

Full Length Research Paper

# Recovery of $^{15}\text{N}$ labelled rice and soybean residues by maize grown on an inceptisol of Malang, Indonesia

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A study was conducted to evaluate the N recovery of  $^{15}\text{N}$  labelled crop residues (rice straw=RS; soybean=SY) with  $^{15}\text{N}$  isotope dilution technique on growth of maize. The  $^{15}\text{N}$  labelled crop residue from the rice and soybean crops that were grown in glass-house under three  $^{15}\text{N}$  concentrations, that is, 0.625 mM N (N1), 2.5 mM N (N2) , and 10 mM N (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 h and analyzed for polyphenol, lignin, N, C, C/N and %  $^{15}\text{N}$ -abundance. The seven treatments (six types of crop residues and one treatment with no added residues) were arranged in a completely randomized block design with four replicates. The maize was plant pots containing 10 kg of soil and placed in a glass-house. The varying supply of  $^{15}\text{N}$  concentrations resulted in varying quality of crop residues. The amounts of mineral N in the soil supplied with crop residues were significantly greater than in the control at 2, 4 and 8 weeks. The amounts of mineral N in soils amended with residues of SY and RS with a 10 mM N supply were significantly ( $P<0.05$ ) greater than those grown with 2.5 mM and 0.625 mM N supplies during the incubation time. The N recovery by maize shoot from residues with 10.0 mM N was greater (80.86% on average) than that from residues with 2.5 mM N (70.06% on average) and that from residues with 0.625 mM N (58.43% on average). On average, the %N recovery of crop residue N by maize estimated by the  $^{15}\text{N}$  method was 10.73% more than that estimated by the difference method.

**Key words:** Soybean,  $^{15}\text{N}$  labelled, N recovery, rice straw, N mineralization.

## INTRODUCTION

The N-use efficiency from crop residues depends on the mineralization rate and thus on the time when they are available relative to crop requirements. If the crop residues have low N, high lignin and high polyphenol contents they are mineralized slowly thus little crop residues N applied will be used by the succeeding crop although it remains in the soil. On the contrary, crop residues having high N, low lignin and low polyphenol contents which mineralize rapidly can supply a large amount of nutrients during the early periods of crop growth, but may not contribute much to the maintenance of soil organic matter and soil physical conditions. In order to have a better synchronization of N

release and crop N demand, one should be able to control the rate of N release by changing the quality of the prunings. Results of previous study reported by Sholihah et al. (2012) showed that crop residues quality can be altered by manipulation of soil fertility where the crops are to be grown. The efficiency of fertilizer N can be enhanced by synchronizing fertilizer application with plant demand (Keeney and Nelson, 1982). Synchronizing of N mineralization from crop residue, fertilizer N application time, and subsequent crop demand for N can improve N use efficiency of crops grown (Reeves et al., 1993).

In most studies of crop residue application to the soil, the amount of mineral N released from the crop residues recovery in the soil was obtained by comparing the value observed to untreated soil. However, several recent studies have used the soil mineral  $^{15}\text{N}$  content or the

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**Table 1.** Chemical composition of crop residues used for the study (Sholihah et al., 2012).

Residues <sup>15</sup> N concentrations	C (%)	N (%)	C/N ratio	<sup>15</sup> N abundance (%)	Lignin (%)	Polyphenol (%)
Rice Straw (RS) *)						
N1	35.46 <sup>a</sup>	1.70 <sup>a</sup>	21.00 <sup>b</sup>	0.44 <sup>a</sup>	18.76 <sup>b</sup>	5.98 <sup>b</sup>
N2	35.08 <sup>a</sup>	1.94 <sup>ab</sup>	18.00 <sup>a</sup>	1.24 <sup>b</sup>	5.34 <sup>a</sup>	5.34 <sup>a</sup>
N3	32.38 <sup>a</sup>	2.04 <sup>b</sup>	16.00 <sup>a</sup>	3.14 <sup>c</sup>	4.24 <sup>a</sup>	4.74 <sup>a</sup>
Soybean (SY) *)						
N1	39.03 <sup>b</sup>	3.61 <sup>a</sup>	10.88 <sup>c</sup>	1.29 <sup>b</sup>	9.96 <sup>b</sup>	3.21 <sup>b</sup>
N2	32.25 <sup>a</sup>	3.78 <sup>a</sup>	8.63 <sup>b</sup>	2.38 <sup>c</sup>	9.82 <sup>b</sup>	2.94 <sup>a</sup>
N3	31.58 <sup>a</sup>	4.95 <sup>b</sup>	6.41 <sup>a</sup>	4.34 <sup>d</sup>	6.28 <sup>a</sup>	2.63 <sup>a</sup>

\*) N1 = 0.625 mM <sup>15</sup>N concentration ; N2 = 2.5 mM <sup>15</sup>N concentration; N3 = 10 mM <sup>15</sup>N concentration; different letters mean different at LSD 5%.

recovery of crop residues thus isotopic studies using <sup>15</sup>N to estimate crop N uptake from organic N input (Azam et al., 1985; Safwat et al., 2002; Miller et al., 2009).

This paper reports the effects of manipulation of crop residues quality as reported by Sholihah et al. (2012) on N mineralization of the crop residues and uptake of N released by maize. Estimation of N mineralized from the crop residues as measured by different method was compared with that estimated by direct <sup>15</sup>N recovery method.

## MATERIALS AND METHODS

### Crop residues

Crop residues used for this study were <sup>15</sup>N labelled rice (RS) and soybean (SR) residues that were obtained by growing the crops in 30 cm diameter plastic pots containing 5 kg of quartz sand medium supplied with 0,625 mM N (N1), 2,5 mM N (N2), and 10 mM N (N3) supplied as CO(<sup>15</sup>NH<sub>2</sub>)<sub>2</sub> 10% atom excess in a complete nutrient solution (Sholihah et al., 2012). The crops were grown for 2 months (5 June 2010 to 5 September 2011) in a glass-house of the Faculty of Agriculture, Brawijaya University, Indonesia (7°.48'.50" S and 112°.37'41" E). At harvest, the crops were pruned and oven dried at 60°C for 72 h for polyphenol, lignin, N, C, C/N and % <sup>15</sup>N-abundance analyses. The polyphenol was extracted in hot 50% aqueous methanol and determined colorimetrically using the Folin-Denis method (Anderson and Ingram, 1992). Lignin was determined as acid-detergent lignin (Goering and Van Soest, 1970). The C concentration was determined by Walkley and Black method, and the N concentration was determined by Kjeldahl method (Keeney and Nelson, 1982). <sup>15</sup>N enrichment of the residues was determined using a Micromass 622 (UK) mass spectrometer at the National Nuclear Agency of Indonesia, Jakarta. Chemical quality parameters of two crop residues used for this study are presented in Table 1.

### Maize growth experiment

Fresh chopped (1 to 2 mm) of each residue was incorporated into 10 kg of soil in a 15 cm diameter plastic pot at the rate of 20 t crop residues ha<sup>-1</sup> (90.2 g crop residues kg<sup>-1</sup> of soil). The seven treatments (six types of crop residues and one treatment with no added residue) were arranged in a completely randomized block design with four replicates. All pots received basal fertilizers consisting of 28 mg P kg<sup>-1</sup> as SP36, 25 mg K kg<sup>-1</sup> as K<sub>2</sub>SO<sub>4</sub> and 2.5 mg Zn kg<sup>-1</sup> as ZnSO<sub>4</sub>. Soil moisture content was adjusted and maintained at the approximate water holding capacity. Five pre-germinated seeds of maize were planted in each pot and thinned to one plant after 1 week. The experiment was conducted for 8 weeks. At 1 to 8 weeks after planting, total leaf area (green leaves) was determined by measuring the width and length of the leaves. The measured (width × length) of the leaves were then transformed to leaf area using a standard curve. The standard curve was obtained by plotting (width × length) of 10 maize leaves (obtained from separate pots) against their actual leaf area. At the same time, soil samples were collected using a 0.5 cm diameter stainless steel core. At harvest (8 weeks after planting), maize shoots were harvested at the height of 1 cm above soil surface. Roots were separated manually from the soil by sieving and rinsing with water. The shoots and roots were then oven dried at 60°C for 72 h, weighed and ground to pass through a 1 mm sieve. The soil samples were then extracted with 2 M KCl and amounts of mineral N in the KCl soil extract were determined using the Kjeldahl distillation method. Amounts of mineral-N in the soil were determined by Kjeldahl method (Keeney and Nelson, 1982). N concentration and <sup>15</sup>N enrichment of the harvested shoots and roots were determined using a Micromass 622 (UK) mass spectrometer at the National Nuclear Agency of Indonesia, Jakarta.

Recovery of pruning N by maize was estimated using the difference method and the direct <sup>15</sup>N method (Cadisch et al., 1998) as follows;

$$\text{Difference method \%N recovery} = \frac{N_{fr}}{AN_{fr}} \times 100$$

Where  $N_{fr}$  = N in maize derived from crop residues and was estimated as the difference in the N yield of maize which crop residues as compared to the control with no added N, and  $AN_{fr}$  = the amount of crop residue N added.

(ii)  $^{15}\text{N}$  recovery method:

$$\%N \text{ recovery} = \frac{R_{maize} \times \text{Total maize N}}{R_{crop \text{ residue}} \times \text{Cop residue N added}} \times 100$$

Where R = atom %  $^{15}\text{N}$  excess.

Microbial biomass N at the end of experiment was measured using chloroform fumigation and extraction method (Brookes et al., 1985). N biomass content was determined using Kjeldahl method with a constant value  $k_{EN} = 0.45$  (Jenkinson, 1988). N recovery in the microbial biomass was calculated using a method used by Ehaliotis et al. (1998).

## RESULTS AND DISCUSSION

### Effect of varying N supply on quality of the crop residues

The results showed that chemical quality parameters of the residues varied depending on N concentration supplied during plant growth (Table 1). In both crop residues, the N content increased with increasing N concentration in the nutrient solution. In contrast, organic carbon, polyphenol and lignin content decreased. Lignin and polyphenol content were significantly affected by the 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 N supplied. Polyphenols were much reduced when N supply in both species were limited. Increasing concentration of 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.

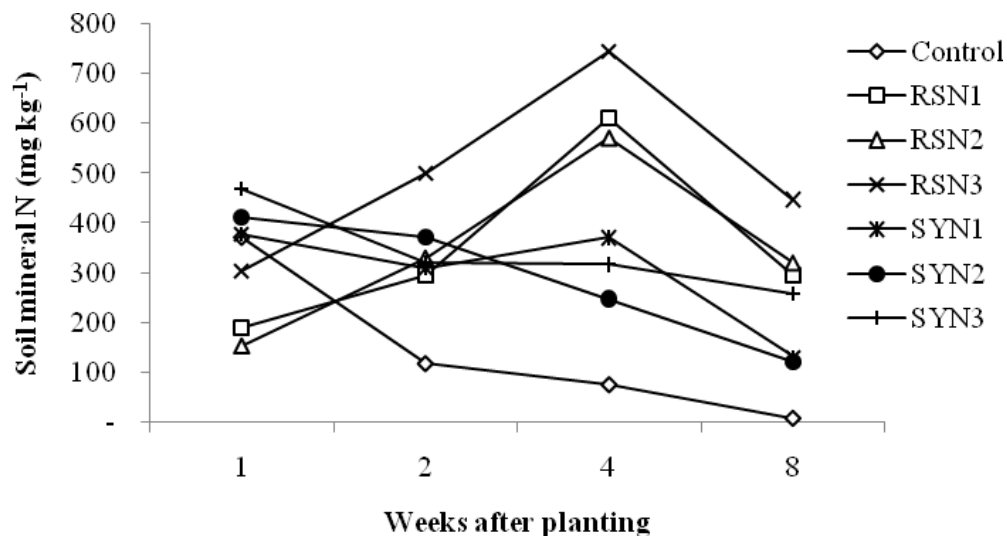
Increasing of N content and decreasing of organic carbon, lignin, and polyphenol content, consequently resulted in the lower ratio of C/N, lignin/N, polyphenol/N. These ratios are commonly used to determine the quality of the organic matter, that is, the higher ratio means the higher quality. The results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

### Soil mineral-N, maize growth and dry matter yield of maize

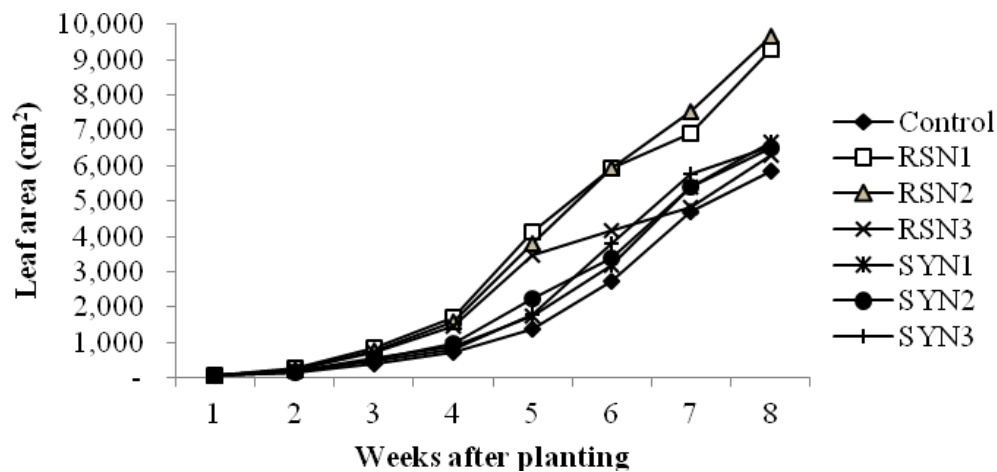
The amounts of mineral N in the soil supplied with crop residues were significantly greater than in the control at 2, 4 and 8 weeks (Figure 1). At 2 weeks after planting, the amounts of mineral-N in soil receiving crop residues ranged from 323.22 mg kg<sup>-1</sup> soil (SYN3) to 499.88 mg kg<sup>-1</sup> soil (RSN3). However, the amounts of soil mineral N in soil amended with either SR or RS, except for SYN2, increased after 2 weeks (Figure 1). The amounts of mineral N in soils amended with residues of SY and RS with a 10 mM N supply were significantly ( $P < 0.05$ ) greater than those grown with 2.5 mM N and 0.625 mM N supplies during the incubation time. High soil mineral N with application of higher levels of N has also been reported by Rozas et al. (2004) who reported that mineral N was significantly for the highest N rates applied at six leaf stage at the end of the growing season and indicated that high N rates applied at this stage exceeded crop requirements. In this study, the amounts of soil mineral N in the control treatment decreased from 372.86 to 169.79 mg kg<sup>-1</sup> throughout the experimental period and application of crop residues increased the mineral N concentration in the soil. This mineral N flush addition of crop residue has been reported in other studies (Kuzyakov et al., 2000; Hartz et al., 2000; Douxchamps et al., 2010; Roy et al., 2011).

At every week, maize growth as measured by leaf area generally reflected the availability of soil mineral N. Growth was vigorous during 8 weeks in the treatments containing 10 mM N concentration (Figure 2). Addition of SYN2 (soybean residue supplied with 2.5 mM N) produced significantly ( $P < 0.05$ ) greater leaf area than all crop residues at final harvest (8 weeks).

The weight of total maize dry matter (shoots and roots) in all crop residue treatments was greater than in the control (Figure 3). Application of crop residues, however, did not significantly increase root dry weights. Treatment with addition of crop residue produced maize dry matter of



**Figure 1.** Soil mineral N amended with crop residue of soybean (SY) and rice straw (RS) with different <sup>15</sup>N concentration during growth by maize (8 weeks).



**Figure 2.** Total leaf area of maize amended with crop residue of soybean (SY) and rice straw (RS) with different <sup>15</sup>N concentration.

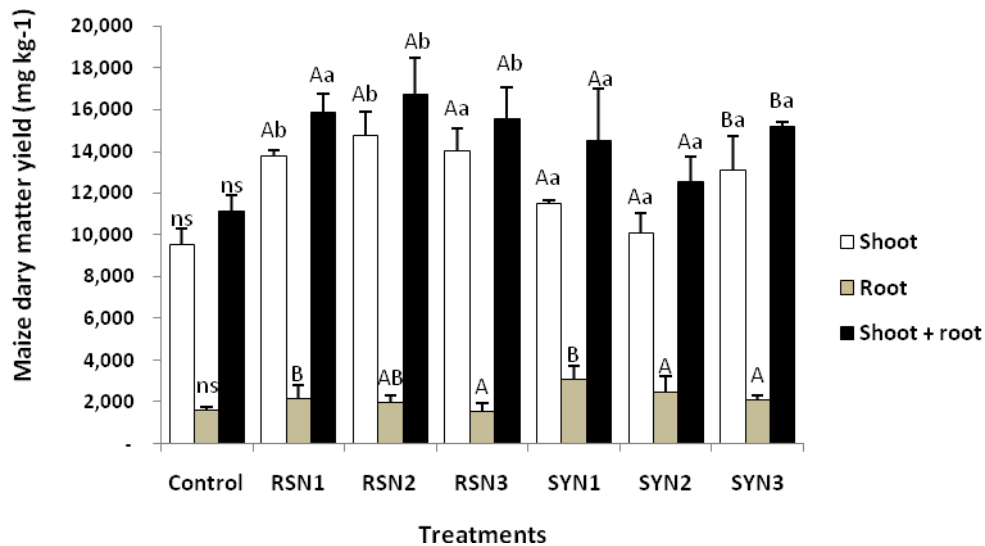
43% (RSN1), 50% (RSN2), 40% (RSN3), 31% (SYN1), 13% (SYN2) and 36% (SYN3) above that produced in the control.

Nitrogen uptake by maize was improved by application of crop residues (Figure 4). Compared to the control treatment, application of crop residues increased N uptake by maize shoot from 53% (RSN1) to 280% SYN2). Jung et al. (1972) reported that N content in grain and tissue increased significantly with increasing rate of applied N. Furthermore, they also reported that N concentration in

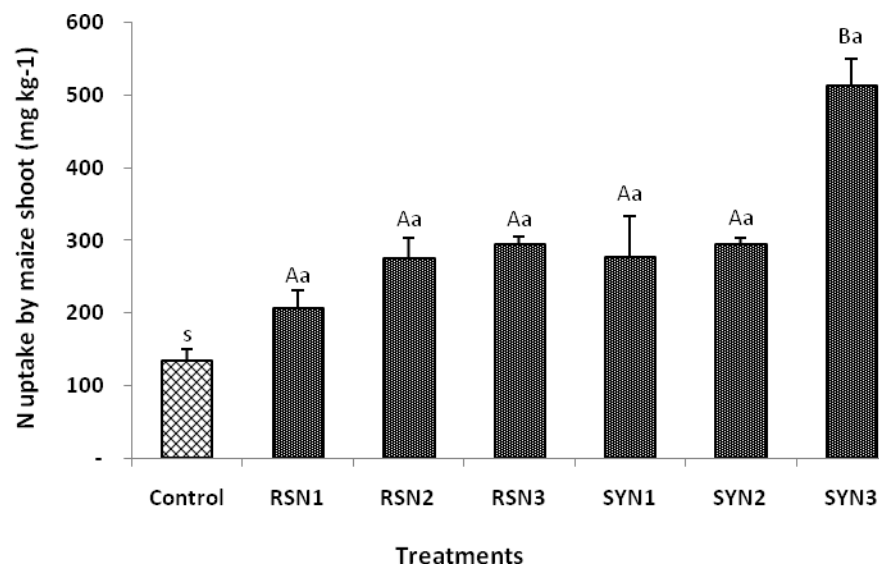
grain and tissue generally increased as the time of N application was delayed.

Application of crop residue with different <sup>15</sup>N concentration clearly demonstrated the important role of N content crop production. The different quality of crop residue influenced soil mineral N, maize growth, dry matter yield and N uptake.

Soybean residue with high N supply (SYN3) contributed the highest N uptake due to a high quality and large amount of N released. In addition, the



**Figure 3.** Dry matter yield of maize amended with crop residue of soybean (SY) and rice straw (RS) with different <sup>15</sup>N concentration during growth by maize (8 weeks). Different letters mean different at LSD 5%, capital letters between the concentrations <sup>15</sup>N=N and lower case letter between the residues. ns= no significant.



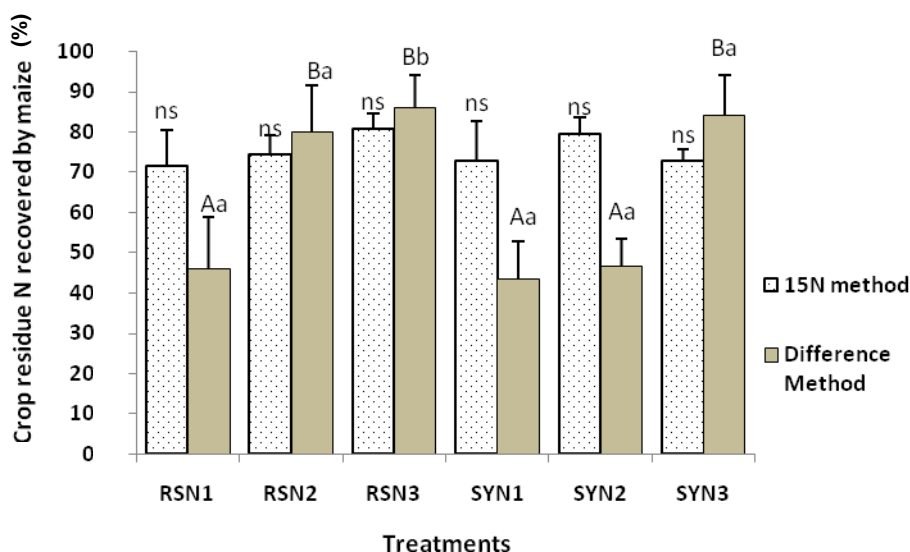
**Figure 4.** N uptake by maize amended with crop residue of soybean (SY) and rice straw (RS) with different <sup>15</sup>N concentration during growth by maize (8 weeks). Different letters mean different at LSD 5%, capital letters between the concentrations <sup>15</sup>N=N and lower case letter between the residues. s = significant.

crop residue application may favour the activity of microorganism, resulting in a greater N uptake by maize.

#### Recovery of N by maize

The N recovery by maize shoot from residues with 10.0

*mM* N was greater (80.86% on average) than that from residues with 2.5 *mM* N (70.06% on average) and that from residues with 0.625 *mM* N (58.43% on average). The contribution of crop residue N to maize plant N estimated by the difference method ranged from 43.6% (SYN2) to 85.99% (RSN3), while that estimated by the <sup>15</sup>N recovery method ranged from 71.36% (RSN1) to 80.64% (RSN3)



**Figure 5.** The recovery of N by maize amended with crop residue of soybean (SY) and rice straw (RS) with different  $^{15}\text{N}$  concentration during growth by maize (8 weeks) estimated by the difference method and  $^{15}\text{N}$  method. Different letters mean different at LSD 5%, capital letters between the concentrations  $^{15}\text{N}=\text{N}$  and lower case letter between the residues. ns= no significant.

**Table 2.** Coefficient of determination ( $R^2$ ) for linier regressions between initial composition of crop residue of RS and SY with different  $^{15}\text{N}$  concentration and %N uptake by maize after 8 weeks.

Initial composition of crop residues	N uptake by maize				
	Coefficient of correlation (r)	Coefficient of Determination ( $R^2$ )			
		Linier	Sig	Inverse	Sig
N (%)	0.808*	0.653	0.052	0.497	0.118
C: N ratio	-0.755*	0.570	0.083	0.753	0.025
Polyphenol (%)	-0.686*	0.470	0.133	0.549	0.092
Polyphenol:N ratio	-0.672*	0.451	0.144	0.684	0.042

\*.Correlation is significant at the 0.05 level (1-tailed).

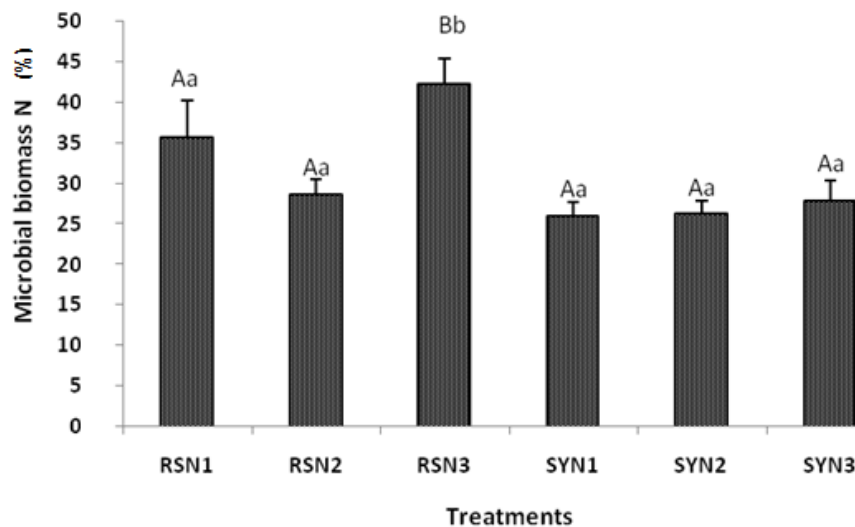
(Figure 5). On average, the %N recovery of crop residue N by maize estimated by the  $^{15}\text{N}$  method was 10.73% greater than that estimated by the difference method. This difference was probably associated with mineralization-immobilization-turnover and other pool substitution processes involving added crop residue  $^{15}\text{N}$  (Jenkinson et al., 1985; Fox et al., 1990). The patterns of N recovery between the two methods, however, were almost similar.

Douxchamps et al. (2010) reported that combined total  $^{15}\text{N}$  recovery in maize and soil at harvest was highest for the canavalia residue treatment with 98% than mineral fertilizer treatment with 83%. Vanlauwe et al. (1997) reported 9% *Leucaena* N recovery by maize. Toomsan et al. (1995) reported 15 to 23% soybean N recovery in rice and 8 to 22% groundnut N recovery in rice. Dourado-Neto

et al. (2010) reported an average N recovery from residues of 7% in crops and 71% in soil.

Increasing  $^{15}\text{N}$  concentration increased %N recovery by maize shoot from residues. However, addition of  $^{15}\text{N}$  concentration increased total mineral N released, since the treatment increased total N content in the crop residues applied to the soil (Sholihah et al., 2012). Rozas et al. (2004) reported that the N recovery by maize was greater when N was applied at six leaf stages than when N was applied at planting time.

The amount crop residue N taken up by maize was significantly correlated with the initial N content, polyphenol content, C:N ratio, and polyphenol:N ratio of the crop residues (Table 2). By plotting the a residue quality parameters against the N uptake by maize, the strong



**Figure 6.** Microbial biomass N amended with crop residue of soybean (SY) and rice straw (RS) with different  $^{15}\text{N}$  concentration during growth by maize ( 8 weeks). Different letters mean different at LSD 5% , capital letters between the concentrations  $^{15}\text{N}=\text{N}$  and lower case letter between the residues.

relationships were observed for C:N ratio and polyphenol:N ratio ( $p < 0.05$ ), and were best described by an inverse function (Table 2). Therefore, the improvement of fertility condition of the soil by increasing the supply N did not only increase the crop residue N available for mineralization but most importantly also reduced the relative proportion of polyphenols in the crop residue.

### Microbial biomass N

The different  $^{15}\text{N}$  concentration in RS and SY residues significantly affected the soil microbial biomass N (Figure 6). The microbial biomass N content in RS was higher than SY during growing period (8 weeks). This was in contrast with the incubation experiment which showed the higher microbial biomass N content in SY than RS. This was because of N mineralization rate of SY residue was faster than RS.

The mineral N was then quickly taken up by maize, soon after it was released. Cadisch et al. (1998) reported that microbial biomass N in residue-amended soils was higher than in untreated soil at the end of first crop cycle although no significant pruning species effect were observed. Pruning N at the end of the first crop cycle contributed between 9% (low quality *Calliandra* and *Peltophorum*) and 19% (*Leucaena*) of total microbial biomass-N. The % pruning N recovered in the microbial biomass as estimated by the  $^{15}\text{N}$  method was low with *Calliandra* and *Peltophorum* prunings (3.1 to 4.0%) and greatest in *Leucaena* prunings (6.6%).

### Conclusion

Application of different  $^{15}\text{N}$  concentrations of added crop residues into soil-plant systems affected maize growth and N recovery by maize. The recovery of N release from crop residue increased with the increasing  $^{15}\text{N}$  concentration in the crop residues, ranging from 58 to 81% in rice straw and from 44 to 81% in soybean residue. The amount of N crop residue taken up by maize was significantly correlated with the initial N content, polyphenol content, C:N ratio, and polyphenol:N ratio of the crop residues. The %N recovery of crop residue by maize estimated by the  $^{15}\text{N}$  method was 11 % higher than that estimated by the difference method.

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