RESEARCH ARTICLE

# Improving maize residue use in soil fertility restoration by mixing with residues of low C-to-N ratio: effects on C and N mineralization and soil microbial biomass

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#### **Abstract**

The application of organic residues with wide C-to-N ratio on soils is known to cause nitrogen immobilization unless applied with nitrogen fertilizer. Considering that fertilizer usage is limited in low input agricultural systems in Africa, we determined whether it was possible to alleviate N immobilization of *Zea mays* (maize) by applying together with *Tithonia diversifolia* or *Vicia faba* green manure with low C-to-N ratio. The effect of sole *Z. mays* application on soil microbial biomass and carbon mineralization were also compared with when mixed with *T. diversifolia* or *V. faba*. The objectives were achieved using laboratory incubation experiments conducted over 84 days. As expected, the application of sole *Z. mays* residues resulted in an initial net N immobilization that lasted for 28 days. Relative to sole *Z. mays*, the application of *Z. mays* with either *V. faba* or *T. diversifolia* increased N mineralization by 58% and 55% respectively. It was also evident, that in comparison with sole *Z. mays*, soil microbial biomass and C mineralization were significantly higher in soils that received residues of *V. faba* and *T. diversifolia* either alone or in combination with *Z. mays*. The study showed that *V. faba* and *T. diversifolia* either alone or in combination with *Z. mays* residues had relatively high N concentration and narrow C-to-N ratio, which accounted for the increased N mineralization and improved microbial biomass and C mineralization. We inferred from the results of our study that N supplies from *V. faba* and *T. diversifolia* could be substantial in alleviating delayed decomposition and N immobilization of *Z. mays* residues.

**Keywords:** Soil biogeochemistry, microbial activities, soil fertility, plant residue quality

# 1 Introduction

While there is significant evidence that the addition of organic residues (obtained from trees/shrubs and crops) to soils can improve overall soil fertility, smallholder farmers are increasingly challenged in the selection of appropriate plant materials for soil nutrient management practices (Partey, 2011). In 2001, Palm and her colleagues formulated a simple

decision tool for managing organic resources. This system distinguished organic resources based on their chemical characteristics and decomposition patterns suggesting how each can be managed for short-term nutrient release within cropping systems (Palm *et al.*, 2001; Vanlauwe *et al.*, 2005). According to this decision support system, high quality organic residues (generally

high in nitrogen and low in lignin and polyphenols) can be solely incorporated into soils with no N fertilizer additions while low quality organic residues would have to be applied in combination with N fertilizers (Palm *et al.*, 2001). The incorporation of low quality organic resources with low N concentration and wide C-to-N ratio could result in initial net N immobilization unless supplementary N is provided through the application of N fertilizers (Bhupinderpal-Singh and Rengel, 2007).

In Africa, most of the available organic residues have competitive uses and are often low in nutrient concentrations (Vanlauwe et al., 2005) to be used as sole nutrient sources for crops. As in most parts of the tropics, residues from cereal crops such as maize (Zea mays) are among the most abundant but low quality organic resources in Sub-Saharan Africa, which although potential in soil management practices, are often burnt before cropping. In the past, farmers had complained about delayed decomposition of maize residues when left on farmlands, which cause N immobilization in the short term (Partey et al., 2013a). While the application of maize residues with inorganic fertilizers is a viable option (Smaling et al. 2002), regular application of inorganic fertilizers is seldom practiced in the region (Mateete et al., 2010) due to several socioeconomic constraints (Partey et al., 2013b). The low level of fertilizer use will mean that farmers will continuously crop farmlands without adequate nutrient replenishment. This therefore necessitates the exploration of suitable high quality organic residues, which can serve as alternatives to inorganic fertilizers. In several parts of Africa, wide ranges of experiments have confirmed the fertilizer equivalency values and nutrient supply capabilities of the green manures of the Mexican sunflower (Tithonia diversifolia) and faba bean (Vicia faba) to be comparable to that of inorganic fertilizers (Gachengo et al., 1999; Jensen et al., 2010). Mixing low quality maize residues with these available high quality organic resources with high N supply capabilities is therefore seen as a potential agroecological innovation that promote a better utilization of maize residues in agroecosystems for soil fertility improvement. In our current study, we determined whether N immobilization associated with maize residue application could be improved using *Tithonia diversifolia* and *Vicia faba* green manure with relatively low C-to-N ratio. We also compared the effect of sole *Z. mays* application and mixed *T. diversifolia* + *Z. mays* and *V. faba* + *Z. mays* application on soil microbial biomass and C mineralization. We hypothesized that when maize residues are mixed with high quality organic plant materials, the effect of the mixture on N mineralization, soil microbial biomass and activity will be significantly higher than sole maize residue application.

#### 2. Materials and Methods

## 2.1. Plant residue characterization

Plant materials used in the study were the aboveground portions of V. faba, T. diversifolia and Z. mays. All plant materials were of African origin obtained from previously established greenhouse experiments at the botanical gardens of the University of Manchester, England. The plants had not received any fertilizer treatments during establishment. V. faba and T. diversifolia were three and eight months old respectively during residue characterization. To characterize for quality parameters, the plant materials were oven-dried at 65 °C till constant weight, grounded with a pestle and mortar and sieved to 0.5 mm size. The sieved plant materials were then analyzed for total N, P, K, Ca, Mg, C, lignin and polyphenols in four replicates. The plant materials were either analyzed solely or in a mixture (i.e. T. diversifolia + Z. mays; and V. faba + Z. mays in a 1: 1 w/w ratio). Nitrogen and C were determined simultaneously by dry combustion using LECO TruSpec<sup>TM</sup> CN autoanalyzer (LECO Corporation). Total K, Ca, and Mg were determined by the dry ashing and atomic absorption spectrophotometery method as described by Eneji *et al.* (2005) and Motsara and Roy (2008). Phosphorus was also determined in an ash solution by the ammonium phosphomolybdate method (Motsara and Roy, 2008) whilst lignin was determined according to the acid detergent fiber method (van Soest, 1963). Polyphenols were determined by the method described by Gachengo et al. (1999).

The chemical characteristics of the plant materials are shown in Table 1 as reported previously by Partey *et al.* (2013a).

Table 1. Chemical characteristics of sole and mixed plant residues used in experiment

Chemical element/compound (g/kg)								C/N
N	P	K	С	Ca	Mg	Lig	Poly	-
28.1 (0.8)	5.2 (0.2)	46.2 (1.4)	400.6 (4.2)	13.0 (1.1)	8.3 (0.2)	58.0 (1.8)	18.0 (0.7)	14.3 (0.3)
54.7 (1.0)	2.5 (0.2)	17.6 (0.3)	427.4 (2.4)	27.0 (1.1)	3.0 (0.3)	41.0 (1.4)	14.0 (0.8)	7.8 (0.2)
10.8 (0.6)	2.9 (0.1)	20.6 (0.7)	401.3 (4.4)	4.2 (0.1)	2.9 (0.1)	57.0 (1.9)	5.6 (0.3)	37.2 (2.7)
25.4 (1.2)	4.3 (0.1)	33.4 (0.7)	417.6 (5.1)	8.2 (0.6)	6.3 (0.1)	56.7 (1.3)	10.2 (1.0)	17.8 (0.8)
31.3 (0.8)	2.7 (0.2)	19.4 (0.3)	436.2 (1.5)	19.7 (1.0)	2.8 (0.2)	48.0 (1.5)	8.1 (0.4)	13.9 (0.4)
	N 28.1 (0.8) 54.7 (1.0) 10.8 (0.6) 25.4 (1.2)	N P  28.1 (0.8) 5.2 (0.2)  54.7 (1.0) 2.5 (0.2)  10.8 (0.6) 2.9 (0.1)  25.4 (1.2) 4.3 (0.1)	N P K  28.1 (0.8) 5.2 (0.2) 46.2 (1.4)  54.7 (1.0) 2.5 (0.2) 17.6 (0.3)  10.8 (0.6) 2.9 (0.1) 20.6 (0.7)  25.4 (1.2) 4.3 (0.1) 33.4 (0.7)	N P K C  28.1 (0.8) 5.2 (0.2) 46.2 (1.4) 400.6 (4.2) 54.7 (1.0) 2.5 (0.2) 17.6 (0.3) 427.4 (2.4) 10.8 (0.6) 2.9 (0.1) 20.6 (0.7) 401.3 (4.4) 25.4 (1.2) 4.3 (0.1) 33.4 (0.7) 417.6 (5.1)	N         P         K         C         Ca           28.1 (0.8)         5.2 (0.2)         46.2 (1.4)         400.6 (4.2)         13.0 (1.1)           54.7 (1.0)         2.5 (0.2)         17.6 (0.3)         427.4 (2.4)         27.0 (1.1)           10.8 (0.6)         2.9 (0.1)         20.6 (0.7)         401.3 (4.4)         4.2 (0.1)           25.4 (1.2)         4.3 (0.1)         33.4 (0.7)         417.6 (5.1)         8.2 (0.6)	N         P         K         C         Ca         Mg           28.1 (0.8)         5.2 (0.2)         46.2 (1.4)         400.6 (4.2)         13.0 (1.1)         8.3 (0.2)           54.7 (1.0)         2.5 (0.2)         17.6 (0.3)         427.4 (2.4)         27.0 (1.1)         3.0 (0.3)           10.8 (0.6)         2.9 (0.1)         20.6 (0.7)         401.3 (4.4)         4.2 (0.1)         2.9 (0.1)           25.4 (1.2)         4.3 (0.1)         33.4 (0.7)         417.6 (5.1)         8.2 (0.6)         6.3 (0.1)	N         P         K         C         Ca         Mg         Lig           28.1 (0.8)         5.2 (0.2)         46.2 (1.4)         400.6 (4.2)         13.0 (1.1)         8.3 (0.2)         58.0 (1.8)           54.7 (1.0)         2.5 (0.2)         17.6 (0.3)         427.4 (2.4)         27.0 (1.1)         3.0 (0.3)         41.0 (1.4)           10.8 (0.6)         2.9 (0.1)         20.6 (0.7)         401.3 (4.4)         4.2 (0.1)         2.9 (0.1)         57.0 (1.9)           25.4 (1.2)         4.3 (0.1)         33.4 (0.7)         417.6 (5.1)         8.2 (0.6)         6.3 (0.1)         56.7 (1.3)	N         P         K         C         Ca         Mg         Lig         Poly           28.1 (0.8)         5.2 (0.2)         46.2 (1.4)         400.6 (4.2)         13.0 (1.1)         8.3 (0.2)         58.0 (1.8)         18.0 (0.7)           54.7 (1.0)         2.5 (0.2)         17.6 (0.3)         427.4 (2.4)         27.0 (1.1)         3.0 (0.3)         41.0 (1.4)         14.0 (0.8)           10.8 (0.6)         2.9 (0.1)         20.6 (0.7)         401.3 (4.4)         4.2 (0.1)         2.9 (0.1)         57.0 (1.9)         5.6 (0.3)           25.4 (1.2)         4.3 (0.1)         33.4 (0.7)         417.6 (5.1)         8.2 (0.6)         6.3 (0.1)         56.7 (1.3)         10.2 (1.0)

Values are the means of four replicates. Td = T. diversifolia, Vf = V. faba, M = Z. mays. Lig = lignin, Poly = polyphenol. Values in parentheses are standard error of means

#### 2.2. Initial soil characterization

Sandy-loam soil used for the experiment was collected from the Botanical grounds of the University of Manchester, UK, located on lat 53° 261 N and long 2° 131 W in England. The soil was collected using a stainless steel auger from 20 locations in a 5 m x 5 m plot within 20 cm of the topsoil layer. The soil samples were composited and homogenized by hand mixing. They were then air-dried till constant weight and passed through a 2 mm sieve and analyzed for physicochemical properties using five replicate sub-samples. Soil pH was analyzed using a glass electrode with a soil/water ratio of 1: 2, total N by dry combustion using LECO TruSpec<sup>TM</sup> CN autoanalyzer (LECO Corporation), organic carbon by the dichromate oxidation method (Motsara and Roy, 2008), cation exchange capacity using ammonium acetate extract (Motsara and Roy, 2008), and available P by Olsen's method (Motsara and Roy, 2008). The physicochemical properties of the soil were: Sand (59.4%), Clay (3.8%), pH (6.7), Total N (1.2 g/kg), organic C (13.8 g/kg), cation exchange capacity (6.5 cmol\_/kg), available P (2.4 mg/kg).

## 2.3. Incubation experiment

Incubation experiment was performed using closed chambers under laboratory-controlled conditions. Briefly, 125 mg (equivalent to 5 t ha<sup>-1</sup>) of 0.5 mm sieved ground plant material of *V. faba* (Vf), *T. diversifolia* (Td) and *Z. mays* (M) either applied alone or in a mixture [Vf (62.5 mg) + M (62.5 mg); and Td (62.5 mg) + M (62.5 mg)] were mixed with 50 g of pre-conditioned 2 mm sieved sandy-loam soil in 1 L jars and incubated in the dark at 28 °C for 84 days. Unamended soil was used as a control. Further controls

were included using jars without soils. The moisture content was kept constant at 55% water holding capacity of the soil. Inside the chamber, a 50 ml beaker containing 10 ml of 0.5 M NaOH was placed on the soil to absorb CO2. The CO2 evolved was collected, after 1, 7, 14, 28, 56, and 84 days of incubation in the 10 ml 0.5 M NaOH and determined by titration with 0.1 M HCl against a phenolphthalein indicator after precipitation with BaCl, (0.5 M). The CO, evolved was used in determining microbial activities by soil respiration. In addition, nitrogen mineralization was determined by measuring the production of mineral N  $(NH_4^+ + NO_5^-)$  at 7, 14, 28, 56, and 84 days of incubation. Ammonium and nitrate were determined by extracting 25 g of moist soil with 2 M KCl at a 1: 4 soil and extractant ratio. Ammonium and nitrate in the KCl extract were determined by the indophenol blue and phenoldisulphonic acid methods respectively (Motsara and Roy, 2008). All measurements were done in four replicates. Net N mineralized (Nm) from the different treatments was calculated by subtracting the inorganic N of the unamended control from amended soils at each sampling time (Abbasi and Khizar, 2012; Sistani et al., 2008). Moreover, soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined at the end of the incubation period using the chloroform fumigation and extraction method (Ladd and Amato, 1989). Soluble carbon in the 0.5 M K, SO, extract of fumigated and unfumigated soils was determined colorimetrically as described by Motsara and Roy (2008) whilst mineral N in the KCl extract in fumigated and unfumigated soils was determined by the indophenol blue method (Motsara and Roy, 2008). All measurements were done in four replicates. For biomass C and N calculations, k factors of 0.35 (Sparling et al., 1990) and 0.45 (Ross and Tate, 1993) were used respectively. The following equation according to Sparling and West (1998) was used to estimate the microbial C:

$$MBC = E_C/k$$

Where  $E_C$  = the extracted C after fumigation – extracted C before fumigation, k = the fraction of the killed biomass extracted as carbon under standardized conditions.

Biomass N was calculated using the equation by Moore *et al.* (2000):

$$MBN = E_N/k_{IN}$$

Where  $E_N = NH_4$ -N extracted after fumigation – extracted  $NH_4$ -N before fumigation and  $k_{IN}$  = the proportionality factor to convert  $E_N$  to MBN.

## 2.4. Statistical analysis

We used one-way analysis of variance (ANOVA) test to demonstrate the effect of treatments on soil parameters at each sampling period. Where there was significant effect, the means of treatments were compared using Tukey test at 5% probability level. Correlation and regression analysis were used to demonstrate significant relationships among soil parameters. All statistical analyses were conducted using GENSTAT 11 (VSN International, 2008).

## 3 Results

## 3.1. Nitrogen mineralization

Analysis of variance test revealed significant (p < 0.05) effect of treatments on mineral N (NH<sub>4</sub><sup>+</sup> + NO<sub>3</sub><sup>-</sup>) at all sampling periods (Table 2). All treatments generally showed increase in mineral N through time, peaking on the 56th day of incubation and declining drastically after. Throughout the experiment, N mineralization in mixed T. diversifolia + Z. mays was significantly (p < 0.05)higher than sole T. diversifolia or Z. mays treatments. Similarly, soils that received mixed application of V. faba + Z. mays recorded significantly high N mineralization rates compared to soils that received either sole V. faba or Z. mays treatments. Nitrogen mineralization rates in sole T. diversifolia and V. faba were higher than the control until the 84th day of incubation. This was consistent with the observation made between sole Z. mays and sole T. diversifolia or V. faba except on the 56th day when N mineralization was significantly higher in sole Z. mays (Table 2).

Treatments	Days after incubation							
	7	14	28	56	84			
Control (no input)	16.0 <sup>a</sup>	45.6 <sup>ab</sup>	43.1ª	57.0 <sup>a</sup>	23.0 <sup>a</sup>			
Td	54.8 <sup>b</sup>	68.8 <sup>b</sup>	108.7 <sup>b</sup>	$303.3^{b}$	50.6 <sup>ab</sup>			
Vf	56.8 <sup>b</sup>	145.9°	213.5°	333.3 <sup>b</sup>	$30.6^{a}$			
M	14.24 <sup>a</sup>	27.2 <sup>a</sup>	56.6 <sup>a</sup>	434.6°	$28.6^{a}$			
Td + M	$99.0^{c}$	120.3 <sup>d</sup>	$324.8^{d}$	544.6 <sup>d</sup>	174.2°			
Vf + M	96.4°	166.8 <sup>e</sup>	435.9 <sup>e</sup>	567.6 <sup>d</sup>	77.9 <sup>b</sup>			
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001			

Table 2. Mineral N (NH<sub>4</sub> + NO<sub>3</sub>) (mg N kg<sup>-1</sup>) at successive incubation periods in soil amended with sole and mixed organic residues over 84 days under controlled laboratory conditions

Values are the means of 4 replicates. Td = T. diversifolia, Vf = V. faba, M = Z. mays. SED = standard error of mean differences. Means in a column with the same letters as superscript do not differ significantly according to Tukey test at 5% probability level.

Table 3. Cumulative net N mineralization (mg N kg<sup>-1</sup> soil) from sole and mixed plant residues at successive incubation periods over 84 days under controlled laboratory conditions

Treatments	Days after in	cubation	Mean (Treatment effects)			
	7	14	28	56	84	_
Td	38.8 <sup>b</sup>	62.0 <sup>b</sup>	127.6 <sup>b</sup>	373.9 <sup>b</sup>	401.5 <sup>b</sup>	200.76 (69.6)
Vf	40.8 <sup>b</sup>	141.1 <sup>c</sup>	316.3°	592.6°	600.2 <sup>c</sup>	338.2 (102.2)
M	-1.76 <sup>a</sup>	-20.21 <sup>a</sup>	-6.7 <sup>a</sup>	$254.9^{a}$	260.5 <sup>a</sup>	260.5 (58.6)
Td + M	83.0°	157.7 <sup>d</sup>	439.4 <sup>d</sup>	$927.0^{d}$	1078.2 <sup>d</sup>	537.1 (179.4)
Vf + M	80.4 <sup>c</sup>	201.6 <sup>e</sup>	594.4 <sup>e</sup>	1105.0 <sup>e</sup>	1159.9 <sup>e</sup>	628.3 (199.3)
Mean (Time effects)	48.2 (15.6)	108.4 (39.2)	294.2 (107.2)	650.7 (161.1)	700.1 (179.8)	()

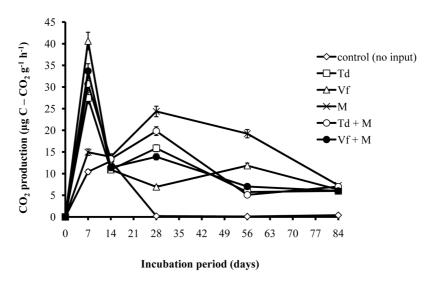
Values are the means of 4 replicates. Td = T. diversifolia, Vf = V. faba, M = Z. mays. Means in a column with the same letters as superscript do not differ significantly according to Tukey test at 5% probability level. Values in parentheses are standard error of means.

Meanwhile, N mineralization with sole Z. mays residue application was comparable to the control except on the 56th day of incubation. Contrary to other organic residue treatments, net N immobilization was recorded in sole Z. mays amended soils during the first 28 days of incubation (Table 3). In most cases, cumulative net N mineralization (CN<sub>m</sub>) was significantly (p < 0.05) higher in sole V. faba than T. diversifolia treatments. Consistently, CN, was higher in mixed T. diversifolia + Z. mays than in sole T. diversifolia or sole Z. mays. Similarly, CN<sub>m</sub> was significantly greater in mixed V. faba + Z. mays than when either V. faba or Z. mays were solely applied.

## 3.2. Carbon mineralization and soluble organic C

The application of treatments significantly (p < 0.05) increased soil respiration with comparable rates among the organic residue treatments. The production of carbon dioxide as affected by the treatments during incubation is shown in Figure 1. Generally, all treatments showed similar dynamic patterns of  ${\rm CO_2}$  production while C mineralization flattened in the control after the  $14^{\rm th}$  day

of incubation. Meanwhile, the application of treatments significantly (p < 0.05) increased soluble organic C (SOC) contents of soils with mixed T. diversifolia + Z. mays recording the greatest level. As shown in Table 4, SOC in all the mixed residue treatments (V. faba + Z. mays or T. diversifolia + Z. mays) were greater than when their components were singly applied. Among the plant residue treatments, sole Z. mays had the least impact on SOC.



**Figure 1.** Carbon dioxide production as affected by sole and mixed organic residues over 84 days of incubation. Data points are the means of four replicates. Error bars represent the standard error of mean (SEM). Td = T. diversifolia, Vf = V. faba, M = Z. mays

# 3.3. Soil microbial biomass and metabolic quotient

Soil microbial biomass carbon (MBC) increased significantly (p < 0.05) with treatment application. MBC ranged from 225.7 in the control to 1271.3  $\mu$ g C g<sup>-1</sup> in mixed V. faba + Z. mays treatments (Table 4). Among organic residue treatments, sole Z. mays application had the least impact on MBC. Moreover, MBC was significantly (p < 0.05) higher in soils that received mixed treatments (V. faba + Z. mays or T. diversifolia +

 $Z.\ mays$ ) than either sole  $V.\ faba$ , T. diversifolia or  $Z.\ mays$  amended soils. Meanwhile, ANOVA test revealed significant (p = 0.011) effect of treatments on the soil microbial biomass nitrogen (MBN) with only sole  $Z.\ mays$  differing significantly (p < 0.05) from the control. Conversely, the MBC/MBN ratio increased in all organic residue treatments except in  $Z.\ mays$  amended soils. The MBC/MBN ratio ranged from approximately 2 in the control to 11 in sole  $V.\ faba$ .

Table 4. Soil microbial biomass carbon, microbial biomass nitrogen and microbial activities as affected by sole and
mixed plant residues measured at the end of an 84-day incubation period

Treatments	MBC (μg C g <sup>-1</sup> )	MBN (μg N g <sup>-1</sup> )	MBC/MBN	qCO <sub>2</sub> (10 <sup>5</sup> h <sup>-1</sup> )	Soluble organic C (μg g <sup>-1</sup> )
Control (no input)	225.7ª	106.5ª	2.1ª	5.3 <sup>b</sup>	40.3ª
Td	782.7 <sup>c</sup>	145.0 <sup>ab</sup>	5.4 <sup>bc</sup>	4.8 <sup>b</sup>	154.7°
Vf	1181.9 <sup>e</sup>	112.8 <sup>a</sup>	$10.7^{d}$	3.2ª	199.9 <sup>d</sup>
M	605.8 <sup>b</sup>	187.8 <sup>b</sup>	3.2 <sup>ab</sup>	6.5°	74.2 <sup>b</sup>
Td + M	866.7 <sup>d</sup>	146.0 <sup>ab</sup>	6.1 <sup>bc</sup>	3.8 <sup>a</sup>	$353.0^{\rm f}$
Vf+M	1271.3 <sup>f</sup>	150.9 <sup>ab</sup>	8.5 <sup>cd</sup>	2.8 <sup>a</sup>	298.3 <sup>e</sup>
SED	10.01	15.52	0.97	0.3	4.63
p value	< 0.001	0.011	< 0.001	< 0.001	< 0.001

Values are the means of four replicates. MBC = microbial biomass carbon, MBN = microbial biomass nitrogen, qCO<sub>2</sub> = metabolic quotient, SED = standard error of mean differences. Td = T. diversifolia, Vf = V. faba, M = Z. mays. Means in a column with the same letters as superscript do not differ significantly according to Tukey test at 5% probability level.

MBC/MBN ratio was comparable between sole *Z. mays*, sole *T. diversifolia* and mixed *T. diversifolia* + *Z. mays* treatments. Furthermore, microbial metabolic quotient (qCO<sub>2</sub>) was significantly (p < 0.05) greatest in *Z. mays* treatment and lowest in mixed *T. diversifolia* + *Z. mays*, sole *V. faba* and mixed *V. faba* + *Z. mays* amended soils (Table 4).

## 4. Discussions

While the combined application of maize (*Zea mays*) residues with relatively wide C-to-N ratio and inorganic N fertilizers reportedly improve N immobilization of *Z. mays* residues, regular application of mineral fertilizers with organic residues are too seldom practiced in developing countries due to several socioeconomic constraints. Considering earlier reports

that confirmed *T. diversifolia* and *V. faba* have high N supply capabilities and high decomposition rates (Partey *et al.*, 2013a, Partey *et al.*, 2011), combining maize residues with green manure sources of these plant species was seen as a potential agroecological innovation for maize residue use in soil management practices in low input smallholder agricultural systems. In our current study, we determined whether N immobilization associated with *Z. mays* residue application could be improved by mixing with organic residues of low C-to-N ratio.

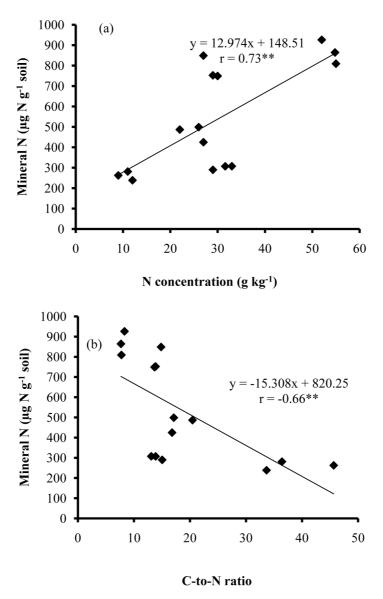
As expected, the results of our study demonstrated differential effects of the plant residues on N mineralization dynamics, C mineralization and soil microbial biomass carbon as a result of their intrinsic chemistry. In contrast to *T. diversifolia* and *V. faba* residues either applied alone or in combination with *Z. mays*, the application of sole *Z. mays* residues resulted

in an initial net N immobilization that lasted for 28 days (Table 3). The lack of initial net N mineralization in sole Z. mays compared with the other organic residues can be attributed to its relatively low N concentration and wide C-to-N ratio. This assertion is supported by the significant positive and negative correlation obtained between mineral N values and plant N concentration (r = 0.73, p = 0.002) and C/N ratio (r = -0.66, p =0.007) respectively (Figure 2). As shown in Table 1, the results on plant residue characterization showed Z. mays residues have relatively low N concentration resulting in a C/N ratio beyond the critical maximum above which initial net N immobilization could be expected (Palm et al. 2001). Compared with sole Z. mays, both T. diversifolia and V. faba residues had low C/N ratios and could therefore supply majority of the N requirement for decomposing microorganisms, thus eliminating any period of net N immobilization (Gentile et al. 2009; Partey et al. 2011; Schroth, 2003). The long phase of N immobilization induced by Z. mays residues in our study supports the assertion that incorporation of large quantities of maize residues in the field would restrict N availability for growing crops (Sakala et al. 2000). Meanwhile, the study showed that applying Z. mays residues in combination with either T. diversifolia or V. faba green manures could alleviate N immobilization of Z. mays residues. Relative to sole Z. mays application, the application of Z. mays in combination with either T. diversifolia or V. faba averagely increased N mineralization by 55% and 58% respectively. We attribute this observation to improved N composition and C/N ratio when Z. mays residues were mixed with T. diversifolia and V. faba residues (Table 1).

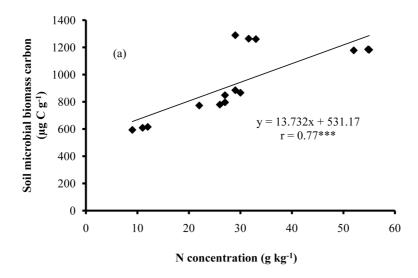
Furthermore, the application of the plant residues (except the case of Z. mays) resulted in an initial flush of  $\mathrm{CO}_2$  that was significantly higher than the subsequent sampling periods (Figure 1). Increased microbial activity and rapid decomposition of the plant residue treatments with relatively high N and narrow  $\mathrm{C/N}$  ratios were expected to be higher at the early stage which is consistent with the patterns of  $\mathrm{CO}_2\text{-C}$  evolution observed. The high  $\mathrm{CO}_2\text{-C}$  evolution due

to accelerated decomposition, might arguably limit possibility for long-term build-up of organic matter and soil fertility (Partey et al. 2012). Meanwhile, the increased initial CO<sub>2</sub>-C evolution in both mixed T. diversifolia + Z. mays, and V. faba + Z. mays relative to sole Z. mays is in agreement with earlier hypothesis that predicts accelerated decomposition of mixed residues of different qualities (Gartner and Cardon 2004; Partey et al. 2013a). The increased microbial activity as reflected in the results on CO<sub>2</sub>-C evolution at the early stages of residue addition further explains why N mineralization was increased in mixed plant residue treatments to overcome the strong immobilization of Z. mays residues.

expected, the application of treatments significantly (p < 0.001) increased the soil MBC with significant variations among the plant residue treatments. Among the plant residue treatments, sole Z. mays and mixed V. faba + Z. mays residue application showed the least and greatest impacts on MBC respectively. The least impact of sole Z. mays residues on MBC and microbial activities was also reflected in the high qCO, values obtained which demonstrated low C utilization by microbes when soils were amended with sole Z. mays residues. As depicted in Figure 3, our study found the soil MBC was significantly related to residue N concentration (r = 0.77, p < 0.001) and C/N ratio (r = -0.77, p < 0.001)0.001). These significant relationships demonstrate that differences in both C and N inputs could significantly impact microbial biomass C (Tu et al. 2006). The significance of C inputs for increased soil microbial biomass was further confirmed by the significant positive correlation (r = 0.84, p < 0.001) observed between MBC and soluble organic C (Table 5). Furthermore, the results of our present study revealed a significant positive correlation (r = 0.60, p = 0.01) between microbial biomass C and available N (Table 5), which affirmed that differences in microbial biomass and activity under different organic amendments would have significant implications for nutrient availability to crops.



**Figure 2.** Relationship between mineral N measured at end of incubation and N concentration (a) and C/N ratio (b) of plant residues used in the experiment. \*\* means significant at 1% probability level. N = 15.



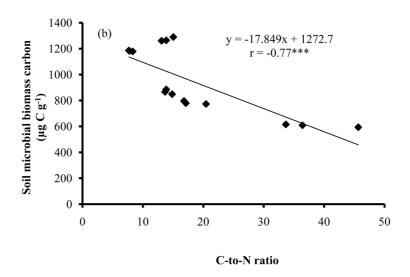


Figure 3. Relationship between soil microbial biomass carbon measured at end of incubation and N concentration (a) and C/N ratio (b) of plant residues used in the experiment. \*\*\* means significant at 0.1% probability level. N = 15.

<b>Table 5.</b> Pearson correlation coefficient (r) for the linear interrelationships among soil properties measured at the end
of an 84-day incubation of soils amended with sole and mixed plant residues

	MBC	SOC	MBN	MBC/MBN	MN
MBC	1				
SOC	0.84***	1			
MBN	ns	ns	1		
MBC/MBN	0.90***	0.78***	-0.57**	1	
MN	0.60**	0.63**	-0.52*	0.76***	1

MBC = microbial biomass carbon, MBN = microbial biomass nitrogen, SOC = soluble organic carbon, MN = mineral nitrogen, N = 24. ns = not significant. \*, \*\* and \*\*\* represent statistical significance at 5%, 1% and 0.1% probability levels respectively.

It is reported that high microbial biomass and activity will often lead to high nutrient availability to crops (Wang et al. 2004), through enhancing both the microbial biomass turnover and the degradation of non-microbial organic materials (Tu et al. 2006). The wide C/N ratio of Z. mays residues should therefore explain why MBC level in sole Z. mays amended soils was comparatively lower. However, the application of the plant residues did not increase the soil MBN with comparable levels among treatments. Meanwhile, the MBC-to-MBN ratio in the soil showed significant (p < 0.05) variations among treatments. MBC/MBN ratio increased in all organic residue treatments except in Z. mays amended soils. Whilst previous reports have mentioned a larger MBC/MBN ratio indicates the chance of more N immobilization by microbes than N availability by mineralization (Abbasi and Khizar 2012) this was not consistent with our results as confirmed by the relatively high net N mineralization values in the plant residue treatments (Table 3) at the end of incubation. The argument is further supported by the significant positive correlation (r = 0.76, p < 0.001) obtained between the MBC/MBN ratio and mineral N after 84 days of incubation (Table 5). We attribute this observation to changes in the microbial community composition. According to Moore et al. (2000), the MBC/MBN ratio is often used to describe the structure and the state of the microbial community and reflect the abundance of either fungi or bacteria in the soil. A high MBC/MBN ratio (7 to 12) indicates that the microbial biomass contains a higher proportion of fungi, whereas a low value (2 to 6) suggests that bacteria predominate in the microbial population (Moore *et al.* 2000). The range of values found in our study fell within that reported by Moore *et al.* (2000) which provides basis for assumption that the plant residue treatments influenced the population dynamics of both bacteria and fungi in the soil based on their intrinsic chemical characteristics.

#### 5. Conclusion

The study has provided significant evidence that the green manures of both *V. faba* and *T. diversifolia* are viable sources of soil N. The application of *V. faba* and *T. diversifolia* green manures in soil is expected to improve soil N economy in cropping systems for improved crop productivity. While the addition of sole *Z. mays* residues to soils resulted in a long phase of N immobilization, the study showed that applying *Z. mays* in combination with either *V. faba* 

or T. diversifolia could increase N mineralization by 58% and 55% respectively relative to sole Z. mays application. The results on N mineralization were also consistent with the differential effects of the plant residue treatments on C mineralization and microbial biomass. Compared with sole Z. mays amended soils, the results generally showed significantly higher soil microbial biomass and activities in soils that received residues of V. faba and T. diversifolia either applied alone or in combination with Z. mays residues. The results on N mineralization, C mineralization and microbial biomass were related to residue chemistry. The study showed that V. faba and T. diversifolia either alone or in combination with Z. mays residues had relatively high N concentration and narrow C-to-N ratio, which accounted for the increased C and N mineralization and microbial biomass observed. Whilst our results did not dispute the potential of sole Z. mays residues for soil fertility improvement, it has demonstrated that maize residue contribution to soil N availability could be significantly improved when applied together with T. diversifolia and V. faba with relatively low C-to-N ratio.

## Acknowledgement

The authors express their sincere gratitude to the Sustainable Consumption Institute, University of Manchester; and Africa Rice Centre, Benin who provided funding for Samuel Partey.

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