agro-systems (Tognetti et al. 2008, Jacob et al. 2009).

Neem cake, the final by-product of oil extraction from neem (*Azadiractha indica* A. Juss) kernels, is used for a number of purposes including crop fertilizer management being a source of nitrogen (N) since its N concentration ranges from 3–5.4% (Rao and Prasad 1980, Joseph and Prasad 1993). However, the natural heterogeneity of this derivative does not allow general unambiguous indications on its use to improve soil fertility.

Melia azedarach L., also known as melia, is a deciduous tree belonging to the Meliaceae family, used as an ornamental plant in Italy. Soil incorporation of melia derivatives was studied as a tool to increase soil fertility (Toselli et al. 2010).

Soil mineral N is essential for plant growth, however excess of N may be leached out and increase hazard for the environment. The aim of the research was to evaluate the dynamics of N and carbon (C) release of different neem cakes of unknown industrial origin, along with ground melia leaves, to provide information on their fertilization potentials.

MATERIAL AND METHODS

In the experiment six different neem cakes (named as neem 1–6) were used that were commercialized by different companies and whose industrial process is unknown. All neem cakes were in a grainy form, except neem 3 which was pelleted. More information on these neem cakes (i.e. limonoids content) can be found in Nicoletti et al. (2012). In addition, leaves of *Melia azedarach* L., harvested in June 2010 from seedlings grown in

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pots and stored frozen at –20°C, were used (treatment named melia). Meliaceae derivatives (neem cakes and melia leaves) were oven-dried at 65°C, ball-milled and analysed (two replicates) for total C and N concentration, with a CHN elemental analyser (Thermo Fisher, mod. EA 1110,Waltham, USA).

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, to improve soil texture, at a ratio soil:sand of 3:1, sieved at 2 mm, air-dried, moistened with distilled water to reach water content of 14% (w:w) and incubated at 20°C at constant soil humidity for one week prior to use. All neem cakes were pulverized with a mortar in order to be used in the same form and incorporated into the soil at a rate of 8 g fresh weight (FW)/kg dry weight (DW) soil. Melia leaves were cut by hand in little pieces (less than 0.5 cm length) and incorporated at a rate of 16 g FW/kg DW soil. A control treatment consisting of unamended soil was also included.

For each fertilization treatment 32 250 mL glass jars were filled with 50 g of amended soil covered with perforated black polyethylene bags to allow aeration and incubated in a growth chamber with a constant temperature of 23 ± 2°C and relative air humidity of 85%. Throughout the incubation period soil moisture was maintained to the initial level of 14% (w:w) by weighing and corrected, if necessary, by adding distilled water.

At days 0 (2 h after the start of the experiment), 1, 2, 6, 12, 26, 54 and 118, four jars per treatment (replicates) were destructively sampled for mineral-N and microbial C determinations. Nitrate- and NH_4^+-N fractions were extracted by shaking 10 g of soil in 100 mL of 2 mol/L KCl for 1 h. After sedimentation, soil extracts were stored at –20°C until the analyses that were performed by autoanalyser (Auto Analyser AA-3; Bran + Luebbe, Norderstadt, Germany).

Microbial biomass C was determined by the fumigation extraction method (Vance et al. 1987). The amount of C extracted with 0.5 mol/L K_2SO_4 from non-fumigated soil was considered as extractable organic C (EOC).

To quantify C mineralisation, CO_2 fluxes were periodically measured during all incubation period. Four glass jars (replicates) per treatment were sealed for approximately 3 h, and the CO_2 accumulated in the headspace of the jar was measured with an infrared gas analyser (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.

Since the amount of N added was different according to the amendment, to compare the mineralisation of different Meliaceae derivatives, apparent net N mineralisation (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 mineralisation 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 occurred).

Data were analysed in a factorial design with two factors: treatments (8 levels: control, neem 1–6, melia leaves) and time (8 levels: day 0, 1, 2, 6, 12, 26, 54, 118). When the analysis of variance showed a statistical significance at $P \leq 0.05$, means were separated by the Student Newman Keuls test. When the interaction between factors was significant, standard error of the means (SEM) was used as a minimum difference between statistically different values (Saville and Rowarth 2008). Pearson correlation coefficient (r) was determined to evaluate (1) the relationship between C (%), N (%) and C/N ratio of the Meliaceae derivatives and total C and N mineralised, and (2) the relationship between EOC, microbial respiration (mg CO_2-C/kg soil/h) and microbial biomass.

RESULTS AND DISCUSSION

The Meliaceae derivatives used in the experiments showed a different mineral composition and dry matter concentration (Table 1). Carbon ranged from 35% (neem 3) to 50% (neem 2) and N 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). These differences can

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Table 1. Chemical characteristics of the Meliaceae derivatives and amounts of carbon (C) and nitrogen (N) added to soil

Treatment	Derivative characteristics				Amount of C and N added	
	dry matter (%)	C (%)	N (%)	C/N	C (ppm soil)	N (ppm soil)
Neem 1	89	47 ± 0.12	2.2 ± 0.02	21	3364	159
Neem 2	94	50 ± 0.80	3.1 ± 0.13	16	3770	234
Neem 3	92	35 ± 0.02	3.7 ± 0.06	9	2592	275
Neem 4	96	41 ± 0.33	1.7 ± 0.05	24	3126	128
Neem 5	91	44 ± 0.56	1.6 ± 0.07	27	3192	119
Neem 6	94	49 ± 0.28	4.4 ± 0.46	11	3692	329
Melia leaves	29	42 ± 0.12	2.4 ± 0.04	17	1982	114

± standard error (n = 2)

be attributed to their different origin in term of species (*Azadiractha indica* vs. *Melia azedarach*), organ (kernels vs. leaves), and industrial processing, since the commercial neem cakes employed in the experiment were produced by different companies and were the by-products of different oil extraction processes.

Ammonium-N was released immediately after soil incorporation of neem 3 (Table 2), and ranged between 48 and 96 ppm in the first 12 days, with a maximum concentrations (96 ppm) at day 1, then it decreased to values < 3 ppm. Also neem 2 and 6 had a peak of release of NH_4^+-N, but it was delayed of a few days and with maximum values of 22 ppm (neem 2 at day 6) and 54 ppm (neem 6 at day 12). In all the other treatments and sampling days, NH_4^+-N was present at low concentrations (< 6.5 ppm) (Table 2).

Nitrate-N concentration in control soils ranged between 30 ppm (at day 1) and 70 ppm at day 118 (Table 2). After incorporation of neem 2 and neem 3, NO_3^--N increased with time until day 54, and reached the maximum values of 120 and 137 ppm, 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 until day

Table 2. Release of ammonium- (NH_4^+) and nitrate- (NO_3^-)-N after soil addition of the Meliaceae derivatives

Treatment	NH_4^+-N (ppm soil)								NO_3^--N (ppm soil)							
	day								day							
	0	1	2	6	12	26	54	118	0	1	2	6	12	26	54	118
Control	5.0	6.5	3.8	1.6	1.8	1.2	0.78	0.85	30	31	34	47	42	48	64	70
Neem 1	2.4	4.1	2.1	6.2	4.7	2.9	0.57	1.1	35	12	19	13	20	33	52	75
Neem 2	9.2	8.0	4.2	22	16	2.9	0.93	1.9	29	30	35	41	58	86	120	104
Neem 3	48	96	88	75	48	1.5	0.82	2.7	33	34	45	54	60	132	137	124
Neem 4	2.5	3.3	2.2	2.9	2.2	1.1	0.76	0.96	35	12	16	8.6	9.4	17	27	62
Neem 5	2.5	2.0	1.8	3.0	2.1	1.5	0.55	0.94	38	19	14	7.1	7.1	13	25	54
Neem 6	2.2	3.1	3.2	38	54	1.7	0.66	3.6	30	28	23	20	44	147	132	141
Melia	4.3	2.8	1.4	2.9	2.1	2.6	0.62	1.1	32	28	33	32	35	45	63	98
Significance	2 SEM = 3.4								2 SEM = 11							
Interaction day × treatment	***								***							

***P ≤ 0.001. Differences between 2 values > 2 standard error of means, indicates statistical difference

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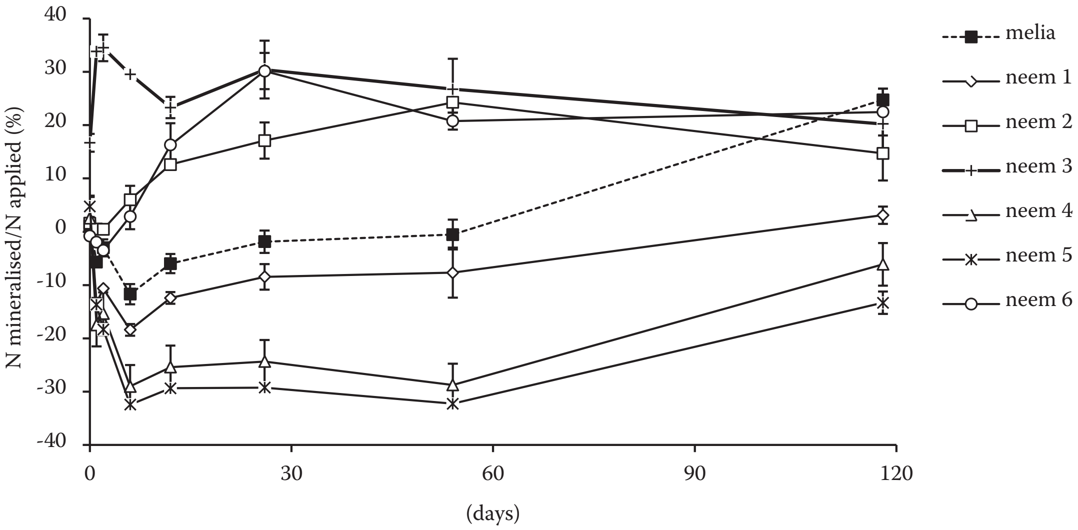

Figure 1. Apparent net nitrogen (N) mineralisation of the Meliaceae derivatives. Interaction time × fertilization treatment significant at $P \leq 0.001$. Minimum difference between statistically different values (2 SEM) = 4.8. Bars represent standard error (n = 4)

12 and in neem 6 until day 6, then, it increased until the end of the incubation and resulted higher than control soils from day 54 in neem 1, and from day 12 in neem 6. In melia-treated soils, NO_3^--N was similar to control, except at day 6 (when it was lower), and at day 118, when it resulted higher (Table 2).

Net apparent N mineralisation (Figure 1) was always positive for neem 2 (C/N:16) and especially neem 3 that, with its lowest C/N ratio (9), showed a maximum N mineralised (34.5% of added-N) at day 2. The other derivatives initially caused a net N immobilisation: in particular, neem 6 (C/N:11) caused a short period of immobilisation (2 days), after which the amount of N mineralised increased and reached values around 20% of added-N at day 54. Net N mineralisation was always negative (net immobilisation) for neem 4 (C/N:24) and neem 5 (C/N:27). Melia leaves (C/N:17) and neem 1 (C/N:21) showed a net N immobilisation during the first 54 days of incubation, followed by a net mineralisation (Figure 1).

Mineralised N was negatively correlated with the C/N ratio of the derivatives and positively corre-

Table 3. Simple correlation coefficient (r) between nitrogen (N) and carbon (C) mineralised and derivative concentration of C, N and C/N ratio during the incubation period

	Day							
	0	1	2	6	12	26	54	118
N mineralised (% added-N)								
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 mineralised (% added–C)								
C (%)	–	ns	ns	ns	ns	0.61***	0.65***	0.65***
N (%)	–	–0.71***	–0.70***	–0.46*	ns	ns	ns	ns
C/N	–	0.79***	0.77***	0.50**	0.39*	ns	ns	ns

ns – not significant, *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. At each day n = 32 (8 treatment × 4 replications)

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lated with the N concentration (Table 3) as already reported by Vanlauwe et al. (1996). In contrast, derivative C% was correlated to mineralised N only immediately after the application of the derivatives when the microbial biomass attacked the new C pools to find energy source; as a consequence, N was immediately immobilised (negative correlation). Later, the presence of C did not control N mineralisation as N became the factor driving N mineralisation. Thus, the use of neem cakes 3, 2 and 6 as a fertilizer is suggested when a rapid N supply to plants is required, whereas neem cakes 4 and 5 could be used to reduce soil nitrate concentration, helping to prevent nitrate leaching, as hypothesized for other organic amendments (Chaves et al. 2008, Jin et al. 2008).

In previous experiments, neem cake, used as a coating agent of urea, was found responsible for nitrification inhibition (Sahrawat 1989). In our conditions no indication of this effect was detected for none of the neem cakes, as high NH_4^+ concentrations have never 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 a previous study in which soil-applied commercial neem cake (10 g/kg) was ineffective in delaying the oxidation of NH_4^+-N to NO_3^--N, after soil application of urea (Toselli et al. 2010). The lack of inhibitory effect on nitrification can be attributed to the high rates and different modality of application of neem cake.

After 118 days of incubation, the amount of C mineralised in the control soil was 224 ppm DW (data not shown). The addition of the derivatives increased C mineralisation, which was 3–5 time higher than control at day 118 (data not shown). The amount of C mineralised by the end of the incubation was rather small and ranged from 25% of added C for neem 1 and neem 2 to 15% for neem 3 (Figure 2) indicating that the addition of these derivatives can have a positive effect on the soil C balance. The observed differences at day 118 resulted mainly from differences in C mineralisation during the first 40 days.

Mineralised C was positively correlated to the C/N ratio in the first 2 days and to the C concentration of the residue from day 26 to the end of the experiment (Table 3). In several studies on residue decomposition, the dynamics of C decomposition was found to be negatively related to C/N (Vanlauwe et al. 1996). This generally happens when soil + residue N availability is not sufficient to microbial needs, thus becoming the limiting factor of the decomposition (Recous et al. 1995). In our experiment, if the initial mineral N concentrations of the unamended soil (35 ppm DW) and the amount of N added with the derivatives are considered, the N availability

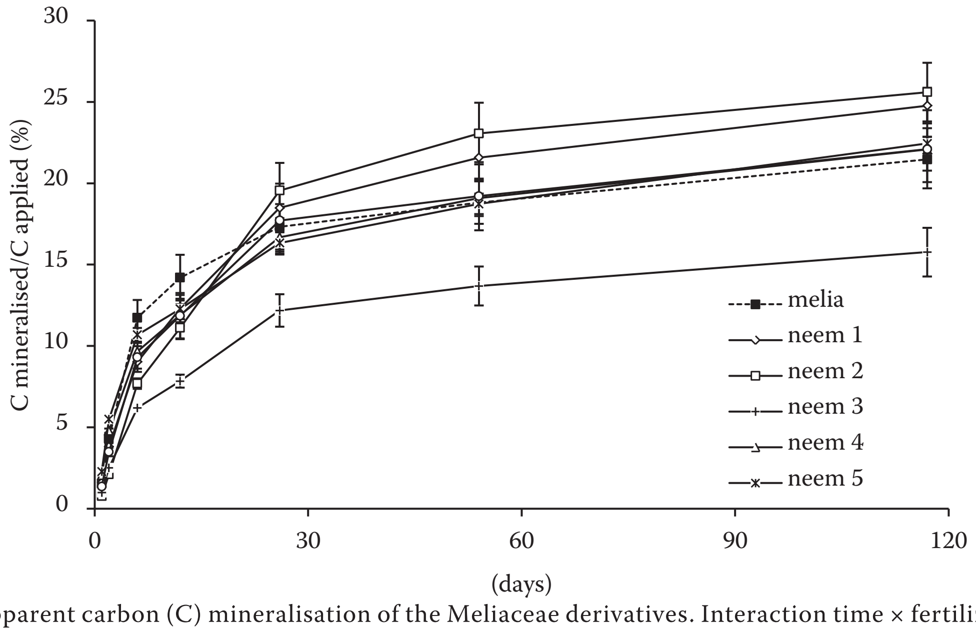

Figure 2. Apparent carbon (C) mineralisation of the Meliaceae derivatives. Interaction time × fertilization treatment not significant. Minimum difference between statistically different values (2 SEM) = 2.62. Bars represent standard error (n = 4)

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Table 4. Concentration of microbial biomass carbon (C) and K_2SO_4-extractable organic C (EOC) after soil addition of the Meliaceae derivatives

Treatment	Microbial biomass C (ppm soil)						EOC (ppm soil)					
	day						day					
	2	6	12	26	54	118	2	6	12	26	54	118
Control	11	46	42	15	25	7.9	165	149	166	124	113	63
Neem 1	68	188	53	82	41	15	235	283	286	193	167	82
Neem 2	99	121	64	66	37	13	267	294	310	217	173	89
Neem 3	94	97	21	42	44	7.2	309	256	303	214	148	93
Neem 4	90	111	36	56	55	32	314	287	261	202	159	86
Neem 5	22	102	9.8	48	43	20	343	272	283	199	159	86
Neem 6	95	178	60	42	34	23	320	264	284	214	156	88
Melia	152	108	42	57	38	14	205	198	236	136	124	67
Significance	2 SEM = 18						2 SEM = 30					
Interaction day × treatment	***						*					

*$P \leq 0.05$; ***$P \leq 0.001$. Differences between 2 values > 2 standard error of means, indicates statistical difference

for the decomposers was probably sufficient. In the first two days of incubation, C mineralisation was rather negatively correlated to the derivative N concentration, indicating that N availability initially inhibited C mineralisation. This is in agreement with the work of Sakala et al. (2000) who found an initial inhibition of respiration proportional to the amount of NH_4^+-N added during the first 10 days of an incubation experiment with maize and pigeon pea residues.

Microbial biomass C peaked during the first 6 days after incorporation of all derivatives (Table 4).

For almost all the incubation period, EOC was lower in untreated control soil compared to the amended soils, which generally showed a peak between day 2 and day 12 (Table 4). The surplus of EOC compared to control, varied between 2.0% (in melia leaves) and 5.5% (in neem cake) of added C. After day 12, EOC decreased with time in all treatments, and correlated with microbial respiration (r = 0.66, $P < 0.001$) and microbial biomass C (r = 0.42, $P < 0.001$). This dynamics of EOC, considered a labile substrate for soil microbial activity, indicates a relationship between EOC and respired CO_2 that was confirmed by the correlation analysis. Also microbial biomass C increased immediately after the application of the derivatives, but the microbial C was not correlated to the EOC as it was to the respired CO_2, indicating that the enhancement of the microbial biomass was not only the consequence of the increase of labile C. Also Spyrou et al. (2009) found an increased microbial biomass C after soil application of pulverized melia fruits and attributed this response to the release of organic C and nutrients in the soil by the amendment.

In conclusion, proper parameters to evaluate the impact of neem cakes on soil fertility are C/N ratio and N concentration. The former is negatively related to the rate of N release, while N concentration is positively related to N mineralisation. Carbon mineralisation was similar for all derivatives accounting for less than 25%, indicating the potential use of the Meliaceae derivatives amendments as a tool to increase C sequestration in soil.

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