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NITROGEN MINERALIZATION IN DENSITY FRACTIONS OF SOIL ORGANIC MATTER FROM MAIZE AND LEGUME CROPPING SYSTEMS

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Summary—Biologically-mediated nutrient availability in the soil is largely dependent on soil organic matter (SOM) decomposition and mineralization processes. The light fractions of SOM obtained by floatation of the sand-size fraction in a silica suspension (LL, 150–3000 μm and < 1.13 g cm^{-3}) and floatation of whole soil in sodium iodide (NAL, < 1.7 g cm^{-3}) were previously found to be strongly affected by maize and legume cropping systems. The objective of this study was to assess the anaerobic and aerobic N mineralization rates of LL, NAL and whole soil after the eighth cropping season (4 y) in seven maize and legume cropping systems. Significantly greater ($P < 0.05$) N mineralization in whole soil, LL and NAL resulted from the seasonal additions of *Gliricidia sepium* prunings to continuous maize than from continuous maize with removal of crop residues and from a gliricidia stand with prunings removed. Aerobic and anaerobic N mineralization in whole soil from the seven cropping systems correlated ($P < 0.05$) with aerobic N mineralization in LL and NAL, which in turn correlated with the N concentration and amount of N in the respective fraction. Amount of LL-N but not LL-C or dry weight of LL correlated with anaerobic and aerobic N mineralization of whole soil. Dry weight, amount of N and amount of C in NAL were not correlated with whole soil mineralization. These results suggest that amount of N in light fraction SOM merits further examination as a sensitive measure of biologically-mediated N availability. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Soil organic matter (SOM) contents in native soils are usually in a state of dynamic equilibrium where organic matter losses are balanced by organic matter inputs (Jenny, 1980). Cultivation of soils, however, alters this equilibrium by increasing SOM losses (Sanchez *et al.*, 1989). Conventional tillage practices are largely responsible for SOM decline due to exposure of aggregate-protected organic matter to microbial decomposition (Elliot, 1986; Cambardella and Elliot, 1992; Beare *et al.*, 1994). In view of the important role of SOM in supplying nutrients, buffering nutrients and water and maintaining soil structure (Woomer *et al.*, 1994), considerable attention has been directed towards the identification of agricultural systems that maintain adequate SOM contents. A common dilemma, however, is the difficulty to define and measure management-sensitive fractions of SOM. The lack of a suitable measure of SOM as an indicator of soil quality has limited the predictive understanding of SOM dynamics (Wander *et al.*, 1994).

The physical fractionation of soil appears to be a promising approach for isolating and quantifying funtional pools of SOM (Christensen, 1992). Organic matter in the sand-size fraction (> 53 μm) or macroorganic matter is often more labile than organic matter in the clay- and silt-size fractions (Tiessen and Stewart, 1983; Gregorich *et al.*, 1988). Light fraction SOM, isolated by floatation of either whole soils or soil particle-size fractions in dense solutions, is more influenced by management practices than total SOM (Strickland and Sollins, 1987; Cambardella and Elliot, 1993; Janzen *et al.*, 1992; Hassink, 1995; Barrios *et al.*, 1996). Light fractions primarily consist of mineral-free organic residues at various degrees of decomposition; and, as compared to the whole soil, they are enriched in C and N (Christensen, 1992).

Models designed to predict SOM dynamics and N mineralization generally divide SOM into several pools with different turnover rates (Jenkinson and Rayner, 1977; Van Veen and Paul, 1981; Parton *et al.*, 1987). Pools with a rapid turnover rate are assumed to have an important role in N availability because SOM dynamics and N cycling are closely linked through the processes of N mineralization and immobilization (Duxbury *et al.*, 1989). A basic problem with the modelling approach, however, has been the inability to isolate and quantify the func-

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tional pools of SOM (Stevenson and Elliot, 1989; Woomer *et al.*, 1994; Hassink, 1995).

In a study on the same experiment Barrios *et al.* (1996) found that light fractions (LF) of SOM differed ($P < 0.05$) among seven maize and legume cropping systems after eight cropping seasons (4 y). Our objectives were: (i) to determine the effect of the cropping systems on N mineralization in whole soil and isolated LF; (ii) to examine the relationship between N mineralization in LF and amounts of N and C in the LF; and (iii) to assess whether LF was related to total N mineralization in the soil.

MATERIALS AND METHODS

Soil was collected in September 1993 from a field experiment at Machakos in Kenya (1°33′S, 37°14′E; elevation: 1600 m). Rainfall at the site is bimodal with an annual mean of 760 mm. The soil is a fine, mixed, isohyperthermic Kandic Rhodustalf. Air-dried soil in the top 15 cm had pH (1:2.5 soil–water suspension) = 6.4, organic C = 9.7 g kg^{-1}, total N = 1.0 g kg^{-1}, KCl extractable Ca = 7 $cmol_c$ kg^{-1}, clay = 32% and sand = 54%.

The experiment was a randomized complete-block design with three replications, seven treatments, and two cropping seasons y^{-1}. It was established in September 1989, and eight annual crop seasons had been completed at the time of soil collection. The treatments were; (i) continuous maize (*Zea mays* L.) with removal of crop residues (M), (ii) one season of cowpea [*Vigna unguiculata* (L.) Walp.] rotated with one season of maize (CP/M), (iii) pigeonpea [*Cajanus cajan* (L.) Millsp.]-cowpea intercrop for one year rotated with maize for two seasons (PP-CP/M), (iv) continuous pigeonpea-maize intercrop (PP-M), (v) continuous maize with prunings from gliricidia [*Gliricidia sepium* (Jacq.) Walp.] added each season (M + G), (vi) gliricidia hedgerow continuously intercropped with maize (G-M) and (vii) gliricidia stand (40 × 90 cm spacing) with prunings removed (G).

Soil samples (0–15 cm) were collected in a grid pattern from 20 locations in each plot after harvest of maize. Soil from each plot was bulked, air dried and sieved (3-mm). The < 3 mm fraction was fractionated by density using sodium iodide, gravimetrically adjusted to 1.7 g cm^{-3} (Strickland and Sollins, 1987). The collected light fraction, referred to as NAL, was washed three times with 50 ml 1 M NaCl to remove NaI, followed by three times with 100 ml deionized water before drying at 50°C for 48 h. The Ludox light (LL), intermediate (LM) and heavy (LH) fractions were obtained by density fractionation of the > 150 μm size fraction in a colloidal silica suspension gravimetrically adjusted at 1.13 and 1.37 g cm^{-3}. Ludox fractions were washed three times with 100 ml deionized water before drying to constant weight at 40°C (Meijboom *et al.*, 1995). We focused on NAL and LL in this study because our earlier research by Barrios *et al.* (1996) had indicated that they were the SOM fractions most affected by maize and legume cropping systems. Further fractionation details are given in Barrios *et al.* (1996).

Anaerobic and aerobic N mineralization were determined with either 10 g of whole soil or 50 mg of SOM fraction mixed with 9.95 g of ashed (500°C for 8 hr) sand and inoculated with 1.5 ml of a common dilute soil solution (5×10^{-5} g ml^{-1}). Fresh soil samples from every plot were pooled, thoroughly mixed, and a subsample diluted with sterile deionized water in order to prepare the common dilute soil solution. All incubations were run in duplicate. In anaerobic incubations, the sample was flooded with 25 ml deionized water in a 30-ml glass bottle. The bottles were gently tapped for 30 s to remove air bubbles, sealed with a rubber stopper and then kept for 7 days at 40°C. After incubation, the samples were transferred to 125-ml extraction bottles and extracted with 25 ml 4 M KCl with shaking for 1 h at 150 reciprocations min^{-1} (30-mm-reciprocal stroke) and subsequent gravity filtering using prewashed Whatman No 5 paper. Ammonium was determined colorimetrically (Anderson and Ingram, 1993). Ten grams of soil and 50 mg of SOM fraction, mixed with 9.95 g of ashed sand and inoculated with dilute soil solution, were similarly extracted with 50 ml 2 M KCl and analyzed for initial ammonium. Nitrogen mineralization was calculated as the difference in ammonium between incubated and unincubated samples.

In aerobic incubations, whole soil was brought to about 50% water filled pore space (WFPS) (Linn and Doran, 1984) in a 60-ml bottle, and sand-SOM fraction mixtures were brought to about 66% WFPS in 60-ml bottles. The bottles were kept at 26 ± 1°C in the dark for 28 days inside sealed 500-ml glass jars with a thin layer of water to maintain humidity and prevent loss of water from the soil. Glass jars were briefly opened twice per week in order to ensure aerobic conditions. After incubation, 50 ml 2 M KCl was added to the incubation bottle, and inorganic N was extracted with shaking for 1 h at 150 reciprocations min^{-1} (30-mm-reciprocal stroke) and subsequent gravity filtering using washed Whatman No. 5 paper. Ammonium was determined colorimetrically (Anderson and Ingram, 1993). Nitrate plus nitrite was determined by cadmium reduction (Dorich and Nelson, 1984) with subsequent colorimetric determination of nitrite (Hilsheimer and Harwig, 1976). No effort was made to separate nitrate and nitrite. Because nitrite is likely small relative to nitrate, the values are reported as nitrate for sake of simplification. Ten grams of soil and 50 mg of SOM fraction mixed with 9.95 g of ashed sand were similarly extracted with 50 ml 2 M KCl and analyzed for initial am-

monium and nitrate. Nitrogen mineralization was calculated as the difference in inorganic N (ammonium plus nitrate) between incubated and unincubated samples. Nitrogen mineralization rates were expressed as: (i) N released by a SOM fraction per unit of whole soil mass per day ($\mu g\ N\ kg^{-1}\ d^{-1}$) and (ii) percentage of the N in a fraction mineralized during incubation.

Analyses of variance (ANOVA) were conducted using the SAS programme (SAS Institute, 1990) to determine the effect of the seven cropping systems (error df = 12) on N mineralization. The F ratio is calculated as TMS/EMS, where TMS is the treatment mean square and EMS the error mean square (Mead *et al.*, 1993). In order to determine whether different F ratios are due to differences in the numerator or denominator, TMS and EMS were examined separately. The coefficient of variation between treatment replicates (CV) is $100(EMS)^{1/2}$/mean, and the coefficient of variation between treatment means (TCV) is $100(TMS)^{1/2}$/mean. The CV and TCV are reported for each ANOVA and used to assess whether a significant effect of cropping system treatments resulted from low random error (low CV) and/or a large separation of treatment means (high TCV) (Barrios *et al.*, 1996). Variation between laboratory replicates indicated that there was no additional plot to plot variation (F ratio comparing plot with laboratory variation was not significant), so correlations were based on treatment means.

RESULTS AND DISCUSSION

N mineralization

The cropping system significantly ($P < 0.05$) affected rates of anaerobic and aerobic N mineralization in whole soil and LL (Table 1). Cropping system affected aerobic, but not anaerobic N mineralization for NAL. The low CV in whole soil and high TCV in LL were responsible for the significant differences observed in the rates of aerobic and anaerobic N mineralization between cropping systems. The lack of significant differences among cropping systems for anaerobic N mineralization in NAL was attributed to high CV, while significant differences for aerobic N mineralization in NAL were attributed to relatively low CV.

Anaerobic and aerobic N mineralization in whole soil (WS) were highest for continuous maize with prunings of gliricidia added each season (M + G) and lowest for the gliricidia stand with prunings removed (G) (Table 2). Nitrogen mineralization, as determined in both whole soil and light fractions, was generally comparable among continuous maize with removal of residues (M), cowpea and pigeonpea systems (CP/M, PP-CP/M, PP-M), hedgerow intercropping (G-M) and gliricidia stand with removal of prunings (G).

Table 1. Probability table for the effect of seven cropping systems on N mineralization in whole soil (WS) and fractions of soil organic matter

Fraction[a]	Anaerobic N mineralization						Aerobic N mineralization					
	Rate[b]			Percentage of total N mineralized[c]			Rate			Percentage of total N mineralized		
	Pr > F	CV[d]	TCV[e]	Pr > F	CV	TCV	Pr > F	CV	TCV	Pr > F	CV	TCV
WS	0.013	29	61	0.037	29	52	0.033	18	34	0.116	20	30
NAL	0.417	167	175	0.515	183	176	0.005	78	187	0.054	92	158
LL	0.020	41	82	0.275	38	45	0.021	71	142	0.062	57	95

[a] NAL = sodium iodide light fraction ($< 1.7\ g\ cm^{-3}$) and LL = Ludox light fraction ($> 150\ \mu m$, $< 1.13\ g\ cm^{-3}$).
[b] Values expressed on the basis of whole soil.
[c] (N mineralized in fraction/N content in fraction) ×100.
[d] CV = coefficient of variation between field replicates.
[e] TCV = coefficient of variation between treatment means.

Table 2. Effect of cropping systems on N mineralization in whole soil (WS) and fractions of soil organic matter

Treatment[a]	Anaerobic N mineralization			Aerobic N mineralization		
	Rate (μg N kg^{-1} d^{-1})[b]		Percentage of total N mineralized[c]	Rate (μg N kg^{-1}d^{-1})[b]		
	WS	LL[d]	WS	WS	NAL	LL
M	3140	76.9	2.5	677	18.9	2.8
CP/M	3261	128.7	2.3	752	89.3	26.2
PP-CP/M	3657	75.4	2.8	626	26.5	9.9
PP-M	3110	134.2	2.3	593	27.4	7.0
M + G	6722	269.7	4.4	918	214.8	45.5
G-M	3850	139.0	2.5	690	93.0	23.1
G	2774	130.7	1.9	494	5.9	11.6
SED[e]	891	46	0.6	102	43.4	10.5

[a]M = continuous maize with removal of crop residues, CP/M = one season cowpea rotated with one season maize, PP-CP/M = pigeonpea-cowpea intercrop for one year rotated with maize for two seasons, PP-M = continuous pigeonpea-maize intercrop, M + G = continuous maize with prunings of gliricidia added each season, G-M = gliricidia hedgerow continuously intercropped with maize and G = gliricidia stand with prunings removed.
[b]Values expressed on the basis of whole soil.
[c](N mineralized/total N) ×100.
[d]LL = Ludox light fraction (> 150 μm, < 1.13 g cm^{-3}) and NAL = sodium iodide light fraction (< 1.7 g cm^{3}).
[e]SED = Standard error of the mean difference.

The percentage of whole soil total N that mineralized showed significant ($P < 0.05$) treatment differences among cropping systems under anaerobic conditions but not under aerobic conditions, (Table 1). It was highest for M + G and lowest for G (Table 2). The percentage of total light fraction N (NAL or LL) that mineralized anaerobically or aerobically was not significantly different ($P < 0.05$) among cropping systems (Table 1).

The percentage of total N mineralized in NAL, but not in whole soil and LL, was higher for aerobic than anaerobic incubations (data not shown) suggesting that traces of sodium iodide may have retarded recolonization of NAL by anaerobic bacteria during short-term anaerobic incubations. This result is consistent with Sollins *et al.* (1984), who found an 85% decrease in whole soil anaerobic mineralization after agitation in a sodium iodide solution. Despite slight differences in anaerobic incubation method between Sollins *et al.* (1984) and our study, the percentage of NAL-N mineralized that Sollins *et al.* (1984) reported for a tropical montane forest soil (2.1%) was comparable to our mean value (2.6%).

The measure of random error (CV) indicates that whole soil treatment replicates were less variable than those of the light fractions (Table 1). The significant treatment effect for light fraction mineralization resulted from a large separation between treatment means (TCV). Barrios *et al.* (1996) similarly found that measurements of N and C in light fraction generally had higher TCVs than for N and C concentration in whole soil.

Anaerobic and aerobic N mineralization for whole soil were significantly correlated ($P < 0.05$) (Table 3). Light fraction anaerobic and aerobic N mineralizations generally correlated well with anaerobic and aerobic mineralization for whole soil. Nitrogen mineralization for LL was highly correlated ($P < 0.01$) with N mineralization for NAL. This is consistent with the concept that organic N in LL is part of the larger NAL fraction (Barrios *et al.*, 1996).

Relationships between LF mineralization, C and N

Barrios *et al.* (1996) found that the amount of LF-N and amount of LF-C were sensitive measures of differences in SOM among cropping system treat-

Table 3. Correlation coefficients between N mineralization for whole soil (WS) and fractions of soil organic matter from seven cropping systems

Parameter[a]	WS		LL	
	Anaerobic N mineralization	Aerobic N mineralization	Anaerobic N mineralization	Aerobic N mineralization
WS				
aerobic N mineralization	0.852			
LL				
anaerobic N mineralization	0.861	0.691		
aerobic N mineralization	0.854	0.830	0.885	
NAL				
aerobic N mineralization	0.929	0.922	0.891	0.965

5 degrees of freedom. $P < 0.05$ when $r > 0.754$, $P < 0.01$ when $r > 0.874$.
[a]LL = Ludox light fraction (> 150 μm, < 1.13 g cm^{-3}) and NAL = sodium iodide light fraction (< 1.7 g cm^{-3}).

Table 4. Correlation coefficients between C, N and C-to-N ratio in soil organic matter fractions from seven cropping systems and N mineralization rates for the respective fraction

Parameter for fraction	Anaerobic N mineralization	Aerobic N mineralization	
	LL[a]	LL	NAL
Dry weight	0.494	0.940	0.453
C concentration	−0.349	−0.101	−0.188
Amount of C in fraction[b]	0.439	0.904	0.429
N concentration	0.611	0.830	0.935
Amount of N in fraction[b]	0.594	0.949	0.806
C-to-N ratio	−0.594	−0.750	−0.824

5 degrees of freedom. $P < 0.05$ when $r > 0.754$, $P < 0.01$ when $r > 0.874$.
[a]LL = Ludox light fraction (> 150 μm, < 1.13 g cm^{-3}) and NAL = sodium iodide light fraction (< 1.7 g cm^{-3}).
[b]mg C or N kg^{-1} of whole soil.

ments. Greater amounts of LF-C and -N resulted from either rotation of cowpea (*V. unguiculata*) with maize or the seasonal addition of *G. sepium* prunings to continuous maize, than from continuous maize with removal of crop residues (Barrios *et al.*, 1996). Our second objective was to assess whether qualities of SOM light fractions (dry weight, C and N concentration, amount of fraction-C and -N, C-to-N ratio), sensitive at detecting differences among cropping system treatments, are related to N-mineralization in the respective light fraction. Anaerobic and aerobic N mineralization in LL and aerobic mineralization in NAL were selected for correlation analysis because they were significantly affected by cropping system (Table 1).

No correlations were found between LL qualities and anaerobic N mineralization in LL (Table 4). However, dry weight of LL, amount of LL-C and -N and N concentration in LL correlated with aerobic N mineralization for LL. N concentration in NAL and amount of NAL-N correlated with aerobic N mineralization for NAL. The C-to-N ratio was inversely correlated with aerobic N mineralization in NAL (Table 4).

Correlations between light fraction qualities and N mineralization in the respective light fraction suggest that N mineralization in light fractions is directly related to N concentration and amount of N in the fraction and inversely related to the C-to-N ratio in the fraction. The lack of correlation between LL qualities and anaerobic N mineralization in LL could indicate that incubation of SOM fractions mixed with coarse sand will likely have unrestricted aeration and thus preferentially colonized by aerobic soil microbes.

Table 5. Correlation coefficients between amount of C and N in fractions of soil organic matter from seven cropping systems and whole soil N mineralization

Parameter			
Fraction[a]	Measure	Anaerobic N mineralization	Aerobic N mineralization
LL	Dry wt	0.672	0.688
LL	C	0.688	0.685
LL	N	0.779	0.820
NAL	Dry wt	0.277	0.374
NAL	C	0.272	0.465
NAL	N	0.675	0.737

5 degrees of freedom. $P < 0.05$ when $r > 0.754$, $P < 0.01$ when $r > 0.874$.
[a] LL = Ludox light fraction (> 150 μm, < 1.13 g cm^{-3}) and NAL = sodium iodide light fraction (< 1.7 g cm^{-3}).

Relationships between LF and whole soil N mineralization

Amount of LL-N, but not dry weight of LL or LL-C, correlated ($P < 0.05$) with anaerobic and aerobic N mineralization in whole soil (Table 5). This observation is consistent with Hassink (1995) who also found a good correlation between the amount of N in LL and N mineralization in temperate grassland soils. Whereas Hassink (1995) also found a correlation between amount of LL-C and N mineralization, these relationships were not significant in our study.

It should be noted, however, that immersion of different litters in Ludox, as compared to a water control, stimulated their N mineralization (Magid *et al.*, 1996). This observation suggests that the Ludox solution may be affecting size-density fractions and thus questions the value of this fraction as a direct measurement of the mineralizing organic pool. Nevertheless, its potential value as a predictive indicator of soil fertility still remains to be demonstrated across multiple locations, soils and cropping systems.

The dry weight of NAL and NAL-C and -N were not significantly correlated ($P < 0.05$) with N mineralization of whole soil (Table 5). Amount of N in NAL, however, had relatively high correlation coefficients, just slightly lower than those for amount of N in LL.

The lowest ash content among light fractions was found in LL (Meijboom *et al.*, 1995; Barrios *et al.*, 1996). This indicates that LL is essentially composed of mineral-free organic matter. The observation by Hassink (1995) that the relationship between amount of LL-N and N mineralization was not affected by soil texture and that amount of LL-N and active microbial biomass are the best predictors for N mineralization further supports the non-

complexed nature of LL and its ready access to microbial consumption.

The mean amount of N in LL was only 1.4% of the total soil N. This finding is consistent with Paul and Juma (1981) who proposed that the small proportion of total N represented by labile SOM fractions was largely the result of their rapid turnover. The LL fraction is likely either a small portion of the labile SOM or closely linked to labile SOM

Conclusions

The relationship between amount of N in light fractions (LL and NAL) and whole soil N mineralization suggests that amount of N in light fraction merits further examination as an indicator of differences in SOM among cropping systems. Amount of N in light fraction, determined as the product of fraction dry weight and its N concentration, is particularly valuable as an indicator because it is not affected by contamination with density agents. Microbial activity, however, may be substantially affected by density agents, suggesting a limited usefulness of N mineralization measurements in light fractions not separated with water. While whole soil N mineralization provides a measure of available N in the soil, amount of light fraction N may complement this information by providing a relative indication of biologically-mediated N availability from labile SOM.

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