

# Soil Microbial Biomass Carbon, Nitrogen and Phosphorus Dynamics under Different Amendments and Cropping Systems in the Semi–deciduous Forest Zone of Ghana

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## Abstract

A field experiment to monitor the dynamics of microbial biomass carbon, nitrogen and phosphorus under amendments and cropping systems were conducted in 2006 and 2007 at the Central Agricultural Station, Kwadaso Kumasi, Ghana. The field experiment was a split plot with three replications. Three different amendments (poultry manure, poultry manure + chemical fertilizer, chemical fertilizer) and a control (no amendment) constituted the sub-plots whereas cropping systems (continuous maize, maize/soybean intercrop and maize/cowpea rotation) were assigned to the main plots. Soil samples under each amendment and cropping system were taken at 3 weeks interval within each cropping season and analysed. The results of the study revealed a general buildup of microbial biomass over the seasons. Microbial biomass carbon ranged from 25 to 248 mg/kg soil in 2006 (major season) to 87 to 713 and 546 to 770 mg/kg soil in 2006 (minor) and 2007 (major seasons), respectively. Biomass carbon showed positive correlations with soil organic carbon with  $r$  values of 0.71, 0.40 and 0.64 in 2006 (major) 2006 (minor) and 2007 (major seasons), respectively. Biomass nitrogen showed more temporal fluctuations than biomass carbon. Negative values (-47.7 to -7.40 mg/kg soil) for microbial biomass phosphorus were observed at 42 and 63 days after amendments application (DAAA), signifying immobilization of phosphorus at the peak of crop growth. The immobilized phosphorus was, however, released 84 DAAA, thus, adding to the available phosphorus content of the soil. The study has shown that microbial biomass could be influenced positively by amendments and cropping systems overtime, and that phosphorus could be immobilized at the peak of crop growth; its release not concurring with peak nutrient demands of crops, hence, the need for synchronization.

## Introduction

Soil fertility decline is a major biophysical problem confronting crop production in Ghana. Most Ghanaian soils contain low organic matter of less than 1.0% (NSFMAP, 1998) which is inadequate to sustain crop production. Above all, most of the soils are developed on thoroughly weathered parent materials. They are old and have been leached over a long period of time (Benneh *et al.*, 1990) and are, therefore, of low inherent fertility (NSFMAP, 1998). It is, therefore, obvious that soil fertility decline in Ghana would be on the increase if

pragmatic actions are not quickly taken to curtail the situation. One way of doing this is the study of soil microbial biomass dynamics as affected by specific amendments in the various cropping systems. Due to the dominant contribution of microbial biomass in soil metabolism, and its importance as a sink and source of nutrients for plants, microbial biomass is considered to be one of the main determinants of soil fertility (Jenkinson & Ladd, 1981).

In Ghana, use of fertilizer and other soil amendments are carried out without taking

into consideration the effects on microbial biomass, which serves as early indicator of changes in soil chemical and physical properties resulting from soil management and environmental stresses in agricultural ecosystems. Determination of microbial nitrogen is important for the quantification of nitrogen dynamics in agricultural ecosystems because it controls soil organic nitrogen availability and loss, especially in high input systems (Moore *et al.*, 2000). However, not much work has been done on microbial biomass carbon, nitrogen and phosphorus as affected by amendments and cropping systems. The few works done were centered on only one or two cropping systems in temperate climates, results of which may be of limited importance and applicability in a tropical country like Ghana. The consequence is poor planning of amendments which eventually results in the reduction in productivity of cropping systems.

According to Grant *et al.* (2002), sustainability of cropping systems requires that nutrients removed from the soil be balanced by nutrient replacement. This, however, cannot be achieved unless the trend of change or dynamics of microbial biomass, which largely controls the availability of crop nutrients in the various cropping systems, is studied. Even though microbial biomass is important in the breakdown of soil organic matter, resulting in the availability of nutrients, little is known about its seasonal variation or changes with crop rotations and other agronomic practices such as intercropping (Stern, 1993). In an attempt to bridge this gap in knowledge, a study was initiated in 2006 to investigate the pattern of change of microbial biomass carbon, nitrogen and phosphorus with time under

different amendments and cropping systems in the semi – deciduous forest zone of Ghana.

### Materials and methods

#### *Study site*

A field experiment was conducted at the Central Agricultural Station, Kwadaso, Kumasi, in 2006 – major, 2006 – minor and 2007 – major seasons. Geographically, the area lies between latitudes 06° 39' and 06° 43' N and longitudes 01° 39' and 01° 42' W of the Greenwich Meridian. It is located in the semi-deciduous forest zone of Ghana (Taylor, 1952) and is characterized by a bimodal rainfall distribution. The major rainy season starts from March to July and the minor season starts from September to November. There is a short dry period in August. The mean annual precipitation is about 1500 mm while mean monthly temperatures range from 24 to 28 °C. Generally, relative humidity is high in the mornings, being about 90% at 0600 h and falling to between 60 and 70% in the afternoon (1500 h). The study was conducted on Asuansi soil series classified by Adu (1992) as Ferric Acrisol according to FAO (1990) and Typic Haplustult according to USDA (1998).

Initial soil analysis of the site in 2006 showed a pH of 6.7 in 1:2.5 suspension of soil and water. Soil texture was determined by the hydrometer method (Boyucos, 1962) and was found to be sandy loam. Organic carbon, which was determined by the modified Walkley and Black procedure, as described by Nelson & Sommers (1982), ranged from 1.08 to 1.36%. Other soil chemical properties of the site were NO<sub>3</sub><sup>-</sup> - N,

3.97 mg/kg soil; NH<sub>4</sub>-N, 4.04 mg/kg soil; total N, 0.07%; available P, 45.13 mg/kg soil; exchangeable K, 0.38 cmol/kg soil.

#### *Experimental design and treatments*

The field experiment was a split plot arranged in a randomized complete block design (RCBD) with three replications. The main plot factor was cropping system and that of the sub-plot was amendment. The cropping systems evaluated were continuous maize (CM), maize/soybean (M/S) intercropping and maize/cowpea (M/C) rotation. Three different amendments and a control (no amendment) were considered. Poultry manure – PM (4 t/ha), chemical fertilizer – NPK 15-15-15 (60-60-60 kg/ha) and poultry + chemical fertilizer (2 t/ha PM + 30-30-30 kg/ha NPK) were applied by side placement 2 weeks after planting (WAP). At 5 WAP, plots amended with poultry manure + chemical fertilizer (PM + CF), and chemical fertilizer (CF) were ‘top dressed’ with nitrogen at the rate 15 kg/ha and 30 kg/ha, respectively. The cultivars of the test crops were maize (Dorke SR), cowpea (Soronko) and soybean (Ahoto). The total land area measured 42.5 m × 14.0 m (595.0 m<sup>2</sup>).

#### *Soil sampling*

Ten plants were selected at random from the middle rows of each plot. Soil samples were taken from the base of each plant at a depth of 0–15 cm (Moore *et al.*, 2000) using hand auger. The 10 auger soil samples were then pooled together (bulked) as representative sample for each plot. The representative samples (36) were subjected to microbial analyses in the fresh state after sieving through a 4-mm mesh. In all, four samplings were made during each season at

intervals of 3, 6, 9 and 12 weeks after application of amendments. However, in order to assess the nutrient status of the soil before cropping, the soil was initially characterized and the physico-chemical properties determined. The microbiological properties (biomass carbon, nitrogen and phosphorus) of the soils were determined in the laboratory of the Soil Research Institute, Kwadaso, Kumasi.

#### *Soil microbial biomass carbon and nitrogen*

Soil microbial carbon and nitrogen were monitored under the different amendments and cropping systems. The chloroform fumigation method and extraction (FE) as described by Ladd & Amato (1989) was used to determine the microbial biomass. Ten grams of field moist soil sample, after passing through a 4-mm mesh, was put in a crucible and placed in a desiccator. A shallow dish containing 30 ml of alcohol-free chloroform was placed by it. A crucible containing a control sample (10 g) was placed in a separate desiccator without chloroform. The desiccators were covered and allowed to stand at room temperature for 5 days (Anderson & Ingram, 1998).

Immediately after fumigation, 50 ml of 0.5 M K<sub>2</sub>SO<sub>4</sub> solution was added to the soil samples to extract microbial carbon and nitrogen from the lysed microorganisms. Total nitrogen in the extract was then determined by the Kjeldahl method. The amount of microbial carbon in the extract was determined using the colorimetric method. An aliquot (5 ml) of the extract was pipetted into 250-ml Erlenmeyer flask. To this were added 5 ml of 1.0 N (0.1667 M) potassium dichromate and 10 ml concentrated sulphuric acid. The resulting solution was allowed to cool for 30 min after which

10 ml of distilled water was added.

A standard series was developed concurrently with carbon concentrations ranging from 0, 2.5, 5.0, 7.5, 10.0-mg/ml C. These concentrations were obtained when volumes of 0, 5, 10, 15 and 20 ml of a 50 mg/ml C stock were pipetted into labelled 100-ml volumetric flasks and made up to the mark with distilled water. The absorbances of the standard and sample solutions were read on a spectronic 21D spectrophotometer at a wavelength of 600 nm.

A standard curve was obtained by plotting absorbance values of the standard solutions against their corresponding concentrations. Extracted carbon concentration of the samples was determined from the standard curve. For biomass C and N calculations, k-factors of 0.35 (Sparling *et al.*, 1990) and 0.45 (Jenkinson, 1988; Ross & Tate, 1993) were used, respectively.

The following equations (Sparling & West, 1988) were used to estimate the microbial C and N from the extracted C and N, respectively:

$$\text{Microbial C (mg)} = E_c/k$$

$$\text{Microbial N (mg)} = E_n/k$$

where  $E_n$  = the extracted nitrogen produced following fumigation;  $E_c$  = the extracted carbon produced following fumigation;  $k$  = the fraction of the killed biomass extracted as carbon or nitrogen under standardized conditions.

#### Soil microbial phosphorus

For microbial biomass P analysis, 5 g of field-moist soil was weighed into a crucible and fumigated in a dessicator with 30 ml of alcohol-free chloroform for 5 days. Both

fumigated and unfumigated soil samples were shaken with 35 ml Bray's No.1 extracting solution (0.03 M NH<sub>4</sub>F + 0.025 M HCl) for 10 min and filtered. Correction for adsorption of P during fumigation was made by simultaneously equilibrating unfumigated soil with a series of P containing standard solutions followed by extraction with the Bray-1 solution. The amount of chloroform released P was determined according to the relationship between P added (from standard solutions or microbial lysis) and P extracted by the Bray-1 solution (Obersson *et al.*, 1997).

Phosphorus adsorption during equilibrium is described by the following equation according to Barrow & Shaw (1975) and adapted by Morel *et al.* (1997):

$$Ext_s = Ext_0 + b_1 P_{ad}$$

where  $Ext_s$  = Pi concentration (mg/l) extracted after equilibration with different amounts of P added;  $Ext_0$  = Pi concentration extracted without P addition;  $b_1$ ,  $b_2$  = coefficients estimated by non-linear regression of mean values of  $Ext_s$  against  $P_{ad}$ ;  $P_{ad}$  = amount of P added (0–20 mg/kg)

Chloroform released P corresponds to a P addition and is calculated from the equation:

$$P_{chl} = [(Ext_{chl} - Ext_0)/b_1]^{1/b_2}$$

where  $P_{chl}$  = chloroform released P (mg/kg);  $Ext_{chl}$  = Pi concentration in extracts of fumigated samples.

The amount of microbial P is estimated by assuming a  $k_p$  factor of 0.4 (Brookes *et al.*, 1982; McLaughlin & Alston, 1986).

#### Statistical analysis

Data on all parameters/response variables were subjected to analysis of variance (ANOVA) using the GenStat statistical package (GenStat, 2007). Separation of

means was done using the least significant difference (LSD) method at  $P = 0.05$ . Regression and correlation analyses were carried out to determine the nature and magnitude of relationships between biomass carbon and soil organic carbon.

## Results

### *Microbial biomass carbon and nitrogen*

The results of trials conducted showed that microbial biomass carbon and nitrogen were generally not significantly influenced by amendments (Fig. 1ace, 2ac). Significant differences ( $P < 0.05$ ) were, however, observed between cropping systems 21 days following amendments application in 2006 - major and minor seasons (Fig. 1b and 2d).

At 63 days after amendments application (DAAA) in 2007 - major season, chemical fertilizer treated plots recorded significantly higher ( $P < 0.05$ ) microbial biomass nitrogen than all plots (Fig. 2e). Microbial biomass nitrogen was significantly affected by sampling periods in both years of study (Fig. 2a–2f). However, biomass carbon was significantly influenced ( $P < 0.05$ ) only in 2006 – minor season (Figs. 1c and 1d).

There were fluctuations in microbial biomass carbon and nitrogen at the sampling periods. The least and highest values were generally obtained at 42 and 63 DAAA, respectively (Fig. 1cd, 2abef). Higher values were recorded during the 2007- major season compared with the major and minor seasons of the previous year (2006). Microbial biomass carbon recorded during the 2006 – major and minor seasons ranged from 25 to 248 mg C/kg soil and 87 to 713 mg C/kg soil, respectively. For the 2007 – major season, a range of 546 to 770 mg C/kg soil was observed. Biomass nitrogen under amendments ranged from

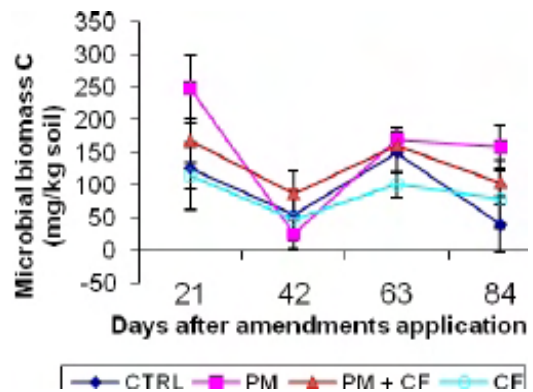


Fig. 1a. Microbial biomass C dynamics under different amendments in 2006 – major season

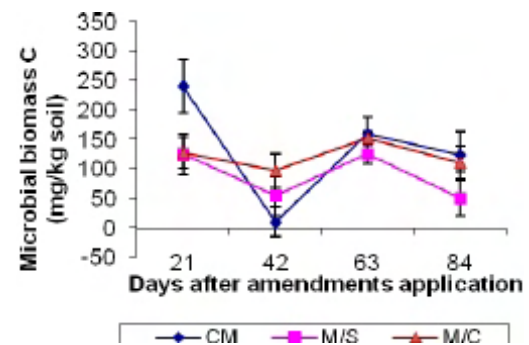


Fig. 1b. Microbial biomass C dynamics under different cropping systems in 2006- major season

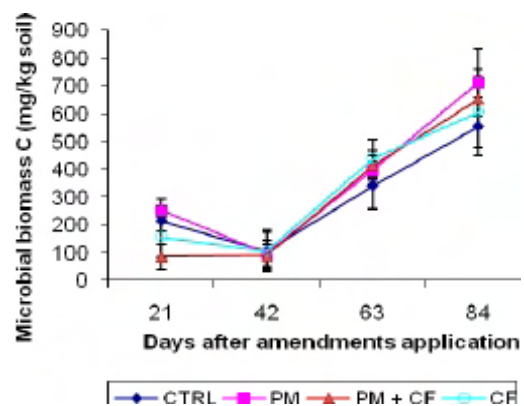


Fig. 1c. Microbial biomass C dynamics under different amendments in 2006 – minor season

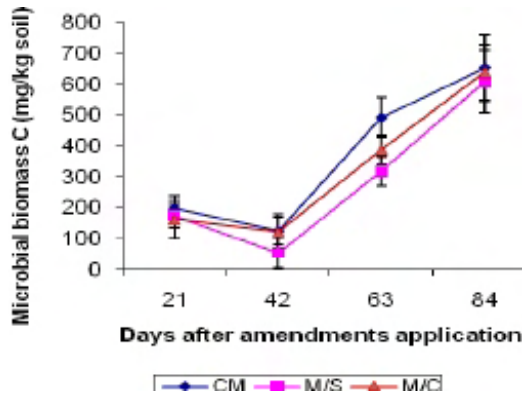


Fig. 1d. Microbial biomass C dynamics under different cropping systems in 2006 –minor season

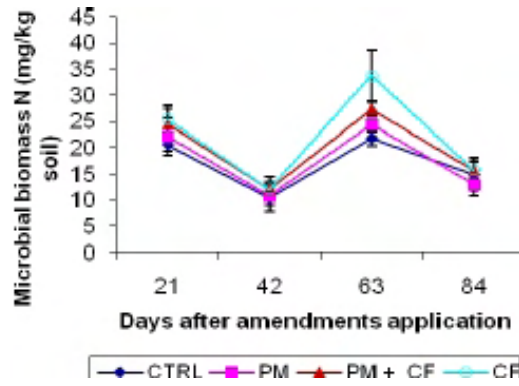


Fig. 2a. Microbial biomass N dynamics under different amendments in 2006 –major season

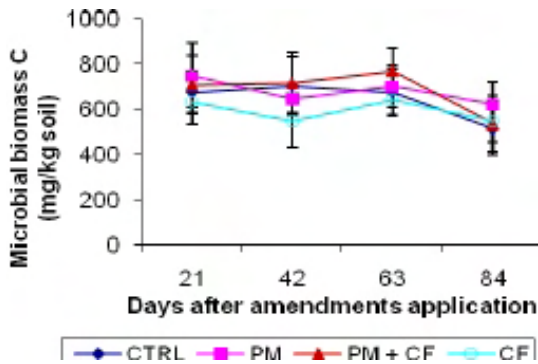


Fig. 1e. Dynamics of microbial biomass C under different amendments in 2007 –major season

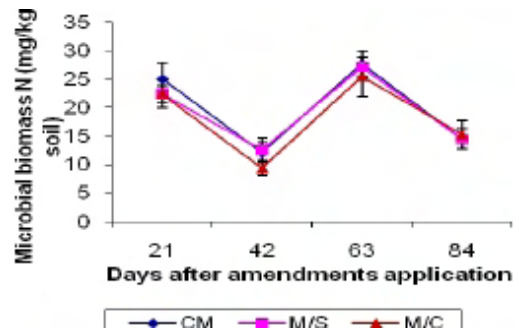


Fig. 2b. Microbial biomass N dynamics under different cropping systems in 2006 -major season

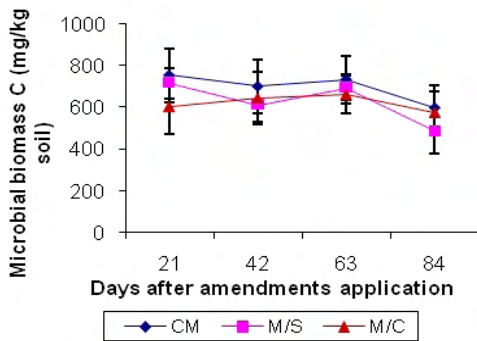


Fig. 1f. Dynamics of microbial biomass C under different cropping systems during the 2007- major season

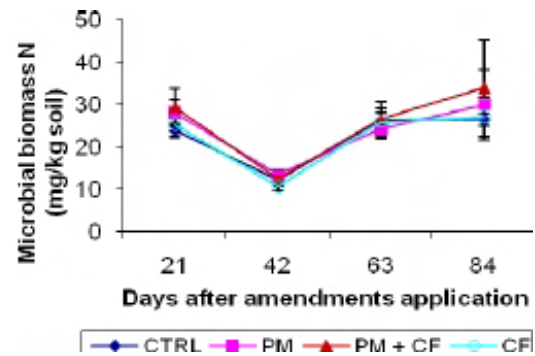


Fig. 2c. Microbial biomass N dynamics under different amendments in 2006 -minor season

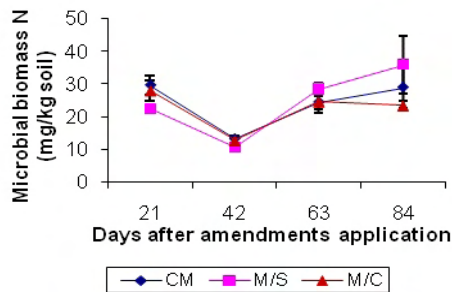


Fig. 2d. Microbial biomass N dynamics under different cropping systems in 2006 - minor season

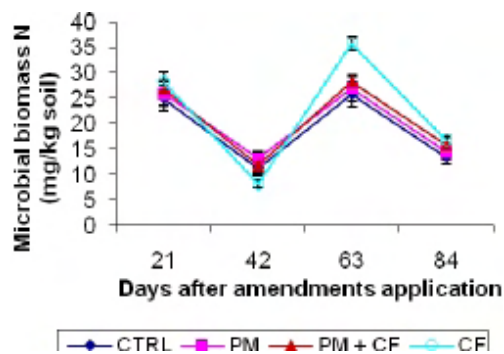


Fig. 2e. Microbial biomass N dynamics under different amendments in 2007 – major season

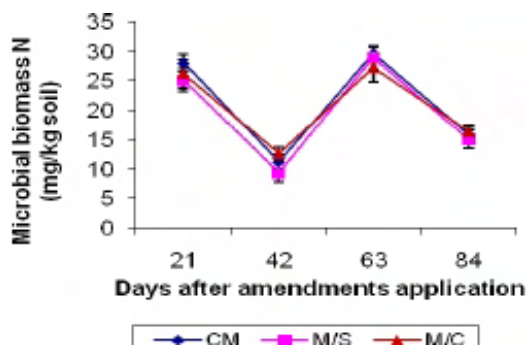


Fig. 2f. Microbial biomass N dynamics under different cropping systems in 2007-major season

10.37 to 33.6 mg/kg soil, 12.10 to 33.9 mg/kg soil and 13.13 to 35.70 mg/kg soil in 2006 – major, 2006 - minor and 2007 – major seasons, respectively. Microbial biomass carbon showed positive correlation with soil organic carbon ( $r = 0.71, 0.40$  and  $0.64$ ) (Table 1). Effective cation exchange capacity (ECEC) showed strong positive correlations with biomass carbon (Table 2) in all seasons.

#### Microbial biomass phosphorus

Soil microbial phosphorus under the different cropping systems and amendments did not follow any specific trend (Fig. 3a–3f). At 21 DAAA in 2006 – major season, biomass phosphorus was similar for all the amendments and cropping systems (Fig. 3ab). Significant differences ( $P < 0.05$ ) were observed between amendments 21 DAAA in 2006 - minor and 2007- major seasons (Fig. 3ce). Negative values were recorded in all plots at 42 and 63 DAAA (Fig. 3cd) signifying immobilization of the phosphorus. Soil microbial P at the 84 DAAA was positive throughout the entire study period. The lowest range of microbial biomass phosphorus was recorded in 2006 – minor season ( $-47.7$  to  $32.6$  mg P/kg soil) whereas the highest was recorded in 2007 – major season ( $-39.62$ – $120.23$  mg P/kg soil).

## Discussion

#### Microbial biomass carbon and nitrogen

Soil microbial biomass, a living part of soil organic matter, is an agent of transformation for added and native organic matter and acts as a labile reservoir for plant-available nitrogen, phosphorus and sulphur (Jenkinson & Ladd, 1981). The activity of the microbial biomass is commonly used to

Table 1  
Relationship between microbial biomass C (y) and SOC (x) for the three seasons

Season	Regression equation	r	r <sup>2</sup>
2006 -major	$y = 326.13x - 227.94$	0.84	0.71
2006 -minor	$y = 368.46x - 96.53$	0.63	0.40
2007 -major	$y = 392.11x + 177.71$	0.80	0.64

SOC: Soil organic carbon.

Table 2  
Relationship between ECEC (y) and microbial biomass carbon (x) for the three seasons

Season	Regression equation	r	r <sup>2</sup>
2006 -major	$y = 94.17x - 697.02$	0.97	0.95
2006 -minor	$y = 34.99x + 14.77$	0.74	0.55
2007 -major	$y = 45.51x - 253.94$	0.98	0.96

ECEC: Effective cation exchange capacity.

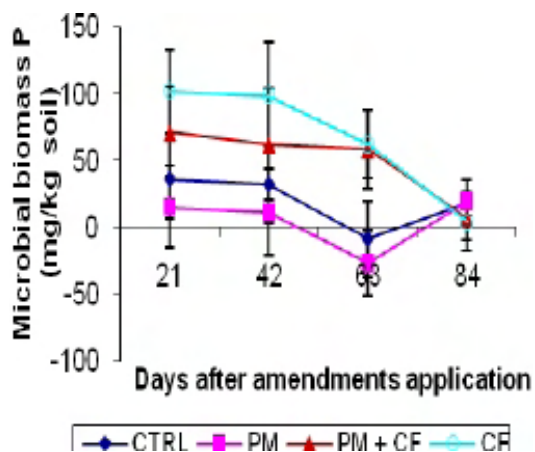


Fig. 3a. Microbial biomass P dynamics as affected by different amendments in 2006 – major season

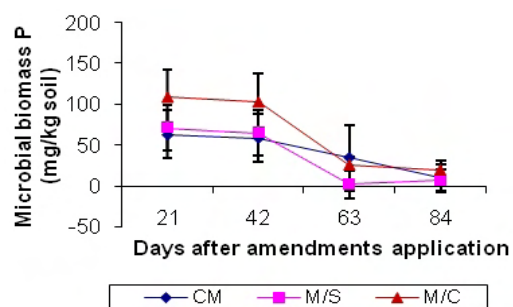


Fig. 3b. Microbial biomass P dynamics as affected by different cropping systems in 2006 – major season



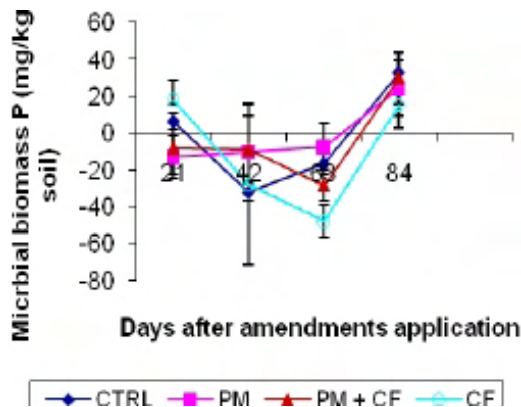


Fig. 3c. Microbial biomass P dynamics as affected by different amendments in 2006 – minor season

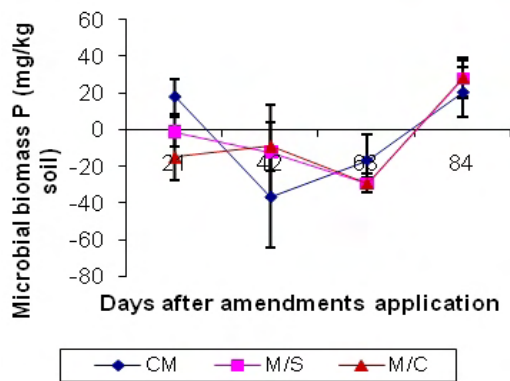


Fig. 3d. Microbial biomass P dynamics as affected by different cropping systems in 2006 – minor season

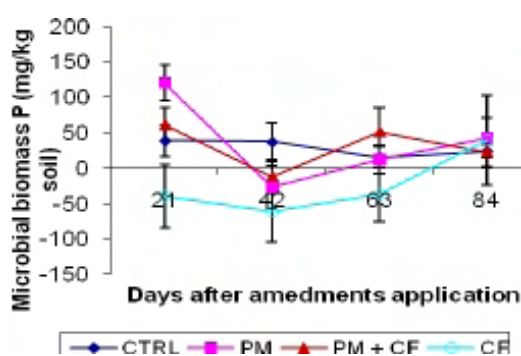


Fig. 3e. Microbial biomass P dynamics under different amendments in 2007 – major season

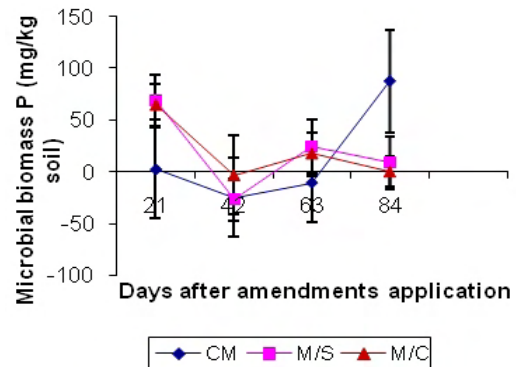


Fig. 3f. Microbial biomass P dynamics under different cropping systems in 2007 – major season

characterize the microbiological status of soil (Nannipieri *et al.*, 1990) and to determine the effects of cultivation (Anderson & Domsch, 1993) and field management (Perott *et al.*, 1992) on soil microorganisms.

Results of the study showed temporal fluctuations in microbial biomass carbon and nitrogen. The fluctuations occurred between sampling periods within the major seasons. There was a general decline in biomass carbon and nitrogen 42 DAAA. This was followed by an increase at 63 DAAA and a decrease at 84 DAAA (Fig. 1ab, 2aef). These fluctuations could be due to variations in soil moisture and temperature, stage of plant growth and available substrate. Similar observations have been reported by several other workers (Insam, 1990; Kaiser *et al.*, 1995; Lovell *et al.*, 1995; Chang & Juma, 1996). Soil microbial properties are influenced by variations in soil moisture and temperature, nutrient supply, etc. (Campbell *et al.*, 1999).

The microbial biomass nitrogen values showed more temporal fluctuations than those of biomass carbon (Fig. 1a–2f).

Joergensen (1995) reported more temporal fluctuations of biomass nitrogen than biomass carbon. Microorganisms differ much more in their nitrogen content than in their carbon content, depending on their stage of growth (Jenkinson & Ladd, 1981). This is one reason for the larger variations in  $k_n$  values (fraction of the killed biomass extracted as N under standardized conditions) compared with  $k_c$  values (fraction of the killed biomass extracted as biomass C) found in literature (Joergensen, 1995). Therefore, small shifts in the structure of the microbial community can result in large changes in N.

Higher microbial biomass carbon was observed in the 2007- major season (Fig. 1ef) than in 2006 - minor (Fig. 1cd) and 2006 - major seasons (Fig. 1ab). This was as a result of the cumulative effect of the amendments, and crop residues left on the field after harvest during the previous seasons. The result was the provision of an extra energy source for microbial growth during the subsequent season (2007). According to Ross (1987), crop residues can have a large effect on soil microbial biomass and activity, which, in turn, affect the ability of soil to supply nutrients to plants through soil organic matter turnover. Efficient nutrient management in cropping systems could, therefore, lead to the buildup of microbial biomass over time.

Good fits of correlation ( $r^2 = 0.71, 0.40$  and  $0.64$ ) (Table 1) were found between microbial biomass carbon and organic carbon in the 2006 – major and 2007 – major seasons, which suggested that microbial biomass concentration depended on the organic matter availability to microbial activity (Anderson & Domsch, 1989). This

is in conformity with findings of Beck *et al.* (1997) and Leiros *et al.* (2000), who reported good correlation between biomass carbon and organic carbon. However, Insam & Domsch (1989) found no correlation between the biomass carbon and organic carbon.

Published data on the relation of microbial biomass carbon to organic carbon are inconsistent, showing either a positive correlation or no correlation as both the organic carbon quality and the microbial community structure are associated with soil type (Jozef, 2004). Results pointed to high correlations between microbial biomass carbon and effective cation exchange capacity. This is in agreement with published data. Wolters & Joergensen (1991) found a close positive relationship between the microbiological parameters and ECEC. The ECEC relationship with nutritional status of soil consists in its role in prevention of nutrient leaching from the soil (Jozef, 2004).

#### *Soil microbial biomass phosphorus*

It may be inferred from the results of the study that microbial biomass phosphorus was sensitive to factors that could have influenced the size and structure of microbial biomass. These include microclimate (soil moisture and temperature) and fertilizer (amendment) (Moore *et al.*, 2000).

The negative microbial phosphorus values obtained under amendments and cropping systems (Fig. 3cdef) were due to immobilization of phosphorus. This corroborates with an observation made by Tetteh (2004), who reported phosphorus immobilization under decomposing organic

materials. This is very important in Ghanaian soils, which are often associated with high phosphorus fixation, thus, reducing its availability to plants (Tetteh, 2004).

The immobilized phosphorus by microbes will be released gradually, thus, protecting the released phosphorus from physico-chemical adsorption reactions. If the immobilized phosphorus will be passed on from one generation of microbes to the other, then there will be a constant competition between microbes and plants for phosphorus. At 84 DAAA in the seasons, results indicated no immobilization of phosphorus in the soil of any of the amended plots (microbial P values were positive). This means that immobilized phosphorus was released, thus, adding to the available phosphorus in the soil. This, however, might have not been used by the crops (asynchrony) since the release did not coincide with the peak nutrient demand of the crops but rather with their physiological maturity, leading to harvest.

The pronounced immobilization of phosphorus, as recorded in this study, could be traced to the fact that the element forms an integral part of the cell nucleus of the microbes and is required in the form of phosphate ( $PO_4^{3-}$ ) radical to combine with adenosine diphosphate (ADP) for energy transfer within the microbial cellular tissue (Barber, 1995). This resulted in higher microbial affinity for the phosphorus, thereby, causing immobilization. From this study, it can be deduced that if the phosphorus immobilized was released 84 DAAA, then management practices should be geared towards making the release concur with peak crop demand. This will enhance

synchrony and improve soil productivity.

### Conclusion

Efficient nutrient management in cropping systems could lead to buildup of microbial biomass over time. Crop residues left on the field after previous seasonal harvest resulted in microbial buildup even in plots under no amendment in subsequent seasons. Biomass nitrogen showed more temporal fluctuations than biomass carbon, which suggested that microorganisms differ much more in their nitrogen content than in their carbon content. Small shifts in the structure of the microbial community can result in large changes in biomass nitrogen. Microbial biomass dynamics show the micro-changes occurring in the soil in the short term. Result indicated that microbial concentration depended on the organic matter availability to microbial activity. The study has established that phosphorus could be immobilized at the peak of crop growth for 21 days and be released within 21 days; the release not concurring with peak nutrient demand of crops. Management practices should, therefore, be geared towards making the release concur with peak crop nutrient demand.

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