Soil microbial community structures and activities in relation to nitrogen cycling in two contrasting soils in Malawi - community responses to added carbon

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Abstract
Fallowing is known as one of the conservative farm management techniques, which results in high crop yields and quality, potentially due to some changes in soil microbial structures and activities. However, few studies have investigated these changes in sub-Saharan Africa, where decreasing soil fertility is a serious issue. In this study, we examined the effects of different farm managements on the soil microbial community structures using soils sampled in Malawi, sub-Saharan Africa. Two sites located next to each other, within 100 m, were selected. One was the conservatively managed soil (maize after bean, followed by 1 year fallow) and another was the intensively farmed soil (maize after maize, continuous). Meanwhile, the addition of crop residues, including rice straw, is known as a technique to prevent the decrease of soil fertility. Thus, we performed incubation studies to investigate soil microbial responses of these soils to rice straw application. Changes in the bacterial diversities in these soils following the addition of rice straw were investigated with 16S rRNA gene approach on Miseq. Similar trends of nitrogen activities, such as the rapid decrease in soil NO_3^--N after rice straw application, were observed in the two soils. Bacterial community structural analyses suggested that the rapid increases in the ratios of *Firmicutes* and *Betaproteobacteria* to added carbon were different in the two soils. Future studies should focus more on functional genes to understand the gap between soil microbial activities and community.

Key Words
andosol, fluvisol, soil type, fallow, continuous cultivation, soil fertility

Introduction
Fallowing used to be a common practice for crop cultivation in Malawi, sub-Saharan Africa. Fallowing often prevents the reduction of soil organic matter and improves soil structure, when compared to continuously cultivated soils (Six et al., 1998). However, due to the increasing global population, the traditional cropping system, including fallowing, has gradually disappeared. Instead, smallholders attempt to produce more crops by intensive cultivation with added chemical fertilizer, which often result in the loss of soil organic matter and structures (West and Post, 2002). On the other hand, the incorporation of crop residue is often an option to improve soil organic matter and structure status in agricultural fields.

Soil microbes are strongly linked to these agricultural management techniques (e.g. fallowing and the addition of crop residues) and are often related to the fertility of soils. For example, soil microbial diversity is known as a factor controlling the decomposition of wheat straw (Baumann et al., 2013).Yet few studies have revealed how soil management systems, particularly fallowing and continuous cultivation, would influence soil microbial community structures and activities following the addition of carbon (C) in the sub-Saharan African soils.

In this incubation study, we investigated the impacts of different managements (fallow vs continuous cultivation) on soil microbial community structures and activity associated with soil nitrogen (N) cycling after an addition of rice straw (as a C source).

Methods
Soil sampling site
Soil sampling was conducted in an experimental farm at Lilongwe University of Agriculture and Natural Science, Malawi (33°46′S, 14°10′ E) in November 2013. Two sites were chosen for the sampling. One was received a 1-year fallow followed after maize and bean cultivations (“fallow” soil). The other was where maize had cultivated continuously (“continuous” soil). The distance between the two sites was within 100 m. In the past three years, N fertilizer (92 kg N ha^{-1} yr^{-1}, which was achieved through the application of 69 N kg

ha^{-1} yr^{-1} of urea and of 23 kg N ha^{-1} yr^{-1} of a basal fertilizer), was applied during maize growth periods.

Addition of carbon as rice straw
A short-incubation experiment was conducted to investigate the changes in soil microbial communities following the addition of rice straw. Finely cut rice straw (*Oryza sativa* L.) was incorporated into soils, which was equivalent to 12.5 g kg^{-1} soil. 80 g of soils was placed into a bottle (ml) and incubated at a room temperature for 33 days. During the incubation period, water filled pore space was adjusted to 55% by adding appropriate amounts of water.

Measurement of inorganic-N
As an index of nitrogen activity, N_2O emission, nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) were measured at day 3, 12, 25 and 33. A closed chamber method was applied for N_2O measurement. The gas sampling was performed immediately before the sampling of the 15 g soils to minimize the effect of soil disturbance. For each gas sampling time, the gas samples were taken from inside the headspace at 0 and 30 min, and placed in evacuated vial containers (30 ml). The gas samples were then analysed using a gas chromatograph equipped with an electron capture detector (GC-2014, Shimadzu) within 48 h after the samplings. NO_3^--N and NH_4^+-N were extracted from 0.1 g of subsampled soils by adding 10 ml of 10% KCl and shaking for 30 min. The extracts were analysed colorimetrically using a flow injection analyser.

Changes in soil microbial community
For samples at day 3 and 33, Illumina Miseq was applied to characterize soil microbial community based on 16S rRNA genes. First genomic DNAs were extracted from soils using a PowerSoil DNA Isolation kit (MoBio Technologies, Carlsbad, CA). The variable region (V3 and V4) of bacterial DNA was amplified by using primers 341F and 805R (Herlemann et al., 2011) with Illumina adaptor sequences. The PCR reaction mixture (25µL) comprised of 4µl of 2.5µM each dNTP, 5µl of 10X EXTag Buffer, 0.4µl of 50µM primers, 0.25µl of EX Tag HotStart and 2.5µl of template DNA. Polymerase chain reaction began with an initial 95°C denaturation for 5 min, followed by 24 cycles of 95°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec and then a final extension at 72°C for 7 min. After purifying PCR amplicons using LaboPass PCR Purification Kit (COSMO Genetech, Seoul, Korea), the purified PCR products were quantified with PicoGreen dsDNA quantification reagent (Molecular Probes, Eugene, OR, USA). Paired-end sequencing was conducted with all tagged amplicons. Sequence reads from samples were analysed using the Quantative Insights Into Microbial Ecology (QIIME) software package (Caporaso et al., 2010) with Silva 119 database.

Data analyses
Two-way analyses of variance (ANOVA) were performed to investigate the effects of soil types, rice straw and their interactions, for each time point. Significant difference was assessed by Turkey test with $p=0.05$. As a measurement of microbial diversity, Shannon index which interprets soil microbial diversity and significant effects of the treatment on the soil microbial community structures, based on permutational multivariate analyses of variance (PERMANOVA), were calculated with a package provided for a statistical software "R" (vegan).

Results
Changes in soil nitrogen
The maximum N_2O emissions from soils with straw occurred at day 3 and the emissions dropped immediately (Fig. 1a). Contrastingly, for soils without straw, the emissions peaked at day 25, and for day 12 and 25 the N_2O emissions were significantly higher for continuous soil ($p<0.01$, Fig. 1a). The addition of straw resulted in the immediate depletion in NO_3^--N concentrations, both in fallow and continuous soils (Fig. 1b). For soils without rice straw, NO_3^--N concentrations also decreased over time but the magnitude was markedly smaller when compared to the soils with rice straw (Fig. 1b). Throughout the experiment, there were not significant effects of soil type ($p>0.05$). The concentration of NH_4^+-N was also measured throughout the current study, but the concentrations were small for all the samples (<5 mg NH_4^+-N kg^{-1} soil, data not shown).

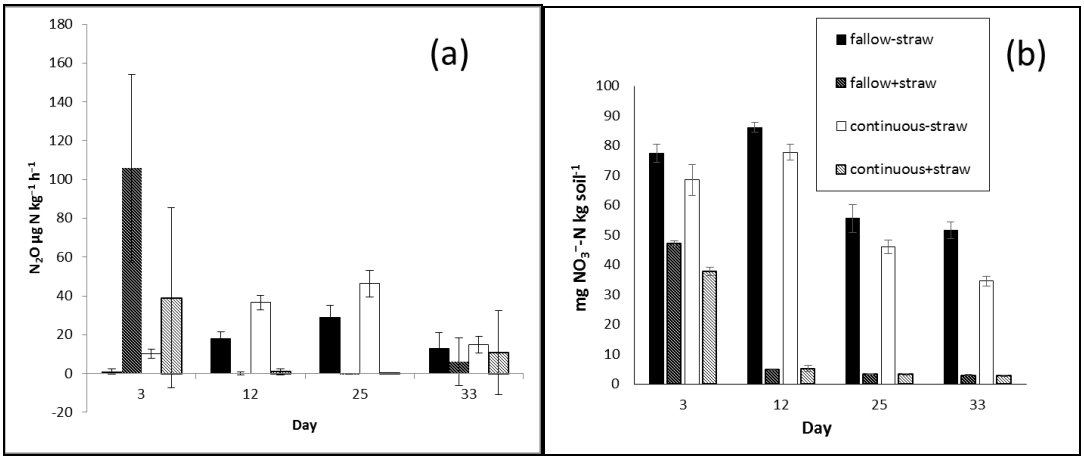

Figure 1. Changes in (a) nitrous oxide (N_2O) emissions from soils and (b) nitrate (NO_3^-N) concentrations in soils over the incubation period. The bars indicate fallow without straw addition, fallow with straw addition, continuously cultivated without straw and continuously cultivated with straw addition, respectively, from the left. The bars indicate standard deviations (n = 4).

Microbial community based on 16S rRNA

Microbial community structures were characterized after the addition of rice straw at day 3 and 33, respectively (Fig. 2). During incubation period, *Actinobacteria*, *Acidobacteria* and *Alphaproteobacteria* were relatively abundant among all the treatments. At day 3, it was obvious that *Firmicutes* and *Betaproteobacteria* dominated under rice straw treatment, 10.48% and 31.44% in fallow soil and 20.76% and 25.00% in continuous soil, respectively. Up to day 33, *Firmicutes* and *Betaproteobacteria* decreased. *Alphaproteobacteria* and *Planctomycetes* markedly increased in the presence of rice straw.

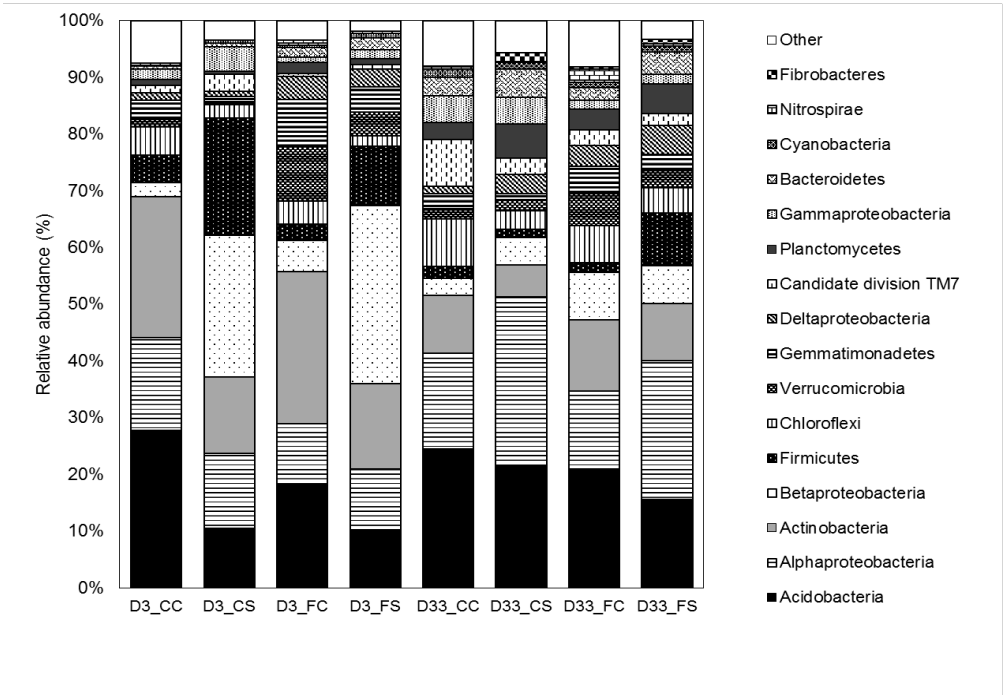

Figure 2. Changes in soil microbial community structures based on 16S rRNA analyses. D3 and D33 mean 3 and 33 days after the commencement of the incubation, respectively. CC, CS, FC and FS mean "continuously cultivated soil without the addition of carbon", "continuously cultivated soil with the added rice straw", "fallowed soil without the addition of carbon" and "fallowed soil with the added rice straw", respectively.

Microbial diversity

Control/fallow soils had more members of phyla than soils with rice straw/continuously cultivated at day 3 while the differences in richness of the soils were not evident at day 33 (Table 1). ANOVA revealed that shannon index and richness were impacted by soil, straw and time and that there were effects of soil x time and straw x time on richness (Table 2).

Table 1. Different diversity indexes for the continuously cultivated and fallowed soil with and without the added rice straw at two timings (3 and 33 days after the start of the incubation).

	Fallow soil				*Continuous soil*			
	control		*straw*		*control*		*straw*	
	Day3	*Day33*	*Day3*	*Day33*	*Day3*	*Day33*	*Day3*	*Day33*
Shannon	2.32 ± 0.021	2.59 ± 0.030	2.20 ± 0.061	2.39 ± 0.17	2.13 ± 0.020	2.47 ± 0.033	2.12 ± 0.13	2.35 ± 0.12
Richness	36.3 ± 2.7	38.8 ± 1.3	33.8 ± 0.96	39.8 ± 2.1	31.0 ± 0.81	36.8 ± 0.96	26.8 ± 2.8	36.8 ± 1.5
Pielou's evenness	0.65 ± 0.008	0.71 ± 0.009	0.63 ± 0.017	0.65 ± 0.041	0.62 ± 0.009	0.69 ± 0.007	0.64 ± 0.030	0.65 ± 0.034

Table 2. ANOVA p-value based on diversity indices at day 3 and 33.

Effects	*Shannon*	*Richness*	*Pielou's evenness*
Soil	<0.01	<0.001	0.43
Straw	<0.01	<0.05	<0.05
Time	<0.001	<0.001	<0.001
Soil x Straw	0.20	0.27	0.05
Soil x Time	0.41	<0.01	0.66
Straw x Time	0.17	<0.01	<0.01
Soil x Straw x Time	0.82	0.76	0.55

Discussion

Generally, the response of the two soils to the addition of straw was similar, in terms of the decrease in NO_3^--N concentrations and N_2O emissions (Fig. 1), suggesting the occurrence of immobilization and denitrification. However, the responses of some microbes, such as *Firmicutes* and *Betaproteobacteria*, to added C were different in the two soils (Fig. 2).

The information on the soil microbial community structures are known as an indicator of soil quality (Mbuthia et al., 2015) but it is difficult to qualitatively analyse the relationship between soil microbial structures and the activity in soils. Our study indicated members of some of the phyla abundant at day 3 with the presence of C (straw) markedly decreased at day 33. Thus, only a few phyla might be contributing to the decomposition of the added straw at the beginning of the decomposition processes while after a few weeks the diversity of soil microbes might influence the decomposition of the relatively stable C. Our data is still preliminary and further experiments are required to confirm this theory but fallowing might be a beneficial method to increase the diversity of soil microbes especially when C is depleted in soils. This experiment compared a maize/legume/fallow system and a continuous maize system. Thus, continuous work is needed to identify specific factor(s) controlling the microbial diversity in soils.

Conclusion

In this study, the evidence was provided that the added rice straw induced a rapid immobilization of N and some changes in soil microbial community structures. However, it was difficult to link the microbial activities with microbial community structures or with their diversities. The studies which focus on specific processes using functional genes will help us understand the gap. Based on the comparison of soil microbial community structures between continuously cultivated and fallowed soils in Malawi, Africa, there were significantly more diverse community structures in the fallowed soil. The diversity of soil microbial community structure was reduced at 3 days after the application of rice straw, suggesting that only a limited number of phyla responded to the added C. However, there are uncertainties in our understanding of microbial diversity related to soil fertility and quality. Longer term field-based experiments will be needed to evaluate the effect of microbial diversity which results from different soil managements.

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