Nitrogen mineralization by maize from previously added legume residues following addition of new legume residues using ^{15}N labelling technique

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Abstract

Although organic matter is applied repeatedly over crop cycles, the relationship between quality of legume residues and N release has been generally determined over single crop cycles (8 to10 weeks). To evaluate the N recovery by maize (*Zea mays* L.) from legume residues over two planting cycles, freshly chopped (1 to 2 mm) and ^{15}N labeled residues of *Centrosema pubescens* Benth. and *Calopogonium mucunoides* Desv. were applied at the rate of 100 mg $N{\cdot}kg^{-1}$ soil in a glasshouse study. After the harvest of the maize plants (8 weeks), soil in the pots was split into two halves, one of which recieved a further addition of unlabelled legume residues (100 mg $N{\cdot}kg^{-1}$ soil), while the other did not. Recovery of N from the residues ranged from 12 to 44% , most of which was from the first application. Repeated addition of unlabelled N rich and low pholyphenol *Centrosema* residues, led to a stimulation effect on the previously applied ^{15}N labelled legume residues, whereas new addition of N poor, high polyphenol legume residues led to a retardation effect. There was no compensation for initial N release from N poor, high polyphenol legume residues at second application.

Keywords: *Calopogonium mucunoides*, *Centrosema pubescens,* ^{15}N recovery, retardation

Introduction

It is well known that N release from soil applied organic matter depends on the physical and chemical characteristics of the substrate – especially its N, lignin and polyphenol contents, the environmental conditions, and the community of decomposer organisms (Handayanto et al., 1994; Heal et al., 1997; Kumar, 2008). The relationship between chemical characteristics and decomposition of crop residues has been mainly determined using cumulative decomposition or N release data over relatively short periods of time (8 to 10 weeks), or over single crop cycles. Less attention has been paid on the long-term N mineralization patterns. Yet, this may be of practical interest as low quality organic matter (low N, high pholyphenol contents) that decompose slowly contribute to accumulation of soil organic matter. In most low external input agriculture systems in Indonesia, organic matter is applied before planting, and after harvest also to benefit the subsequent crop. New organic matter application, however, may alter the decomposition rates of previously applied materials (Jenkinson, 1981). Ehaliotis et al. (1998) indicated that application of N rich legume residues to soil that was previously applied with ^{15}N labelled maize residues having high C: N ratio significantly increased the recovery of the maize residue N during five subsequent plantings. This paper reports results of a study on N mineralization of legume residues of different quality over two planting sequences involving maize (*Zea mays* L.) crop in Indonesia using the ^{15}N labelling method. Our objectives were to evaluate the stimulatory or retardation effects, if any, of repeated application of organic matter on N recovery from previously applied legume residues. Such information

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is generally not available in the Indonesian context and, if available, would enable the farmers to effectively manage the N economy of the maize crop more efficiently.

Materials and Methods

Production of ^{15}N labelled legume residues

^{15}N labelled *Centrosema pubescens* Benth. and *Calopogonium mucunoides* Desv. residues were obtained by growing the legumes in 30 cm diameter plastic pots containing 6 kg of quartz sand in a glasshouse at the Faculty of Agriculture, Brawijaya University, Malang, Indonesia for three months (24 May 2005 to 27 August 2005). Each species was grown under two N concentrations, i.e., 1.25 m*M*, and 10.0 m*M* N, supplied as $K^{15}NO_3$ 5% atom excess, in solution at the rate of 400 ml $pot^{-1} \cdot day^{-1}$. Other nutrients (Ca, K, P, S, Mg, Cl, Fe, Mn, Zn, B, Mo and Co) were also supplied in solution (Handayanto et al., 1995) to ensure that they would not be limiting. After three months, the aboveground materials of the plants were pruned and oven dried at 60°C for 72 h and analyzed for polyphenol, N, C, and protein binding capacity. Polyphenols were extracted in hot 50% aqueous methanol and determined colorimetrically using the Folin-Denis method (Anderson and Ingram, 1992). Protein binding capacity of polyphenol in the legume residue extract was measured by a reaction with bovine serum albumin (Dawra et al., 1988). Carbon was determined by the Walkley and Black method, and the N concentration was determined by Kjeldahl method (Keeney and Nelson, 1982). Nitrogen concentration and ^{15}N enrichment of the legume residues were determined using a Micromass 622 (UK) mass spectrometer at the National Nuclear Agency of Indonesia, Jakarta. Results showed that chemical quality parameters of the four legumes varied depending on N concentration supplied during plant growth (Table 1). When N was added in substantial amounts during the plant growth, legume N content increased but the total polyphenol content and protein-binding capacity of polyphenols decreased.

Pot Culture Experiment 1

Freshly chopped (1 to 2 mm) legume residues (*Centrosema* 1.25 mM N, *Centrosema* 10.0 mM N, *Calopogonium* 1.25 mM N, and *Calopogonium* 10.0 mM N; Table 1) were incorporated into 2 kg of soil in a 20 cm diameter plastic pot at the rate of 100 mg $N \cdot kg^{-1}$ soil. The soil used for this study was air dried and ground (< 2mm) top soil (0 to 15 cm) collected from the dry land area of South Malang, East Java, Indonesia. It is a Typic ustorthent and has the following characteristics: pH (H_20) 7.51; 1.7% organic C; 0.14% total Kjeldahl N; 20 $mg \cdot kg^{-1}$ P (Bray II), 6.8 $cmol^{(+)} \cdot kg^{-1}$ cation exchange capacity; 0.4, 0.3, 2.9 and 1.1 $cmol^{(+)} \cdot kg^{-1}$ of Na^+, K^+, Ca^{2+} and Mg^{2+}, respectively in ammonium acetate (pH 7); and 74% sand, 20% silt and 14% clay. The four treatments were randomized in a complete block design with four replicates. All pots received basal fertilizers consisting of 22 mg P kg^{-1} soil as superphosphate, and 50 mg K kg^{-1} soil as KCl. Three

Table 1. Chemical composition of organic matters (legume residues) used for the study.

Legume residues	Composition			
	N (%)	Polyphenol (%)	C:N ratio	PBC[1] (µg $BSA \cdot mg^{-1}$)
Centrosema-1.25 mM N	2.65	5.90	16.5	110
Centrosema-10.0 mM N	3.95	4.26	11.4	87
Calopogonium-1.25.mM N	2.09	6.20	17.6	315
Calopogonium-10.0 mM N	2.86	2.30	14.5	125

[1]PBC = protein-binding capacity of polyphenols.

pre-germinated seeds of maize (local variety) were planted in each pot on 5 September 2005. Pots were irrigated daily to keep the soil moisture content at the approximate water holding capacity. At eight weeks, maize plants were harvested, shoots and roots were separated, and after oven-drying at 60°C for 72 h, weighed and ground to pass through a 1 mm sieve. N content and ^{15}N enrichment of the harvested maize shoots and roots were determined as described above. The soil samples were extracted with 2 *M* KCl and amounts of mineral N in the KCl soil extract were determined using the Kjeldahl distillation method. Recovery of legume residue N by maize was calculated following the direct ^{15}N recovery method,

$$\% \text{ N recovery} = \frac{R_{maize} \text{ x Total maize N}}{R_{legume\ residue} \text{ x Legume residue N added}} \text{ x } 100$$

where R is atom % ^{15}N excess

Pot Culture Experiment 2

Two kilograms of the soil originally used for each treatment in the first experiment was air dried, and subdivided into two halves and placed in separate pots (10 cm diameter). One set of the pots was used to evaluate the effects of addition of new legume residues (unlabelled, supplied with 10 *mM* N KNO_3 for three months from 24 May 2005 to 27 August 2005) on N mineralization of previously added legume residues. The remaining pots were used to evaluate the residual effects of the ^{15}N-labelled legume residues previously added to the soil on N uptake by maize. The pots (four treatments with addition of new legume residues and four treatments without its addition) were arranged in a randomized complete block design and replicated four times. The experimental procedures for the first and second experiment were similar. Difference between N recovery of treatments with or without addition of new unlabelled legume residues at the second experiment (% N recovery with addition of new legume residues – % N recovery without addition of new legume residues), reflects the stimulatory or retardation effect on N recovery by the maize crop.

Results and Discussion

Recovery of legume residue N by maize

Cumulative recovery of ^{15}N from legume residues by the two maize crops ranged from 11.3% (*Calopogonium* grown under 1.25mM N concentration) to 43.8% (*Centrosema* grown under 10.0 mM N concentration) (Fig.1). Most of the N recovery occurred during the first crop cycle (9.9 to 35.3%), while its recovery during the second planting cycle was only 0.3 to 5.1%. Sisworo et al. (1990) also reported relatively small amount of legume residue N recovery by crops for long terms. There was also no evidence to show that slow decomposing organic matter improved N mineralization potential of soil organic matter in 16 weeks.

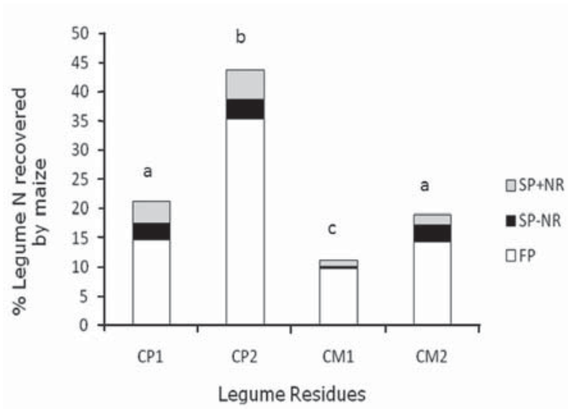

Figure 1. Percentage legume N recovered by maize during two crop plantings. FP = first planting, ^{15}N labelled legume residue was added. SP+NR = second planting: new legume residue was added after FP (8 weeks). SP-NR = no added legume residues after FP. CP = *Centrosema pubescens*; CM = *Calopogonium mucunoides*, grown under 1.25 mM N (1) and 10.0 mM N (2) concentrations. Letters on bars are comparison of total N recovery (LSD, á = 5%).

Although organic matter that released high amount of N at first crop cycle also released high amount of N at second planting, at the end of second planting, there was no difference in N release pattern. For example, *Calopogonium* (1.25 mM N) residue that decomposed slowly during the first planting still released small amount of N during the second crop (Fig. 2). It is probable that during 16 weeks of organic matter decomposition, there would only be a small amount of N released from low quality legume residues.

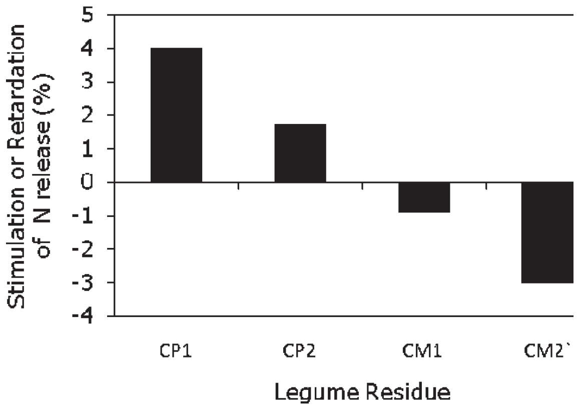

Figure 2. Effect of addition of new legume residues on ^{15}N release from previously added legume residues at second planting. CP = *Centrosema pubescens*; CM = *Calopogonium mucunoides*, grown under 1.25 mM N (1) and 10.0 mM N (2) concentrations.

Stimulation and retardation of N mineralization

Addition of new unlabelled *Centrosema* during the second cycle increased N uptake by maize and recovery of *Centrosema* ^{15}N previously added at first planting (Fig. 2). Ehaliotis et al. (1998) also showed a similar pattern, that addition of N rich legume residues increased recovery of residue N by maize. This positive interaction could be interpreted as priming effect or added nitrogen interactions. In general, priming effect may be stimulation or retardation of decomposition and recovery of N following addition of fresh organic matter. Vanlauwe et al. (1994) reported a priming effect of 7 to 10% from maize residue fractions due to the increase breakdown of recalcitrant organic matter, which in turn, was explained based on the increase activity of catabolic enzymes from the newly added substrate.

In contrast to the positive interaction of *Centrosema,* addition of new *Calopogonium* residue during the second planting cycle decreased N recovery from labelled *Calopogonium* by maize. This negative effect of legume residue addition on the release of ^{15}N from previously added legume residues can be due to complexation of protein with polyphenols from new organic materials, or the increase of stabilization of ^{15}N organic matter into soil organic matter. Palm and Sanchez (1991) have showed negative effect of polyphenol on N released from organic matter and analysis of protein binding capacity of polyphenol could be used to characterize the amount of active polyphenol that bind protein (Handayanto et al., 1994). Handayanto et al. (1997) also reported binding of N from high quality legume tree pruning by polyphenols in treatments of direct mixing. Therefore, application of polyphenol-rich legume residues resulted in a stronger N binding that led to more N left in the soils and relatively smaller amounts of N available for plant uptake, at least in the short term.

Although this study indicated the occurrence of priming effect, the maximum effect was only less than 5% (positive or negative) from the initial added N that was recovered after sixteen weeks. Handayanto et al. (1997), however, showed when organic matters of different quality were mixed, interaction of quality would occur, especially during the first stage of decomposition process. Ehaliotis et al. (1998) also reported that the highest increase of recovery of N from maize residues when N rich legume resides was added along with maize residues, compared to when legume residue was solely added at final planting.

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