In: Soil Fertility ISBN: 978-1-62081-087-3
Editors: B. Adewuyi and K. Chukwu

Chapter 1

EFFECTS OF ORGANIC RESIDUE CHEMISTRY ON SOIL BIOGEOCHEMISTRY: IMPLICATIONS FOR ORGANIC MATTER MANAGEMENT IN AGROECOSYSTEMS

Samuel T. Partey,[1,2*] Richard F. Preziosi,[2] and Geoffrey D. Robson[2]

[1]Sustainable Consumption Institute, The University of Manchester, Waterloo Place, Oxford Road, Manchester, UK
[2]Faculty of Life Sciences, The University of Manchester, Michael Smith Building, Oxford Road, Manchester, UK

ABSTRACT

While there is significant evidence that the addition of organic residues to soils can improve overall soil fertility, it is unclear as to what organic residues will have the most beneficial impact. The use of residue chemistry as an index for assessing organic resource quality is highly reported and has been linked to organic matter decomposition and nutrient release patterns. However, less is reported on the direct linkages between organic residue chemistry and soil biogeochemistry and how the impact varies between sole and mixed organic residues. In this research,

* Corresponding Author
Email: sammtech147@yahoo.co.uk; samuel.partey@postgrad.manchester.ac.uk
Tel: +44(0) 7550487534

we investigated the residue chemistries of *Tithonia diversifolia*, *Vicia faba* and *Zea mays* (either applied alone or in combination) and how they affected and related to soil microbial activities and biochemical properties. The results revealed significant differences in chemical composition among the species and affirmed that organic materials impact differently on soil microbial and biochemical properties based on their chemistry. Carbon, polyphenol, C: P and lignin: N ratios had the greatest impact on soil microbial activities and biochemical properties. The levels of biological activities were comparable in sole and mixed amendments but N mineralization and availability increased significantly in *V. faba* and *T. diversifolia* amendments. The results also demonstrated that basal respiration, microbial biomass carbon and the activities of β-glucosidase and β-glucosaminidase are sensitive indicators of nitrogen availability and organic C levels in soils.

Keywords: soil biogeochemistry; microbial activities; soil fertility; organic matter

1. INTRODUCTION

Whilst global agriculture is increasingly challenged to meet the world's rising demand for food sustainably, declining soil fertility and mismanagement of plant nutrients have made this difficult even when improved varieties of crops have been made available (Pinstrup-Andersen, 2000). The impact of declining soil fertility on crop production are particularly visible in developing countries, where the most serious food security challenges exist both currently and in the future (Badiane and Delgado 1995; Rosegrant et al., 1995). Commonly, farmers in the developing world practise low-input agriculture that depends on organic matter in the soil to sustain production. The soil organic matter plays an important part in improving the intrinsic quality of soils and optimizing crop production (Gruhn et al., 2000). Therefore with the limited use of conventional fertilizers and declining soil organic matter levels (owing to continuous cropping), declining soil fertility could be a perpetual constraint to food production in developing countries (Kumwenda et al., 1996).

While there is significant evidence that the addition of organic residues (obtained from trees/shrubs and crops) to soils can improve overall soil fertility (Nziguheba et al., 2000; Sanchez et al., 1997), it is unclear as to what organic residues will have the most beneficial impact. The selection of organic materials for soil fertility improvement and maintenance has therefore remained a difficult challenge for many farmers (George et al., 2002).

Numerous studies characterizing and assessing the suitability of organic materials for soil fertility improvement have employed residue chemistry (mainly N, P, C, C/N, lignin and polyphenols) and its consequential effect on decomposition and nutrient release patterns (Partey et al., 2011; Brady and Weil, 2004; Palm et al., 2001; Young, 1997). Although there is significant information that organic residues with low C/N ratios, high N and low lignin and polyphenolic contents will enhance microbial activities and increase N mineralization (Troeh and Thompson, 2005; Brady and Weil, 2004), this has mostly been confirmed with sole organic inputs (Partey et al., 2011; Chander et al., 1997; Azmal et al., 1996;) rather than mixed organic residues. In most of sub-Saharan Africa where legumes are commonly intercropped with cereal crops, the addition of nutrient inputs from senesced legume leaves make viable contributions in increasing residual N for subsequent cropping. Because crops are grown in association in these intercropping systems, the residues of different crops will inevitably mix and decompose simultaneously within the same soil volume (Sakala et al., 2000). The interactions between decomposing residues can be complex and may result in N mineralization and biochemical pathways of the separate components of the mixture (Singh et al., 2007; Sakala et al., 2000; Handayanto et al., 1997). Understanding the interactions between organic residues of different chemistries is therefore essential, since leaf litter does not segregate neatly into individual species types in ecosystems, and the composition of plant communities changes over time (Gartner and Cardon, 2004).

Considering that microbial biomass, activity and nutrient status, combined with indices of microbial community such as microbial metabolic quotient and microbial biomass C: N ratio are useful indicators of soil fertility or sustainability changes (Dinesh, 2004), we related these parameters to the residue chemistries of *Zea mays* (Maize), *Vicia faba* (Faba bean) and *Tithonia diversifolia* (Mexican sunflower) to determine their implications on nutrient management in organically managed agroecosystems.

2. MATERIALS AND METHODS

2.1. 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^{o} 26^{1} N and long 2^{o} 13^{1} 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 or stored at 4 ^{o}C for microbiological analysis 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^{TM} 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: pH (6.7), Total N (1.2 g/kg), organic C (13.8 g/kg), cation exchange capacity (6.5 $cmol_c$/kg), available P (2.4 mg/kg).

2.2. Plant Residue Characterization

Plant materials used in the study were: the green biomasses of Faba bean (*Vicia faba*), Mexican sunflower (*Tithonia diversifolia*); and maize (*Zea mays*) stover. Both *T. diversifolia* and *V. faba* were raised under greenhouse conditions and were 5 months and 1.5 months old respectively at the time of sampling. *Z. mays* stover was obtained from previously raised maize crops (Konsort variety sourced from Huntseeds, UK) in the greenhouse. All plants did not receive any fertilizer treatments when raised. The plant materials (fresh leaves and tender stems in the case of *T. diversifolia* and *V. faba*) were characterized for chemical composition. The plant materials were either analyzed solely or in a mixture (i.e. *Z. mays* + *T. diversifolia*; and *Z. mays* + *V. faba* in a 1: 1 w/w ratio). The plant materials were oven dried at 65 ^{o}C till constant weight, ground with a pestle and mortar and sieved to 0.5 mm size. The sieved plant materials were analyzed for total N, P, K, Ca, Mg, C and lignin in four replicates. Nitrogen and C were determined simultaneously by dry combustion using LECO TruSpec^{TM} 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).

2.3. Incubation Experiment

Fifty grams of air-dried 2 mm sieved sandy-loam soil (described above) was put in 250 ml conical flasks. The flasks were moistened to 55% water holding capacity and pre-incubated at 28 ^{o}C for 7 days. After pre-incubation, 125 mg (equivalent to 5 t ha^{-1}) of *V. faba* (Vf), *T. diversifolia* (Td) and *Z. mays* stover (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)] was added to the soil and adjusted to 55% water holding capacity. Unamended soil was used as a control (C). Moisture levels were maintained with distilled water at 55% water holding capacity throughout the experiment by monitoring the weight of the flasks daily. The treatments (C, Td, Vf, M, Td + M and Vf + M) were arranged in a complete randomized design with four replications and kept in the dark at 28 ^{o}C. Destructive sampling was done at 7, 14, 28, 56 and 84 weeks after incubation to monitor changes in various soil microbial and biochemical properties using four replicates.

2.4. Soil Carbon and Nitrogen Mineralization

Microbial respiration or carbon mineralization was determined at 28 ^{o}C in closed chambers under laboratory-controlled conditions. Briefly, 125 mg (equivalent to 5 t ha^{-1}) of *V. faba* (Vf), *T. diversifolia* (Td) and *Z. mays* stover (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 sandy-loam soil in 1 L jars and incubated in the dark at 28 ^{o}C for 84 days. Unamended soil was used as a control (C). 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 CO_2. The CO_2 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 $BaCl_2$ (0.5 M). The CO_2 evolved during the 84th day of incubation was used as the basal respiration (Bresp) value. All measurements were done in four replicates. Nitrogen mineralization was determined by measuring the production of mineral N (NH_4^+ and NO_3^-) during incubation.

N mineralization was determined at 1, 7, 14, 28, 56, and 84 days of incubation 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.

2.5. Soil Microbial Biomass

Soil microbial carbon and nitrogen were determined at the end of the incubation period using the chloroform fumigation and extraction method (Ladd and Amato; 1989; Brookes et al., 1985). 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: Microbial biomass C = E_C/k.

Where E_c = the extracted carbon after fumigation – extracted carbon before fumigation, k = the fraction of the killed biomass extracted as carbon or nitrogen under standardized conditions.

2.6. Enzyme Assays

β-glucosidase activity was assayed at the end of incubation by the method described by Eivazi and Tabatabai (1988). One gram (1 g) of moist sieved (2 mm) soil was placed in an erlenmeyer flask (50 ml) and treated with 0.25 ml of toluene, 4 ml of tris buffer (pH 6.0) and 1 ml of *p*-nitrophenol-β-D-glucoside as a substrate. After stoppering the flask, the contents were thoroughly mixed and incubated for 1h at 37^{o}C. After incubation, 1 ml of $CaCl_2$ solution, 4 ml of Tris buffer (pH 12) were added to stop the reaction. The flask was then swirled and the soil suspension filtered through a millex filter (0.25 μm). Controls were set-up without the addition of soil. The colour intensity of the yellow filtrate was measured with a digital ultraviolet spectrophotometer (Cecil elegant technology, England) at 400 nm. All measurements were carried out in triplicate with one control. The *p*-nitrophenol contents of the filtrates were then calculated by comparing the results to a standard curve for p-nitrophenol developed as described by Tabatabai and Bremner (1969). β-glucosidase activity was expressed as released p-nitrophenol per unit dry soil weight and incubation time.

Phosphomonoesterase activity was assayed at the end of incubation using the procedure by Tabatabai and Bremner (1969) and Eivasi and Tabatabai (1977). One gram (1 g) of moist sieved (2 mm) soil was placed in an erlenmeyer flask (50 ml) and treated with 0.25 ml of toluene, 4 ml of Tris buffer (pH 6.5 for acid phosphatase and pH 11 for alkaline phosphatase) and 1 ml of *p*-nitrophenol phosphate solution. After stoppering the flask, the contents were thoroughly mixed and incubated for 1h at 37^{o}C. After incubation, 1 ml of $CaCl_2$ solution, 4 ml of Tris buffer (pH 12) were added to stop the reaction. The flask was then swirled and the soil suspension filtered through a Millex filter (0.25 μm). Controls were set-up without the addition of soil. The colour intensity of the filtrate was measured with a spectrophotometer at 400 nm. All measurements were carried out in triplicate with one control. The p-nitrophenol contents of the filtrates were then calculated by comparing the results to a standard curve for p-nitrophenol developed as described by Tabatabai and Bremner (1969). Phosphomonoesterase activity was expressed as released p-nitrophenol per unit dry soil weight and incubation time.

N-acetyl-β-D-glucosaminidase activity was determined at the end of the incubation period using the method of Parham and Deng (2000) as described by Ekenler and Tabatabai (2002). β-glucosaminidase activity was assayed by placing 1.0 g of soil into a 50 ml Erlenmeyer flask, and then adding 4 ml of 0.1 M acetate buffer (pH 5.5) and 1 ml of 10 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide (pNNAG) solution. The slurries were mixed thoroughly, stoppered, and placed in an incubator at 37 ^{o}C for 1h after which 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added to stop the reaction. The samples were swirled and filtered through a Millex filter (0.25 μm). The colour intensity of the filtrate was measured at 405 nm with a spectrophotometer. Controls were performed with the substrate being added after the reactions were stopped. Additional controls were performed by following the procedure described but without addition of soil to the reaction mixtures. The controls were designed so that they allowed for subtraction of the soil background colour and any trace amount of *p*-nitrophenol produced by chemical hydrolysis of pNNAG during the incubation. The *p*-nitrophenol contents of the filtrates were then calculated by comparing the results to a standard curve for p-nitrophenol developed as described by Tabatabai and Bremner (1969). β-glucosaminidase activity was expressed as released p-nitrophenol per unit dry soil weight and incubation time.

2.7. Statistical Analysis

All parameters measured were subjected to Analysis of Variance (ANOVA) test. Treatment means were compared using Tukey test at 5% probability level. Correlation and regression analysis were used to demonstrate the relationship between organic residue chemistry and soil microbial activities and biochemical properties. All statistical analyses were conducted using GENSTAT 11 (VSN International, 2008).

3. RESULTS

3.1. Organic Residue Chemistry

Differences in residue chemistry were evident in both the single and mixed organic materials (Table 1). In most cases, mixing *Z. mays* with either *V. faba* or *T. diversifolia* reduced their elemental composition levels. While nitrogen levels ranged from 10.8 g/kg in *Z. mays* to 54.7 g/kg in *V. faba* (Table 1), mixing *V. faba* (Vf) and *Z. mays* (M) brought N level 43% below that recorded in the sole *V. faba* treatment. In addition, mixed *T. diversifolia* (Td) and *Z. mays* treatment recorded N level 17% below that recorded with *T. diversifolia* alone. Carbon-to-nitrogen ratio was highest in M (37.2) and was more than four times that recorded in Vf (7.8). C/N ratio was increased in Vf + M and Td + M mixes compared to Vf and Td residues alone. Meanwhile, Td showed the highest concentrations of P, Mg and K whilst Vf showed the greatest concentration of Ca. Both N/P and C/P ratios were highest in Vf due to its relatively low P concentration. Lignin composition ranged from 41 g/kg in Vf to 58 g/kg in Td whilst polyphenolic concentration increased in the order: M < Vf + M < Td + M < Vf < Td. Furthermore, the high N content in Vf resulted in a significantly low lignin/N and (lignin + polyphenol)/N ratios (Table 1) compared to the other treatment.

3.2. Soil Nitrogen Mineralization

Analysis of variance test revealed significant ($p < 0.05$) effect of treatments on all soil nitrogen parameters (Table 2). The application of treatments increased nitrogen availability.

Table 1. Chemical composition of sole and mixed organic residues used in the experiment

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

Treatment	**Chemical element (g/kg)**								**C/N**	**C/P**	**N/P**	**Lig/N**	**(Lig + Poly) N**
	N	P	K	C	Ca	Mg	Lig	Poly					
Td	28.1	5.2	46.2	400.6	13.0	8.3	58.0	18.0	14.3	77.0	5.4	2.1	2.7
Vf	54.7	2.5	17.6	427.4	27.0	3.0	41.0	14.0	7.8	171.0	21.9	0.7	1.0
M	10.8	2.9	20.6	401.3	4.2	2.9	57.0	5.6	37.2	138.4	3.7	5.3	5.8
Td + M	23.4	4.3	33.4	417.6	8.2	6.3	56.7	10.2	17.8	97.1	5.4	2.4	2.9
Vf + M	31.3	2.7	19.4	436.2	19.7	2.8	48.0	8.1	13.9	161.6	11.6	1.5	1.8

Total inorganic nitrogen (TIN) (measured as the cumulative sums of ammonium and nitrate levels) ranged from 300.7 in the control to 1167.1 µg N g^{-1} in *V. faba* treatments. A higher TIN production was recorded in sole *V. faba* and *T. diversifolia* treatments than when mixed with *Z. mays*.

Meanwhile, there were distinct differences in the dynamic patterns followed by ammonium and nitrate in the 84-day incubation period. Generally, ammonification was higher than nitrification. Ammonium levels generally increased up to the 56th day where the highest levels were recorded but declined drastically up to the 84th day (Figure 1). Conversely, nitrate levels declined through time until the 56th day when levels increased gradually and peaked on the 84th day (Figure 2). At the end of the incubation period, the nitrate-to-ammonium ratio ranged from 0.06 in *V. faba* and *T. diversifolia* treatments to 0.15 in maize treatments. This ratio increased with maize addition in *V. faba* and *T. diversifolia* treatments (Table 2).

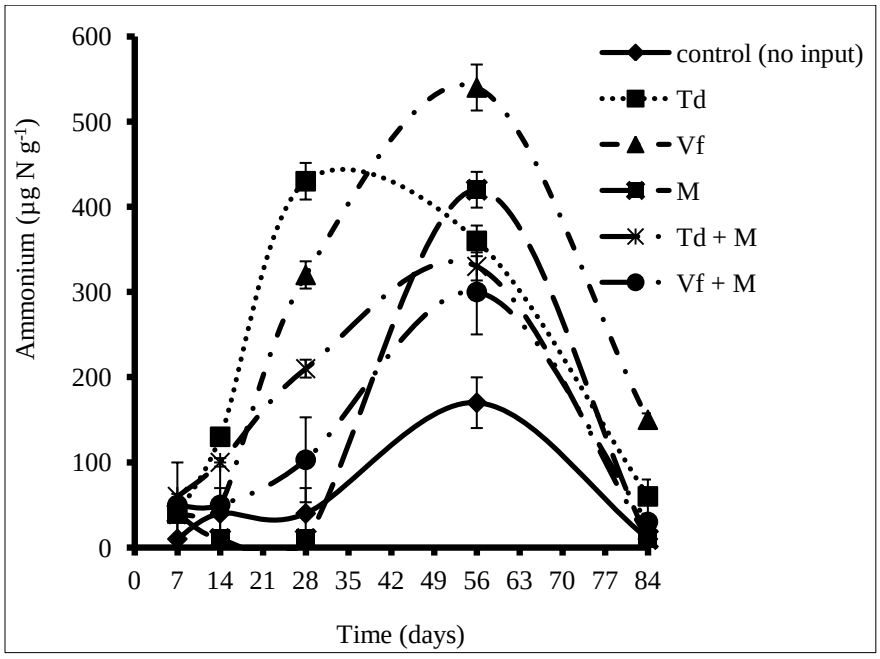

Figure 1. Temporal dynamics in ammonium levels as affected by sole and mixed organic residues over 84 days of incubation. Data points are the means of four replicates. Error bars represent the standard error of mean (SEM). Vf = *V. faba*, Td = *T. diversifolia*, M = *Z. mays*.

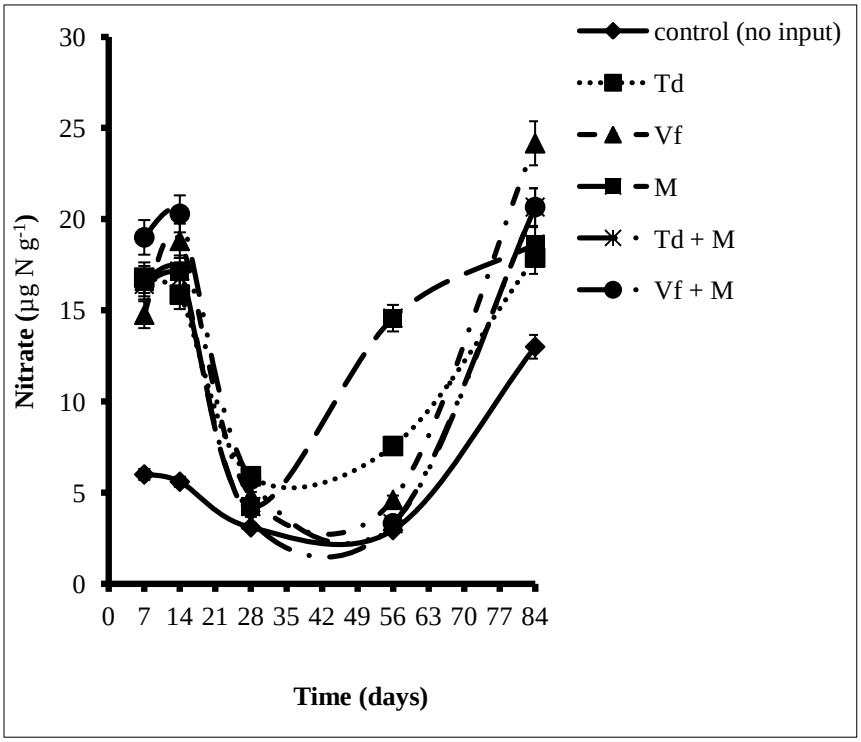

Figure 2. Temporal dynamics in nitrate levels as affected by sole and mixed organic residues over 84 days of incubation. Data points are the means of four replicates. Error bars represent the standard error of mean (SEM). Vf = *V. faba*, Td = *T. diversifolia*, M = *Z. mays*.

3.3. Soil Carbon Mineralization and Microbial Respiration

The application of treatments had significant ($p < 0.05$) effect on soil organic C (SOC) and microbial respiration. SOC levels were the same in both mixed and single treatments but differed significantly ($p < 0.05$) from the control (Table 2). The mineralization kinetics of C measured as cumulative CO_2 evolved during incubation is shown in Figure 3. All treatments showed similar increasing kinetic patterns while C mineralization flattened in the control after the 14^{th} day. Basal respiration significantly ($p < 0.05$) increased with treatment application with comparable rates among treatments.

Table 2. Fractions of soil carbon and nitrogen as affected by sole and mixed organic residues after 84 days of incubation

Treatments	MBN ($\mu g N g^{-1}$)	N_m ($\mu g\ N\ g^{-1} 84\ d^{-1}$)			NO_3^-/NH_4^+	SOC (g/kg)	MBC ($\mu g\ C\ g^{-1}$)	MBC/SOC (%)	Basal respiration ($\mu g\ C - CO_2\ g^{-1}\ h^{1}$)	qCO_2 ($10^5\ h^{-1}$)	MBC/ MBN
		NH_4^+	NO_3^-	TIN							
Control (no input)	136.5^{ab}	270.0^{a}	30.7^{a}	300.7^{a}	0.11^{c}	14.0^{a}	225.7^{a}	1.6^{a}	0.012^{a}	5.3^{b}	1.7^{a}
Td	146.0^{ab}	1020.0^{d}	64.0^{bc}	1084.0^{d}	0.06^{a}	19.0^{b}	866.7^{d}	4.6^{b}	0.033^{b}	3.8^{a}	6.1^{bc}
Vf	112.8^{a}	1100.0^{d}	67.1^{cd}	1167.1^{e}	0.06^{a}	18.7^{b}	1181.9^{e}	6.3^{c}	0.038^{c}	3.2^{a}	10.7^{d}
M	187.8^{b}	490.0^{b}	71.2^{e}	561.2^{b}	0.15^{d}	19.9^{b}	605.8^{b}	3.0^{b}	0.040^{c}	6.5^{c}	3.2^{ab}
Td + M	145.0^{ab}	710.0^{c}	60.4^{b}	770.6^{c}	0.09^{b}	19.6^{b}	782.7^{c}	4.0^{b}	0.038^{c}	4.8^{b}	5.4^{bc}
Vf + M	150.9^{ab}	533.0^{b}	69.0^{de}	602.0^{b}	0.13^{c}	19.7^{b}	1271.3^{f}	6.5^{c}	0.036^{bc}	2.8^{a}	8.5^{cd}
SED	15.52	24.14	1.04	24.02	0.005	0.38	10.01	0.09	0.002	0.3	0.97
p value	0.011	<0.001	<0.001	<0.001	<0.001	< 0.001	< 0.001	< 0.001	< 0.001	<0.001	<0.001

Values are the means of 4 replicates. Td = *T. diversifolia*, Vf = *V. faba*, M = *Z. mays*, MBN = microbial biomass nitrogen, MBC = microbial biomass carbon, TIN= total inorganic nitrogen, SOC = soil organic carbon, qCO_2 = metabolic quotient, N_m = cumulative nitrogen mineralization, 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% proability level.

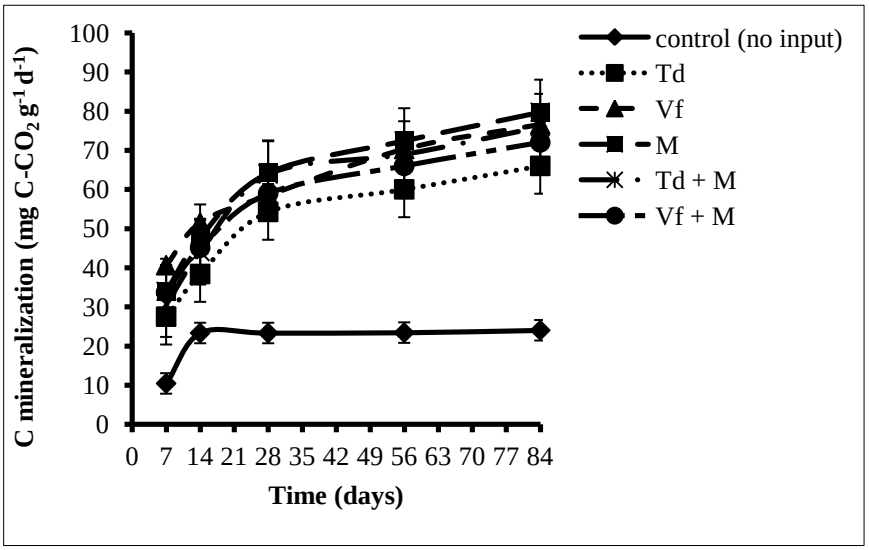

Figure 3. Carbon mineralization kinetics as affected by sole and mixed organic residues over 84 days of incubation. Data points are the means of four replicates. Error bars represent the standard error of mean (SEM). Vf = *V. faba*, Td = *T. diversifolia*, M = *Z. mays*.

3.4. Soil Microbial Biomass and Metabolic Quotient

The application of treatments did not increase the microbial biomass N (MBN) compared to the control although significant ($p < 0.05$) differences occurred between *Z. mays* and *V. faba* treatments. Meanwhile, treatments significantly increased the soil microbial biomass C (MBC). MBC ranged from 225.7 in the control to 1271.3 µg C g^{-1} in Vf + M treatments. Contrary to observed results between treatments Td and Td + M, the addition of M to *V. faba* increased MBC by 7% compared to sole *V. faba* application. The MBC: SOC ratio was the same in *V. faba* and *V. faba* + *Z. mays* treatments as it was between *T. diversifolia*, *Z. mays* and *T. diversifolia* + *Z. mays* treatments. Treatments significantly ($p < 0.001$) affected the MBC/MBN ratio with the greatest levels recorded in *V. faba* and *V. faba* + M treatments. Moreover, microbial metabolic quotient (qCO_2) was significantly ($p < 0.05$) greatest in *Z. mays* treatment and lowest in *T. diversifolia*, *V. faba* and *V. faba* + *Z. mays* amended soils (Table 2).

3.5. Soil Enzyme Activities

At the end of incubation, the application of treatments increased both β-glucosidase and β-glucosaminidase activities. β-glucosidase activity was significantly ($p < 0.05$) greatest in *Z. mays* and *V. faba* + *Z. mays* treatments (Figure 4a). The results also showed a higher β-glucosidase activity in mixed organic residues compared to single treatment applications. β-glucosaminidase activity was lower than β-glucosidase activity and was comparable among the organic residue treatments (Figure 4b). While treatments had no significant effect ($p > 0.05$) on acid phosphatase and alkaline phosphatase activities, alkaline phosphatase activity was predominant compared with acid phosphatase activity (Figure 5).

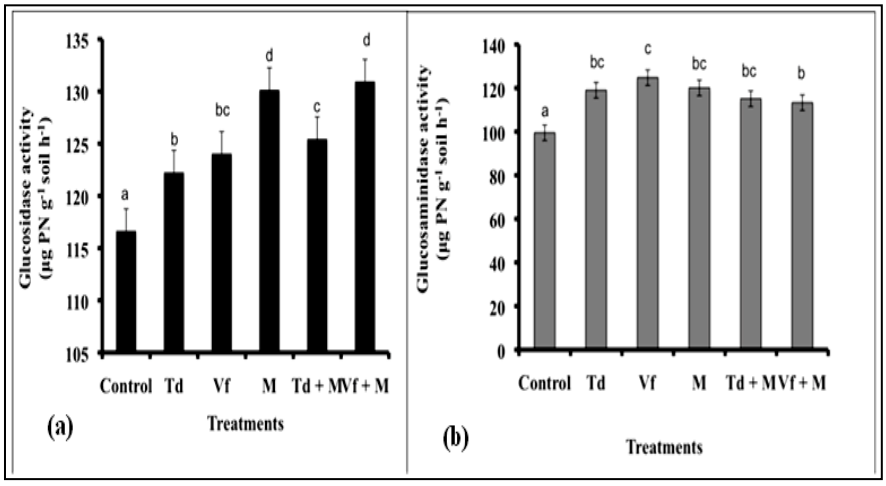

Figure 4. Effects of sole and mixed organic residues on β-glucosidase (a) and β-glucosaminidase (b) activities. Data points are the means of four replicates. Error bars represent the standard error of mean (SEM). Bars with the same letters do not differ significantly according to Tukey test at 5% probability level. Vf = *V. faba*, Td = *T. diversifolia*, M = *Z. mays*.

3.6. Relationship between Soil Parameters

Interrelationships between soil microbial and biochemical properties were observed. Both ammonium and nitrate were significantly correlated to each other and to the total inorganic N (Table 3). Total inorganic N was negatively

($r = -0.53$, $p < 0.05$) correlated to microbial metabolic quotient but showed strong positive correlation with soil organic C ($r = 0.50$, $p < 0.05$), microbial biomass C ($r = 0.64$, $p < 0.05$), basal respiration ($r = 0.56$, $p < 0.05$) and β-glucosaminidase activity ($r = 0.76$, $p < 0.05$). Various soil parameters were also significantly dependent on the soil organic carbon: basal respiration ($r = 0.94$, $p < 0.001$), microbial biomass C ($r = 0.68$, $p < 0.01$), β-glucosaminidase ($r = 0.75$, $p < 0.001$) and β-glucosidase ($r = 0.83$, $p < 0.001$).

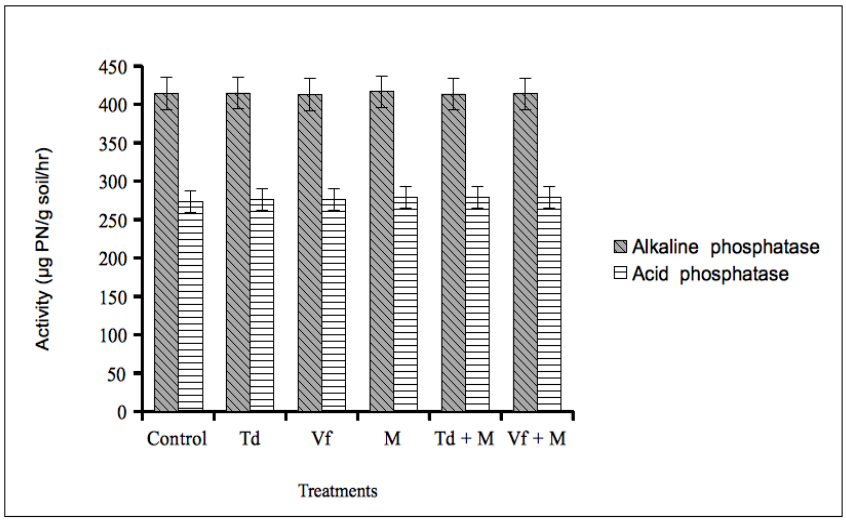

Figure 5. Effects of sole and mixed organic residues on phosphomonoesterase activities. Data points are the means of four replicates. Error bars represent the standard error of mean (SEM). Vf = *V. faba*, Td = *T. diversifolia*, M = *Z. mays*.

The activities of β-glucosaminidase and β-glucosidase were significantly correlated with each other and with basal respiration and the microbial biomass C. Furthermore, the microbial biomass N showed positive correlation with alkaline phosphatase activity ($r = 0.45$, $p < 0.05$) (Table 3).

3.7. Relationship between Organic Residue Chemistry and Soil Parameters

Several significant relationships were observed between the soil parameters monitored and the chemical composition of the treatments (Table

4). Soil organic C levels were significantly dependent on lignin ($r^2 = 0.94$, $p < 0.001$), C ($r^2 = 0.94$, $p < 0.001$) and lignin-to-nitrogen ratio ($r^2 = 0.86$, $p \leq 0.01$). Plant nitrogen was significantly correlated to ammonium ($r^2 = 0.71$, $p \leq 0.05$), total inorganic nitrogen ($r^2 = 0.72$, $p \leq 0.05$) and the soil microbial biomass carbon ($r^2 = 0.77$, $p \leq 0.05$). It was evident from the results that carbon, carbon-to-phosphorus ratio and the lignin-to-nitrogen ratio were mostly correlated to the soil parameters monitored (Table 4).

Considering that one soil parameter was significantly dependent on several plant chemical properties, we deduced linear models using stepwise regression to remove all indirect variables and determine the set of chemical parameters that most closely influence the soil parameters and include them in the multiple regression equation. Only significant regression models were reported (Table 5).

4. DISCUSSION AND CONCLUSION

The biochemical characteristics of organic residues are known to influence their suitability for nutrient management in agroecosystems (Partey et al., 2011; Troeh and Thompson, 2005; Palm et al., 2001, Young, 1997). According to Brady and Weil (2004), organic substrates of high N, low lignin, low C: N ratio and polyphenols have fast decomposition and nutrient release rates hence of high quality for soil fertility improvement practices. The results obtained showed fairly high macronutrient concentrations and relatively low composition of secondary compounds in both the single and mixed treatments. With the exception of maize, all plant residues recorded N levels beyond the critical minimum of 25 g/kg below which initial net immobilization of N could be expected (Palm et al., 2001).

Similarly, C/N ratio recorded for *Z. mays* was above the critical maximum beyond which initial net immobilization of N could be expected. Carbon-to-phosphorus ratios were all below the 200: 1 threshold for initial net mineralization of P (Schroth, 2003). Furthermore, polyphenol concentrations in all organic residues were below the critical maximum (30 g/kg) above which microbial degradation activities could be impaired (Brady and Weil, 2004). Nitrogen concentration in the organic residues was negatively correlated to lignin ($r = -0.87$, $p = 0.053$) and (lignin + polyphenol)/N ($r = -0.89$, $p = 0.045$) which is similarly reported by Vahdat et al. (2011).

Table 3. Correlation matrix showing the correlation coefficients for the relationship between soil microbial and biochemical properties

	qCO_2	**SOC**	**NO_3^-**	**NH_4^+**	**$NO_3^- + NH_4^+$**	**GSMD**	**MBC**	**Bresp**	**MBN**	**ALKP**	**GLD**
qCO_2	1										
SOC	Ns	1									
NO_3^-	Ns	0.95^{***}	1								
NH_4^+	-0.54^{*}	0.47^{*}	0.53^{*}	1							
$NO_3^- + NH_4^+$	-0.53^{*}	0.50^{*}	0.56^{*}	1.00^{***}	1						
GSMD	Ns	0.75^{***}	0.81^{***}	0.75^{***}	0.76^{***}	1					
MBC	-0.79^{***}	0.68^{**}	0.75^{***}	0.62^{**}	0.64^{**}	0.62^{**}	1				
Bresp	ns	0.94^{***}	0.95^{***}	0.53^{*}	0.56^{*}	0.82^{***}	0.68^{**}	1			
MBN	0.49^{*}	ns	Ns	ns	ns	ns	ns	ns	1		
ALKP	ns	ns	Ns	ns	ns	ns	ns	ns	0.45^{*}	1	
GLD	ns	0.83^{***}	0.84^{***}	ns	ns	0.49^{*}	0.59^{**}	0.80^{***}	ns	ns	1

GLD = β-glucosidase, GSMD = β-glucosaminidase, Bresp = basal respiration, ALKP = alkaline phosphatase, MBN = microbial biomass nitrogen, MBC = microbial biomass carbon, SOC = soil organic carbon, qCO_2 = metabolic quotient, ns = not significant. *, ** and *** refers to significance at 5%, 1% and 0.1% probability levels respectively. N = 18.

Table 4. Correlation coefficients for the relationship between organic residue chemistry and some soil parameters

	PN	**C**	**Lig**	**Poly**	**C: PN**	**C: P**	**Lig: PN**	**(Lig + Poly): PN**
SOC	ns	0.97***	0.97***	ns	ns	0.80*	0.93**	ns
NO_3^-	ns	0.97***	0.90**	ns	ns	0.89*	0.97***	ns
NH_4^+	0.84*	ns	Ns	0.95**	ns	ns	ns	ns
MBC	0.88*	0.81*	Ns	ns	ns	0.84*	ns	ns
Bresp	ns	0.97***	0.92**	ns	ns	0.87*	0.95**	ns
GLD	ns	ns	Ns	ns	ns	0.82*	0.92**	ns
GSMD	ns	0.87*	Ns	ns	ns	0.80*	0.82*	ns
ALKP	ns	ns	Ns	ns	ns	ns	ns	0.80*
ACP	ns	0.82*	0.81*	ns	ns	ns	0.86*	ns
$NO_3^- + NH_4^+$	0.85*	ns	Ns	0.95**	ns	ns	ns	ns
MBN	ns	ns	Ns	ns	0.86*	ns	ns	0.86*

Lig = lignin, poly = polyphenol, GLD = β-glucosidase, GSMD = β-glucosaminidase, PN = plant nitrogen, Bresp = basal respiration, ACP = acid phosphatase, ALKP = alkaline phosphatase, MBN = microbial biomass nitrogen, MBC = microbial biomass carbon, ns = not significant. *, ** and *** refers to significance at 5%, 1% and 0.1% probability levels respectively. N = 18.

Table 5. Stepwise regression models for the relationship between organic residue chemistry and some soil parameters

Soil property	Equation	R^2	p value
NO_3^-	Y = 0.12Poly + 1.83 Lig: N + 30.84	0.99	0.001
NH_4^+	Y = 9.64Poly + 238.07	0.90	0.004
Basal respiration	Y = 1.19 x 10^{-5}C + 0.012	0.95	0.001
β-glucosidase	Y = 0.69Lig: N + 115.88	0.85	0.009
β-glucosaminidase	Y = 0.009C + 99.77	0.77	0.023
Soil microbial biomass C	Y = 3.60N + 377.21	0.77	0.021
Soil microbial biomass N	Y = 1.54C: N – 0.12N + 137.88	0.94	0.015
Soil organic C	Y = 0.003C – 0.006N – 0.005Poly + 14.0	1.00	<0.001
$NO_3^- + NH_4^+$	Y = 9.93Poly + 285.04	0.91	0.004
Acid phosphatase	Y = 0.26Lig: N + 273.86	0.74	0.028

Lig = lignin, poly = polyphenol. N = 18

Differential effects of the various treatments on the soil microbial and biochemical properties reflected their apparent differences in chemical composition. Most treatments recorded increased NH_4^+ - N mineralization until the 56^{th} day when levels fell drastically as a result of possible N immobilezation. The increased N mineralization during the early stages of incubation can be attributed to the high N and low polyphenolic levels in the treatments. Among the plant chemical properties, total inorganic N was significantly correlated to plant N composition and polyphenols (Table 4). Several studies (Troeh and Thompson, 2005, Vanlauwe et al., 2005, Young, 1997) have demonstrated that initial N levels in plants and polyphenol composition can influence their decomposition and N release rates. The results of our study showed fairly high N and low polyphenol concentrations (Table 1) in the organic amendments demonstrating possible accelerated decom-position and N release which may have resulted in the increased available N levels recorded. Initial N and polyphenol levels were best predictors of N mineralization (Table 5), confirming that initial plant N concentration influences soil N levels. From the regression models, the results of our present study set a 28 g kg^{-1} critical maximum of polyphenol for net N mineralization to be realized in decomposing plant litter. This threshold value is consistent with the recommendations of Brady and Weil (2004). Whilst initial net N mineralization was observed in all treatments, cumulative net N mineralization at the end of the incubation period was significantly higher in sole treatments (particularly *V. faba* and *T. diversifolia*) than mixed treatments. Results may be related to different decomposer communities which may have developed on plant materials based on their intrinsic qualities (Cobo et al. 2002). The relative increase in available N compared to the control was approximately 86%, 100%, 156%, 260% and 288% in *Z. mays*, *V. faba* + *Z. mays*, *T. diversifolia* + *Z. mays*, *T. diversifolia* and *V. faba* respectively. Similarly, the proportion of total N mineralized or available after incubation was 40%, 43%, 45%. 60% and 69% in *Z. mays*, *V. faba* + *Z. mays*, *T. diversifolia* + *Z. mays*, *T. diversifolia* and *V. faba* respectively which recommends the use of these amendments (particularly *V. faba* and *T. diversifolia*) for nitrogen fertilization (especially in places where inorganic fertilizer use are limited).

The application of treatments significantly ($p < 0.001$) increased the soil microbial biomass C. The results showed significant differences between treatments as a result of their varying biochemical composition. Significant correlations between microbial biomass and plant N and C compositions were observed which demonstrated that differences in both C and N inputs (including quantity and quality) could significantly impact microbial biomass

C and activity (Tu et al., 2006). The significance of C inputs for increased soil microbial biomass was further confirmed by the significant positive correlation observed between microbial biomass C and organic C. The results of our present study revealed a significant positive correlation between microbial biomass C and available N. This affirmed that differences in microbial biomass and activity under different organic amendments would have significant implications for nutrient availability to crops. 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). Despite the significant correlation obtained between microbial biomass C and nitrogen availability, nitrogen mineralization was comparatively lower in mixed (which recorded the highest microbial biomass) than sole treatments. We expected that with higher C additions and increased microbial biomass in mixed treatments, higher N availability could be obtained which was not so. We attributed this to the synergistic influence of both C and N in the residues. As shown in Table 2, we recorded comparable C utilization efficiencies (shown by the qCO_2 values) between mixed (*V. faba* + M and *T. diversifolia* + M) and sole (*V. faba* and *T. diversifolia*) but significant variations in N compositions. Therefore N composition should have influenced the microbial biomass more than C composition. This is further explained by the higher correlation coefficient value recorded for N (Table 4) and the inclusion of N in the stepwise regression model generated for microbial biomass C (Table 5).

Moreover, our results demonstrated increased basal respiration (C mineralization) with treatment application. All amended soils showed comparable kinetic patterns in microbial respiration (Figure 3), probably because organic C levels were comparable (Table 2). This observation is also explained by the strong positive correlation observed between basal respiration and organic C, which is similarly reported by Kaiser et al. (2010) and Vanlauwe et al. (1999). Increased enzymatic activities in amended soils were also observed. The effect of treatments on soil enzyme activities was attributed to tissue quality of the organic residues used. In most cases, the C: P and lignin: N ratios were influential in predicting enzyme activities (Table 4). Among the enzymes, β-glucosidase and alkaline phosphatase activities were highest. Other studies have also found alkaline phosphatase and β-glucosidase activities predominant in soils from other regions (Acosta-MartÍnez and Tabatabai 2000; Bandick and Dick, 1999). Although enzyme activities are affected by soil properties, research findings have indicated that the

predominance and ecological role among enzymes do not change in different soils and vegetation (Acosta-MartÍnez and Tabatabai 2000). The significant correlation established between the two carbon cycling enzymes supports soil ecological claims that the overall activity of a single enzyme may depend on enzymes in different locations including intracellular enzymes from viable proliferating cells, and accumulated or extracellular enzymes stabilized in clay minerals and/ or complexed with humic colloids (Nannipieri et al. 2002). The results showed a significant close relationship between microbial biomass size and enzyme activity, which is consistent with the findings of Stark et al. (2008). N mineralization rate also showed strong correlations with the two carbon cycling enzymes and the microbial biomass C which is in agreement with the findings of Bengtsson et al. (2003) and Stark et al. (2008). In addition, both β-glucosidase and β-glucosaminidase were strongly correlated to the soil organic C and microbial respiration which confirms the two enzymes as sensitive indicators of soil organic matter and C cycling in agroecosystems (Dornbush, 2007).

In conclusion, our study confirmed organic residue chemistry differs significantly in sole and mixed organic residues and have implications on the levels of biological activities and N mineralization. The results demonstrated comparable levels of many biological activities in sole and mixed amendments but N mineralization and availability increased significantly in sole *V. faba* and *T. diversifolia* amendments. 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. Furthermore, results demonstrated that basal respiration, microbial biomass carbon and the activities of β-glucosidase and β-glucosaminidase are sensitive indicators of nitrogen availability and organic C levels in soils.

ACKNOWLEDGMENT

The authors express their sincere gratitude to the Sustainable Consumption Institute, University of Manchester who provided funding for Samuel Partey for carrying-out this research.

REFERENCES

Acosta-MartÍnez, V., Tabatabai M.A., 2000. Enzyme activities of a limed agricultural soil. *Biology and Fertility of Soils* 31, 85 – 91

Azmal, A.K.M., Maraumoto, T., Shindo, H., Nishiyama, M., 1996. Mineralization and changes in microbial biomass in water-saturated soil amended with some tropical plant residues. *Soil Science and Plant Nutrition* 42, 483–492.

Badiane, O., Delgado C.L. (eds), 1995. A 2020 vision for food, agriculture, and the environment in Sub-Saharan Africa. Food, Agriculture, and the Environment Discussion Paper 4. Washington, DC: International Food Policy Research Institute.

Bandick, A.K., Dick R.P., 1999. Field management effects on soil enzyme activities. *Soil Biology and Biochemistry* 31, 1471–1479.

Bengtsson, G., Bengtson, P., Mansson, K.F., 2003. Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. *Soil Biology and Biochemistry* 35, 143–154.

Brady, N.C., Weil, R.R., 2004. Elements of the Nature and Properties of Soils, Second Edition. Upper Saddle River, NJ: Prentice – Hall, Inc.

Brookes, P.C, Powlson, D.S., Jenkinson, D., 1982. Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry* 14, 319- 329.

Chander, K., Goyal, S., Mundra, M.C., Kapoor, K.K., 1997. Organic matter, microbial biomass and enzyme activity of soils under different crop rotations in the tropics. *Biology and Fertility of Soils* 24, 306–310.

Cobo, J.G., Barrios, E., Kass, D.C.L., Thomas, R.J., 2002. Decomposition and nutrient release by green manures in a tropical hillside agroecosystem. *Plant Soil* 240, 331–342.

Dinesh, R., 2004. Long-term effects of leguminous cover crops on microbial indices and their relationships in soils of a coconut plantation of a humid tropical region. *Journal of Plant Nutrition and Soil Science* 167, 189–195.

Dornbush, M.E., 2007. Grasses, litter, and their interaction affect microbial biomass and soil enzyme activity. *Soil Biology and Biochemistry* 39, 2241–2249.

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry* 20, 601-606.

Eivazi, F., Tabatabai, M.A., 1977. Phosphatases in soils. *Soil Biology and Biochemistry* 9, l67- 172.

Ekenler, M., Tabatabai, M.A. 2002. β-Glucosaminidase activity of soils: effect of cropping systems and its relationship to nitrogen mineralization. *Biology and Fertility of Soils* 36, 367–376.

Eneji, A. E., Yamamoto, S., Wen, G., Inanaga, S., Honna, T., 2005. *Toxicological and Environmental Chemistry* 87, 147–158.

Gachengo, C.N., Palm, C.A., Jama, B., Othieno, C., 1999. Tithonia and Senna green manures and inorganic fertilizers as phosphorus sources for maize in western Kenya. *Agroforestry Systems* 44, 21–36.

Gartner, T.B., Cardon, Z.G., 2004. Decomposition dynamics in mixed-species leaf litter. *Oikos* 104, 230–246.

George, T.S., Gregory, P.J., Robinson, J.S., Buresh, R.J., Jama, B., 2002. Utilization of soil organic phosphorus by agroforestry and crop species in the field in western Kenya. *Plant and Soil* 246, 53 – 63.

Gruhn, P., Goletti, F., Yudelman, M., 2000. Integrated Nutrient Management, Soil Fertility, and Sustainable Agriculture: Current Issues and Future Challenges. International Food Policy Research Institute, 2033 K Street, N.W. Washington, D.C. 20006 U.S.A, pp 31.

Handayanto, E., Giller, K.E., Cadisch, G., 1997. Regulating N release from legume tree prunings by mixing residues of different quality. *Soil Biology and Biochemistry* 29, 1417–1426.

Kaiser, M., Wirth, S., Ellerbrock, R.H., Sommer, M., 2010. Microbial respiration activities related to sequentially separated, particulate and water-soluble organic matter fractions from arable and forest topsoils. *Soil Biology and Biochemistry* 42, 418 - 428

Kumwenda, J.D.T., Waddington, S.R., Snapp, S.S., Jones R. B., Blackie, M.J., 1996. Soil fertility management research for the maize cropping systems of smallholders in southern Africa: A review. Natural Resources Group Paper 96 -02. Mexico City: International Maize and Wheat Improvement Center (CIMMYT).

Ladd, J.N., Amato, M., 1989. Relationship between microbial biomass carbon in soils and absorbance of extracts of fumigated soils. *Soil Biology and Biochemistry* 21, 457- 59.

Motsara, M.R., Roy, R.N., 2008. Guide to Laboratory establishment for plant nutrient analysis. FAO Fertilizer and Plant nutrition bulletin. Food and Agriculture Organization, Rome. 219pp.

Nannipieri, P., Kandeler, E., Ruggiero, P., 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Burns R. G, Dick

R. P (eds) *Enzymes in the environment: activity, ecology and applications*. Dekker, New York, pp 1–33.

Nziguheba, G., Merckx, R., Palm, C.A., Rao, M.R., 2000. Organic residues affect phosphorus availability and maize yields in a Nitisol of western Kenya. *Biology and Fertility of Soils* 32, 328–339.

Palm, C.A., Gachengo, C.N., Delve, R.J., Cadisch, G., Giller, K., 2001. Organic inputs for soil fertility management in tropical agroecosystems: application of an organic resource database. *Agriculture, Ecosystems and Environment* 83, 27-42.

Parham, J.A, Deng, S.P., 2000. Detection, quantification and characterization of β-glucosaminidase activity in soil. *Soil Biology and Biochemistry* 32:1183–1190

Partey, S.T., Quashie-Sam, S.J., Thevathasan, N.V., Gordon, A.M., 2011. Decomposition and nutrient release patterns of the leaf biomass of the wild sunflower (*Tithonia diversifolia*): a comparative study with four leguminous agroforestry species. *Agroforestry Systems* 8, 123–134.

Pinstrup-Andersen, P. 2000. Foreword. In: Gruhn, P., Goletti, F., Yudelman, M. *Integrated Nutrient Management, Soil Fertility, and Sustainable Agriculture: Current Issues and Future Challenges*. International Food Policy Research Institute 2033 K Street, N.W. Washington, D.C. 20006 U.S.A, pp 31

Rosegrant, M.W., Agcaoili-Sombilla, M., Perez, N.D., 1995. Global food projections to 2020: Implications for investment. Food, Agriculture and the Environment Discussion Paper 5. Washington, DC: IFPRI.

Ross, D.J., Tate, K.R., 1993. Microbial carbon and nitrogen, and respiratory activity, in litter and soil of southern beech (Nothofagus) forest distribution and properties. *Soil Biology and Biochemistry* 25: 477- 483.

Sanchez, P.A., Shepherd, K.D. Soule, M.J., Place F.M., Buresh, R.J., Izac, A.N., 1997. Soil fertility replenishment in Africa: an investment in natural resource capital. In: Buresh R.J., Sanchez P.A., Calhoun F. (eds), *Replenishing Soil Fertility in Africa*: SSSA Special Publication Number 51. ASA and SSSA, Madison, USA, pp. 1 – 46.

Sakala, W.D., Cadisch, G., Giller, K.E., 2000. Interactions between residues of maize and pigeonpea and mineral N fertilizers during decomposition and N mineralization. *Soil Biology and Biochemistry* 32, 679–688.

Schroth, G., 2003. Decomposition and Nutrient Supply from Biomass. In: Schroth G, and FL Sinclair (Eds). 2003. Trees, Crops and Soil fertility Concepts and Research Methods. CABI Publishing, UK. pg 131.

Singh, S., Ghoshal, N., Singh, K.P., 2007. Variations in soil microbial biomass and crop roots due to differing resource quality inputs in a tropical dryland agroecosystem. *Soil Biology and Biochemistry* 39, 76–86.

Sparling, G.P., Feltham, C.W., Reynolds J., West, A.W., Singleton, P., 1990. Estimation of soil microbial carbon by fumigation – extraction method. Use on soils of high organic matter content, and a reassessment of the KEc- factors. *Soil Biology and Biochemistry* 22: 301-07.

Sparling, G.P., West, A.W., 1998. A direct extraction method to estimate soil microbial carbon: Calibration in situ using microbial respiration and ^{14}C-labelled cells. *Soil Biology and Biochemistry* 20, 337- 43.

Stark, C.H., Condron, L.M., O'Callaghan, M., Stewart, A., Di, H.J., 2008. Differences in soil enzyme activities, microbial community structure and short-term nitrogen mineralisation resulting from farm management history and organic matter amendments. *Soil Biology and Biochemistry* 40, 1352–1363.

Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry* 1, 301–307.

Troeh, F.R., Thompson, L.M., 2005. Soils and soil Fertility, Sixth Edition. Blackwell Publishing Professional, USA. 489pp.

Tu, C., Jean, B., Ristaino, J.B., Hu, S., 2006. Soil microbial biomass and activity in organic tomato farming systems: effects of organic inputs and straw mulching. *Soil Biology and Biochemistry* 38, 247–255.

Vahdat, E., Farshid, N., Basiri M., 2011. Lignin content of range plant residues controls N mineralization in soil. *European Journal of Soil Biology* 47, 243 – 246.

Vanlauwe, B., Gachengo, C.N., Shepherd, K., Barrios, E., Cadisch, G., Palm, C.A., 2005. Laboratory validation of a resource quality-based conceptual framework for organic matter management. *Soil Science Society of America Journal* 69, 1135 - 1145.

Vanlauwe, B., Nwoke, O.C., Sanginga, N., Merckx, R., 1999. Evaluation of methods for measuring microbial biomass C and N and relationships between microbial biomass and soil organic matter particle size classes in West-African soils. *Biology and Fertility of Soils* 35, 18 - 26.

van Soest, P.J., 1963. Use of detergents in the analysis of fibrous feeds. II: A rapid method for the determination of fibre and lignin. *Journal of the Association of Agricultural Chemists* 46, 829–835.

VSN International Ltd., 2008. GENSTAT for Windows. Eleventh edition. VSN International, 5. The Waterhouse, Waterhouse Street, Hemel Hempstead, Hertfordshire HP1 1ES,

Wang, W.J, Smith, C.J, Chen, D., 2004. Predicting soil nitrogen mineralization dynamics with a modified double exponential model. *Soil Science Society of America Journal* 68, 1256–1265.

Young, A., 1997. Agroforestry for Soil Management. CAB International, Wallingford, UK. pp 320.