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Analysis of Microbial Activity and Community Structure in Organically and Chemically Fertilized Soils

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The aim of this research was to observe differences in beta-glucosidase activity and changes in the community structure of soil microbes in chemically and organically fertilized soils. Beta-glucosidase is one of the most important enzymes produced by soil microbes because it plays a key role in the decomposition of cellulose debris added to soil as fertilizer. A soil rich in bacterial diversity is stable and suitable for optimal plant growth. Soils with very low nitrogen and carbon content were examined under natural weather conditions in Japan. Significant differences ($P<0.05$) were observed in beta-glucosidase activity between the different treatments as measured by colorimetric analysis. Beta-glucosidase activity increased in all pots but at different rates. After 60 days, composted manure (treatment 1) and the combination of chemical and fertilizers and composted manure (treatment 3) showed the highest and second highest levels of beta-glucosidase activity, respectively. Treatment 1 showed 30% higher beta-glucosidase activity as compared to treatment 3 and 75% higher beta-glucosidase activity as compared to treatments 2 and 4 (control).

The community structure of the soil bacteria was assayed by the PCR-denaturing gradient gel electrophoresis (PCR-DGGE) technique. We captured the banding pattern of soil microbes on the gel images following DGGE of PCR-amplified 16S rDNA using the 357F and 518R primers. Although the gross trend was the same, the four different treatments showed differences in the DNA banding pattern, suggesting differences in the soil bacterial species community structure among the four different soil treatments. Treatment with chemical fertilizers gave a few intense bands, whereas treatment with composted manure and treatment with a combination of chemical fertilizer and composted manure yielded more bands that were less intense. These findings suggest that chemical fertilizers promote the colonization of specific dominant bacterial species, whereas composted manure promotes diversity in the soil bacterial population. Principal component analysis confirmed that the treatment 1, 2 and 3 results varied markedly from the treatment 4, control results. We conclude that exclusive use of chemical fertilizers in soils with low or moderate carbon levels would lead to low beta-glucosidase activity and a reduction in the diversity of the soil microbe community structure. Low beta-glucosidase activity and reduced soil bacterial diversity might be detrimental to the condition of the soil and lead to an increase in the soil's postharvest recovery period and a reduction in the preseason decomposition rate of plant residues in the soil.

Key words: soil microbe activity, soil microbe community structure, chemical fertilizer, compost manure

Introduction

The importance of organic matter in agriculture soils cannot be overstated. Organic materials influence nutrient availability by adding nutrients to the soil, through patterns of mineralization and immobilization, as an energy source for microbial activities, as precursors of soil organic matter (SOM), and by reducing the number of P-sorption sites (Cheryl *et al.,* 1997). In the sub-Saharan region, the need for soil organic input is increasing because of deteriorating soil conditions caused by

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continuous cultivation and because of the increasing price of chemical soil fertilizers. In Malawi, the unit price of chemical fertilizers has almost doubled over the past few years. For this reason, the Ministry of Agriculture and other agricultural organizations have been promoting the use of organic composted manure, mixing crops with legumes, and incorporating plant residues as alternatives to chemical fertilizers. However, for economic and biological reasons, the adoption and implementation of such methods by smallholder farmers has been minimal and inadequate. For instance, although fertilizers are used over much of sub-Saharan Africa, the amounts applied are insufficient to meet crop demands (Smaling *et al.,* 1997). Organic input is often proposed as an alternative to mineral fertilizers. However, traditional organic input and animal manure cannot meet crop nutrient demands over large areas because of the limited quantities available, the low nutrient content of the materials, and the high labor demands for processing and application (Cheryl *et al.,* 1997). A recent study in Kenya showed that 1 kg of nitrogen (N) and phosphorus (P) from compost costs USD 0.50 and 1.20, respectively, compared with USD 0.42 and 1.18 for N and P purchased as inorganic fertilizer (N = 200 g/kg and P = 90 g/mg (Nandwa, 1996 and unpublished data). Crop residues also have competing uses, primarily as feed for livestock, which reduces the amount available for managing soil fertility. An average of 700 kg/ha, is available for sub-Saharan Africa. This is much less than the 3 to 7 mt/ha recommended for replenishing nutrients removed by crop harvesting (Fernandez-Rivera *et al.,* 1995).

Apart from the known challenges of using organic compost and plant residues discussed above, smallholder farmers in the traditional area of Simlemba in Kasungu district have constantly expressed dissatisfaction with the practice of using organic compost and plant residues, claiming that slow residue decomposition leads to damaging termite attacks on crops, especially maize. The author has been working for over three years in the traditional area of Simlemba, promoting sustainable agriculture technologies in the framework of the Simlemba Community Initiative for Rural Livelihood Improvement Project (SCIRLIP). The project's midterm evaluation report revealed that termite attacks accounted for 30% of maize grain losses among the target farmers. The traditional authority (TA) of the Simlemba area is located in the northern part of Kasungu district, in the central region of Malawi, Africa. This area is covered within the Kaluluma EPA (Extension Planning Area) of the Kasungu District Agriculture Development Office (DADO) within the Ministry of Agriculture extension system. The area includes 23,241 households and a population growth rate of 3% (Population Census, 1998). Sandy loam soils with patches of katondo (latosols/weathered ferrallitic soils) and a topography of gentle and moderate gentle slopes dominate the area. The annual rainfall is > 800 mm and is usually erratic, with medium to long dry spells (Kasungu Socioeconomic Profile, 2005). The rainy season starts on 5 December and ends by 30 March. The elevation ranges from 800 to 1,600 m above sea level, the annual temperature ranges from 12℃ to 30℃, and the relative humidity falls within 40% to 83%, (Kasungu SEP, 2005). High temperatures are experienced from August to October, whereas low temperatures persist from May to July. Major crops grown in the area include maize, groundnuts, tobacco, and beans.

For many years the farmers in this area have been employing very unsystematic/traditional care practices for the soils, including techniques such slash and burn, tilling, and ridging. Furthermore, there has been inconsistent but continuous use of chemical fertilizers, and even after many years of chemical fertilizer use the fields in this area have rarely been amended with lime. Termites are an important part of the fauna in tropical agroecosystems. Termites work on wood and crop residues that contain lignin and cellulose, converting them into organic matter, and they are considered ecosystem engineers, together with other fauna such as earthworms. The activity of termites helps to develop the soil structure through bioturbation and aggregate formation, thus improving soil quality (Uys, 2002; Susilo *et al.,* 2004). On the other hand, soil microbe activity and community structure have a strong impact on the soil's ability to decompose organic input with high cellulose content (Schwarz, 2001). Microbial diversity in soils is considered important for maintaining the sustainability of agricultural production systems (Stark *et al.,* 2007;

Sekiguchi *et al.,* 2008). Soil microbes play very important roles in the carbon (C) and N cycles and are instrumental in mineral transformations of phosphorous sulfur, iron and other elements in the soil (Alexander, 1977).

The common perception of smallholder farmers that composted manure input and residue incorporation lead to increased crop damage by termites arises from many possible reasons, including the loss of the ecological quality that allows quick decomposition of residues in the soil owing to continuous cultivation, and minimal sustainable soil improvement amendments. While literature on termite damage to crops in Malawi is lacking, many studies in the sub-Saharan region have suggested that soil management practices and cropping systems have a significant influence on termite attacks on crops. In Malawi, a study conducted from 1989 to 1996 by the biology department of the University of Malawi in collaboration with the Natural Resources Institute at the University of Greenwich found that termites can damage up to 25% of the maize crop (the staple cereal) and up to 30% of the cotton crop. The study examined several causative factors and concluded that there was considerable variation in damage according to season (moisture availability) and location. Another study in Zambia, a neighboring country, observed that 50% to 75% of the variance in the maize yield could be explained by the presence preseason inorganic N and termite damage. However, termite damage to maize was not influenced by inorganic N, which represents the N readily available to maize. The decomposition rate of biomass (which is related to the lignin polyphenol to N ratio) and water retention during fallow periods also appeared to influence termite damage (Sileshi *et al.,* 2000).

In a study in Lesotho, maize stalks and leaves that had been ploughed into the soil three years previously reappeared unaltered when the field was ploughed again because there was no biological activity in the soil (Shaxson, 2006). In another study in Uganda, field experiments during two cropping seasons at the Namulonge Agriculture Research Institute assessed the effect of intercropping maize with soybean, groundnuts, and common beans on termite damage to maize, the activity of common predatory ants, and maize yields. Intercropping caused a significant ($P > 0.01$) reduction in termite attacks, a reduced loss of maize yield, and increased nesting of predatory ants in maize fields. In many instances, termite attack severity was significantly lower ($P > 0.05$) with maize-soya intercropping than when maize was intercropped with groundnuts and beans. Species of the genera *Mymicaria* and *Leposita* were the dominant ant predators recorded. The study revealed that soya beans and groundnuts are more effective in suppressing termite attacks than common beans, suggesting that it is necessary to identify suitable legumes for each cropping situation. Overall, intercropping might form a component of an integrated management strategy for termites in smallholder cropping systems in East Africa (Sekamatte *et al.,* 2003).

Given the literature summarized above, it is likely that a lack of soil microbial activity contributes to the inability of soils to decompose plant residues. Termite attacks on crops are aggravated by several factors, including dry soil conditions and the presence of dry plant residues. Some common soil care practices of farmers in the Simlemba area include the application of chemical fertilizers to soils, the incorporation of residues, and the application of composted manure mainly derived from crop residues with a minimal content of livestock manure. Considerable research has focused on the factors affecting the decomposition rates of residues. By comparison, few studies assess the effects of chemical fertilizers and organic manure on the activity and community structure of soil microbes and how these may affect the soil ecosystem with respect to residue decomposition and termite attack. The author has been working and interacting with farmers in the Simlemba area for at least 3 years and hence pursued this research to explore and clarify the effect of chemical fertilizers and organic compost on the community structure and activity of soil microbes. The objectives of the present study were (1) to assess the effects of chemical fertilizers on the activity of soil microbes, (2) to determine the diversity of the microbe community structure in chemically and organically fertilized soils, and (3) to explore appropriate low-cost proposals for improving soil fertility and soil conditions. Given the complex nature of the soil environment and the experiences of smallholder farmers, the findings from this research may contribute to the development of an integrated management strategy for termites and soil fertility in smallholder cropping systems and soil care regimes in general.

Materials and Methods

Enzymatic methods were used to assess the microbial activity in soil samples. For the assessment of the microbial community structure in soil, PCR-denaturing gradient gel electrophoresis (PCR-DGGE) technique was used.

Experimental Design

A completely randomized experiment (Montgomery, 1991) was performed under natural conditions at the University of Tsukuba. Soils from the Ishigaki Islands, sampled by scientists from the Japanese International Research Centre for Agriculture Sciences (JIRCAS) in Okinawa prefecture, were used for the experiment, which was conducted in 7.6-L planting pots. These soils are similar to the typical tropical soils from the Simlemba area in Malawi and to the soils used in 2004 by Nagumo *et al.* (2006). Duplicate pairs of planting pots were prepared with homogenized soil, and each pair was exposed to one of four different treatments. The different soil treatments were as follows: treatment 1, compost manure; treatment 2, chemical fertilizers containing the elements nitrogen and phosphorus; treatment 3, soil containing 50% chemical fertilizer and 50% composted manure, having the same composition as the fertilizers used for treatment 1 and 2, respectively; and treatment 4 'control' containing soil with no added composted manure or chemical fertilizer. Silver sweet corn cultivars pre-germinated in sterile nutrient media were then planted in all pots. Table 1 summarizes some of the characteristics of the experimental soils. Four treatments were prepared in duplicate as described in Table 2.

Beta-glucosidase activity in the soil samples was determined colorimetrically using a spectrophotometer (SmartSpec Plus, Bio-Rad). We used a method based on the cleavage of the substrate *p*-nitrophenyl-β-D-glucoside (buffered at pH 6) by beta-glucosidase to release *p*-nitrophenol. In this method, the released *p*-nitrophenol is extracted and the yellow color due to the presence of *p*-nitrophenol under alkaline conditions is quantified by measuring the absorbance at 410 nm (Dick *et al.,* 1996). Soils samples were obtained from the top of the pots and from a level 15 cm deep using clean soil spades. The moisture content of the air-dried samples was determined prior to conducting the enzyme assay to ensure that equivalent 1-g soil samples were used for each analysis. The enzyme assay involved the incubation of 1-g soil samples in 50-mL Erlenmeyer flasks with number 2

Table 1. Characteristics of the soil used for experiments

Level (cm)	CEC (Cation Exchange Capacity), cmol/kg	Total C (%)	Total N (%)	C/N (Ratio)
0	7.7	1.45	0.13	10.9
10	5.8	0.90	0.09	10.5
31	7.9	0.48	0.06	7.9

Table 2. Description of soil treatments

Treatment 1 (T1)	Treatment 2 (T2)	Treatment 3 (T3)	Treatment 4 (T4)
100% composted manure (90% plant residues, 1 kg compost per pot)	100% chemical fertilizer (11 g N and 2 g P per pot)	50% compost (500 g per pot) and 50% chemical fertilizer (5.5 g N and 1 g P per pot)	Control (no fertilizer or compost added)

stoppers for 1 h at 37℃ with 0.25 mL toluene, 4 mL modified universal buffer (MUB) pH 6, and 1 mL of *p*-nitrophenyl-β-D-glucoside (PNG), photometric grade (Sigma Chemical Co., Osaka). Samples were then treated with 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M tris(hydroxymethyl) aminomethane (THAM) buffer, pH 12 (Dick *et al.,* 1996). The samples were then centrifuged at high speed for 15 min. The absorbance at 410 nm was measured with the spectrophotometer. The *p*-nitrophenol content of the filtrate was calculated by reference to a standard calibration curve generated with solutions containing 0, 20, 40, 60, 80 and 100 μg of *p*-nitrophenol, assay grade (Wako Pure Chemical Industry, Osaka).

PCR-DGGE Analysis

The soil microbial community structure was assayed by PCR-DGGE. For each treatment, duplicate 1 g soil samples were taken from each pot from a depth of 15 cm. DNA was extracted from each sample and then purified using a Fast DNA Spin KIT (MP Biomedicals, LLC, Solon, Ohio). DNA sample verification was performed by 1.5% agarose gel electrophoresis. The DNA samples were balanced, and the final DNA concentration in the samples was made equivalent by adding the appropriate amount of distilled water. The samples were then subjected to PCR amplification followed by DGGE.

For PCR amplification, 200 μL DNA samples consisting of 2 μL DNA, 1.6 μL of 2.5 mM dNTP, 2 μL loading buffer, 0.1 μL EX Taq, 2 μL each of the primers (357F and 518R), and 10.3 μL of distilled water were used. PCR amplification was conducted by using a Bio-Rad iCycler (Bio-Rad Laboratories, USA). For PCR amplification of the 16S rDNA of the soil microbes, the forward primer, 357F (5′-GC clamp-CCTACGGGAGG-CAGCAC-3′), and the reverse primer, 518R (5′-GTATTACCGCGGCTGG-3′), were used. Thermal cycling conditions were 94℃ for 5 min followed by 20 cycles of 94℃ for 30 s, 65℃ for 30 s and 72℃ for 15 s, then 8 cycles of 94℃ for 30 s, 55℃ for 30 s and 72℃ for 15 s and finally, at 72℃ for 2 min. Verification of PCR amplification was performed by 1.5% agarose gel electrophoresis.

DGGE analysis was performed using a DCode™ Universal Mutation Detection System (Bio-Rad Laboratories) in accordance with the manufacturer's instructions. DGGE was performed at a constant voltage of 36 V at 60℃ for 18 h on a 30% to 60% denaturing gradient gel. The banding pattern of soil microbes was captured on DGGE gel images, and noise was removed by using Adobe Photoshop software. The DNA peak and band strength measurements were analyzed again by IMAGE MASTER 1D software to generate numerical values for the density and migration of the bands. The Pirouette software package was used for principal component analysis (PCA) of band strength numerical data profiles.

Soil Analyses

Air-dried soil samples from all treatments, in duplicate, were passed through a 2-mm and 0.5-mm sieve prior to analysis of pH, C, and N. The C and N contents of the soil were analyzed with a fully automatic highly sensitive NC analyzer (Serigraph NC 900, Osaka Japan). The NC analyzer was calibrated and standardized using acetanilide with C at 71.09% and N at 10.36%. For analysis of soil pH, 10 g soil samples of the < 0.5 mm fraction were prepared and mixed with 25 mL of distilled water, stirred with a glass rod, and then left to settle for 30 min. The soil pH was measured by using HM-30R pH meter (DKK-TOA Corporation). The soil moisture content of air dried samples was determined by measuring the weight loss of soil samples, taken from a depth of 15 cm, after 20 h of oven drying at 105℃.

Data Analysis

Statistical analysis of the data was performed by using Microsoft Excel. Single-factor ANOVA, frequency bars, and standard deviations were calculated from the numerical data obtained from soil analyses and colorimetric analyses. Adobe Photoshop, IMAGE MASTER 1D, and Pirouette software were used to analyze DGGE gel images and numerical band-length data.

Results

Beta-Glucosidase Activity

Figure 1 presents an overview of the weather conditions that prevailed during the course of the experiment. Figure 2 shows the time course for the change in absorbance at 410 nm due to *p*-nitro-

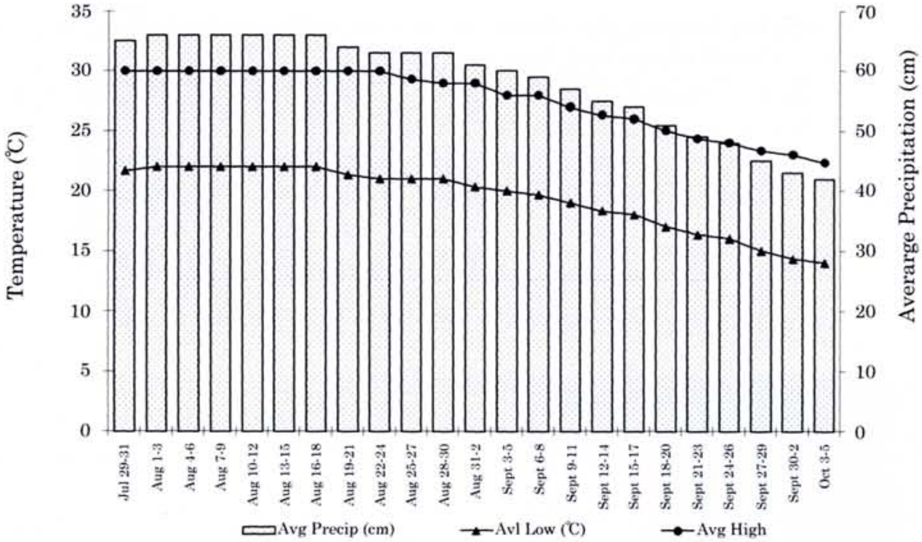

Fig. 1. Local weather conditions during the experiment. Figure generated using data collected through the website, www.weather-forecast.com/locations/Tsukuba.

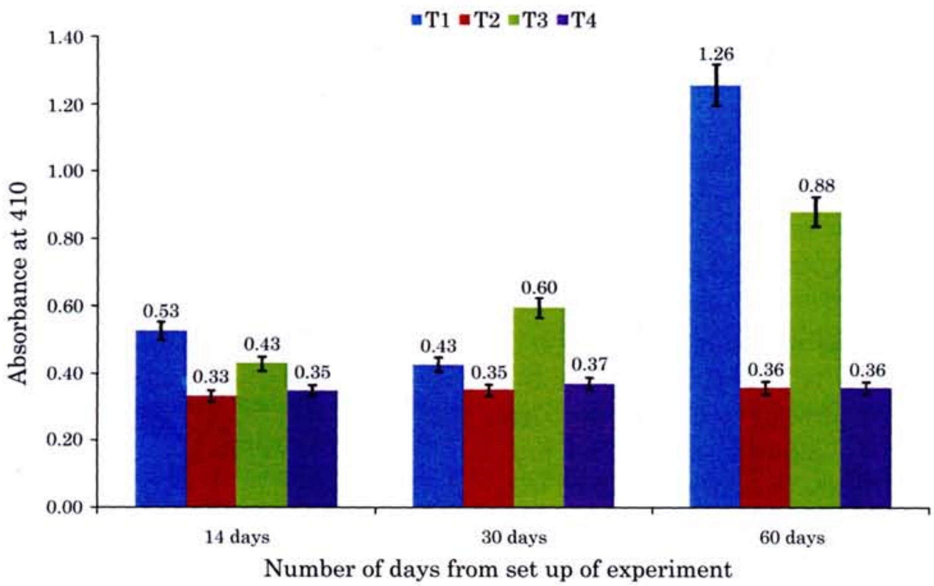

Fig. 2. Time course for the change in absorbance at 410 nm due to *p*-nitrophenol formation.

phenol formation in soil samples taken at 2 weeks, 30 days and 56 days. Table 3 shows the beta-glucosidase activity in the soil samples over the time course of the experiment as indicated by the amount of *p*-nitrophenol released, calculated by reference to a standard calibration curve. After 60 days, composted manure (treatment 1) resulted in significantly ($P > 0.01$, one-way ANOVA) higher beta-glucosidase activity than the combination of chemical fertilizers and composted manure (treatment 3). Treatments 2 and 4 resulted in the lowest levels of beta-glucosidase activity, but the difference was not significant ($P > 0.05$, one-way ANOVA). Treatment 1 resulted in 30% higher beta-glucosidase activity compared with treatment 3 and 75% higher beta-glucosidase activity compared with treatments 2 and 4 (control). The average high and low temperatures (Fig. 1) that prevailed during the experiment were consistent with the annual weather conditions in the Simlemba area of

Table 3. Beta-glucosidase activity in soil samples from all treatments at different time points

	T1	T2	T3	T4
14 days	29.22857	18.06286	23.62857	19.05714
30 days	23.48952	19.15619	33.18476	20.26095
56 days	71.06857	19.58286	49.46857	19.48

Note: values represent amounts of PNP in $\mu g\ g^{-1}$ of soil hr^{-1}.

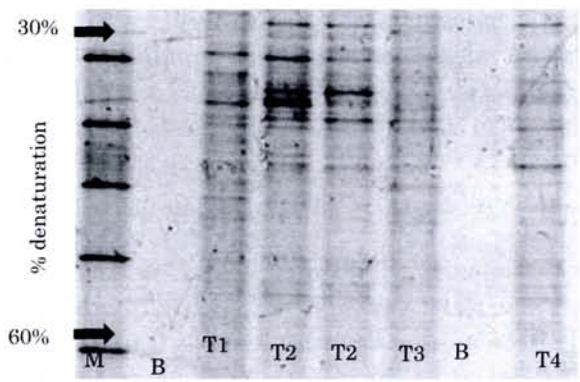

Fig. 3. DGGE gel image, gray scale. Microbe community structure changed and developed dominant microbe populations in T2 while those in T1 and T3 became more complex as well as those in T4.

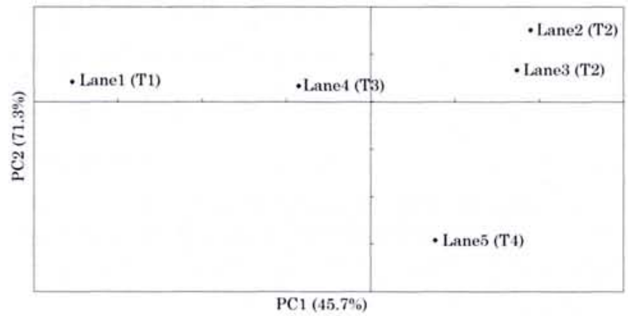

Fig. 4. PCA (principal component analysis) of microbe variability; Lane 1=T1, Lanes 2 and 3=T2, Lane 4=T3 and Lane 5=T4 (Control). The values in parentheses indicate the percentage of variability accounted for by each principal component axis.

Kasungu district, where the average high is 30℃ and the average low is 14℃ from July to October which is the second growing season in the area (Kasungu SEP, 2005). Similarly, the average precipitation recorded in the present experiment was also consistent with the average precipitation of > 800 mm for Simlemba area from July to October (Kasungu SEP, 2005).

Soil Community Structure

Figures 3 and 4 show the DGGE gel images (gray scale) and the results of the PCA, respectively. The band profiles shown in the DGGE images indicate that the microbial community structure of the soil for treatments 1, 2 and 3 varies relative to the control (treatment 4). Muyzer *et al.* (1993) demonstrated that DGGE analysis of bacterial DNA that was PCR-amplified with universal bacterial primers is suitable for determining bacterial species diversity, which is indicated by the band profile; a more complex pattern of bands indicates a higher level of diversity. Treatments 1 and 3 gave a more complex pattern of bands with lower intensity compared with treatment 2 and the control. The PCA results also indicated variability in the microbial community structure among the different treatments, especially when compared to the control. Changes in soil pH were significantly ($P > 0.05$) different across all treatments, but not between treatment 2 and the control. Table 4 shows the changes observed in the different treat-

Table 4. Changes in pH, total carbon, total nitrogen, and maximum plant height attained during the experimental period

	pH	Total Carbon%	Total Nitrogen%	Max. Plant Height (cm)
T-1	7.5	7.1	0.5	71
T-2	5.01	0.89	1.8	83
T-3	6.69	3.7	0.6	93
T-4	6.36	0.9	0.09	44

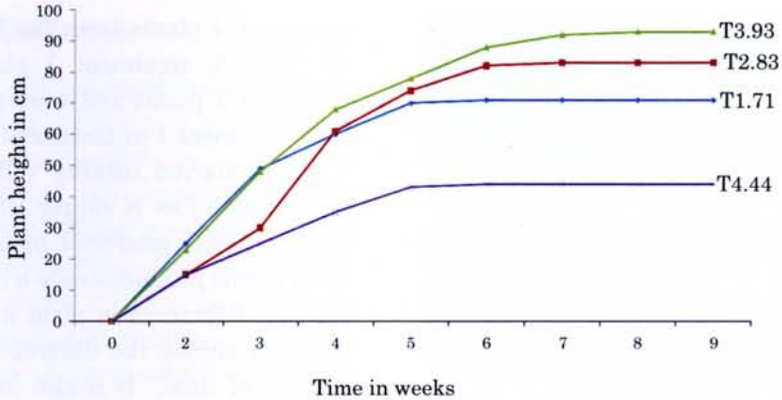

Fig. 5. Maize plant growth/height trend for the different treatments.

ments during the experiment for total C, total N, pH and the maximum height attained by the maize plants. Figure 5 shows the growth/height trend of the maize plants for the different treatments.= Total C was significantly ($P > 0.05$) higher in treatment 1 than in the other treatments. Treatment 2 gave the highest level for total N and was significant ($P > 0.01$).

Discussion

In light of the benefits and challenges of composted manure and soil organic inputs on the soil ecosystem, this experiment was pursued to investigate the effects of common chemical fertilizers (N and P) on the activity of soil microbes by measuring soil beta-glucosidase activity, and on the community structure of soil microbes by bacterial DNA analysis. The measurements of beta-glucosidase activity in soils exposed to different treatments revealed that chemical fertilizer alone results in the lowest level of beta-glucosidase activity, compared with composted manure or a combination of compost manure and chemical fertilizer. Since beta-glucosidase activity is associated with the activity of soil microbes and plays a key role in the hydrolysis of cellulose, these results suggest that the exclusive or combined use of compost in low-C soils may enhance the ability of soils to decompose plant residues with a high cellulose content. The reduced level of beta-glucosidase activity in soils treated exclusively with chemical fertilizers suggests that chemical fertilizers impair the ability of soil to decompose plant residues, which may be exacerbated under low-moisture conditions. Conversely, the higher level of beta-glucosidase activity associated with treatments 1 and 3 might be caused by the rich microbe and organic matter content of composted manure. This scenario is consistent with the findings of Stark *et al.*, 2007, who showed that the addition of green manure to lupine (*Lupinus angustifolius L.*) crops improves soil biology by increasing the biomass and activity of microbes. Taken together, these results confirm the importance of increasing the organic content of soils in order to improve soil conditions, enhance the activity of microbes, and increase beta-glucosidase activity. Moreover, the exclusive use of chemical fertilizers (treatment 2) caused no significant ($P > 0.05$) increase, or even slight improvement, in the soil beta-glucosidase activity. This result suggests that chemical fertilizers alone should not be used as a second soil treatment option. The prolonged exclusive use of chemical fertilizers may have a damaging effect on soil ecosystems and the ability of soils to break down crude plant residues with high cellulose content.

PCR-DGGE analysis showed that lanes 2 (Treatment 2) and 3 (Treatment 2) had few intense bands, whereas lanes 1 (Treatment 1) and 4 (Treatment 4) had numerous faint bands. Muyzer *et al.* (1993) demonstrated that a more complex band pattern from PCR-DGGE analysis indicates a higher level of bacterial species diversity. The PCR-DGGE analysis results of this study suggest that the treatment of soils with chemical fertilizers favored the growth of a small number of specific microbes. By comparison, treatments 1 and 3, which included composted manure, promoted the

Fig. 6. Different growth outlook of maize plants during the experiment. At 5 weeks, maize in T3 exhibited the best growth followed by T2. T1 maize growth stagnated but was better than growth in T4.

growth of numerous types of microbes, thus suggesting a more diversified soil microbial community structure, which is essential for rich soil conditions and desirable for sustainable agriculture. Although the DGGE gel images indicated the presence of microbes in the soil treated with chemical fertilizer, these microbes did not produce beta-glucosidase, most likely because the soil composition did not require the production of beta-glucosidase for bacterial survival. PCA analysis confirmed the differences in microbe community structure between the control and the different treatments.

Figure 6 summarizes the growth patterns of the maize plants with the different soil treatments. The growth/height of all plants slowed from week 5, and the plants started to turn yellow. At this point, the plants had achieved heights of 67 to 71 cm, 80 to 84 cm, 92 to 94 cm and 42 to 46 cm with soil treatments 1, 2, 3 and 4, respectively. During the first 2 weeks, treatment 1 plants grew the fastest and greenest, followed by treatment 3 plants. Treatment 2 and 4 plants were shorter, with yellow leaves, than treatment 1 and 3 plants. Treatment 1 plants continued to be the healthiest and fastest growing until the end of week 3. From week 3, the treatment 3 plants became the greenest and fastest growing. Treatment 2 plants had not grown very high by the end of week 3, compared with the other plants, but became greener than treatment 1 and treatment 4 plants from the beginning of week 4. By week 5, treatment 2 plants were as tall as treatment 1 plants and were greener and healthier than treatment 1 or treatment 4 plants. Treatment 1 plants started turning yellow from week five, suggesting a low N supply. Moreover, only treatment 2 plants produced maize cobs, whereas the other plants produced only a tassel, thus suggesting marked differences in plant nutrient availability in the soils among the different treatments with the passage of time. It is also important to note that plant growth in all soil treatments was generally poor, probably because of soil compaction in the plastic planting pots.

In the different soil treatments, markedly different soil acidity (pH) levels were observed 60 days into the experiment; for treatments 1, 2, 3 and 4, the soil pH was 7.5, 5.01, 6.69 and 6.36, respectively. These findings show a shift in the soil pH from the control (treatment 4) in soil treatments 1, 2 and 3. In treatment 2 the soil pH shifted to acidic levels, whereas in treatment 1 the soil pH shifted to alkaline conditions. These conditions are not favorable, as acidic and alkaline soils tend to bind fundamental plant nutrients. Treatments 3 and 4 (control) maintained the soil pH within the acceptable range of 5.5 to 7.0 (Hodges, 2008). This observation highlights the need for the combined use chemical fertilizers and composted manure to achieve a good soil environment and favorable crop performance with respect to soil acidity. While the objectives of the present study did not include investigation of specialized enzyme activity with respect to constitutive and adaptive enzymes, a worthwhile future study would be to explore the effects of chemical and organic fertilizers on such enzymes (Paul *et al.,* 1996). Further information may be gained by extending the experimental time frame from the short observation time period of 60 days to a longer term field experiment. For instance, at the end of the growth period of the maize plant, plant residues could be chopped and buried in the soil to investigate the decomposition rates associated with different soil treatments. Mineral cellulose could be used to investigate differences in the cellulose decomposition rate between soil treatments.

Conclusion

The present study was conducted to analyze the activity and community structure of soil microbes based on beta-glucosidase activity in soils treated with chemical fertilizers and farmyard organic compost manure. PCR-DGGE analysis and PCA were used to assess the diversity of the soil microbe population in soil exposed to different treatments and to assess changes in soil microbe community structure. The results of the present study clearly confirmed that addition of organic compost fertilizers to soil improved the soil environment with respect to beta-glucosidase activity. The present study also demonstrated that beta-glucosidase activity was markedly compromised in soils treated exclusively with chemical fertilizer. The PCR-DGGE analysis results suggest that organic compost yielded a more diverse soil microbe soil environment than chemical fertilizer, which appeared to promote the dominance of a few specific microbes. The findings of the present study also suggest that the prolonged exclusive use of chemical fertilizers may adversely affect the activity of soil microbes and reduce soil beta-glucosidase activity. A reduction in soil beta-glucosidase activity would reduce the ability of soils to quickly decompose crude plant residues with high cellulose content, because beta-glucosidase plays a key role in the hydrolysis of cellulose (Schwarz, 2001). A further disadvantage of low beta-glucosidase activity is the increased possibility of termite attacks on crops. A previous report revealed that slow decomposition of plant residues is associated with invasion by termites (Sileshi *et al.,* 2000). The present study demonstrated that the exclusive use of chemical fertilizer creates acidic soil conditions, whereas the exclusive use of organic manure creates alkaline soil conditions. This observation highlights the need for the combined use chemical fertilizers and composted manure to achieve a good soil environment and favorable crop performance with respect to soil acidity. The exclusive use of chemical fertilizers in soils with a low or modest C level can lead to low beta-glucosidase activity and diminished soil microbe community structure in terms of diversity. This tendency may be detrimental to the soil condition and the ability of the soil to support quick post-harvest or preseason decomposition of plant residues.

Recommendations

Given the low and erratic rain associated with the Simlemba area and the tendency for termite attacks to increase in dry conditions coupled with presence of crude plant residues, farmers in the Simlemba area should use fully fermented and matured composted manure instead of raw plant residues in order to improve agriculture productivity and minimize crop losses due to termite attacks, especially under low-rainfall conditions. Considering the shortfalls of typical composted manure in terms of its ability to support maize growth throughout the growth cycle, the application of appropriate composted manure enriching techniques, such as the use of locally made bio-extracts, could be employed by farmers. The combined use of composted manure and chemical fertilizers would also provide a marked advantage over the exclusive use of chemical fertilizers. Researchers and development workers should also work with farmers to explore various appropriate economically viable and ecologically sustainable composted manure improvement techniques. Future experiments should investigate the effects of the soil treatments applied in the present study under field conditions, especially in the Simlemba area, to determine outcomes applicable to the local environment.

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