Responses of CO_2 Emissions and Soil Microbial Community Structures to Organic Amendment in Two Contrasting Soils in Zambia

Toru Hamamoto (hamamotoru@gmail.com)
Tohoku University
Nhamo Nhamo
International Center for Biosaline Agriculture
David Chikoye
International Institute of Tropical Agriculture
Ikabongo Mukumbuta
Hokkaido University
Yoshitaka Uchida
Hokkaido University

Research Article

Keywords: drylands, organic amendments, soil microbes, CO2 emissions

Posted Date: December 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1121060/v1

Version of Record: A version of this preprint was published at Scientific Reports on April 16th, 2022. See the published version at https://doi.org/10.1038/s41598-022-10368-9.

Abstract

In sub-Saharan Africa, efforts have been made to increase soil carbon (C) content in agricultural ecosystems, due to severe soil degradation. The use of organic materials is one of the realistic methods to recover soil C. However, the impacts of organic amendments on soil microbial community and C cycles under limited soil C conditions are still unknown. We conducted field experiments using organic amendments in two sites with contrasting C content in Zambia. At both sites, temporal changes of soil carbon dioxide (CO_2) emissions, bacterial and archaeal community structures were monitored during crop growing season (126 days). The organic amendments increased CO_2 emissions with increased bacterial and archaeal abundance in the Kabwe site, while no impacts were shown in the Lusaka site. We also observed larger temporal variability in soil microbial community structure in Kabwe than in Lusaka. These contrasting results between the two soils might be due to the gap in microbial community stability. However, organic amendments have a significant potential to enhance microbial abundance and consequently sequester soil C in the Kabwe site. Site-specific strategies are needed to deal with the issues of soil C depletion in drylands.

Introduction

The productivity of agricultural soils is positively correlated to their Carbon (C) content [1, 2]. However, soil C depletion is observed globally within agricultural systems. In sub-Saharan Africa, many soils have low soil C content below values, typically 1.1%, which is the critical limit for agricultural productivity in this region [1, 3–5]. In southern Africa, soils are fragile due to over-cultivation and soil C ranges from 0.5–2.5% [3, 6–9]. Organic amendments (e.g. animal manure and crop residue) are have the potential to maintain or increase soil C, activating soil microbial decomposition process [10–12]. It has also been suggested that the combined use of organic amendments and inorganic fertilizers, such as urea, increases fertilizer use efficiency [13, 14]. The ability of organic amendments to improve soil C levels is partly controlled by the soil's inherent properties (such as texture, mineralogy, etc.) and soil types [15, 16]. Soils with relatively higher clay content tend to store soil C more effectively when compared to soils with relatively higher sand content [17–21]. At continental scale, the soil C content in African soils is positively related to the amount of clay and silt [6, 8, 22]. Therefore, effects of organic amendment on C dynamics might largely depend on original soil C status and related soil properties in sub-Sharan Africa. Most of studies in Africa have focused on C budgets and agricultural production in different soils. However, the effects of organic amendments on soil microbial communities and how these drive soil C cycles to impact applied-C decomposition and CO_2 emissions remain unknown.

To fully understand the link between the organic amendments and soil C cycle, the release of C by microbes and changes in soil microbial community need to be studied. In addition to soil characteristics, factors; the types of organic amendments [23], environmental conditions (e.g. soil moisture) [24], and land management (e.g. plant residue application, fertilizer application, and tillage) [4, 25], also contribute to the magnitudes of microbial decomposition after organic amendments. Many studies in sub-Saharan Africa have reported that the low rate of C stock in soils might be due to rapid decomposition of soil

organic matter and organic amendments due to high temperatures and activity of macro- and micro-fauna [26–31]. However, few studies have been conducted to observe the microbiome in C-limited agricultural soils in sub-Saharan Africa [3, 32, 33]. Information regarding soil microbes in crop production systems is critically important to establish site-specific strategies to deal with the issue of C depletion in this area.

In this study, we assessed CO_2 emissions, litter bag decomposition rates, bacterial and archaeal abundance, and taxonomic diversity under different organic amendments at two different experimental sites in Zambia. Our main objectives were (1) to investigate the impact of different types of organic amendments on CO_2 emissions and litter bag decomposition, (2) to evaluate the responses of soil microbes to different organic amendments, and (3) to compare the interactions between soil C dynamics and bacterial and archaeal communities under two contrasting soils in dry tropical agroecosystems in Zambia. We hypothesized that organic amendments increased CO_2 emissions by activating soil microbes. However, the magnitude of the response of organic amendments on soil microbes contrasts even in soils due to soil's inherent properties such as clay content and soil C levels.

Results

Effects of soils and fertilizer treatments on soil moisture

Gravimetric soil moisture content at the Lusaka site was significantly higher than at the Kabwe site throughout the experimental period, when averaged across the fertilizer treatments ($p < 0.05$, Fig. 1). In general, soils in the Lusaka site maintained around 5% higher moisture content than soils in the Kabwe site. Soil moisture in CM and MR treatments was generally higher than the other treatments at both sites when averaged across the experimental period. The rate at which soil moisture deceased during short dry spells (e.g. day 10 to 42) was more moderate in CM and MR treatments compared to the other treatments.

Effects of soils and fertilizer treatments on CO_2 emissions, Litter bag decomposition rate, and soil bacterial and archaeal abundance

The CO_2 emission rate from soils without any fertilizer treatment (NF) was within the same range between the Lusaka (18.8 to 222.8 CO_2-C m^{-2} h^{-1}) and Kabwe (22.3 to 270.8 CO_2-C m^{-2} h^{-1}) sites, and there was no significant difference in the cumulative CO_2 emission in the NF treatment between the sites; 2.5 and 2.1 t CO_2-C ha^{-1} from Lusaka and Kabwe sites, respectively (Figs. 2 and 3).

At the Lusaka site, mixed model results showed significant differences only among sampling timings (Fig. 2a). The CO_2 emission rates from CM, PM, and MR treatments peaked at 447, 345, and 305 mg CO_2-C m^{-2} h^{-1} on days 52, 84, and 63, respectively. However, organic amendments did not significantly increase CO_2 emissions at the Lusaka site (Fig. 3). At the Kabwe site, there was a significant effect of

fertilizer treatments ($p < 0.05$, Fig. 2b). The CO_2 emissions peaked at the beginning of the experiment and relatively small peaks were observed from day 52 to 63 (e.g., 139 and 167 mg CO_2-C m^{-2} h^{-1} from CM and MR treatments). There was also a significant effect of fertilizer treatments on the cumulative CO_2 emissions at the Kabwe site ($p < 0.01$, Fig. 3). CM and MR treatments had higher cumulative CO_2 emission compared to the other fertilizer treatments ($p <0.05$). CO_2 emission rate was significantly correlated with soil moisture at both the Lusaka ($r = 0.59$, $p < 0.001$) and Kabwe ($r = 0.39$, $p < 0.001$) sites (Fig. 4). The correlation was stronger at the Lusaka site.

Approximately 60% of initially added maize litter decomposed within 120 days at both Lusaka and Kabwe sites (Fig. S2). Fertilizer treatments had a significant effect on litter bag decomposition rate only at the Kabwe site ($p < 0.05$). At the Kabwe site, 40%, 43%, 46%, 56%, and 67% of litter remained in MR, NF, CM, PM, and CF treatments, respectively, 120 days after buried.

Lusaka soils showed higher bacterial and archaeal abundance than Kabwe soils, when averaged across fertilizer treatments and sampling dates ($p < 0.05$). The soil microbial abundance was positively correlated with CO_2 emission ($r = 0.67$, $p <0.001$, Fig. S3). At the Lusaka site, the effects of the organic amendments on the abundance of soil bacteria and archaea were unclear (Fig. 5a). In contrast, there was a significant interaction between organic amendment types and time-course changes in soil bacteria and archaea abundance at the Kabwe site ($p < 0.05$, Fig. 5b). The CM and MR treatments had higher bacterial and archaeal abundance throughout the experimental period, particularly from day 52 onwards after the application of the amendments. The MR treatment showed a continuous increase in the bacterial and archaeal abundance during the experimental period at the Kabwe site.

Effects of soils and fertilizer treatments on bacterial and archaeal community

The microbial taxonomic composition showed a total of 61 phyla (Bacteria and archaea domains). The bacterial and archaeal community mainly consisted of Chloroflexi and Actinobacteria which accounted for 27% and 31% and 21% and 16% of the relative abundance at the Lusaka and Kabwe sites, respectively (Fig. 6). Lusaka soils had a significantly higher relative abundance of Acidobacteria and Actinobacteria, but a significantly lower relative abundance of Chloroflexi, Firmicutes, and Verrucomicrobia when compared to Kabwe soils (Fig. 6). PERMANOVA showed that the bacterial and archaeal community structure was significantly affected by two different sites ($p < 0.001$). NMDS also clearly separated samples depending on the sites (Fig. S4).

The response of bacterial and archaeal relative abundance to fertilizer treatments differed in the Lusaka and Kabwe sites throughout experimental period (Fig. 7). In the Luska site, the CF treatment showed a significant higher relative abundance of Firmicutes and decreased that of Acidobacteria (Table S1). Different organic amendments affected different phyla in the Lusaka site. For example, the relative abundance of Thaumarchaeota in the CM treatment was higher compared to the MR treatment, a higher

proportion of Chlorflexi was observed in CF, CM, and NF treatments, whereas a higher proportion of Actinobacteria was observed in PM and MR treatments until 52 days after organic amendments (Fig. 7). The relative abundance of Planctomycetes gradually increased in all fertilizer treatments towards the end of experiments. Although we did not find clear effect of the fertilizer treatments on the soil microbial community structure at the OTU levels in the NMDS plot, cluster analysis showed the separation of CM and NF treatments from other treatments (Fig. S6). In the Kabwe site, the relative abundances of Thaumarchaeota and Chlorflexi decreased towards the end of experiments, when averaged across the fertilizer treatments (Fig. 7 and Table S2). In contrast, Acidobacteria, Planctomycetes, and Proteobacteria increased the relative abundance on 126 days after organic amendments, compared to Day 0. The CF treatment decreased Acidobacteria than other treatments in the Kabwe site, similar to the trend observed in the Lusaka site. However, the NMDS plot showed no clear effect of the fertilizer treatments on the soil microbial community structure in Kabwe soils (Fig. S5). Cluster analysis in the Kabwe site showed that the soil bacterial and archaeal community structure was affected by the sampling times rather than the fertilizer treatments (Fig. S6).

Diversity indices for the bacterial and archaeal community structures differed between the two sites. Lusaka soils had significantly higher shannon diversity, simpson diversity, and evenness, when averaged across fertilizer amendments and sampling dates (Table 1). The numbers of bacterial and archaeal OTUs (richness) did not differ between the Lusaka and Kabwe sites. The effect of organic amendments on soil bacterial and archaeal diversity was unclear, although these values fluctuated significantly during the experimental period at both Lusaka and Kabwe sites (Fig. S7, Table S3). The coefficient of variation (CV) of microbial diversity was lower at the Lusaka site than the Kabwe site (Fig. S8).

Table 1
Alpha diversity indices averaged across the fertilizer treatments and sampling dates (mean ± SD). The level of significance was determined by Tukey's test. Different letters indicate significant differences ($P < 0.05$) between Lusaka and Kabwe sites.

Area	Shannon diversity	Simpson diversity	Richness	Evenness
Lusaka	9.04 ± 0.32 a	0.996 ± 0.001 a	965 ± 244	0.92 ± 0.01 a
Kabwe	8.67 ± 0.52 b	0.993 ± 0.005 b	921 ± 323	0.89 ± 0.03 b

Discussion

The Lusaka site had generally higher soil moisture status, even though the precipitation was much lower than the Kabwe site. This was largely reflecting differences in the water holding capacities of the two different soils. CO_2 emissions were positively correlated with soil moisture in both Lusaka and Kabwe sites. This positive relationship was previously reported in sub-Saharan African soils and suggests that soil moisture is an important factor controlling microbial activities [4, 24, 34]. Organic amendments also significantly increased soil moisture in both Lusaka and Kabwe sites. Contrastingly, organic amendments stimulated CO_2 emissions only in the Kabwe site. The litter bag decomposition rates and the soil bacterial

and archaeal abundance were also positively influenced by organic amendments only in Kabwe soils. This suggested that the positive impacts of organic amendments on soil microbial condition and then decomposition process appeared stronger in the Kabwe site. According to Thomsen et al. [35], soil texture indirectly affected soil microbial decomposition through water holding capacity. Organic amendments are therefore critical to maintaining soil microbes' ability to decompose organic matter. At the same time, this could have been relevant only in sand-rich soils with limited soil moisture than in clay-rich soils, similar to the Kabwe site.

The PM amendment did not increase the CO_2 emission rates compared to the control in the Kabwe site. Poultry manure used in the current research had a relatively higher C concentration than other organic materials and the C might be more recalcitrant than other organic materials, reducing the decomposition rates and the consequent C emissions. Another reason could be due to the smaller positive moisture retention effects on the PM treatment, compared to other organic amendments. We adjusted the organic material application rates based on their C content. Therefore, the relatively smaller amount of poultry manure applied could have less impacts on soil microbes and did not result in the increased CO_2 emission rates, in the Kabwe soils.

In the clay-rich Lusaka site, soil moisture was more important in controlling soil respiration rather than fertilizer treatments. Soil faunal abundance collected using pit traps was higher in the Lusaka site compared to the Kabwe site (data not shown). Soil fauna could have played a key role in physical breakdown of organic materials and ultimately led to higher decomposition rates in the Lusaka site [30]. This possibility suggests that the applied C in the Lusaka site was less recalcitrant than the Kabwe site, and it is more susceptible to loss from soil through decomposition. Thus, the organic amendments might not be expected to be a sole method to improve soil C content at the Lusaka site. Further studies are needed to understand interactions among soil biological community (faunal-microbial interaction) and soil C dynamics after organic amendments.

The soil bacterial and archaeal phyla was mainly dominated by Chloroflexi and Actinobacteria in both Lusaka and Kabwe sites. These dominant phyla show typical soil microbial community structures in nutrient-limited soils in tropics [3, 36]. There were also clear differences between the two sites in relative abundances of soil bacteria, mainly derived from some oligotrophs. Edaphic factors, in particular soil pH, soil C content, and moisture condition, are largely associated with soil microbial community structures [37]. Lusaka soils had a higher abundance of Acidobacteria but lower abundances of Firmicutes and Verrucomicrobia compared to Kabwe soils. These bacteria phyla are strongly associated with soil C contents and moisture. Acidobacteria can be described as slow-growing oligotrophs and their abundance is negatively correlated with soil C contents [38]. Higher relative abundance of Verrucomicrobia was previously observed in the subsurface (>10 cm depth) soil, suggesting an oligotrophic life strategy dependent on lower C availability [39–41]. Members of the Firmicutes are Gram-positive bacteria and should be favored by limited soil water holding capacity [3, 36, 42].

Within individual experimental sites, the temporal changes of bacterial and archaeal communities were highly variable in all fertilizer treatments. De la Cruz-Barrón et al. [32] also reported that bacterial community structure variation was more significant temporally (over time) and less influenced by fertilizer treatments in Zimbabwean soils largely due to short-term effect of organic materials. However, in the Lusaka site, soil microbial community structures clearly showed two different clusters throughout the experimental period, with one cluster having the CM and NF treatments and the other having the treatments that received both organic and inorganic fertilizers. Therefore, the combined use of chemical and organic N fertilizers largely affects the soil microbial community structure, compared to single use of organic materials or no fertilization.

The temporal variation of bacterial and archaeal diversity indices in Kabwe soils was higher and values were significantly lower than in Lusaka soils. This strongly reflects that the magnitudes of stability and response of soil microbial community structure to edaphic factors and soil physical disturbance events (e.g. weeding management and rainfall fluctuation) rather than organic amendments. Clay particles protect soil microbes from environmental stress such as predation, drought, and heat [43–45]. Also, the soil C content to limited levels could decrease both microbial biomass and diversity [46]. Therefore, it is possible that Kabwe soils are more vulnerable to environmental stress and such conditions consequently destabilize microbial community structure due to enhanced microbial facilitation and niches overlapping [47]. The vulnerability of microbial ecosystems in Kabwe soils could potentially be reduced by using organic amendments. Increased bacterial and archaeal abundance due to organic amendments was observed only in the Kabwe soils. A similar trend was observed for soil respiration and litter bag decomposition rates. These results are consistent with a previous study conducted in loamy sand soils, in which 16S rRNA gene abundance increased in organic farm soils compared to conventional farm soils in India [10]. Organic amendments altered soil bacterial and archaeal communities to those more suitable to decompose complex organic substances. Previous studies stated that long-term organic amendments in sandy soils in the tropical arid regions have a potential to increase soil C and microbial diversity [10, 11, 48]. Therefore, organic amendments can contribute to maintaining the abundance of soil microbiome, and consequently increase C sequestration in the Kabwe soils. However, it should be emphasized that the rate of C sequestration depends on the capacity of the soil to protect the C.

Conclusion

We conducted a field experiment using organic amendments in two soils with contrasting soil C contents (Lusaka and Kabwe) in Zambia. CO_2 emissions are significantly controlled by soil moisture rather than organic amendments in Lusaka soils. However, organic amendments increased bacterial and archaeal abundance, and therefore reduced the vulnerability of microbial ecosystems, only in Kabwe soils which have low (<1%) soil C content. These findings indicate that the stability of the microbial community structure depend on factors controlling the and through changes in soil holding capacity, and hence was the reason for the different impacts of organic amendments between Lusaka and Kabwe sites. Further

studies will be required to determine the efficiency of organic amendments to improve soil C contents in this area.

Materials And Methods

Description of the field study site

The field experiment was conducted at two sites (Lusaka and Kabwe) from December 2017 to April 2018 (Table 2). The Lusaka site is located within the agricultural experimental field of International Institute of Tropical Agriculture (14°23'44.6"S, 28°29'39.9"E). The Kabwe site is located within the agricultural experimental field of Zambian Agricultural Research Institute (15°18'09.4"S, 28°18'17.6"E). The dominant cropping system was maize without any organic amendments in both sites more than five years. The soil in Lusaka is classified as Luvisol, whereas the soil in Kabwe is classified as Acrisol [49]. Zambia is generally divided into three major agro-ecological zones, based on total annual rainfall received in a unimodal pattern between October and April (e.g., region I, IIa and IIb, and III). According to those zones, both Lusaka and Kabwe sites are in region IIb (annual rainfall of between 800 and 1200 mm). However, the rainfall was lower at the Lusaka site (232.6 mm) than at the Kabwe site (734.4 mm), during the experimental period from December to April (Fig. S1).

Table 2
Soil characteristics in Lusaka and Kabwe sites (mean ± SD). The level of significance was determined by Tukey's test. Different letters indicate significant differences ($P < 0.05$) between Lusaka and Kabwe sites.

	Lusaka	Kabwe
pH_KCl	5.0 ± 0.0 a	4.0 ± 0.0 b
P (mg kg^{-1} soil)	6.7 ± 0.5 b	32.7 ± 0.9 a
K (mg kg^{-1} soil)	98.3 ± 5.9 a	52.7 ± 1.7 b
Na (mg kg^{-1} soil)	4.3 ± 1.7	3.3 ± 1.2
Ca (mg kg^{-1} soil)	852.0 ± 48.7 a	91.7 ± 6.9 b
Mg (mg kg^{-1} soil)	260.7 ± 11.1 a	20.7 ± 1.2 b
CEC (cmol kg^{-1} soil)	6.7 ± 0.3 a	1.4 ± 0.0 b
Total C (g kg^{-1} soil)	14.2 ± 0.1 a	5.1 ± 0.0 b
Total N (g kg^{-1} soil)	0.8 ± 0.0	1.0 ± 0.0
Clay (%)	19 ± 0.9 a	12 ± 0.0 b
Silt (%)	19 ± 4.1	12 ± 1.6
Sand (%)	62 ± 3.4 b	76 ± 1.6 a

Experimental design

For each site, 5 treatments plots were established with each treatment replicated 3 times as sub-plots, resulting in a total of 15 sub-plots. Each sub-plot measured 2 m × 3 m in size. The experimental design was a randomized complete design. The 5 treatments were;

1. Chemical fertilizer treatment; hereafter referred to as 'CF'
2. Cattle manure treatment; hereafter referred to as 'CM'
3. Poultry manure compost treatment; hereafter referred to as 'PM'
4. Maze residue (chopped into 20 cm pieces) treatment; hereafter referred to as 'MR'
5. Control, where no fertilizer was applied; hereafter referred to as 'NF' treatment.

All organic materials were collected from local farms. The C and N contents in each organic materials were measured by Zambian Agriculture Research Institute Mount Makulu Research Station. The organic amendments were applied at 2.5 t C ha^{-1} and the actual amounts applied are shown in Table 3. The CM treatment had the highest N content. Therefore, the fertilizer application rate of the CF treatment was applied to have the same amount of N as that of the CM treatment. In contrast, additional N was applied

as inorganic fertilizer in the PM and MR treatments to have the same amount as the CM treatment. The application rates of organic amendments are similar with what has been applied in other studies in Africa and within recommended rates to increase soil C [7]. 200 kg ha^{-1} of D-compound fertilizer (10-20-10-NPK) was applied as basal fertilizer in CF and MR treatments. Urea (46% N content) was applied in CF, PM, and MR treatments to adjust the N rate to that of the CM treatment [50]. Organic materials were incorporated into the soil (15 cm) by using hand hoes 3 days before planting, whereas all chemical fertilizers were applied at planting. Field experiments were run from 21st and 28th December 2017 (Day 0) to 16th and 23rd April 2018 (Day 126) at Lusaka and Kabwe sites, respectively.

Table 3
Applied organic material application rate (dry biomass basis) and their nutrient contents. The brackets in the PM and MR treatments show the additional chemical fertilizer application rate to provide the same amount of N as the CM treatment and avoid nutrient deficiency. CF: chemical fertilizer treatment, CM: cattle manure treatment, PM: poultry manure treatment, MR: maize residue treatment, and NF: no fertilizer treatment.

	CF	CM	PM	MR	NF
Biomass (t ha^{-1})	0	11.8	3.8	18.0	0
C (t ha^{-1})	0	2.5	2.5	2.5	0
N (kg ha^{-1})	108	108	72 (36)	51 (57)	0
P (kg ha^{-1})	40	12.4	3.2	23.3 (40)	0
K (kg ha^{-1})	20	300.8	32.3	213.3 (20)	0

Soil sampling and analysis

Soils were collected at 0 to 5 cm depth every 2 weeks during the experimental period resulting in a total of 13 sampling events. From the 13 sampling events, soil DNA extraction was done for only 5 of them. At each sampling event, soils were collected from four points inside each plot and mixed to make a composite sample. Soil moisture content of each soil sample was measured by oven-drying fresh soils at 100°C > 24 hr immediately after soil sampling and reweighing the dried soils.

Measurement of CO_2 emissions

The CO_2 emission rates were measured using an automated closed-chamber system at both sites every 2 weeks during the experimental period. The chamber was a portable soil respiration chamber attached to a non-dispersive infrared CO_2 analyzer (DIK-0450; DAIKI, Japan). Gas flux was calculated as rate of CO_2 change inside the chamber between 0 min and 5 min after inserting the chamber on the soil surface. The linearity of the increase in CO_2 concentration in the chamber was confirmed prior to starting the

experiment by measuring CO_2 concentration every minute for 5 min at both the Lusaka and Kabwe sites. All gas field measurements were conducted between 9:00 and 13:00.

Measurement of decomposition rate using litter bag

To evaluate the decomposition rate of organic materials, three litter bags with 100 μm mesh-size were filled with maize residue and buried vertically at 10 cm depth in each treatment sub-plot and site (9 bags per treatment per site resulting in 90 litter bags in total). Each bag was 10 cm × 12 cm (length × width) and contained 6 g of dried maize residue (chopped to within 2 cm length). The distance between individual litter bags was > 20 cm. Litter bags were retrieved after 1, 2, and 4 months. The soils were carefully brushed off from the litter bags, and the remaining residues were taken out and gently shaken to eliminate the interfusion of soil. The residues were then washed to remove soil particles, air-dried for 48 h, and then dried at 60°C for 48 h. The dried samples were weighed for the remaining mass. Litter bags were lost in some treatments and thus the number of replicates decreased from three to two due to environmental disturbance (e.g. by wildlife).

Measurement of bacterial abundance

DNA was extracted from the air-dried soils with a modified method according to Sagova-Mareckova et al. [51] and Miller et al. [52]. The extracted DNA was purified with Agencourt AMPure XP (Beckman Coulter, USA) according to a predetermined protocol. The concentration of the purified DNA was measured with Qubit dsDNA HS Assay Kit (Invitrogen, USA). The purified DNA was then diluted 50 times with nuclease-free water for qPCR analysis. We used Mx3000P/Mx3005P QPCR Systems (Agilent, USA). For primers, 515F/806R [53] were chosen for V4 region of 16S rRNA quantitative analysis. For qPCR, samples were prepared with 15 μL of the KAPA SYBR Fast qPCR kit (Kapa Biosystems, USA) and, 1.2 μL of forward primer, 1.2 μL of reverse primer, 0.18 μL of bovine serum albumin (100 mg mL^{-1}) and 3 μL of DNA extract. Nuclease-free water was added to make up to a final volume of 30 μL. Cycling conditions were 30 s at 95°C, 35 cycles at 95°C for 30 s, 58°C for 30 s and 72°C for 1 min, followed by 95°C for 1 min, 55°C for 30 s and 95°C for 30 s. All reactions were run in duplicate.

Amplification and sequencing of 16S rRNA in soils

Bacterial community analysis was performed on all DNA samples extracted from soil. The first PCR was conducted using primers same as for qPCR to amplify the V4 region of 16S rRNA. For PCR, samples were prepared with 10 mL of AmpliTaq Gold® 360 Master Mix (Applied BiosystemsTM, USA), 0.4 μL of forward primer, 0.4 μL of reverse primer, and 1 μL of DNA extract. Nuclease-free water was added to make up to a final volume of 20 μL. The first PCR cycle was 95°C for 10 min, then 25 cycles at 95°C for 30 s, 57°C for 30 s, and 72°C for 1 min, followed by 72°C for 7 min. The first PCR products were then purified with Agencourt AMPure XP (Beckman Coulter) according to the manufacturer's protocol. Using the amplicon obtained from the first PCR procedure, another PCR was performed to make it Ion Torrent sequencing sample-specific. To achieve this, a forward primer of 515F attached with the sequence of Ion Xpress Barcode Adapters Kit (Life Technologies), and reverse primer of 806F attached with the sequence of Ion P1 adaptor (Ion Torrent Life Technologies, USA) were used. The PCR sample contained 10 μL of

AmpliTaq Gold® 360 Master Mix (Applied Biosystems, USA), 0.4 µL of forward primer, 0.4 µL of reverse primer, and 4 µL of purified first PCR product. Nuclease-free water was added to make up to a final volume of 20 µL. The second PCR cycle was 95°C for 10 min, then 5 cycles at 95°C for 30 s, 57°C for 30 s, and 72°C for 1 min, followed by 72°C for 7 min. The second PCR products were purified with the same method as above. The concentration of purified DNA was measured using Qubit dsDNA HS Assay Kit (Invitrogen, USA). The final length and concentration of the amplicons were confirmed using a Bioanalyzer DNA 1000 Kit (Agilent Technologies, USA). The library was diluted to 50 pM and loaded into the Ion 318 chip (Ion Torrent Life Technologies, USA) using the Ion Chef Instruments (Ion Torrent Life Technologies, USA) with the Ion PGM™ Hi-Q Chef kit.

Analysis of the 16S rRNA based bacterial community structures

The barcoded 16S rRNA gene sequences were de-multiplexed, denoised, quality-filtered, and assessed using the Quantitative Insights Into Microbial Ecology (QIIME2) workflow (https://qiime2.org/, [54]). Operational Taxonomic Units (OTUs) were prepared by eliminating all the OTUs that matched the SILVA 132 reference sequence with 97% similarity. On average, 54,995 reads were mapped per sample for 16S rRNA, ranging from 18,754 to 690,339. Rarefaction was performed and the sequence data were subsampled to minimum sequences per sample to ensure fair comparisons between the samples [55]. Sequence data were deposited in the Sequence Read Archive at NCBI under accession number PRJNA694222.

Statistical analysis

All statistical analyses were performed using R ver. 3.6.1. Soil moisture contents, CO_2 emissions, litter bag decomposition rates, relative amounts of soil bacterial DNA, Shannon-Wiener diversity index, species richness, and Pielou's evenness for each category, were analyzed using a mixed model for repeated measurements approach. The cumulative CO_2 emission was analyzed using one-way ANOVA and then Tukey's test was performed for the analysis of significant differences for each treatment. Permutational multivariate analysis of variance (PERMANOVA) was performed to test for differences between samples. Nonmetric multidimensional scaling (NMDS) of community structure based on the Bray-Curtis dissimilarity index was also performed. We used the Mahalanobis distance to identify multivariate outliers in 16S rRNA community datasets, and one sample of MR treatment on Day 52 in the Lusaka site identified as outliers ($p < 0.01$) and discarded from subsequent analysis.

Declarations

Contributions

T.H., N.N., D.C., and Y.U. planned and designed the research. T.H., I.M., and N.N. conducted soil sampling and survey. T.H. and Y.U. performed soil microbial analysis. T.H. conducted analyzed the data, wrote the main manuscript, and prepared figures and tables. All authors reviewed the manuscript.

Acknowledgments

We thank Dr. Suzuki, Mr. Hamazakaza, Mr. Simkamba, and the staff of the International Institute of Tropical Agriculture Zambian office and Zambia Agricultural Research Institute for their kind technical support in the field experiment in Zambia. We also thank Ms. Kurazono for technical support. This work was financially supported by JSPS Grant-in-Aid for JSPS Fellows (18J10924) and Africa Society.

References

1. Aune, J. B. & Lal, R. Agricultural productivity in the tropics and critical limits of properties of Oxisols, Ultisols, Alfisols. *Tropical Agriculture (Trinidad and Tobago)***74**, 96–103 (1997).
2. Bauer, A. & Black, A. L. Quantification of the Effect of Soil Organic Matter Content on Soil Productivity. *Soil Science Society of America Journal***58**, 185–193 (1994).
3. Hamamoto, T., Chirwa, M., Nyambe, I. & Uchida, Y. Small-Scale Variability in the Soil Microbial Community Structure in a Semideveloped Farm in Zambia. *Applied and Environmental Soil Science* (2018).
4. Mapanda, F., Wuta, M., Nyamangara, J. & Rees, R. M. Effects of organic and mineral fertilizer nitrogen on greenhouse gas emissions and plant-captured carbon under maize cropping in Zimbabwe. *Plant Soil***343**, 67–81 (2011).
5. Lal, R. Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. *Science***304**, 1623–1627 (2004).
6. Swanepoel, C. M., van der Laan, M., Weepener, H. L., du Preez, C. C. & Annandale, J. G. Review and meta-analysis of organic matter in cultivated soils in southern Africa. *Nutr Cycl Agroecosyst***104**, 107–123 (2016).
7. Zingore, S., Manyame, C., Nyamugafata, P. & Giller, K. E. Long-term changes in organic matter of woodland soils cleared for arable cropping in Zimbabwe. *European Journal of Soil Science***56**, 727–736 (2005).
8. Sakala, W. D., Cadisch, G. & Giller, K. E. Interactions between residues of maize and pigeonpea and mineral N fertilizers during decomposition and N mineralization. *Soil Biology and Biochemistry***32**, 679–688 (2000).
9. Lal, R. & Stewart, B.A. (ed). Food security and soil quality. CRC Press, Taylor and Francis Group, New York (2010)
10. Aparna, K., Pasha, M. A., Rao, D. L. N. & Krishnaraj, P. U. Organic amendments as ecosystem engineers: Microbial, biochemical and genomic evidence of soil health improvement in a tropical arid zone field site. *Ecological Engineering***71**, 268–277 (2014).
11. Dhull, S., Goyal, S., Kapoor, K. & Mundra, M. Microbial biomass carbon and microbial activities of soils receiving chemical fertilizers and organic amendments. *Archives of Agronomy and Soil Science***50**, 641–647 (2004).

12. Zhong, W. *et al.* The effects of mineral fertilizer and organic manure on soil microbial community and diversity. *Plant Soil***326**, 511–522 (2010).
13. Janssen, B. H. Simple models and concepts as tools for the study of sustained soil productivity in long-term experiments. I. New soil organic matter and residual effect of P from fertilizers and farmyard manure in Kabete, Kenya. *Plant Soil***339**, 3–16 (2011).
14. Ge, G. *et al.* Soil biological activity and their seasonal variations in response to long-term application of organic and inorganic fertilizers. *Plant Soil***326**, 31 (2010).
15. Farina, R., Marchetti, A., Francaviglia, R., Napoli, R. & Bene, C. D. Modeling regional soil C stocks and CO2 emissions under Mediterranean cropping systems and soil types. *Agriculture, Ecosystems & Environment***238**, 128–141 (2017).
16. Muñoz-Rojas, M. *et al.* Impact of Land Use and Land Cover Changes on Organic Carbon Stocks in Mediterranean Soils (1956–2007). *Land Degradation & Development***26**, 168–179 (2015).
17. Gentile, R., Vanlauwe, B., Kavoo, A., Chivenge, P. & Six, J. Residue quality and N fertilizer do not influence aggregate stabilization of C and N in two tropical soils with contrasting texture. *Nutr Cycl Agroecosyst***88**, 121–131 (2010).
18. Amato, M. & Ladd, J. N. Decomposition of 14C-labelled glucose and legume material in soils: Properties influencing the accumulation of organic residue C and microbial biomass C. *Soil Biology and Biochemistry***24**, 455–464 (1992).
19. Spain, A. V. Influence of environmental conditions and some soil chemical properties on the carbon and nitrogen contents of some tropical Australian rainforest soils. *Soil Res.***28**, 825–839 (1990).
20. Schimel, D. S., Coleman, D. C. & Horton, K. A. Soil organic matter dynamics in paired rangeland and cropland toposequences in North Dakota. *Geoderma***36**, 201–214 (1985).
21. Schimel, D., Stillwell, M. A. & Woodmansee, R. G. Biogeochemistry of C, N, and P in a Soil Catena of the Shortgrass Steppe. *Ecology***66**, 276–282 (1985).
22. Giller, K. E. *et al.* Building Soil Nitrogen Capital in Africa. in *Replenishing Soil Fertility in Africa* 151–192 (John Wiley & Sons, Ltd, 1997).
23. Ray, R. L. *et al.* Soil CO_2 emission in response to organic amendments, temperature, and rainfall. *Scientific Reports***10**, 5849 (2020).
24. Sugihara, S., Funakawa, S., Kilasara, M. & Kosaki, T. Effects of land management on CO2 flux and soil C stock in two Tanzanian croplands with contrasting soil texture. *Soil Biology and Biochemistry***46**, 1–9 (2012).
25. Chatskikh, D. & Olesen, J. E. Soil tillage enhanced CO2 and N2O emissions from loamy sand soil under spring barley. *Soil and Tillage Research***97**, 5–18 (2007).
26. Martinsen, V., Shitumbanuma, V., Mulder, J., Ritz, C. & Cornelissen, G. Effects of hand-hoe tilled conservation farming on soil quality and carbon stocks under on-farm conditions in Zambia. *Agriculture, Ecosystems & Environment***241**, 168–178 (2017).

27. Mazzilli, S. R., Kemanian, A. R., Ernst, O. R., Jackson, R. B. & Piñeiro, G. Priming of soil organic carbon decomposition induced by corn compared to soybean crops. *Soil Biology and Biochemistry***75**, 273–281 (2014).
28. Lal, R. Carbon sequestration. *Philosophical Transactions of the Royal Society B: Biological Sciences***363**, 815–830 (2008).
29. Ouédraogo, E., Brussaard, L. & Stroosnijder, L. Soil fauna and organic amendment interactions affect soil carbon and crop performance in semi-arid West Africa. *Biol Fertil Soils***44**, 343–351 (2007).
30. Ouédraogo, E., Mando, A. & Brussaard, L. Soil macrofaunal-mediated organic resource disappearance in semi-arid West Africa. *Applied Soil Ecology***27**, 259–267 (2004).
31. Powlson, D. S., Hirsch, P. R. & Brookes, P. C. The role of soil microorganisms in soil organic matter conservation in the tropics. *Nutrient Cycling in Agroecosystems***61**, 41–51 (2001).
32. De la Cruz-Barrón, M. *et al.* The Bacterial Community Structure and Dynamics of Carbon and Nitrogen when Maize (Zea mays L.) and Its Neutral Detergent Fibre Were Added to Soil from Zimbabwe with Contrasting Management Practices. *Microb Ecol***73**, 135–152 (2017).
33. Wood, S. A. *et al.* Agricultural intensification and the functional capacity of soil microbes on smallholder African farms. *Journal of Applied Ecology***52**, 744–752 (2015).
34. Hamamoto, T., Uchida, Y., von Rein, I. & Mukumbuta, I. Effects of short-term freezing on nitrous oxide emissions and enzyme activities in a grazed pasture soil after bovine-urine application. *Science of The Total Environment***740**, 140006 (2020).
35. Thomsen, I. K., Schjønning, P., Jensen, B., Kristensen, K. & Christensen, B. T. Turnover of organic matter in differently textured soils: II. Microbial activity as influenced by soil water regimes. *Geoderma***89**, 199–218 (1999).
36. Rughöft, S. *et al.* Community Composition and Abundance of Bacterial, Archaeal and Nitrifying Populations in Savanna Soils on Contrasting Bedrock Material in Kruger National Park, South Africa. *Frontiers in Microbiology***7**, 1638 (2016).
37. Xue, L. *et al.* Long term effects of management practice intensification on soil microbial community structure and co-occurrence network in a non-timber plantation. *Forest Ecology and Management***459**, 117805 (2020).
38. Naether, A. *et al.* Environmental Factors Affect Acidobacterial Communities below the Subgroup Level in Grassland and Forest Soils. *Applied and Environmental Microbiology***78**, 7398–7406 (2012).
39. Fierer, N. *et al.* Reconstructing the Microbial Diversity and Function of Pre-Agricultural Tallgrass Prairie Soils in the United States. *Science***342**, 621–624 (2013).
40. Fierer, N., Allen, A. S., Schimel, J. P. & Holden, P. A. Controls on microbial CO2 production: a comparison of surface and subsurface soil horizons. *Global Change Biology***9**, 1322–1332 (2003).
41. Bergmann, G. T. *et al.* The under-recognized dominance of Verrucomicrobia in soil bacterial communities. *Soil Biology and Biochemistry***43**, 1450–1455 (2011).

42. Moreno-Espíndola, I. P. *et al.* The Bacterial Community Structure and Microbial Activity in a Traditional Organic Milpa Farming System Under Different Soil Moisture Conditions. *Frontiers in Microbiology***9**, 2737 (2018).
43. Elliott, E. T., Anderson, R. V., Coleman, D. C. & Cole, C. V. Habitable Pore Space and Microbial Trophic Interactions. *Oikos***35**, 327–335 (1980).
44. Bushby, H. V. A. & Marshall, K. C. Water Status of Rhizobia in Relation to their Susceptibility to Desiccation and to their Protection by Montmorillonite. *Microbiology,***99**, 19–27 (1977).
45. Bitton, G., Henis, Y. & Lahav, N. Influence of clay minerals, humic acid and bacterial capsular polysaccharide on the survival of Klebsiella aerogenes exposed to drying and heating in soils. *Plant Soil***45**, 65–74 (1976).
46. Bastida, F. *et al.* Soil microbial diversity–biomass relationships are driven by soil carbon content across global biomes. *The ISME Journal* 1–11 (2021).
47. Hernandez, D. J., David, A. S., Menges, E. S., Searcy, C. A. & Afkhami, M. E. Environmental stress destabilizes microbial networks. *The ISME Journal* 1–13 (2021).
48. Chivenge, P. P., Murwira, H. K., Giller, K. E., Mapfumo, P. & Six, J. Long-term impact of reduced tillage and residue management on soil carbon stabilization: Implications for conservation agriculture on contrasting soils. *Soil and Tillage Research***94**, 328–337 (2007).
49. Jones, A. et al. (eds.) Soil Atlas of Africa, European Commission. Publication Office of the European Union, Luxembourg (2013)
50. Hadas, A., Kautsky, L., Goek, M. & Erman Kara, E. Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. *Soil Biology and Biochemistry***36**, 255–266 (2004).
51. Sagova-Mareckova, M. *et al.* Innovative Methods for Soil DNA Purification Tested in Soils with Widely Differing Characteristics. *Appl. Environ. Microbiol.***74**, 2902–2907 (2008).
52. Miller, D. N., Bryant, J. E., Madsen, E. L. & Ghiorse, W. C. Evaluation and Optimization of DNA Extraction and Purification Procedures for Soil and Sediment Samples. *Appl. Environ. Microbiol.***65**, 4715–4724 (1999).
53. Carini, P. *et al.* Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nat Microbiol***2**, 1–6 (2016).
54. Bolyen, E. *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology***37**, 852–857 (2019).
55. Mickan, B. S. *et al.* Soil disturbance and water stress interact to influence arbuscular mycorrhizal fungi, rhizosphere bacteria and potential for N and C cycling in an agricultural soil. *Biol Fertil Soils***55**, 53–66 (2019).

Figures

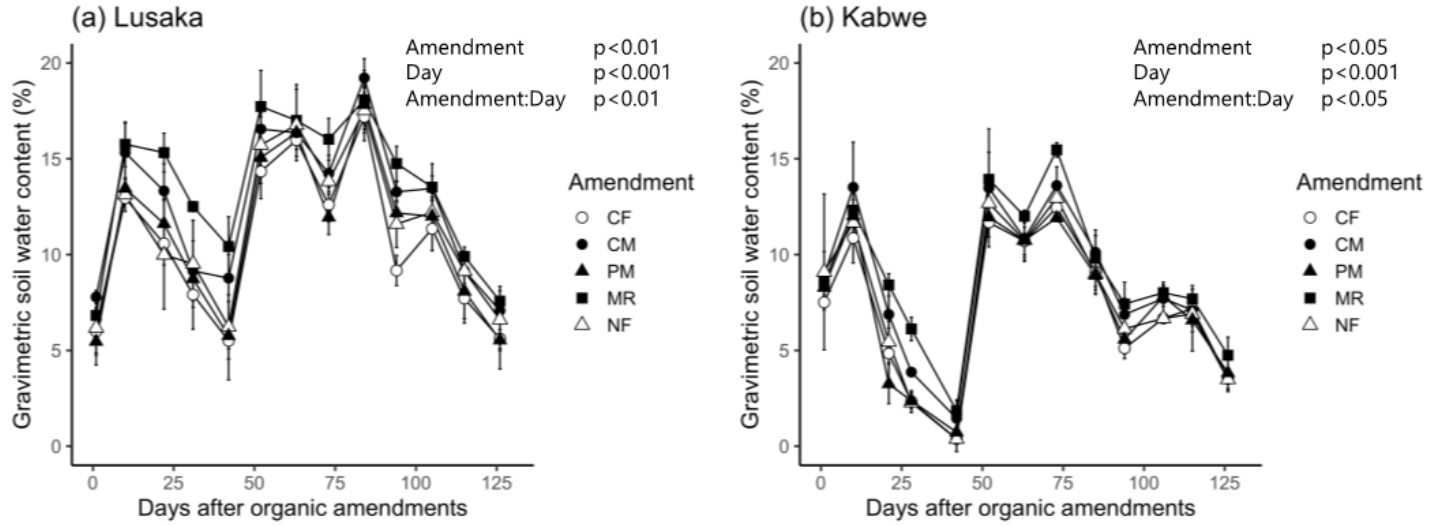

Figure 1

Temporal changes of soil gravimetric moisture at (a) Lusaka and (b) Kabwe sites. Levels of significance were based on mixed model results for repeated measurements. Error bars represent standard deviation (n = 3). CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

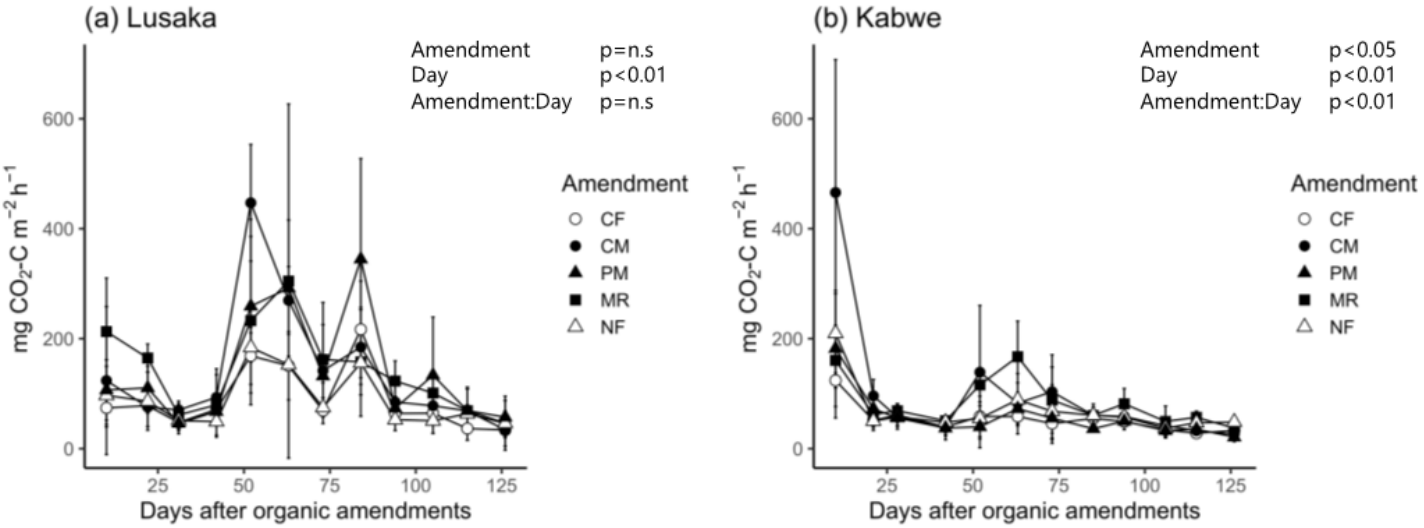

Figure 2

Temporal changes of CO2 emissions rate at (a) Lusaka and (b) Kabwe sites. Levels of significance were based on mixed model results for repeated measurements. Error bars represent standard deviation (n = 3). CF: chemical fertilizer treatment, CM: cattle manure treatment, PM: poultry manure treatment, MR: maize residue treatment, and NF: no fertilizer treatment.

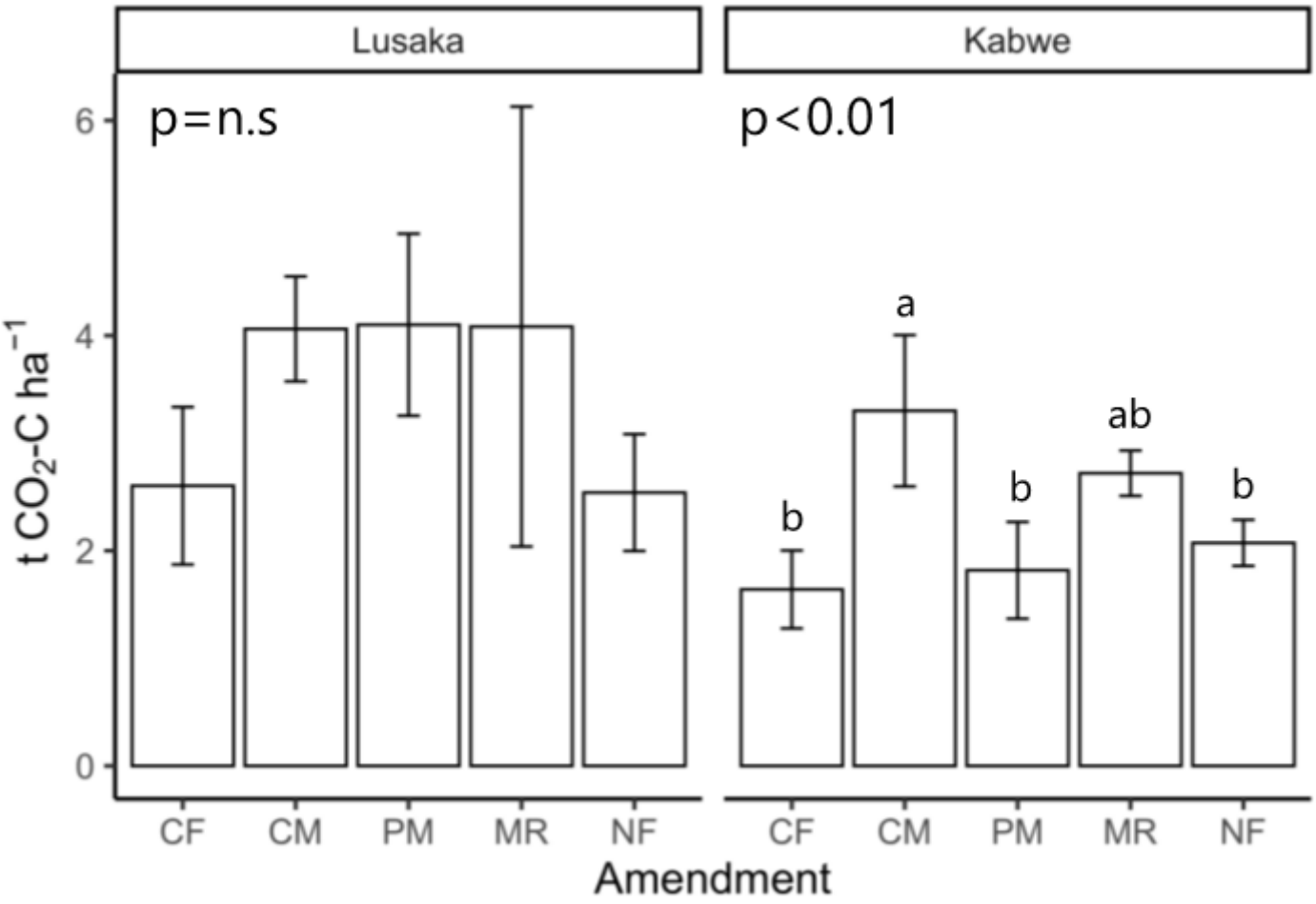

Figure 3

The cumulative CO2 emissions at (a) Lusaka and (b) Kabwe sites. The level of significance was determined by one-way ANOVA, followed by Tukey's test. Different letters on bars represent significant differences among treatments means within the same site. Error bars represent standard deviation (n = 3). CF: chemical fertilizer treatment, CM: cattle manure treatment, PM: poultry manure treatment, MR: maize residue treatment, and NF: no fertilizer treatment.

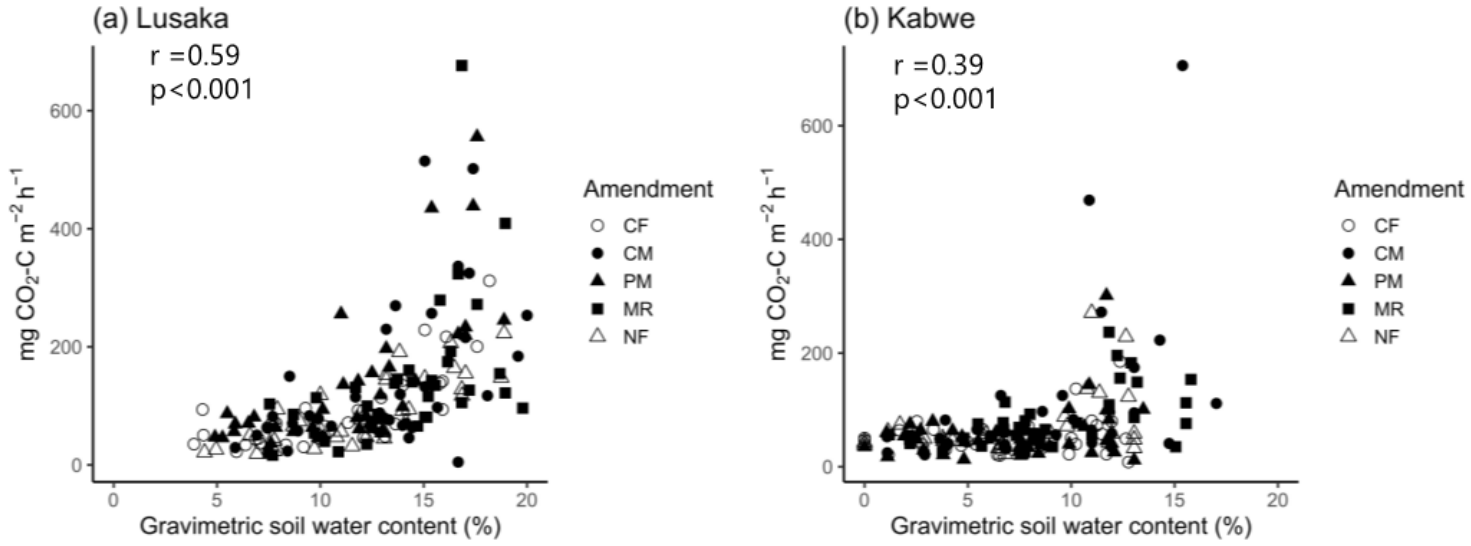

Figure 4

Relationships between CO2 emission rates and soil moisture at (a) Lusaka and (b) Kabwe sites. CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

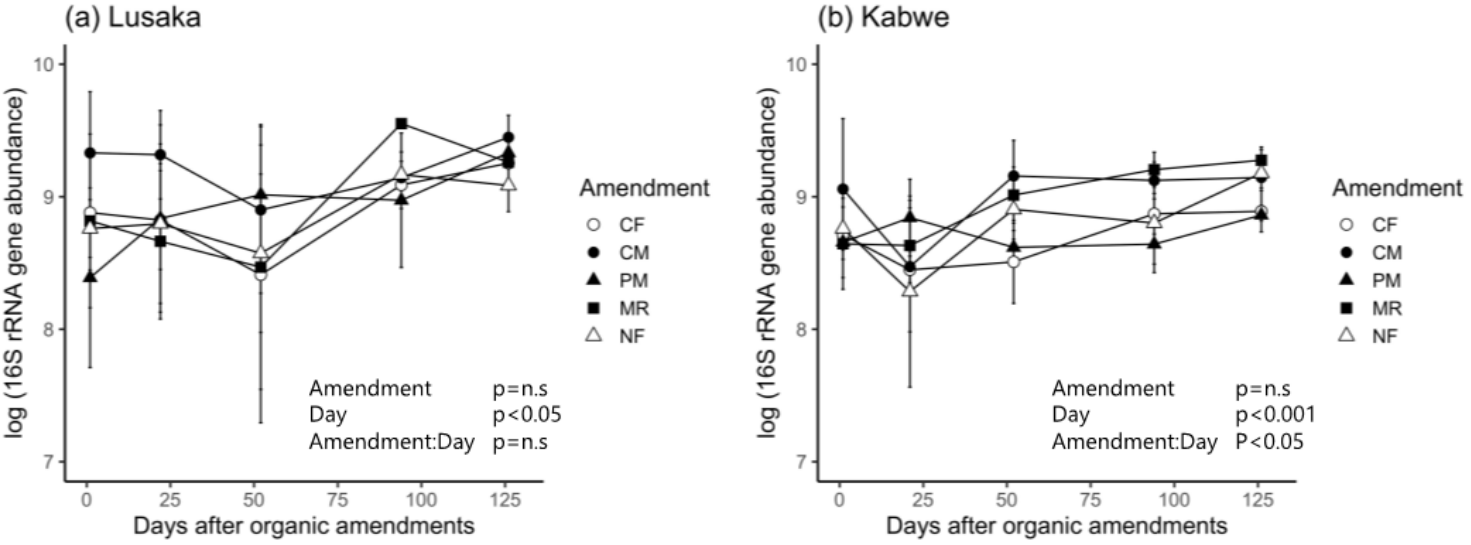

Figure 5

Temporal changes of bacterial and archaeal abundance at (a) Lusaka site and (b) Kabwe site. Levels of significance were based on mixed model results for repeated measurements. Error bars represent standard deviation (n = 3). CF: chemical fertilizer treatment, CM: cattle manure treatment, PM: poultry manure treatment, MR: maize residue treatment, and NF: no fertilizer treatment.

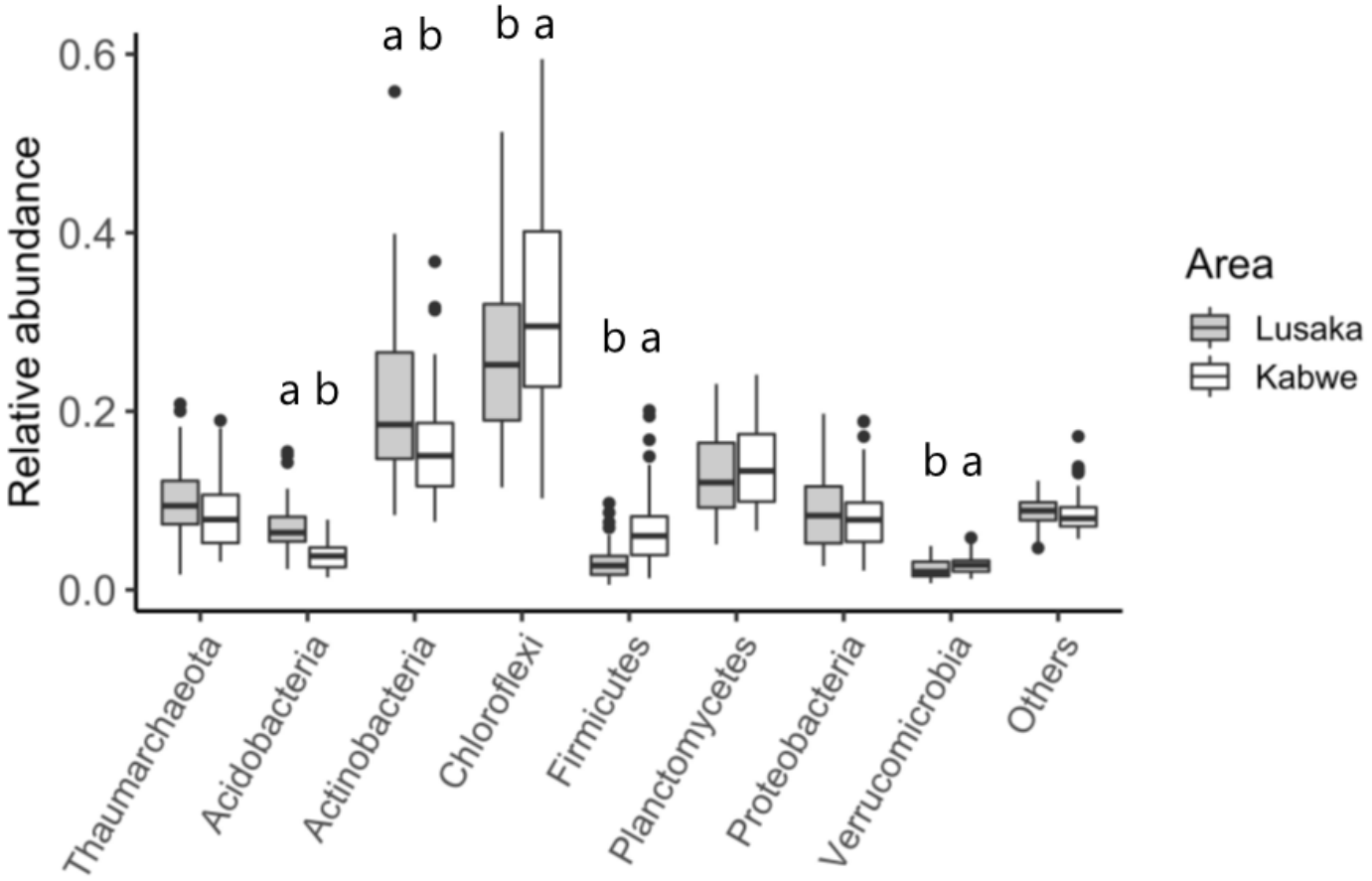

Figure 6

Relative abundances of bacterial and archaeal phyla in the Lusaka and Kabwe sites when averaged across the treatments. Phyla with an abundance of < 2% were grouped as “others.”. Different letters indicate significant differences ($P < 0.05$) between Lusaka and Kabwe sites.

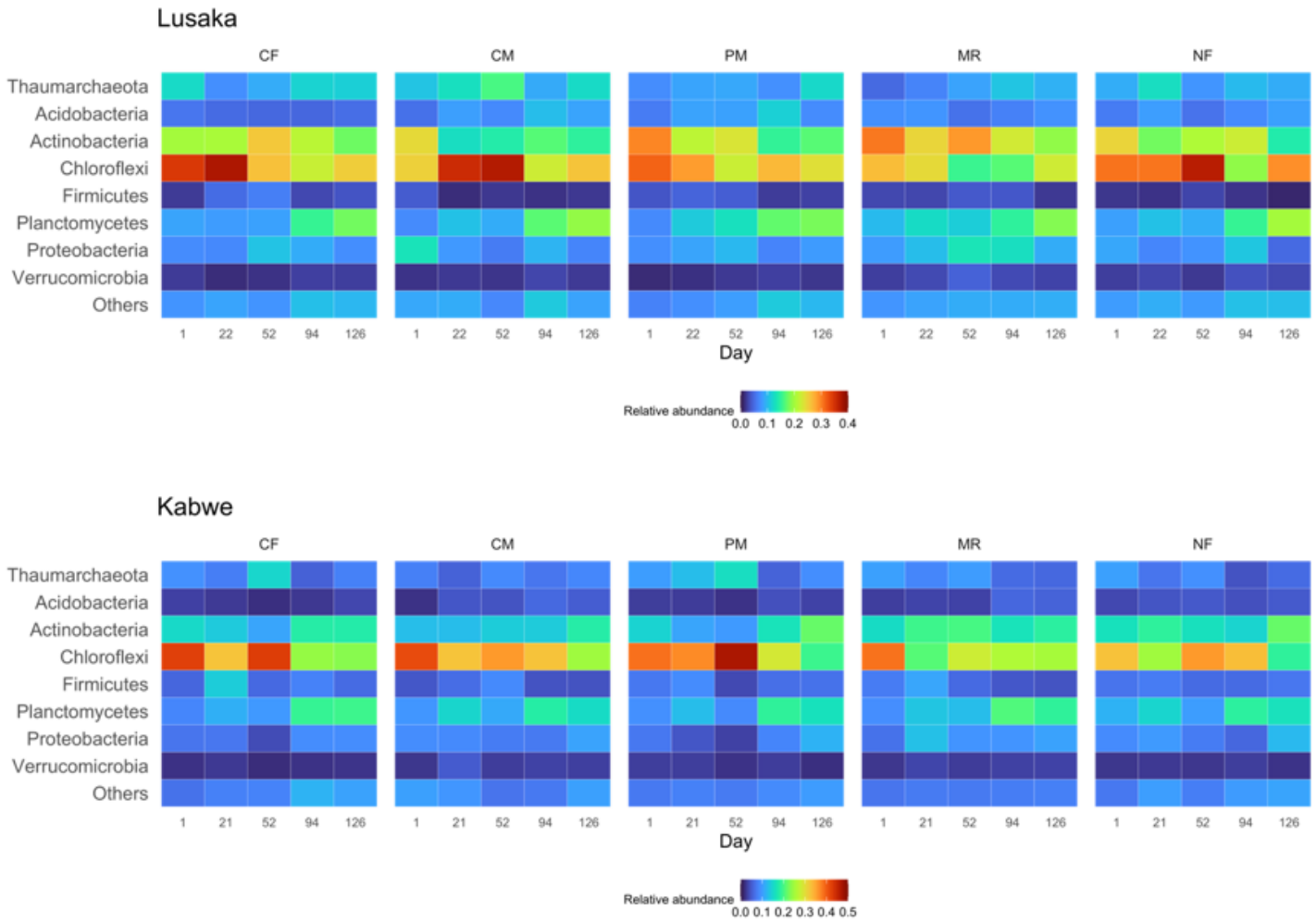

Figure 7

Distribution of the abundances of bacterial and archaeal phyla in Lusaka and Kabwe sites under different fertilizer treatments and sampling dates visualized by heatmaps. CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment. The color intensity of the scale indicates the relative abundance of each phylum. Phyla with an abundance of <2% were grouped as “others.”

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuppleFiguresIITA.docx
- SuppleTablesIITA.xlsx