

Soil microbial biomass carbon and nitrogen as influenced by organic and inorganic inputs at Kabete, Kenya

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Abstract

Soil microbial biomass is the main driving force in the decomposition of organic materials and is frequently used as an early indicator of changes in soil properties resulting from soil management and environment stresses in agricultural ecosystems. This study was designed to assess the effects of organic and inorganic inputs on soil microbial biomass carbon and nitrogen overtime at Kabete, Kenya. *Tithonia diversifolia*, *Cassia spectabilis*, *Calliandra calothyrsus* were applied as organic resources, and Urea as inorganic source. Soil was sampled at 0–10 cm depth before incorporating the inputs and every two months thereafter and at harvesting in a maize-cropping season. Soil microbial biomass carbon and nitrogen was determined by Fumigation Extraction method (FE) while carbon evolution was measured by Fumigation Incubation (FI) method. The results indicated a general increase in soil microbial biomass carbon and nitrogen in the season with the control recording lower values than all the treatments. Microbial biomass carbon, nitrogen and carbon dioxide evolution was affected by both quality of the inputs added and the time of plant growth. *Tithonia* recorded relatively higher values of microbial biomass carbon, nitrogen and carbon evolution than all the other treatments. A significant difference was recorded between the control and the organically treated soils at the end of the season for the microbial biomass nitrogen and carbon dioxide evolution. Both the microbial biomass C and N showed a significant difference ($P \leq 0.05$) in the different months of the season.

Key words: microbial biomass carbon, microbial biomass nitrogen, Carbon evolution, chloroform fumigation extraction method

Introduction

Microbes constitute about one quarter of all living biomass on earth and are responsible for significant nutrient transformations involving both macro and micro nutrients (Alexander 1977) and therefore influencing nutrient availability and ultimately soil health and quality. Soil microbial biomass is the main driving force in the decomposition of organic materials and is frequently used as an early indicator of changes in soil chemical and physical properties resulting from soil management and environment stresses in

agricultural ecosystems (Brookes 1995; Jordan et al. 1995; Transar-capeda et al. 1998). Though 1–3% of total soil C and 5% total soil N is soil microbial biomass C and N respectively, they are the most labile pools in soils (Jenkinson and Ladd 1981) and therefore the nutrient availability and productivity of agro ecosystems mainly depends on the size and activity of the microbial biomass (Friedel et al. 1996). Turn over of microbial biomass is a dynamic process, and responds relatively quickly to changes in environmental conditions, i.e., climate, input of nutrients, and disturbance. In undisturbed ecosystems, nutrient cycles tend to be

more closed and less “leaky” than agro ecosystems. However, an important characteristic of agro ecosystems is that they export large inputs, in the crop biomass and, therefore, addition of large amounts of organic materials to replenish the soils is needed. Sustainable agro ecosystems will probably require more and better-informed management of all ecosystems components including soil biota. The determination of microbial biomass provides estimates of the net flux of carbon and nitrogen through microbial pools and thus reflects the contribution of soil microorganisms as both a source and a sink of carbon and nitrogen in soil ecosystems. Several authors have reported the identification of biological indicators of soil quality as critically important (Doran and Parkin 1994; Elliott et al. 1996), and the rationale for the use of microbial and biochemical parameters as soil fertility indicators is their central role in the cycling of C and N (Visser and Parkison 1992) and their sensitivity to change (Brookes 1995). With the addition of organic waste into the soil becoming a wide spread practice, due to the fact that they are a source of nutrients (Perucci et al. 2000), their effects on soil microbial biomass (SMB) should be taken into account. However, the role of macro and microorganisms in soil productivity, especially transformations and availability of nutrients remains to be fully understood (Zhenli et al. 2003). Because soil microorganisms carry out many below ground process, estimates of microbial biomass may be useful for comparisons of ecosystems function of sites with similar climate, geology, and land use histories. This study was therefore set to evaluate the effects of organic and inorganic resources on soil microbial biomass carbon and nitrogen.

Materials and methods

Study site and experimental design

This experiment was carried out at the National Agricultural Research laboratories (NARL), at Kabete station (36° 46' E – 01° 15' S, 1,650 m asl). The climate is sub-humid, with annual rainfall bi-modal falling in two distinct seasons: the long rainy season (mid March to June) and the short rainy season (mid October to December). Average rainfall is 937 mm. The soils are trachyte geological material typically Humic Nitosols (according to FAO, UNESCO), deep and well weathered and with moderate amounts of carbon (C), calcium (Ca), magnesium (Mg), and potassium (K),

but low available phosphorus (P). The experiment was a Randomized Complete Randomised Block Design (RCBD) with 10 treatments replicated four times. In the study, five treatments were considered as Control, Fertilizer, *Tithonia diversifolia*, *Senna spectabilis* and *Calliandra calothyrsus*. Organic inputs were broadcasted and incorporated before planting as fresh leaves. They were applied on dry matter basis in order to obtain 60 kg N/ha applied, whereas urea was split in two applications (at planting and five weeks after) to give a rate of 60 N kg/ha. Organic materials were chosen to reflect contrasting amount of lignin, polyphenols and the rate of decomposition of each which has been summarized as calliandra (14.4%, 11.1%), senna (10.9%, 2.6%) and tithonia (5.2%, 2.2%) respectively (Mutuo et al. 1999; Lehmann et al. 1999). The rate of decomposition has also been observed to follow the sequence tithonia > senna > calliandra (Palm et al. 2001). Maize (*Zea mays*) was used as test crop and was planted at 0.75 × 0.25 m between and within rows respectively in each of the 5 plots measuring 5.25 m by 5 m.

Soil sampling

Soil samples were collected at planting (before incorporating materials), every two months within the season and at harvesting. 5 cores samples were collected at 0–10 cm depth, pooled together, mixed thoroughly and sieved to remove stones, plant debris and soil fauna. The samples were placed in polythene papers and transported to the laboratory for analysis. In the laboratory, the soil samples were stored at 4°C prior to analysis. The water holding capacity of each sample was determined and the moisture content adjusted to 45% for microbial analysis. The samples were then incubated at 25°C for 7 days in the dark to permit uniform rewetting and allow microbial activity to equilibrate after initial disturbances.

Laboratory analysis

All laboratory analysis was done as described in the laboratory methods of soil and plant analysis (ICRAF 1995; Anderson and Ingram 1993). Soil sub samples (25g equivalent dry weight) were weighed in duplicates for fumigation extraction (FE) and fumigation and incubation (FI) (Jenkinson and Powlson 1976a, b). Fumigation was done by placing soil sub samples in

desiccators with ethanol-free chloroform for 24 hrs in a darkroom. Fumigated samples were removed after evacuating the desiccators using a vacuum pump to free off the chloroform. Microbial biomass carbon and nitrogen was determined after fumigation by the FE. Fumigation incubation method was carried out after soil fumigation. This was done by adding 1 g of fresh soil to 25 g of the fumigated soil. It is expected that the microorganisms in the fresh soil will utilize the killed cells as substrate and therefore grow vigorously releasing a lot of carbon dioxide. Incubation was done by placing fumigated and inoculated soils in to a 250 ml jar with the bottom lined up with 10 ml of water and containing 10 ml of 1N NaOH in a separate small glass vial. The jars were then air-tightened and incubated for 10 days. A second set of unfumigated soil was incubated in the same way. Carbon evolution was determined after the incubation.

Microbial biomass Carbon and Nitrogen

Microbial biomass was determined by extracting fumigated and unfumigated soils with 0.5M K₂SO₄ after shaking on an orbital shaker at 150 rpm for 1 hour. Microbial biomass C was analysed by dichromate method while microbial biomass N was determined using the salicylic method. Microbial C & N were calculated as follows Microbial C = C_(fumigated) – C_(non-fumigated)

$$\text{Microbial N} = \text{N}_{(\text{fumigated})} - \text{N}_{(\text{non-fumigated})}$$

Carbon dioxide evolution

Carbon dioxide evolution was estimated by measuring CO₂ respired from the soil over a period of 10 days. The CO₂ trapped in 1N NaOH was analysed by back titration with 1N HCL after addition of excess 3N BaCl₂. The amount of CO₂ respired from fumigated and unfumigated was used to calculate soil microbial biomass in the equation:

$$\text{Biomass C} = (\text{F}_c - \text{Uf}_c)/\text{k}_c$$

Where

F_c = CO₂ flush from the fumigated sample

Uf_c = CO₂ produced by the control

k_c = Constant

Data analysis

Data was analyzed using analysis of variance (ANOVA) with Genstat 6 for Windows (Release 4.1). Least significance difference was used at 0.05-probability level to detect significant differences among treatments.

Results

Microbial biomass carbon

Control gave lowest values of microbial biomass carbon over the season as compared to the treated soils (Table 1). Among the sole organic treatments, calliandra was highest followed by senna and Tithonia treatments respectively (Table 1). However, there was no significant difference (P < 0.05) recorded among the treatments. Microbial biomass recorded across the months in the season was found to be significantly differently. An increase of about 140.4% in microbial biomass carbon was recorded eight weeks after input application (Table 1), which coincides with the peak plant growth. Microbial biomass carbon decreased within the season reaching its lowest level at the end of the season.

Microbial biomass nitrogen

The control treatment gave the lowest level of microbial biomass nitrogen (Table 2), implying an increase with input addition. Among the organically treated soils, calliandra recorded the lowest level of microbial biomass nitrogen over the season. However, the sequence was not consistent. Tithonia gave the highest values of microbial biomass nitrogen eight weeks after inputs application while senna treatment recorded highest at

Table 1. Microbial biomass nitrogen values in mg N kg⁻¹ of soil

Sampling month	April	June	August	October
<i>Treatment</i>				
Control	25.63	15.93	1.97	6.42
Tithonia	35.00	26.20	3.18	15.65
Fertilizer	37.13	25.13	2.55	13.49
Senna	29.50	22.40	5.27	18.78
Calliandra	27.00	21.82	2.57	13.49
SED	11.28	6.97	1.28	1.68
P ≤ 0.05	0.53	0.06	0.19	0.01

Table 2. Microbial biomass Carbon values in mg C kg⁻¹ of soil

Sampling month	April	June	August	October
<i>Treatment</i>				
Control	85.06	226.68	121.99	45.29
Tithonia	111.40	260.10	126.11	47.79
Fertilizer	114.76	253.42	126.65	46.86
Senna	107.61	265.90	130.55	48.27
Calliandra	116.68	273.30	147.33	50.50
SED	33.00	24.67	21.61	18.91
P ≤ 0.05	0.86	0.30	0.35	0.53

16 weeks and at the end of the season (Table 2). At the end of the season, control treatment was found to be significantly ($P \leq 0.05$) lower than all the other treatments (Table 2). Fertilizer and calliandra treatments were also found to be significantly lower than Senna. A decrease in microbial biomass nitrogen was observed eight weeks after input addition, which continued to 16 weeks within the season and coincided with the peak of plant growth.

Cumulative carbon dioxide evolution

Carbon dioxide evolved by the organically amended soils was found to be higher than that recorded for the control and fertilizer throughout the season (Table 3). Tithonia gave significantly ($P \leq 0.05$) higher values of carbon dioxide than fertilizer and control treatments at the end of the season. Among the organically treated soils, calliandra tended to evolve the least carbon dioxide compared to senna and tithonia treatments (Table 3). Evolved carbon dioxide decreased with time reaching a minimum eight weeks after addition. At the end of season, an increase in CO₂ evolution was observed across all treatments except for calliandra 100% (Table 3).

Table 3. Carbon dioxide evolution.

Sampling month	April	June	August	October
<i>Treatment</i>				
Control	163.12	82.13	101.23	111.23
Tithonia	243.06	189.43	221.23	299.35
Fertilizer	177.03	142.08	103.05	111.09
Senna	271.15	172.06	230.09	260.15
Calliandra	295.09	125.43	187.19	170.28
SED	80.30	54.20	44.41	81.30
P ≤ 40.05	0.60	0.13	0.63	0.03

Discussion

The results suggest that addition of organic inputs increase soil microbial biomass as compared to control and fertilizer treatments and that the size of the microbial biomass is dependent on the type of organic material added. For example, we found higher values of microbial biomass carbon for organically treated soils as opposed to either control or fertilizer. Similar results were reported by Leita et al. (1999), Smith et al. (1993) and Tunlid and White (1982). The sequence of microbial biomass carbon among the organic inputs was calliandra < senna < tithonia. This trend can be attributed to the difference in decomposition rate among the organic materials. Tithonia has been shown to decompose rapidly as compared to senna and calliandra (Gachengo et al. 1999) due to the low level of lignin and polyphenols in the leaves, therefore readily providing food for microbial growth. Recorded increase in microbial biomass carbon eight weeks after addition of inputs can be attributed to the readily available carbon for microbial growth. This also coincided with the peak of plant growth suggesting that plant growth stimulates microbial biomass carbon. The results concurs with the findings of Kaiser and Heinemeyer (1993), Fraser et al. (1988), Mc Gill et al. (1986) and Lynch and Panting (1982), that crop growth often stimulates an increase in the size of microbial biomass during growing season.

The findings of microbial biomass nitrogen indicate that addition of inputs increases the size of nitrogen biomass in the soil. However, this depended on the type of the input and stage of plant. The high levels of microbial biomass nitrogen recorded with tithonia treatment could be attributed to its faster release of nitrogen as compared to senna and calliandra treatments. The decrease in microbial biomass nitrogen coincided with the peak of plant growth (8 to 16 weeks after input addition). Competition for mineral nitrogen by plants and microbes in the soil has been reported (Kaye and Hart 1997; Schimel et al. 1989). This decrease can be explained by the peak demand for nitrogen and therefore presenting a competitive nature between plants and the microbes.

The data on carbon dioxide evolution indicate that addition of organic inputs increase microbial activity or microbial biomass. Carbon dioxide evolution also seems to depend on the material added. Among the organic inputs calliandra gave the lowest value of carbon dioxide and this implies that not only the amount of organic resources added to the soil affect

carbon evolution but also the quality. Possible explanation for this could be the slow decomposition rate for calliandra. The higher carbon dioxide evolved for tithonia can be related to its higher decomposition rate and therefore releasing nutrients for microbial growth faster than the other organic resources. The decrease in carbon evolution recorded eight weeks after input application could present a less stressed microbial biomass, as a pool of carbon is available from the added inputs.

Conclusion

Soil microorganisms are very important for nutrient transfer in low input systems, where crops largely depend on nutrient release from organic materials rather than from inorganic fertilizers. Addition of organic materials was found to boost microbial biomass, which would mean that nutrients were made readily available plant than in the unamended soils. Microbial biomass was greatly influenced by the quality of the organic inputs and time. However, no significance difference was among the treated soils, which would be attributed to the quantity of organic material, added. This calls for more research on microbial biomass under different application rates and since microbial biomass is very dynamic, it would also be important to consider sampling for shorter periods within the season and for more seasons.

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