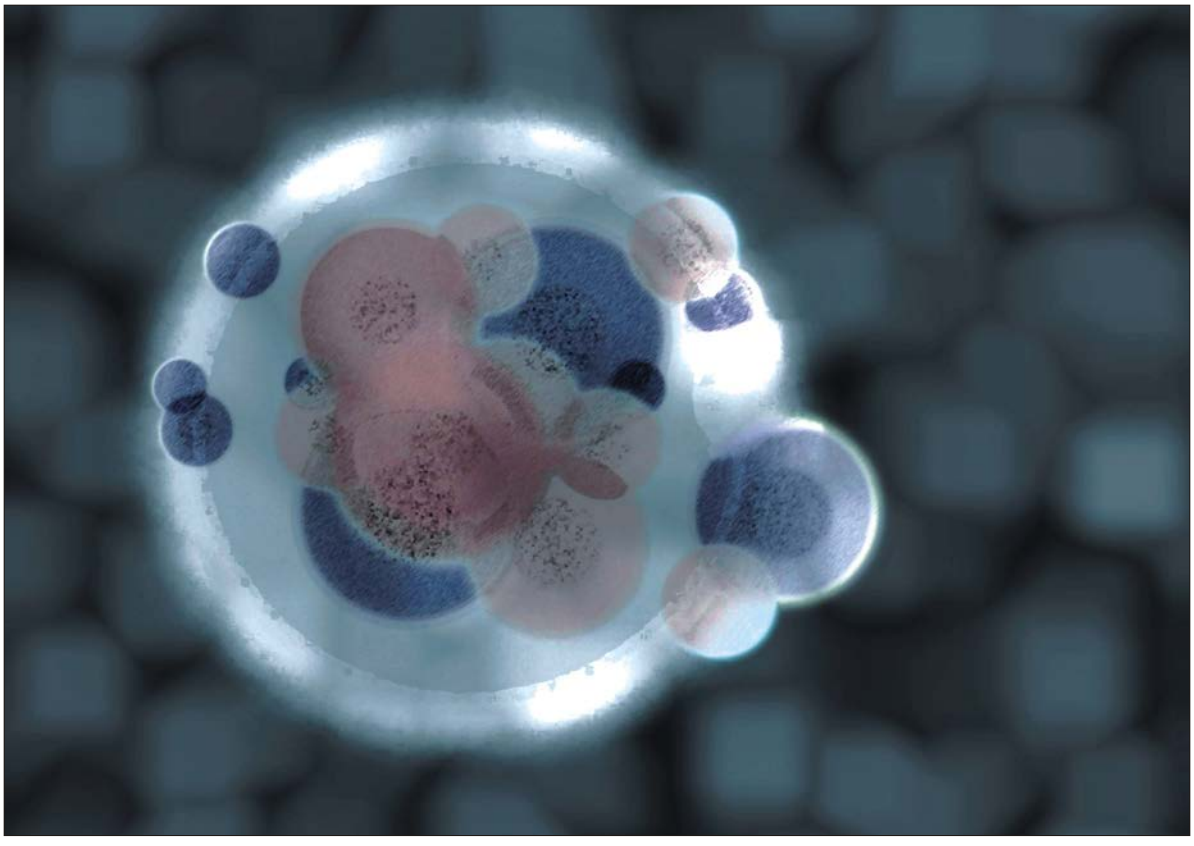

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Mineralization and Microbial Biomass Dynamics During Decomposition of Four Leguminous Residues

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Abstract: Decomposition of Sunn hemp (*Crotalaria juncea*), Devil bean (*Crotalaria retusa*), Calopo (*Calopogonium mucunoides*) and Velvet bean (*Mucuna pruriens*) in litterbag field incubation experiment was compared with mineralization of the same leguminous cover crops in a laboratory incubation study to assess N release and synchrony. Initial rapid N release followed by slower and curvilinear release was observed with the litterbag approach. The laboratory incubation approach however, showed a significant initial N immobilization in all cover crop amended soils, particularly with devil bean. The highest immobilization occurred four days after residue incorporation and coincided with the highest microbial biomass-N flush of 250% above the un-amended soil. Residues with higher quality in terms of lower percentage cellulose and lower Lignin/N ratio mineralized faster with lower N immobilization. Legumes with C/N ratio less than 20 and percent N above 1.7 may immobilize N. High N immobilization particularly in devil bean and mucuna may result in poor synchrony between N release and cereal N requirements and could result in low initial stimulation of succeeding cereal crop and lower yields. Cover crops left to mature and dry should thus be incorporated at least 2 weeks at the beginning of the following season before planting the cereal. Care must be taken in interpreting N release data using litterbag approach.

Key words: Cover crop, fallow management, residue quality, nitrogen mineralization, semi, arid, Ghana

INTRODUCTION

Low soil fertility and nutrient mining are the main causes of stagnation or decline in crop yield and productivity in Africa. Despite the prevalence of poor soils, fertilizer application rate in sub-Sahara Africa is only 9 kg ha^{-1} compared with global mean of 101 kg ha^{-1} (Camara and Heinemann, 2006). High cost and limited access have been cited as contributing factors. According to Weber (1991), 400 scientists meeting in Ouagadougou in 1989 issued a communiqué to the effect that tropical and African countries can produce enough food with the help of organic agriculture. In the recent past, considerable efforts have been made through widespread screening exercises to identify leguminous cover crops for short fallow management to improve agricultural production in small-holder farming systems. Most of these screening exercises assessed mainly total biomass production of cover crops but a considerable number also compared decomposition rates. Decomposition studies often involved the use of litterbag in which some amount of residue was enclosed and the bags buried or left on the soil surface. They were subsequently sampled periodically and examined for loss in litter-weight as an index of decomposition (Carsky, 1989). Inherent in the litterbag decomposition approach is the inevitable escape of N not mineralized but lost from the litterbag during the washing process. This often results in overestimation of N release. The litterbag approach also does not account for N that is immobilized and thus unable to accurately define the time course or pattern of N release. We hypothesized that residues of low quality (high C/N ratio, high lignin/N ratio, high percentage cellulose etc.) will immobilize N that can be captured with a mineralization study.

The objective of this study was to compare the litterbag decomposition method with mineralization study for assessment of cover crops for soil fertility

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improvement. Since decomposition and mineralization are microbially mediated we also monitored changes in the microbial biomass during mineralization.

MATERIALS AND METHODS

Fractionation and biochemical characterization of cover crops: Fractionation was done according to Van Soest (1963) method to obtain percent cellulose and lignin contents.

Total N was obtained by Kjeldahl method (Bremner and Keeney, 1965). About 0.2 g milled plant sample (<250 μm) was digested with concentrated H_2SO_4 (98%) and selenium mixture and 20 mL aliquot of the digest distilled with 40% NaOH into mixed boric acid indicator solution. The distillate was titrated with 0.005 M H_2SO_4 Titrisol.

Total C was determined using Carlo-Erba C and N analyser.

Decomposition of cover crops by litterbag approach: The field decomposition study was carried out in 1997 at Nyankpala on loamy sand Ferric Lixisol. Litter bags with dimensions of 20×20 cm (0.04 m^2) were made by sewing a UV-stabilized polyester net of 2-mm-mesh size, with a mono-filament plastic thread. The bags were filled with dried and chopped cover-crop residues at the following rates: sunn hemp (20 g litter-bag$^{\bullet 1}$), devil bean (56 g litter-bag$^{\bullet 1}$), calopo (34 g litter-bag$^{\bullet 1}$) and velvet bean (28 g litter-bag$^{\bullet 1}$). These rates correspond to biomass of 5, 14, 8.5 and 7 t ha$^{\bullet 1}$, respectively, for the cover crops representing amounts used in experiment in which the cover crop with the highest biomass incorporated did not necessarily give rise to the highest yield of a succeeding cereal (Fosu *et al*., 2004).

The litter-bags were buried 5 cm deep and sampled periodically to estimate decomposition.

Mineralization in controlled environment: About 80 g (oven dry basis) of soil samples (<2 mm) taken from the field where the litterbags were buried was weighed into polyethylene bags of dimension 10×15 cm. The soil samples were wetted to 60% of Water Holding Capacity (WHC) and pre-incubated at 35°C for 14 days. The maximum WHC at – 10 kPa was determined with pressure plate using disturbed samples. At the end of the 14-day pre-incubation, mineral N was determined in the soils and the time designated as day 0. About 0.3 g (approx. 8.5 t ha$^{\bullet 1}$), of sunn hemp, devil bean, velvet bean and calopo residues (<250 μm) dried at 70°C for 48 h were weighed into the polyethylene bags. After thoroughly mixing the residues with the soil, the moisture content of the soils was brought to 100% WHC by pipetting the required amount of deionised water into the bags and the bags were heat sealed. The soil was homogenized by gently massaging between the fingers and the bags transferred into the incubator at 35±0.5°C. Moisture loss was replenished every week by injecting the required amount of deionised water through the polyethylene bag with syringe and closing the hole made by the needle with a self-adhesive material. On average 7.5% moisture was lost per week. There were three replications of each cover crop for each sampling time in a batch system. At day 4, 10, 17, 38, 52 and 66 after incorporation (DAI), three bags of each cover crop were removed at random and analyzed for mineral N.

Microbial biomass nitrogen: Microbial biomass analysis followed the chloroform fumigation extraction method (Brookes *et al*., 1985). Two sets of 25 g samples (oven-dried basis) of soils were weighed, one set into 50 mL glass beakers and the other into 250 mL polyethylene extraction bottles. The latter set was extracted immediately after weighing with 100 mL of 0.5 M K_2SO_4 and filtered through No. 42 Whatmann filter paper and the extract stored frozen until analysis. The samples in the 50 mL beakers were fumigated with alcohol-free chloroform and kept in the dark for 24 h. After removal of the chloroform, the samples were treated as the first set. Thirty milliliter aliquots of 0.5 M K_2SO_4 soil extracts (fumigated and unfumigated) were digested using the modification of Pruden *et al*. (1985) to include $NO_3^{\bullet}$-N. Total N was determined on 20 mL aliquots of the digest by distilling with 40 mL of 10 M NaOH into 5 mL boric acid mixed indicator using Vapodest distillation unit.

Mineral nitrogen (Nmin) analysis: The remaining unfumigated K_2SO_4 extracts that were stored frozen were used for Nmin analysis. The samples were analysed for mineral N on the SKALAT Segmented Flow Analyzer System (Model 5100) in which nitrate was reduced to nitrite while passing a cadmium filled column. The nitrite was coupled with naphtylelthylendiamindihydrochloride (NEDC) to a coloured diazo-complex and the absorbance was read at 540 nm. The sample for NH_4^+ determination was diluted in a buffer solution to complex cations. Hereafter, salicylate, a catalyst and active chlorine were added to form a green coloured complex with the ammonium ion at 40°C and the absorbance read at 660 nm (Henriksen and Selmer-Olsen, 1970).

Calculations and statistical procedures: Microbial biomass N (B_N) was calculated as: $B_N = 2.22\ E_N$.

Where E_N = (total N in K_2SO_4 extract of fumigated soil) minus (total N in K_2SO_4-extract of non-fumigated soil) and 2.22 is the inverse of the k_N factor of 0.45.

Total mineral N (Nmin) = NH_4^+-N+NO_3•-N.

Net Cumulative-N mineralised (Cum-Nmin) = Nmin minus soil N at day 0.

Residue N mineralised was computed as $Nmin_{(r)} = Nmin_{(+r)} - Nmin_{(-r)}$

Where $Nmin_{(r)}$, $Nmin_{(+r)}$ and $Nmin_{(-r)}$ are residue N mineralised, mineral N in residue amended and un-amended soils, respectively.

Cum-Nmin, $Nmin_{(r)}$ and B_N were subjected to ANOVA and significantly different means separated with LSD test using Statistix (1996).

RESULTS AND DISCUSSION

Soil and plant materials: The soil was classified as Greyi-ferric lixisols (FAO). It had the following characteristics: pH ($CaCl_2$) = 6.2, OM = 1.5, % N = 0.05, CEC = 6.6, % sand = 61.3, % silt = 31.2, % clay = 7.5.

The legume residues had the following characteristics: Sunn hemp - % C = 44, % N = 2.5; Devil bean - % C = 42.5, % N = 2.1; Calopo - % C = 40, % N = 2.1; Mucuna - % C = 43.2, % N = 2.2.

Decomposition in litterbag during field incubation: A rapid decrease in ash-free dry matter during the first two weeks was observed for all residue types (Fig. 1). This decrease was greatest in sunn hemp which had the highest residue quality. Decomposition was fairly linear within the first two weeks and also between 10 66 DAI and 66 135 DAI. Within the first two weeks of application, 47, 26, 29 and 31% of dry matter was lost from sunn hemp, velvet bean (mucuna), calopo and devil bean, respectively. This represents N release of 50% in sunn hemp and 25% on average for calopo, mucuna and devil bean. By the 38 DAI the dry matter loss was 67, 53, 59 and 49% from sunn hemp, velvet bean, calopo and devil bean. At the end of the season, 80% of the dry matter of velvet bean, calopo and devil bean were lost while sunn hemp lost 91% of its dry matter in the litter-bag. This represents N release of 90% in sunn hemp and 80% in the other three legume residues. This result is similar to the finding of Güsewell and Verhoeven (2006) who reported positive correlation between litter mass loss and N and P concentrations.

The N release pattern depicted by the litterbag data suggests synchrony for cereal production since all cover crops released their N faster at the beginning when it is most needed by cereals.

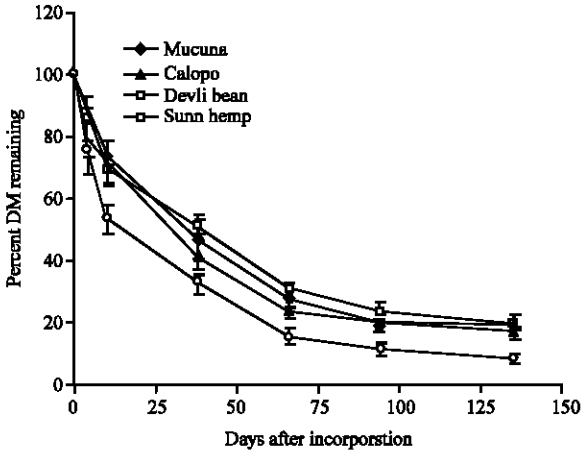

Fig. 1: Decomposition of sunn hemp, mucuna, calopo and devil bean residues in litter-bags at Cheshegu. Bars represent standard deviation

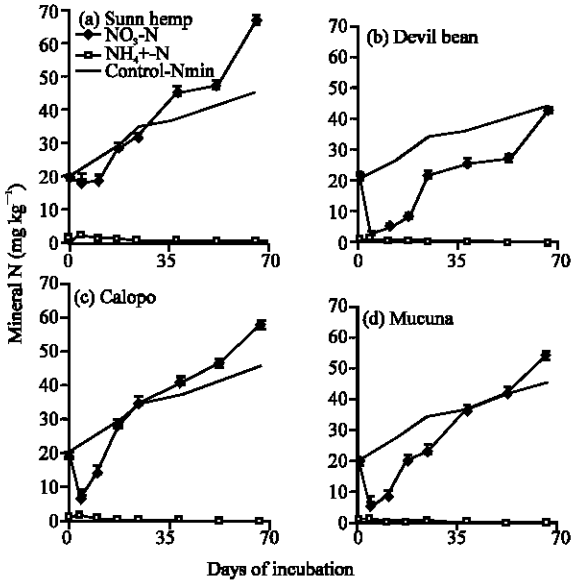

Fig. 2: Evolution of mineral N from sunn hemp, devil bean, calopo and mucuna during a 66-day incubation. Control-Nmin is total mineral N in control (un-amended) soil. Bars represent standard deviation

Mineral N release during laboratory incubation: The mineral N measured in the soil at the end of the incubation ranged from a high of 71.8 μg g•^{1} for sunn hemp to a low of 46.6 μg g•^{1} for devil bean (Fig. 2). In all treatments, most of the mineral N was in the form of NO_3•-N. The relative NH_4^+-N concentration was 6% of total mineral N at the beginning of the incubation and fell to 0.6% on average at the end. The low level of NH_4^+-N implies that

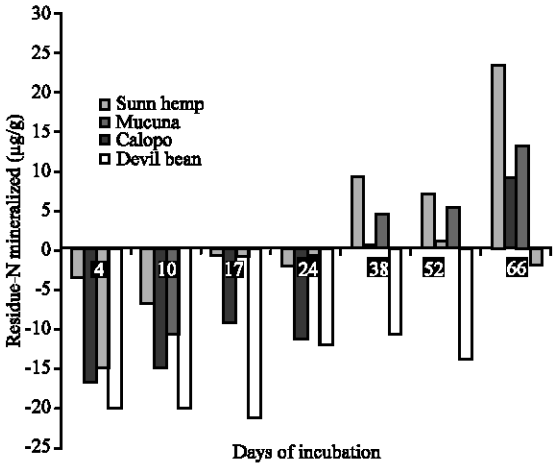

Fig. 3: Residue N mineralized and immobilized from cover crops during a 66-day incubation. Negative values indicate immobilization

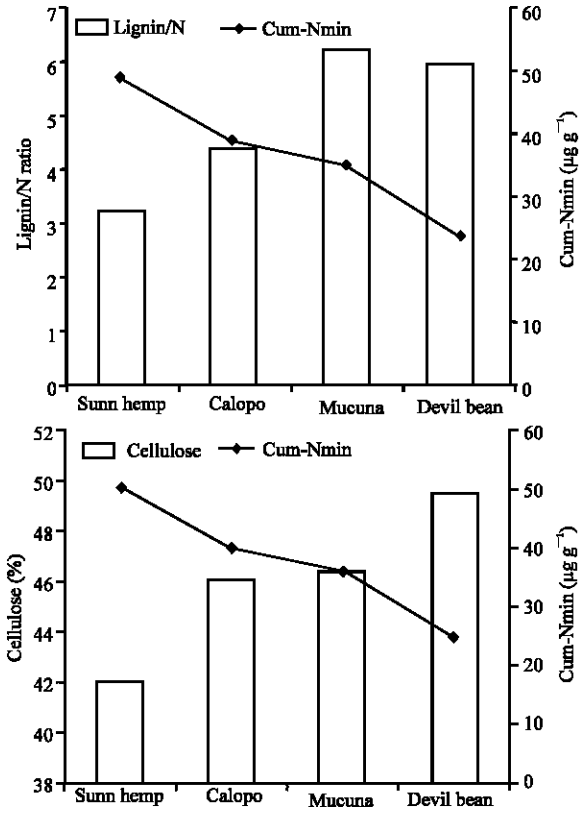

Fig. 4: The relationship between cover crop residue quality and cumulative N mineralized

nitrification was rapid in this soil. In all cover crop-amended soils except sunn hemp, there was a significant depression of soil mineral N after four days of incorporation compared with the control (no cover crop amendment). The residue-N mineralised as assessed by the difference method was negative in all cases up to 24 DAI (Fig. 3) indicating immobilization of N during a significant part of the incubation period. Sall *et al.* (2006) found large N immobilization in a laboratory incubation experiment in which they amended sandy and ferruginous Oxisol with residues of *Faidherbia albida* litter. The least immobilization in our experiment was observed with sunn hemp which had the highest residue quality (lowest cellulose and % lignin/N ratio, Fig. 4). On the other hand the highest immobilization was observed with devil bean whose residue quality was lowest. In general, residue quality was negatively correlated with immobilization and cumulative-N mineralised (Fig. 4 a and b). No net nitrogen release was observed in devil bean during the entire period of the incubation (Fig. 3). Verkaik *et al.* (2006) reported high N immobilization in organic layer of a soil from northern New Zealand when it was amended with high molecular tannins and cellulose.

N immobilization may cause temporary nitrogen starvation in cereals growing following cover crop incorporation. The laboratory incubation data shows that most of the cover crops did not release any N until after 24 DAI and in the case of devil bean no N was released even after 66 days. Cereals require N at the early part of growth and the most critical time for full season varieties of maize is considered to be 42 days after planting. The data suggests that there may be a lack of synchrony between cover crop N release and full season maize N requirements particularly from mucuna and devil bean. It is however, debatable whether N release pattern in a laboratory incubation experiment can be directly applied to field situation since the conditions are different.

Comparing the litterbag incubation data (Fig. 1) and laboratory mineralization data (Fig. 3) showed marked discrepancy in N release from the two approaches. While the litterbag approach showed rapid initial N release by all legume residues followed by slow release after 35 days, the laboratory incubation data showed no N release up to 38 days of incorporation with large amounts of the N immobilized in all the cover crops except sunn hemp. This discrepancy in the N release data between field incubation and laboratory incubation approach was due to the fact that the litterbag approach could not estimate N tied up in the microbial biomass while the laboratory incubation analysis measured mineral N only thus allowing for the estimation of N that was immobilized. In the field incubation studies, residue particles <2.0 mm passed through the litterbags during the washing process whether they were decomposed or not. This could lead to loss of N that was not mineralised from the litterbag but recorded as released thus leading to overestimation of the actual N that might be available to succeeding plants at a particular point on an N release curve.

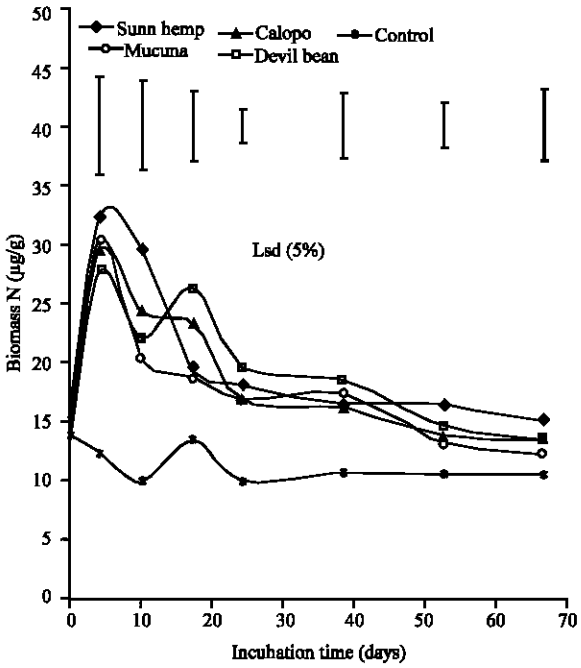

Fig. 5: Microbial biomass changes during the mineralization of 4 cover crops in a 66-day incubation

Microbial biomass N: Biomass N increased rapidly and significantly in soils amended with cover crop residues after 4 days of incubation (Fig. 5). This increase was about 250% above the biomass N measured in un-amended (control) soil and coincided with the N immobilization observed at this time. This result is in agreement with other studies that showed flush in microbial biomass due to incorporation of easily degradable organic residues (Sall *et al*., 2006; 2003). Sall *et al*. (2006) measured up to 400% increase in microbial biomass in a cultivated soil amended with *Faidherbia albida* compared with the un-amended soil after 10 days of incubation.

The biomass N measured for all cover-crop-amended soils after 4 days of incubation were similar. However, at day 10 of incubation, significant differences in biomass N were observed between sunn hemp and mucuna treatments and at day 17, between devil bean and mucuna treatments. The peak biomass N increase that occurred at day 4 in this experiment was found to have occurred earlier (Nicolardot *et al*., 1994). Change in microbial biomass N in the un-amended soil was small throughout the incubation period as a result of low Organic Carbon (OC) content of the soil (OC<0.7%). At the end of the experiment biomass N in the un-amended soil was similar to that in all cover-crop-amended soils.

Table 1: Microbial-N pool (B_{Np}, µg g^{-1}) in residue amended soils, calculated following subtraction of microbial-N from un-amended soil and microbial-N as a % of residue-N added (R_{pc})

Day	Residue	Suun hemp	Mucuna	Calopo	Devil bean	Mean	SE
4	B_{Np}	20.2	18.1	17.1	15.5	17.7	1.2
	R_{pc}	21.0	20.9	20.8	18.8	20.4	
10	B_{Np}	19.7	10.4	14.4	12.2	14.2	0.8
	R_{pc}	20.5	12.0	17.5	14.7	16.2	
17	B_{Np}	6.1	5.3	9.9	13.0	8.6	0.5
	R_{pc}	6.3	6.2	12.1	15.7	10.1	
24	B_{Np}	8.1	6.9	6.9	9.7	7.9	0.4
	R_{pc}	8.4	8.0	8.3	11.7	9.1	
38	B_{Np}	5.6	6.7	5.5	7.8	6.4	0.5
	R_{pc}	5.9	7.7	6.7	9.5	7.5	
52	B_{Np}	5.8	2.6	3.3	4.0	3.9	0.4
	R_{pc}	6.1	3.0	4.0	4.9	4.5	
66	B_{Np}	4.7	1.7	2.9	3.1	3.1	0.5
	R_{pc}	4.8	2.0	3.5	3.7	3.5	

There was a decline of about 83% of the mean microbial N pool from day 4 to 66 and the microbial N as a percent of cover crop N incorporated fell from 20 to 3.5% within the same period (Table 1). This may have resulted from the turning over and remineralization of the biomass. No significant relationship was found between microbial biomass N and net N mineralization. This indicates that biomass N measurements may not give a precise indication of N release from mineralization of organic residues though it may give an early indication of the improvement of C and N content (at least) of soils.

CONCLUSIONS

Litterbag decomposition data showed rapid N release quite different from the high immobilization and slow N release data from the laboratory incubation experiment thus confirming our hypothesis. This result indicates that a rapid residue decomposition measured with litterbag procedure may not necessarily imply rapid mineral N release. Some amount of residue N immobilization occurred during decomposition of legume residues regardless of residue quality. However, the amount of N immobilized and the period of immobilization increased with decreasing residue quality. Care must thus be taken in interpreting litterbag data for screening leguminous cover crops for soil fertility management in cereal production.

Rapid microbial biomass increase will occur when dry legume residue is introduced into the soil. This may lead to immobilization and temporary N starvation for crops. Organic residues must thus be applied for at least 14 days before following with a cereal crop. The size of microbial N pool during decomposition is not a precise measure of N release from mineralization of organic residues. There is need for further investigation using field mineralization technique and comparing that with the litterbag data.

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