

N Mineralization from Residues of Crops Grown with Varying Supply of ^{15}N Concentrations

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Abstract

A laboratory study was conducted to evaluate the effects of varying ^{15}N concentrations on crop residue quality (rice straw=RS and soybean=SY) and N mineralization. Two crops (rice and soybean) were grown in a glass-house under four ^{15}N concentrations, i.e., 0 mM (N0), 0.625 mM (N1), 2.5 mM (N2), and 10 mM (N3) supplied as $\text{CO}(^{15}\text{NH}_2)_2$, in 30 cm diameter plastic pots containing 5 kg of quartz sand. Eight weeks after planting the above-ground biomass was pruned and oven dried at 60° C for 48 hours and analyzed for polyphenol, lignin, N, C, C/N, organic matter and % ^{15}N -abundance. Each of the eight crop residues produced from this experiment was then incorporated into 10 g of soil in a 50 ml plastic bottle. Mineralization of N from the residues was measured over a 14-week period under controlled non-leaching conditions. The results showed that increasing N concentration in the nutrient solution (0 to 10 mM) increased total N but decreased C, lignin and polyphenol content in the crop residues. The results suggested that N supply may increase the quality of the crop residues, as indicated by decreasing of C/N, lignin/N, polyphenol/N ratios. SY residue released N about 2x faster than RS residue, and the amount N released increased with increasing supply of the N concentration. At the 10 mM N supply, RS and SY residues released respectively 3734,857 mg kg⁻¹ and 4352.34 mg kg⁻¹ cumulative amount of mineral N with the mineralization rate about 7x faster than without ^{15}N in the solution. Differences in the %N mineralized were determined by the quality of the crop residues. Regression analyses showed that the N, lignin and polyphenol contents were the residues quality factors which could be used to predict N mineralization of the crop residues, and the relationship was best described by a linear regression.

Keywords: ^{15}N concentration, residue, nitrogen release, microbial biomass N

1. Introduction

Farming system with low input is highly dependent on the use of soil organic matter inputs. Inputs of organic matters have different effects to plant growth depending on the rate of decomposition and mineralization of organic matter. Farmers commonly use varying quality of crop residues available around their farm lands as sources of organic matters. A major consideration for the selection of crop residues is the rate and amount of N mineralized from the residues and hence the N benefit obtained by crops. Nitrogen is the most important nutrient for plant growth, and N fertilizer represents the main energy input in cropping systems (Zentner et al., 2003). To optimize the use of soil available N by crops, it is important to understand the pattern of plant N accumulation and soil N mineralization. Understanding the pattern of N release from fertilizer, relative to plant growth, is essential to maximize N use efficiency and prevent unnecessary loss of N to the environment. This is particularly challenging when organic fertilizers are applied because the N release from such materials depends on the microbially-mediated processes of N mineralization and nitrification, which are influenced by environmental conditions, i.e., temperature, moisture (Rathke, Behrens, & Diepenbrock, 2006), soil properties (pH, texture, organic matter content) and organic matter characteristics like the C:N ratio, lignin content, polyphenol content and particle size (Myrold, 1998; Van Kessel & Reeves, 2002; Stadler et al., 2006).

The rate of N mineralization of a specific type of plant residue is largely determined by its quality (Swift & Sanchez, 1984). High N, low lignin and low polyphenol contents are known as factors contributing to the high quality crop residues (Haynes, 1986). Handayanto, Cadisch, & Giller (1994) demonstrated that variation in nitrogen release patterns among different plant residues were strongly related to differences in (lignin + polyphenol):N ratio and protein-binding capacity of polyphenols in the plant residues. However, when comparing the decomposition of residues from several different species, several quality factors, including physical structure, vary at the same time so that the determination of key factors regulating decomposition is difficult. Therefore, the objective of this study was to manipulate the quality of residues of a species. In order to manipulate quality of residues, rice straw was fertilized with varying rates of N. The decomposition and nitrogen mineralization behaviour of the residues were then tested upon application to the soil. Earlier experiments reported by Handayanto et al. (1994) indicated that the rate of N mineralization was more closely related to the activity of soluble polyphenols than the total polyphenol content. The effect of the polyphenols appeared to be more pronounced under non-leaching conditions. Therefore in the work reported here measurement of N mineralization was carried out under controlled leaching.

2. Materials and Methods

2.1 Experiment 1. Effect of Different N Supply during Crop Growth on the Quality of Crop Residues

¹⁵N labeled rice and soybean residues were obtained by growing in 30 cm diameter plastic pots containing 5 kg of quartz sand and placed in glass-house of the Faculty of Agriculture, Brawijaya University, Malang, Indonesia (7°48'.50" South and 112°37'41" East) for 2 months (5 June 2010 to 5 August 2011). During the experimental period the daily temperature varied from 21°C to 33°C with relative humidity ranged from 45 to 82 %, and light intensity ranged from 365 to 1997 lux). To every pots, four ¹⁵N concentrations, i.e., 0 mM (N0), 0.625 mM (N1), 2.5 mM (N2), and 10 mM (N3) were added as CO(¹⁵NH₂)₂, and 10% atom excess supplied as CO(¹⁵NH₂)₂ in solution at the rate of 400 ml/pot/day. Others nutrients (Ca, K, P, S, Mg, Cl, Fe, Mn, Zn, B, Mo and Co) were also supplied in solution of Hammer, Tibbitts, Laughens, & McFarlane (1978). After 2 months, the above-ground materials of the plants were pruned and oven dried at 60°C for 48 hours and analyzed for polyphenol, lignin, N, C, and organic matter contents and % ¹⁵N-abundance. The polyphenol was extracted in hot 50% aqueous methanol and determined colorimetrically using Folin-Denis method (Anderson & Ingram, 1992). Lignin was determined as acid-detergent lignin (Goering & Van Soest, 1970). The C and N content were determined respectively by Walkley and Black method and Kjeldahl method (Keeney & Nelson, 1982). Nitrogen content and ¹⁵N abundance of the legume residues were measured using a Micromass 622 (UK) mass spectrometer at the National Nuclear Agency of Indonesia, Jakarta.

2.2 Experiment 2. Non Leaching Incubation Experiment

An incubation experiment was conducted in a laboratory with temperature ranging from 26 to 29°C. Each fresh residue was chopped and incorporated into 10 g of soil in a 50 ml plastic bottle. Soil used for the experiment was collected from upland area in North Malang. The soil was classified as Inceptisol (Soil Survey Staff, 2010), having loamy texture, pH (H₂O) 6.20, pH(KCl) 5.40; cation exchange capacity 28.95 cmol kg⁻¹ soil (NH₄OAc pH7); and containing 1.91% organic C; 0.20% total N (Kjeldahl); 22.16 mg P kg⁻¹ soil (Bray II); 0.053 mg N mineral kg⁻¹ soil; 0.0035522 mg microbial biomass N kg⁻¹ soil; and 0.28; 0.5; 1.53 cmol.kg⁻¹ soil of respectively Na⁺, K⁺, Mg²⁺, Water content in the bottle was maintained at 70% field capacity. The eight treatments were arranged in a randomized block design with three replicates. Measurements of soil N content were made at 1, 2, 4, 8 and 14 weeks. Soil mineral N content was determined by the Kjeldahl method (Keeney & Nelson, 1982). Soil pH was determined in 1: 2.5 ratio of soil: water. Soil microbial biomass N was determined by fumigation chloroform and extraction methods, and the rate of N mineralization was calculated using a method Wieder & Lang (1982) by employing the following equation.

$$Y = \exp^{-kt}$$

where :

Y= amount of crop residue N remaining (%) at time 't'

t= time (week)

From these calculations, regression equation was obtained at which the 'Y' is the 'ln' and the value of 'k' is the slope, indicating N mineralization rate.

2.3 Statistical Analysis

Analysis of variance was used to study the effect of ¹⁵N concentration and crop residues and their interaction on the quality of residues, cumulative mineral N and microbial biomass N. If there is a significant influence, a

further Least Significant Different (LSD) test was then performed. Regression analyses were used to determine relationships between N release rate constants (kN), %N released from crop residue as mineral N in soil and the initial composition of crop residues. All analyses were performed with Minitab 15.

3. Results and Discussion

3.1 Effect of Varying N Supply on Quality of the Crop Residues

The results showed that chemical quality parameters of the residues varied depending on ^{15}N concentration supplied during plant growth (Table 1). In both crop residues, the N content increased with increasing ^{15}N concentration in the nutrient solution. In contrast, organic carbon, polyphenol and lignin content decreased. Lignin and polyphenol content were significantly affected by the ^{15}N treatment. Analysis of variance revealed that the N treatment had a significant ($P < 0.01$) influence on the N content of the residues. The lignin and polyphenol contents, however, were more significantly different between the crop species than between the different concentration of ^{15}N supplied. Polyphenols were much reduced when N supply in both species were limited. Increasing concentration of ^{15}N supply increased N content but reduced polyphenol content of the crop residues. Previous studies found that trees often produce higher contents of polyphenolic substances in their leaves when supplied with N at a low rate (Davies et al., 1964; Gershenzon, 1983). N fertilization of particular plant species has been shown to cause a decrease in the production of polyphenols (Bryant et al., 1987). Margna (1977) noted that nutrient shortage could result in increasing activity of enzymes such as phenylalanine ammonialyase or increasing the supply of precursors of polyphenol synthesis. When nitrogen is in short supply, the phenylalanine could then be directly deaminated to reclaim its nitrogen for other purposes such as for conversion to more complex phenolics (Gershenzon, 1983). In addition, as the rate of protein synthesis slows under conditions of N starvation, the unused carbohydrate could be diverted to phenolic synthesis.

Increasing of N content and decreasing of organic carbon, lignin, and polyphenol content, consequently resulted in the lower ratio of C/N, lignin/N, polyphenol/N. These ratios are commonly used to determine the quality of the organic matter, i.e. the higher ratio means the higher quality. The results suggested that ^{15}N supply may increase the quality of the crop residues, as indicated by decreasing of C/N, lignin/N, polyphenol/N ratio.

Table 1. Characteristics of crop residues used in the experiment

Residues ^{15}N concentration	C (%)	N (%)	C/N ratio	^{15}N abundance (%)	Lignin (%)	Polyphenol (%)	Polyphenol : N ratio	Lignin : N ratio	(Lignin + polyphenol) : N ratio
Rice Straw (RS)									
N0	38.85b	1.05a	37.00c	0.37a	23.54c	6.78c	6.46c	22.42c	28.98c
N1	35.46a	1.70b	21.00b	0.44a	18.76b	5.98b	3.52b	11.04b	14.85b
N2	35.08a	1.94bc	18.00a	1.24b	5.34a	5.34a	2.75a	2.75a	5.52a
N3	32.38a	2.04c	16.00a	3.14c	4.24a	4.74a	2.32a	2.08a	4.45a
Soybean (SY)									
N0	36.85b	2.72a	13.55d	0.37a	10.58b	5.98c	2.20c	3.89c	6.11c
N1	39.03b	3.61b	10.88c	1.29b	9.96b	3.21b	0.89b	2.76bc	3.68b
N2	32.25a	3.78b	8.63b	2.38c	9.82b	2.94a	0.78b	2.60b	3.39b
N3	31.58a	4.95c	6.41a	4.34d	6.28a	2.63a	0.53a	1.27a	1.82a

*) N0 = 0 mM ^{15}N concentrations; N1 = 0.625 mM ^{15}N concentrations; N2 = 2.5 mM ^{15}N concentrations; N3 = 10 mM ^{15}N concentrations; different letters mean different at LSD 5%

3.2 N Mineralization of the Crop Residues

3.2.1 Total Amount of Mineral N Released

The amount of N released from soybean residue was significantly higher than that from the RS residue in week 1 to 14 (Table 2). Consistently from week 1 to week 14, the amount of N released increased with increasing concentration of N supplied. There was a significant difference (LSD 5%) between the treatment ^{15}N concentrations. At the end of the incubation period, N mineralization varied from the lowest in RSN0 (15.4%) to the highest in SYN3 (458%). These indicated that initial N content in the crop residues determined the amount of N potentially released to the soils. As the initial N content in the crop residues increased after the application of N in soil solution, increasing N supply therefore would increase the amount of mineral N released.

Table 2. Amount of N released from RS and SY residues during 14 weeks of incubation

Crop residue	N released (%)					N released rate constant, kN (week ⁻¹)
	Incubation time (weeks)					
	1	2	4	8	14	
Rice Straw (RS)						
N0	41.3a	29.4a	27.6a	19.3a	15.4a	0.004a
N1	49.3b	40.6b	36.7b	28.6b	28.8b	0.007a
N2	96.8c	67.7c	55.6c	52.9c	49.2c	0.014a
N3	124.3d	89.4d	95.4d	94.0d	85.3d	0.029a
Soybean (SY)						
N0	186.8a	52.0a	37.7a	31.3a	26.7a	0.006a
N1	210.2b	96.5b	71.8b	60.3b	53.5b	0.015b
N2	314.7c	126.3c	98.9c	85.3c	73.8c	0.023b
N3	458.8d	182.7d	155.7d	128.6d	115.5d	0.041b

*) different letters mean different at LSD 5%

During 14 weeks incubation, the cumulative amount of mineral N released in soils amended with SY were always greater than those amended with RS (Figure 1). This can be related to the lower ratio of C/N, lignin/N, polyphenol/N in SY than RS residue. The higher quality of SY than RS residue resulted in the higher kN, meaning that N mineralized from SY faster than from RS residue. After 14 weeks incubation, 3734.86 mg N kg⁻¹ of soil and 4352.34 mg N kg⁻¹ of soil released respectively from RS and SY residues. Addition of ¹⁵N concentration increased total mineral N released, since the treatments increased total N content in the crop residues applied to the soils.

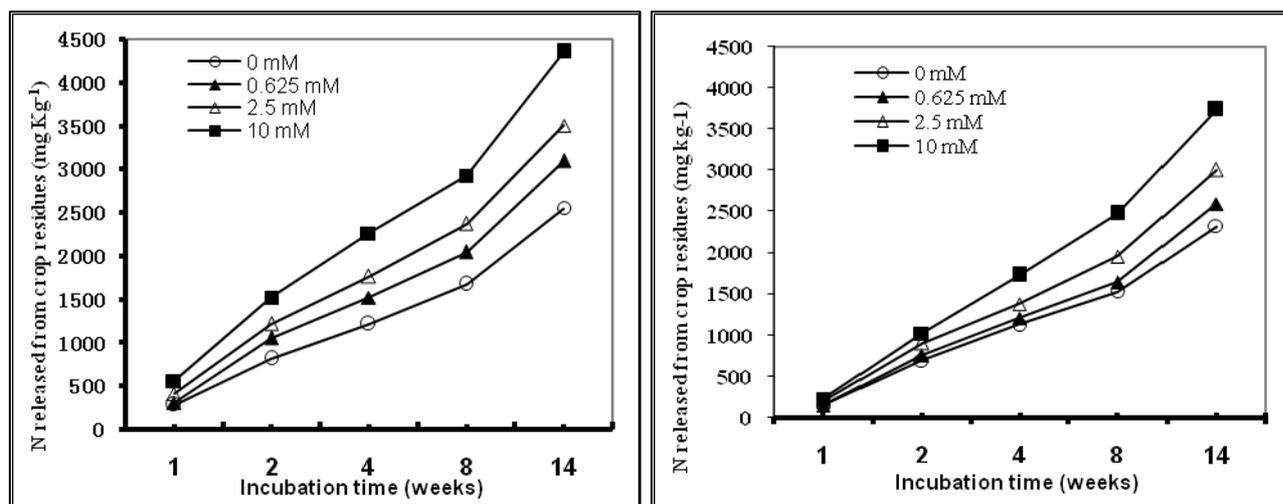


Figure 1. Cumulative amount of mineral N in soil amended with crop residue of soybean (left) and rice straw (right) with different ¹⁵N concentration under non-leaching incubation

3.2.2 Mineralization Rate (kN)

The N mineralization rate (kN) increased as the ¹⁵N concentration increased. Although there was insignificant difference between the N treatments (Table 2), increasing ¹⁵N concentration from 0 to 10 mM in the soil solution tended to speed up mineralization about seven times faster than without ¹⁵N. The mineralization rate (kN) of RS residue (0.004 per week to 0.029 per week) and SY residue (0.006 per week to 0.041 per week), indicated that SY residue released mineral N about two times faster than RS residue.

3.2.3 Factors Affecting % Mineral N and Mineralization Rate (kN)

Regression analyses showed that the amount of mineral N released in the soil did not show regular pattern with the initial composition of the crop residues (Table 3). It was recorded that at 1-2, 4, 8 and 14 weeks incubation, the amount of mineral N had the largest R^2 with respectively C:N ratio (97.5% and 88%), polyphenol (72.5%), % ^{15}N abundance (88.9%) and lignin (68%) content. However, kN values and % ^{15}N abundance had the largest coefficient of determination ($R^2=99.6\%$), followed by polyphenol/N ($R^2=94.1\%$) and (lignin+polyphenol)/N ratio ($R^2=66.6\%$), meaning that mineralization rate could be due to these components in the crop residues. Plant residues with high C/N ratio, lignin and polyphenol contents decompose and release nutrients slowly (Tian, Kang, & Brussaard, 1992; Achakzai & Bangulzai, 2006).

The regression between the kN values and initial composition of crop residue of RS and SY with different ^{15}N concentrations showed significant relationships between N release rate constant (kN) and the initial % ^{15}N abundance, %N, %C, C/N ratio; %lignin, %polyphenol, polyphenol/N ratio, lignin/N ratio and (lignin + polyphenol)/N ratio, which indicated the importance of these parameters in predicting N release from the crop residues. Tian et al. (1992) reported that rate of N release from prunings of various legume hedgerow trees was significantly related N, lignin, and polyphenol contents of the prunings; the release of N increased with increasing N contents and decreased with increasing polyphenol and lignin content.

Table 3. Coefficient of determination (R^2) for linear regressions between initial composition of crop residue of RS and SY with different ^{15}N concentration and %N released from the crop residue as mineral N in soil and release rate constant under non-leaching incubation

initial composition of crop residue of RS and SY with different ^{15}N concentration	Coefficient of determination (R^2)					N released rate constant, kN (week ⁻¹)
	%N released from crop residue as mineral N in soil incubation time (weeks)					
	1	2	4	8	14	
% ^{15}N abundance	0.604*	0.828**	0.459*	0.889**	0.603*	0.996*
N (%)	0.589*	0.764**	0.480*	0.804**	0.587*	0.574*
C (%)	0.572*	0.693**	0.421*	0.794**	0.602*	0.869**
Polyphenol (%)	0.698*	0.538*	0.725**	0.502*	0.645*	0.576*
Lignin (%)	0.326	0.394	0.693*	0.688*	0.680*	0.508*
C/N ratio	0.975**	0.880**	0.120	0.265	0.102	0.582*
Polyphenol/ N ratio	0.496*	0.800**	0.449*	0.860**	0.610*	0.941**
Lignin / N ratio	0.492*	0.599*	0.160	0.47*	0.211	0.585*
(Lignin+Polyphenol) / N ratio	0.519*	0.656*	0.194	0.540*	0.259	0.660*

* significant at $P<0.05$, ** significant at $P<0.01$

3.3 Soil Microbial Biomass N

The different ^{15}N concentrations in RS and SY residues significantly affected the soil microbial biomass N (Figure 2). Increasing of ^{15}N concentrations increased microbial biomass N. The microbial biomass N content in SY was always higher than RS during 14 weeks incubation. Lovell, Cerny, & Balik (1998) stated that variation in microbial biomass N are results of the complex of physical, chemical, biological and anthropogenic factors, and a result of variability in these factor, which along the short generation time microorganisms, is reflected in the large fluctuation of microbial biomass content. Fontaine, Bardoux, Benest, Verdier, Mariotti, & Abbadie (2004) found that supply of cellulose highly stimulated the microbial growth increase of total biomass approximately 350 to 700 mg C kg⁻¹. The increase mainly occurred during the decomposition of cellulose.

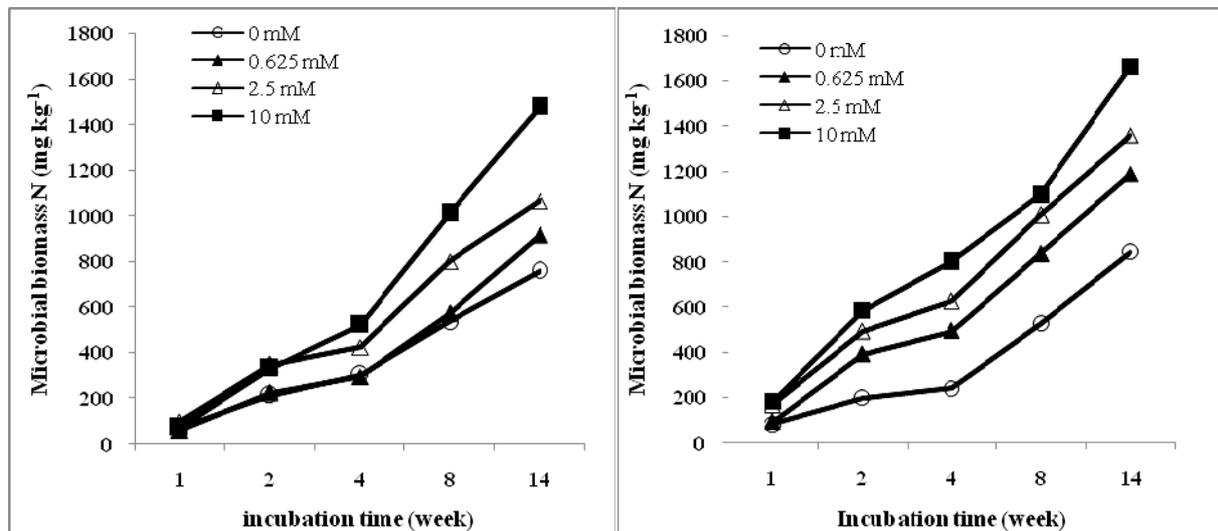


Figure 2. Amount of microbial biomass N in soil amended with crop residue of soybean (left) and rice straw (right) with different ^{15}N concentration under non-leaching incubation

4. Conclusion

Increasing ^{15}N concentration in the nutrient solution (0 to 10 mM) increased total N (0.7 to 1% in RS and 0.9 to 2.2% in SY) but decreased C (3.4 to 6.5% in RS, and 0 to 5.2% in SY), lignin (4.8 to 19.3% in RS, and 0.6 to 4.3% in SY) and polyphenol (0.8 to 2% in RS, and 2.8 to 3.4% in SY) content in the crop residues. The lignin and polyphenol contents, however, were more significantly different between the crop species than between the different concentration of ^{15}N supplied. The results suggested that ^{15}N supply may increase the quality of the crop residues, as indicated by decreasing of C/N, lignin/N, polyphenol/N ratios. As consequences, the total and cumulative amount of mineral N released as well as the microbial biomass N were higher at higher ^{15}N supply. SY residue released N about two times faster than RS residue, and the amount N released increased with increasing supply of the N concentration. At the 10 mM N supply, RS and SY residues released respectively 3734,857 mg kg⁻¹ and 4352.34 mg kg⁻¹ cumulative amount of mineral N with the mineralization rate about seven times faster than without ^{15}N in the solution. Differences in the % N mineralized and its rate were determined by the quality of the crop residues, especially N, lignin and polyphenol content.

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