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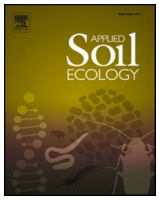

The effect of environmental parameters and fertilization practices on yield and soil microbial diversity in a Kenyan paddy rice field

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ABSTRACT

Rice is gaining importance for nutrition in sub-Saharan Africa, but domestic production can only cover a fraction of the actual needs. Suboptimal fertilization limits production and affordable solutions are needed. It is, however, of utmost importance to minimize negative impacts on the environment and on soil health, which is largely determined by microbial processes.

An agronomic field trial was conducted at the KALRO Mwea research site (Central Kenya) to compare mineral and organic fertilization effects on rice plant parameters and on soil microbial abundance and diversity. Abundance of fungi and bacteria was quantified by ddPCR and the community composition was determined by amplicon sequencing of the ITS2- and 16S-regions, respectively. Mineral fertilizer had a strong positive effect on panicle number, spikelet number, grain yield and straw dry weight, but fertilizer type did not significantly influence soil microbial community abundance or composition. The rice development stage shaped fungal communities with differences between the vegetative and the reproductive stages, whereas the bacterial communities were mainly influenced by soil depth in a range from 0 to 30 cm. Additionally, spatial effects between rows of the experimental field were observed, resulting in row-specific differences in soil organic carbon, total nitrogen and certain fungal taxa, notwithstanding that the field was manually ploughed to a depth of 30 cm before the experiment. This study is the first census of soil fungal and bacterial communities in an African paddy rice field and provides insights into similarities and differences to paddy rice fields in other regions. To increase local African rice production, manure application alone might not be sufficient due to the poor nutrient status of traditional farm-yard manure, yet it can be part of a sustainable and more efficient fertilization strategy.

1. Introduction

Rice is globally one of the most important staple foods and is essential for nutritional security and livelihoods of billions of people worldwide (FAO, 2020). In Africa, rice is the third-largest source of food energy and its demand is rising due to population growth and changes in eating habits (Wopereis et al., 2013). Kenya as an example for sub-Saharan Africa (SSA) can, however, currently only cover ca. 25% of its rice demand through domestic production, while the rest has to be imported (Short et al., 2012). One approach to boost rice production is coordinated irrigation schemes organized by the National Irrigation Board (NIB), such as the Mwea irrigation scheme (MIS) in the highlands of Central Kenya (Mati et al., 2011). At the same time, the price for mineral fertilizer in SSA is high because there is limited fertilizer production in SSA (Balasubramanian et al., 2007) and consequently, fertilization rates are low and limit rice yields (Mueller et al., 2012). In

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addition, chronically low fertilization leads to soil nutrient mining and cropland degradation in the long term, which is especially critical on highly weathered and already nutrient-poor tropical soils (Vitousek et al., 2009). Therefore, sustainable intensification strategies such as using animal manure as a cheap locally-available organic fertilizer have been recommended to ensure high yields, maintain soil health, and make farmers independent from mineral fertilizer imports (Balasubramanian et al., 2007).

Sustainable agriculture fosters efficient soil nutrient cycling by harnessing the abundance and diversity of soil microorganisms. In this regard, management approaches aimed at increasing soil organic C (SOC) and 'feeding' soil microbial communities – such as the addition of animal manure and organic residue – have potential to increase rice yields (Stoop et al., 2002; Bharali et al., 2018; Chen et al., 2018). In fact, soil microbes, being crucial for the mineralization of soil organic matter, can considerably contribute to the nutrient supply of crops, as demonstrated for nitrogen (N) when applied as organically bound N as found in manure (Hua et al., 2020). However, when paddy rice cultivation is considered, the microbiological aspects of flooded soil deserve special attention. In fact, it is well demonstrated that anoxic soil conditions promote microbially mediated release of greenhouse gases like nitrous oxide (N_2O) (Ishii et al., 2011) and methane (CH_4) (Liesack et al., 2000) while emissions of carbon dioxide (CO_2) are reduced (Liu et al., 2013). The majority of the information on soil microbial communities in rice fields comes from experiments carried out in Asia (e.g. Asakawa and Kimura, 2008; Ahn et al., 2012; Xuan et al., 2012; Ahn et al., 2016; Wang et al., 2016; Li et al., 2018; Wang et al., 2018), the Americas (Edwards et al., 2015; Edwards et al., 2018; Maguire et al., 2020; Fernández-Baca et al., 2021) and Europe (Vaksmaa et al., 2017). Few data are available for African rice fields (Pili et al., 2016; Ezeokoli et al., 2021), notwithstanding its crucial relevance for feeding the local population. In addition, regardless of the geographic area, most studies in rice fields have been focused on bacterial communities rather than on the fungal ones (see references above).

For fungal communities in rice fields, a strong dominance of Ascomycota has been observed (Li et al., 2018; Wang et al., 2018; Maguire et al., 2020; Tang et al., 2020). Plant growth stage was found as an important factor driving changes in fungal community composition, while differences in fertilization regimes had a smaller effect (Wang et al., 2018). Bacterial communities in rice fields are generally dominated by Proteobacteria, Acidobacteria and Chloroflexi (e.g. Ahn et al., 2016; Ezeokoli et al., 2021; Fernández-Baca et al., 2021). Plant genotype, developmental stage and especially soil type can have pronounced effects on bacterial communities (e.g. Edwards et al., 2015; Ahn et al., 2016; Wang et al., 2016; Fernández-Baca et al., 2021).

To boost rice production in Kenya and other African countries, several projects have been launched that consider water management (irrigation schemes; Mati et al., 2011), nutrient supply (fertilization regimes; Njinju et al., 2018) and the use of appropriate crop varieties (breeding programs; Nassirou, 2011). However, the implementation of the crop management measures described above is very likely to impact soilborne biodiversity significantly, thereby affecting the rates of microbial processes such as mineralization, nitrification, denitrification, or methanogenesis. Organic farming practices, however variable, generally include the application of plant residues and manure instead of mineral fertilizers; a meta-study provided evidence that organic farming practices generally increase microbial biomass and microbial activity, irrespective of climatic region, land-use, or crop (Lori et al., 2017). Hartmann et al. (2015) described the impact of organic and conventional farming systems on soil fungal and bacterial community composition. The results showed that farmyard manure application is the strongest driver of the changes induced in composition and diversity of microbial communities. In particular, an increase in coprophilic fungi and the bacterial phylum of Firmicutes with known capacities to degrade complex organic matter have been observed as a consequence of manure application. Similarly, manure addition to rice fields was shown to have distinct effects on the composition of soil microbial communities and soil properties, with SOC and total N being the main factors influencing microbial communities (Liu et al., 2020; Tang et al., 2020). Among others, the relative abundance of members of the nitrite-oxidizing Nitrospirae decreased with the addition of manure, while ammonia-oxidizing Nitrosomonadaceae increased upon high manure loads (Liu et al., 2020). Moreover, the supply of plant-derived carbohydrate polymers to agricultural soil was demonstrated to promote nitrate (NO_3^-) assimilation and, consequently, NO_3^- immobilization through induction of fungal NO_3^- reductases (Gorfer et al., 2011). Although this evidence undoubtedly contributes to a better understanding of the relationship between the addition of organic residues including manure and microbial diversity and functionality in cropland soils, the extent to which these findings apply to paddy rice cultivation in tropical Africa remains to be verified.

We aimed to determine whether locally produced farm-yard manure can replace mineral fertilizer without negatively affecting grain yield and soil microbial communities in paddy rice fields in SSA. Therefore, in the present study, we studied how different fertilizer types (mineral and organic) influence rice yield and microbial communities in a continuously flooded Kenyan paddy rice field. Abundance and composition of fungal and bacterial communities were investigated at the vegetative and the reproductive stages of rice plants and at three different soil depths down to 30 cm. We hypothesized that (i) the application of livestock manure would result in similar rice yields as the application of mineral fertilizer at the same N dose, and (ii) fertilization type would have direct and indirect effects on fungal and bacterial communities in the soil, depending on rice growth stage and soil depth.

2. Materials and methods

2.1. Site description and sampling

The agronomic field trial was conducted at the Kenya Agricultural and Livestock Research Organization (KALRO) station in Mwea, Kirinyaga County, Kenya (0°39′01.6"S 37°22′49.9″E, 1162 m above sea level) from August to December 2018. The station is part of the Mwea irrigation scheme (MIS) established by the Government of Kenya in 1954 in the form of a participatory irrigation scheme, which comprises ca. 10,000 ha of paddy rice fields (Muhunyu, 2012).

Air temperature, relative humidity and rainfall were continuously measured with a weather station (WatchDog 2700; Spectrum Technologies, Illinois) on the research farm (Supplementary Fig. 1). The average temperature and humidity from August to December 2018 were 22.3 °C (range 12.4–36.3 °C) and 65.5% RH (range 47.5–86.9% RH), respectively. In the same period, total rainfall was 129.9 mm, with 94.9 mm received between mid-October to mid-November (Supplementary Fig. 1).

On 23rd August 2018, eight plots (2 m × 5 m) were set up spaced 0.5 m apart along both axes in a paddy rice field on a verto-eutric nitisol that had been manually ploughed once to a depth of 30 cm (standard wet tillage and harrowing; Muhunyu, 2012) on 14th August 2018. Details on the exact field layout are shown in Fig. 1. The plots were positioned in four rows with two paired plots per row. Each plot was lined with polyvinyl chloride (PVC) corrugated sheets (inserted 1.5 m deep into the soil, raised 0.4 m above the topsoil surface) and lined with 250-gauge heavy-duty polyethylene sheeting to minimize water flow between treatments. All plots were planted with pre-germinated *Oryza sativa* var. Basmati 370, a pure line aromatic rice variety that was developed from a locally adapted land race in 1933 at the Kala Shah Kaku Rice Research Institute in Punjab (Dey et al., 2019). Pre-germinated rice plantlets were manually transplanted (two plants per hill) spaced 0.3 m × 0.15 m on 27th August 2018. The experiment included two fertilization treatments receiving 75 kg N ha^{-1} each ($n = 4$ for each treatment), either in the form of farm-yard manure (FYM, 8.2 Mg FW ha^{-1}) in a single application of 75 kg N ha^{-1}, or as mineral fertilizer (NPK) in three applications of 25

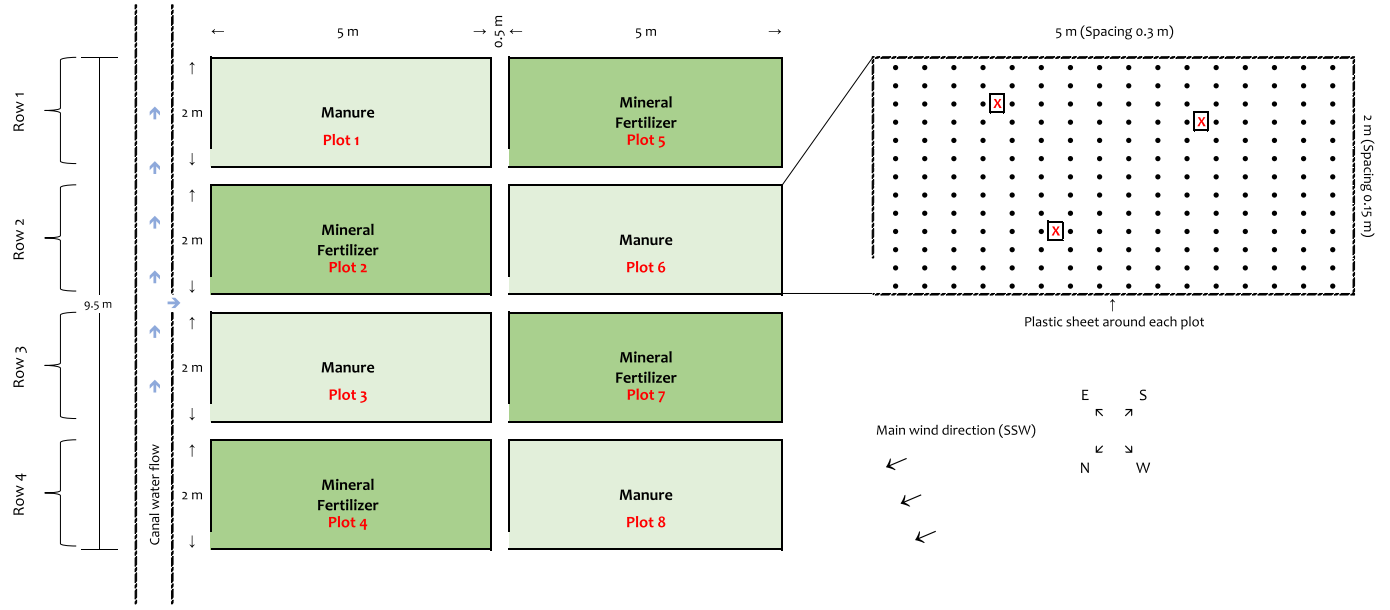

Fig. 1. Field layout at the KALRO – Kenyan Agriculture and Livestock Research Institute – Mwea experimental site. The site is located at 0.650432° S, 37.380523° E at 1162 m above sea level in Kirinyaga County, Central Kenya. Row numbers, plot numbers and fertilizer treatments are indicated. Water supply was facilitated via a canal left from the plots and the plots were surrounded by corrugated plastic panels. Water tables could be regulated through a gate at the lower left corner of each plot. Dots in the single plot indicate positions of individual rice plants on hills (208 hills; 0.3 × 0.15 m) and the "x" demarks approximate positions for DNA sampling. For each depth and plant growth stage, the three replicates per plot were pooled to give a composite sample for soil analyses and DNA isolation.

kg N ha^{-1} each. Inclusion of a combined treatment with FYM and mineral fertilizer was not possible due to space limitations at the experimental field (ca. 9.5 × 10.5 m). On the day of transplanting, the FYM plots were fertilized with cattle manure sourced from a local farm (15.1% C and 1.34% N on a DW basis, pH 8.0) that had been left to decompose in an uncovered heap for one month before application. The NPK treatment received 25 kg N ha^{-1} as basal in the form of an NPK (17:17:17) compound fertilizer on 29th August 2018, two days after transplanting. In addition, 25 kg N ha^{-1} in the form of ammonium sulphate were applied to the NPK treatment as topdressing 21 and 42 days after transplanting. This so-called "75 N-3splits" NPK fertilization regime is standard practice among farmers in the study area (Njinju et al., 2018). The plots were flooded to approximately 0.1 m above the surface three days before transplanting and were kept flooded until one week before harvest for a total of 111 days. Soil samples were collected at two sampling time points, i.e. on 15th October 2018 (vegetative plant stage) and 27th November 2018 (reproductive plant stage), using a soil corer (3 cm inner diameter) to a depth of 30 cm. Three samples per plot were taken within plant row, ca. 7 cm away from the rice plants. Soil cores were cut horizontally into three sub-samples at 0–10 cm, 10–20 cm and 20–30 cm depth. Samples were pooled per plot and depth, immediately sieved in the field (<2 mm) and transported to the laboratory on ice. There the samples were kept in the fridge (+4 °C) overnight as recently suggested by Clasen et al. (2020), and processed the next morning, i.e. within 24 h after sampling, for all further analyses (see below).

Rice was harvested on 18th December 2018 at plant maturity. Shoots were harvested at ground level from 24 hills per plot and sun-dried in paper bags for one month. Determination of grain yield and yield components followed previously described standard procedures (Njinju et al., 2018; Samejima et al., 2021). In brief, panicles were removed from stems and hand threshed. Filled grains were separated from unfilled spikelets by submerging them in tap water. Sunken and floating spikelets were considered filled grain and unfilled spikelets, respectively. Using the panicles and spikelets, grain yield (filled grain weight converted to 14% moisture content) and yield components were determined. The moisture content of the filled grains was measured using a grain moisture tester (Riceter f; Kett Electric Laboratory, Tokyo, Japan). Straw dry matter was determined after oven drying at 70 °C for 24 h.

2.2. Laboratory analyses and soil DNA isolation

Soil moisture was measured gravimetrically after drying at 105 °C until constant weight was reached. For SOC and total N (N_{tot}) determination, soil samples were dried at 50 °C until constant weight, and elemental concentrations were measured via total combustion on an elemental analyser (Vario MAX Cube, Elementar, Langenselbold, Germany). Soil exchangeable ammonium (NH_4^+) and NO_3^- concentrations were measured in KCl extracts [1 mol L^{-1}] using a colorimetric assay as described by Hood-Nowotny et al. (2010). Data from all 48 samples (2 treatments × 4 replicates × 3 soil depths × 2 sampling time points) are shown in the Supplementary Spreadsheet.

DNA was extracted from 250 mg of field-moist soil using the DNeasy® PowerSoil® Pro Kit (Qiagen, The Netherlands) according to the manufacturer's instructions. Each sample was extracted twice, and the extracts were pooled (Gorfer et al., 2021). After extraction, samples were frozen (−20 °C) and shipped to Austria for DNA amplification and sequencing.

2.3. Quantification of total bacteria and fungi via droplet-digital PCR (ddPCR)

For absolute quantification of bacteria and fungi in all 48 samples, droplet-digital PCR (ddPCR) was performed, which enables very precise and highly sensitive quantification of nucleic acids. ddPCR was conducted on a QX200™ droplet digital PCR system (Bio-Rad, Munich, Germany) with the ddPCR™ EvaGreen® Supermix. Assays targeted the bacterial 16S and the fungal 18S rRNA genes. The reaction was performed in 22 µL volumes containing 12.5 µL of EvaGreen® premix, 0.44 µL of each primer (10 µM), 0.44 µL bovine serum albumin (BSA) (2%) and 2 µL of DNA template. Specificity of primers, PCR conditions, primer concentrations and template dilutions were tested in trial runs. Positive, negative, and non-template (sterile water) controls were measured in each analysis as well. The entire reaction mixture was loaded onto 96-

well plates and transferred to the QX200™ droplet generator (Bio-Rad). The droplet emulsions (40 μL) were transferred to a new 96-well PCR plate and amplified in a T100™ Thermal Cycler (Bio-Rad). Total bacteria were quantified by using the primer pair 338f/518r (Ghyselinck et al., 2013; Orschler et al., 2019) and a profile of 5 min at 95 °C, 40 cycles of 30 s at 95 °C, 30 s at 58 °C and 1 min at 72 °C, followed by a hold of 5 min at 4 °C and 5 min at 90 °C for final droplet stabilization and enzyme deactivation as described in Praeg et al. (2020). Total fungi were quantified with the primer set FungiQuant-F/FungiQuant-R (Liu et al., 2012; Unterwurzacher et al., 2018) with a temperature profile of 5 min at 95 °C, 40 cycles of 30 s at 95 °C, 1 min at (52.5 °C) and 2 min at 72 °C, followed by a hold of 5 min at 4 °C and 5 min at 90 °C. The droplets were analyzed in a QX200 droplet reader (Bio-Rad, Munich, Germany) using the QuantaSoft™ Analysis Pro software (Bio-Rad, Munich, Germany). Copy number data from single samples are included in the Supplementary Spreadsheet.

2.4. Microbial community analysis

Microbial community composition was determined for all 48 samples. For characterization of fungal communities, a pre-amplification with the primer pair ITS1F/TW13 was done, followed by amplification of the fungal ITS2-region with primer pair ITS3Mix/ITS4Mix, originally described by Tedersoo et al. (2014, 2015) and modified by Keiblinger et al. (2018). The primer pair was proposed by Tedersoo et al. (2015) to provide good coverage of the fungal kingdom and good taxonomic resolution for amplicon high-throughput sequencing. For bacterial communities, the bacterial 16S V3-V4 region was amplified using primer pair Illumina_16S_341F/Illumina_16S_805R, according to Klindworth et al. (2012). Library preparation for Illumina MiSeq Sequencing was done as described in Deltedesco et al. (2020). All reactions were carried out in quadruplicate and pooled after amplification. For sequencing, samples were sent to the sequencing core facility at the Vienna Biocenter (VBCF-NGS, Vienna, Austria).

Fungal and bacterial raw data generated from the sequencer were checked using FastQC (Andrews, 2010). Next, the data were processed using DADA2 within QIIME2, applying the following steps: preprocessing, quality filtering, and trimming (Callahan et al., 2016; Bolyen et al., 2019). The 'consensus' method was used to remove the chimeras (Callahan et al., 2016). The quality-filtered dataset contained 2,869,218 fungal and 1,057,756 bacterial reads, which were clustered into operational taxonomic units (OTUs) with VSEARCH applying a cut-off of 97% (Rognes et al., 2016). Reads clustered into 1284 fungal OTUs (FOTU) and 3624 bacterial OTUs (BOTU). From both datasets, OTUs that appeared in only one sample and non-specific OTUs (not fungal ITS2 and not bacterial 16S) were removed before further analyses resulting in a final dataset with 649 FOTUs and 2070 BOTUs. Additionally, samples with <1000 quality-filtered reads were removed from the bacterial dataset. Rarefaction curves from all samples kept for further analyses reached a plateau. OTU tables for fungi and for bacteria were normalized according to McMurdie and Holmes (2014). Taxonomy assignment was performed employing Naïve-Bayes classifier in QIIME2 trained on SILVA (ver. 138.1) (Quast et al., 2013) for bacteria and UNITE+INSD (ver. 04.02.2020) (Nilsson et al., 2019; Abarenkov et al., 2020) for fungi. Fungal taxonomy underwent further manual curation to increase phylogenetic accuracy (Deltedesco et al., 2020; Gorfer et al., 2021) to overcome shortcomings of reference databases (Xue et al., 2019). Lifestyle assessment of FOTUs was deduced from taxonomic affiliation (Deltedesco et al., 2020; Gorfer et al., 2021; see Supplementary Spreadsheet). Given the set sequence similarity threshold, species names provided for OTUs must be considered sensu *lato*.

Sequencing and associated data have been deposited at NCBI BioProject PRJNA678482, BioSamples SAMN16803089- SAMN16803136 and GenBank accession numbers MW260635-MW261283 (FOTUs) and KEPX01000001-KEPX01002070 (BOTUs) (see Supplementary Spreadsheet).

2.5. Statistical analyses

For comparison of the fertilizer treatment effect on plant properties, a paired *t*-test with false discovery rate (FDR) correction (Benjamini et al., 2006) was conducted. Significant effects of row and depth on soil parameters were validated using two-way ANOVA by applying a significance level of 0.05. Relationships between soil properties and microbial abundances were evaluated using Pearson correlation analysis.

To infer patterns in bacterial and fungal community composition in relation to the experimental set-up (type of fertilizer, tilling stage, soil depth and row), canonical correspondence analysis (CCA) based on Bray-Curtis dissimilarity distance was performed. The forward selection was applied to select the explanatory environmental variables that best fit the model (Monte Carlo permutation test with 9999 randomisations, $p < 0.05$). Finally, the CCA was tested with a Monte Carlo permutation test (999 permutations) to assess the significance of each environmental variable and the ordination axes. Linear discriminant analysis effect size (LEfSe) algorithm (LDA score ≥ 2 and p-value <0.05) was used to detect the biomarker taxa for each condition considered (Segata et al., 2011). Statistical analyses were conducted with the statistical multi-packages of the R software (R version 3.4.0) (R Core Team, 2017).

3. Results

3.1. Soil chemistry and plant parameters

Plants from the mineral fertilizer treatment had significantly higher numbers of panicles (+57%; $p < 0.001$) and spikelets (+78%; $p < 0.001$), as well as higher grain yield (+69%; $p < 0.01$) and straw dry weight (+95%; $p < 0.01$) compared with the manure treatment (Fig. 2). Rice grain yield was 3.25 t ha^{-1} in the NPK treatment and 1.92 t ha^{-1} in the manure treatment. Fertilizer treatments did not significantly influence filled grain ratio (0.76 ± 0.06; $p = 0.228$) and thousand-seed weight (22.2 ± 0.9 g; $p = 0.222$). No visible roots were present in the deepest soil layer (20–30 cm) collected between neighbouring plants.

In the permanently waterlogged soils, pH was 6.6 ± 0.1, with no significant difference between soil depths or plots of the experimental field. SOC (1.2–1.7%) and N_{tot} (0.08–0.13%) were highly correlated with each other (Pearson; $r^2 = 0.835$; $p < 0.001$). SOC and N_{tot} decreased significantly with sampling depth and showed an unintended spatial effect. Significant differences were observed between rows of plots at the KALRO Mwea experimental site (see Fig. 1). Row 1, which contained one manure and one NPK plot, had significantly higher SOC and N_{tot} contents than the remaining rows, i.e. row 2–4 (see Fig. 3). Effects of depth and row were both significant for SOC and N_{tot} in a two-way ANOVA ($p < 0.001$; see Supplementary Table 1), whereas the interaction was only significant for SOC ($p < 0.05$). Differences between rows were more pronounced for SOC than for N_{tot}. No significant differences in SOC and N_{tot} were found for fertilization treatments or plant stage. Soil C:N ratios, on the other hand, increased by 0.6 units from the vegetative to the reproductive plant stage (from 13.5 ± 0.5 to 14.1 ± 0.37; $p < 0.001$), but did not change significantly with soil depth. Exchangeable NH_4^+-N contents were generally below 4.0 μg g^{-1} dry weight (DW) at the vegetative stage, especially in the deepest soil layer (20–30 cm), where values dropped below 0.8 μg g^{-1} DW. At the reproductive plant stage, exchangeable NH_4^+-N levels increased to 12.3 ± 1.2 μg g^{-1}. No significant differences were observed for exchangeable $NH4^+$-N concentrations at the reproductive stage between different soil depths. NO_3^--N levels were generally low and only reached levels above 0.25 μg g^{-1} at the vegetative plant stage in the deepest soil layer. Statistically significant positive correlations were found between SOC, N_{tot} and exchangeable NH_4^+-N at the vegetative plant stage, whereas at the reproductive plant stage only SOC and N_{tot} were positively correlated with each other. Exchangeable $NH4^+$-N was negatively correlated with SOC and N_{tot} at the reproductive plant stage. Details including p-values and R^2 values are provided in Supplementary Fig. 2.

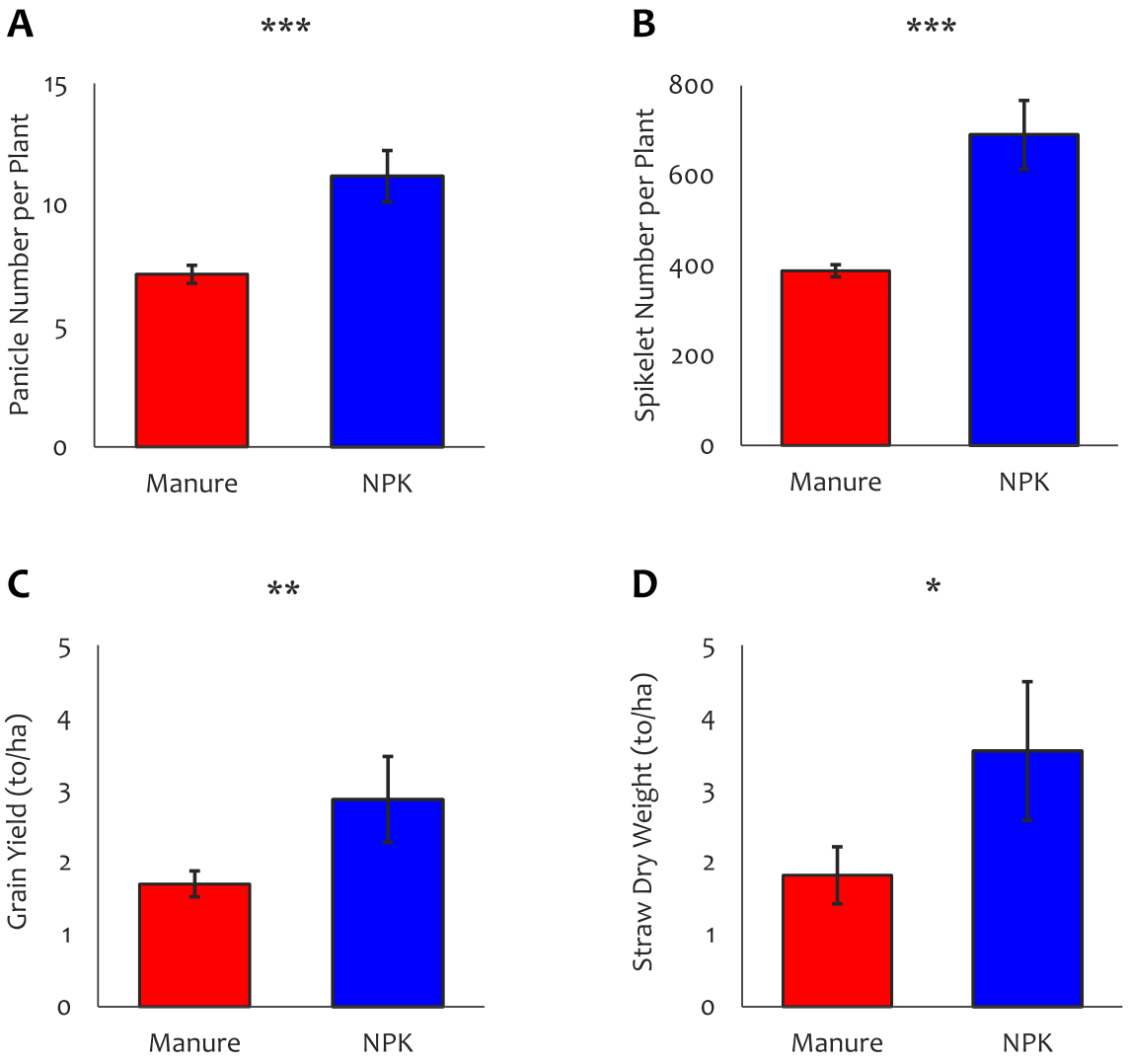

Fig. 2. Panicle number (A), spikelet number (B), grain yield (C) and straw dry weight (D) from a paddy rice field in Central Kenya in 2018. All graphs show means ± SD from four replicate plots for each treatment (manure vs. NPK fertilization), where data from six single plants were collected for each plot. Significance levels of pairwise *t*-tests are indicated (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

3.2. Soil microbial abundance, richness and community composition

Gene abundances of bacteria and fungi were strongly correlated to each other (Pearson; $r^2 = 0.272$; $p = 0.0001$). At the vegetative plant stage, bacterial and fungal abundances decreased with increasing soil depth (Pearson; $r^2 > 0.65$; $p = 0.0001$), whereas no decrease in abundances with increasing soil depth was observed at the reproductive plant stage (Fig. 4). Consequently, positive correlations of fungal and bacterial abundances were established with SOC, N_{tot} and NH_4^+-N at the vegetative stage, but not at the reproductive stage (Supplementary Fig. 2).

The Shannon Diversity Index was 3.66 ± 0.35 for fungi and 5.45 ± 0.62 for bacteria; it did not change significantly with fertilizer treatment, soil depth or plant developmental stage (see Supplementary Spreadsheet).

The fungal communities in all samples from our rice fields were dominated by Ascomycota (73.7–92.6%), followed by Basidiomycota (2.9–24.4%). All other phyla were only rarely detected (<1%). The most abundant orders were Pleosporales (14.9–43.0%), Sordariales (2.8–38.4%), Hypocreales (8.1–28.5%), Eurotiales (3.4–18.3%) and Capnodiales (0–33.1%) of Ascomycota, and Tremellales (1.0–18.9%) of Basidiomycota (Supplementary Fig. 3). This pattern was mainly brought forth by a few dominant FOTUs: *Westerdykella purpurea* FOTU0003 (Pleosporales, 1.0–13-0%), Lasiosphaeriaceae FOTU0005 and FOTU0006 (Sordariales, together 1.4–26.5%), *Emericellopsis* cf. *persica* FOTU0002 (Hypocreales, 0.8–18.9%), *Talaromyces veerkampii* FOTU0007 (Eurotiales, 0–8.3%), *Cladosporium cladosporioides* FOTU0004 (Capnodiales, 0.0–33.1%) and *Papiliotrema* sp. FOTU0001 (Tremellales, 0.7–14.6%). *Emericellopsis* cf. *persica* was with a mean of 9.7% the most abundant FOTU at the site (for details see Supplementary Spreadsheet). The largest proportion of fungal reads was classified as saprotrophic (76.3–84.9%) and among those potentially coprophilous fungi – *Westerdykella* spp. from Sporormiaceae (Zhang et al., 2012) and members of the Lasiosphaeriaceae (Mueller et al., 2004) – were specifically dominant representatives (4.7–37.5%). Furthermore, a substantial fraction could be classified as potentially plant pathogenic (6.3–23.7%), which was mainly attributed to the relative abundance of *Fusarium* spp. and other genera from the Nectriaceae. From fungi that have been associated with the rice sheath rot disease complex (Bigirimana et al., 2015) only two species were detected in our study, notwithstanding the fact that this destructive disease is frequently observed at the research site: *Sarocladium oryzae* and *Rhizoctonia oryzae-sativae*. *Sarocladium oryzae* was found in only two samples with relative abundances of 0.012% and 1.853%; *Rhizoctonia oryzae-sativae* was found in four samples with relative abundances <0.2%. Symbiotic fungi from the arbuscular mycorrhizae-forming Glomeromycota were only rarely detected (0.0–4.1%).

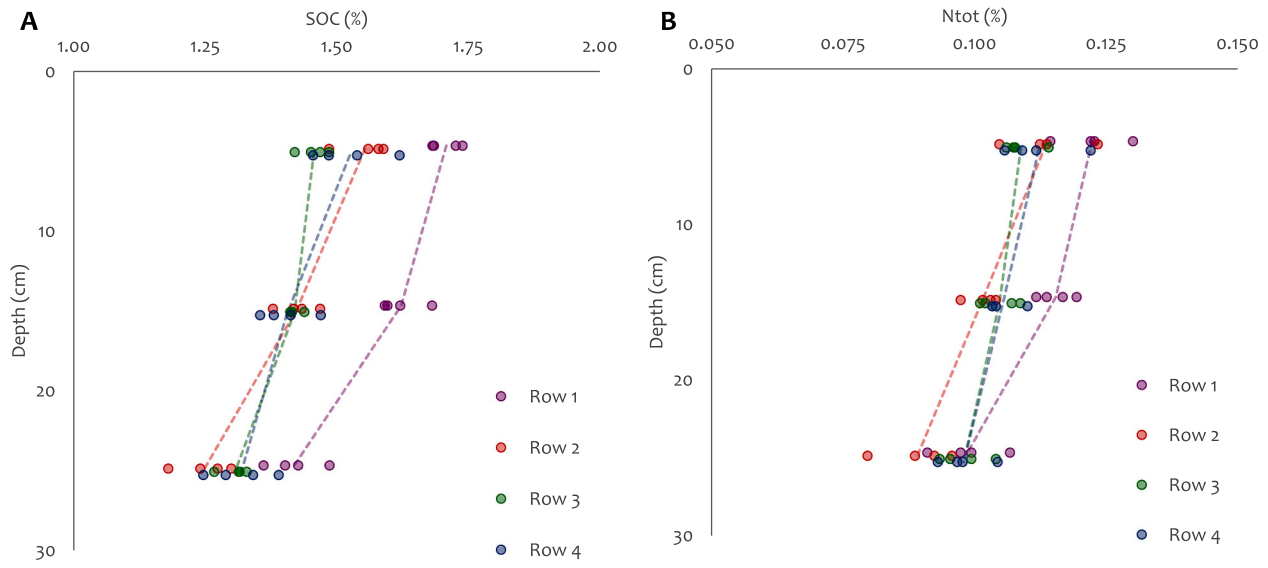

Fig. 3. SOC (A) and N_{tot} (B) in rows and soil depths at a paddy rice field in Central Kenya in 2018. Data from single samples are shown as dots coloured according to row. Means from row × depth, where data from different fertilizer treatments and plant growth stages were combined, are indicated as dashed lines. Differences between rows (row 1 vs. others rows) and depths were highly significant (ANOVA; $p < 0.001$ for each factor). (For interpretation of the references to colour in this figure legend, the reader is referred to the online version of this chapter.)

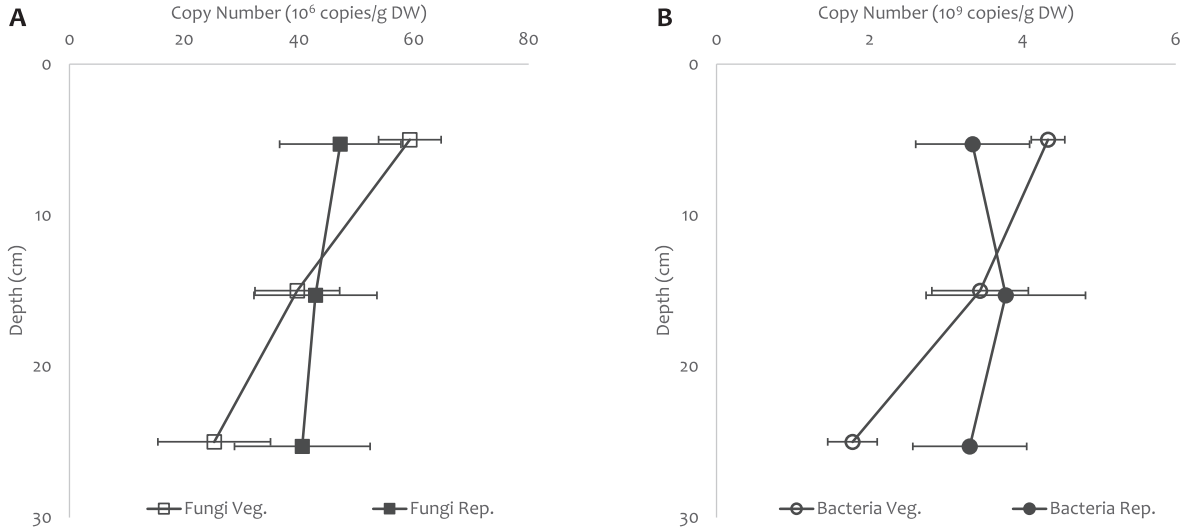

Fig. 4. Fungal (A) and bacterial (B) abundances in a paddy rice field in Central Kenya in 2018. Data from different plant growth stages (open symbols: vegetative stage; closed symbols: reproductive stage) at different soil depths (cm) are shown. Bars denote 95% confidence intervals; fertilizer treatments are not indicated separately. Pearson's correlation of abundance with soil depth is highly significant for both groups at the vegetative stage ($p < 0.001$) but not at the reproductive stage.

The bacterial communities in 34 out of 43 soil samples were dominated by Proteobacteria (18.7–43.5%) (Supplementary Fig. 4), including the highly abundant alphaproteobacterial order Rhizobiales (4.5–10.9%). Dominant BOTUs in Proteobacteria were *Pseudolabrys* sp. BOTU0005 (3.1–6.6%) and gammaproteobacterial taxon *Escherichia/Shigella* BOTU0003, which contributed to over 20% in some samples. The second most abundant phylum was Actinobacteria (13.0–42.8%), with the aerobic group Gaiellales (4.8–27.6%) (Albuquerque et al., 2011) being the most dominant actinobacterial order therein. Additional abundant phyla were Acidobacteria (11.0–17.9%), Chloroflexi (4.6–15.2%) with anaerobic Anaerolineae (2.6–9.5%), and Firmicutes (4.8–14.4%) with aerobic Bacilli (3.8–11.4%) and anaerobic Clostridia (0–3.8%). Furthermore, Betaproteobacteriales (formerly Betaproteobacteria, now included in Gammaproteobacteria; Parks et al., 2018) accounted for 2.6–7.2% of the relative abundances.

The dominant nitrifying bacteria were represented by Nitrosomonadaceae (1.8–5.5%), which convert NH_4^+ to nitrite, and *Nitrospira* spp. (0.44–1.65%) that together with members of the phylum Nitrospinae (max. 0.22%) subsequently convert nitrite to NO_3^-.

3.3. Environmental influences on microbial communities

The fungal community composition was strongly influenced by plant stage as demonstrated in the CCA analysis, showing that the fungal community at the vegetative plant stage was clearly separated from the community at the reproductive plant stage (Fig. 5A, Table 1). This effect was mainly caused by the identified biomarker (LDA = 3.97; $p < 10^{-5}$) *Cladosporium cladosporioides* FOTU0004, which increased in abundance

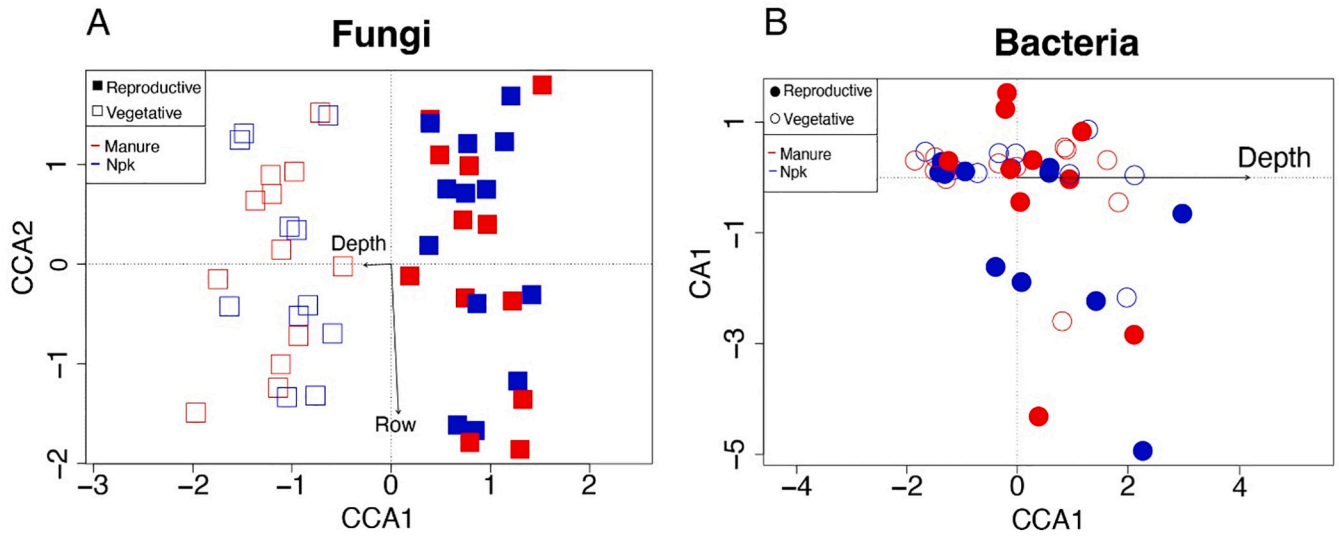

Fig. 5. CCA of fungal (A) and bacterial (B) communities at a paddy rice field in Central Kenya in 2018, showing separation of microbial communities due to ecological factors. Different fertilizer treatments are indicated by different colours (manure in red, NPK in blue) and different plant growth stages by open (vegetative stage) vs. filled (reproductive stage) symbols. Additional influencing factors (sampling depth and row representing the spatial effect) are shown by arrows. The different dots/squares indicate samples from individual plots and soil depths. Significance levels of environmental variables are provided in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
CCA model values explaining the influence of soil depth, plant stage and row effect on bacterial and fungal community composition. Significant influences are shown only ($p < 0.05$).

	Df	Chi-square	F	p-Value
Fungi				
Plant stage	1	0.127	1.515	0.001
Row	1	0.110	1.317	0.002
Depth	1	0.097	1.160	0.013
Residual	44	3.686		
Overall model - com(fungi) ~ plant stage + row + depth)				
Model	3	0.334	1.330	0.001
Residual	44	3.686		
Bacteria				
Depth	1	0.072	1.561	0.001
Residual	41	1.893		

from 2.1 ± 2.4% during the vegetative plant stage to 9.7 ± 6.8% during the reproductive plant stage (Supplementary Fig. 5). *Pyrenochaetopsis microspora* FOTU0029, on the other hand, decreased in relative abundance from 1.14 ± 0.98% during the vegetative stage to 0.35 ± 0.52 during the reproductive stage (LDA = 3.07; $p < 10^{-4}$). All other responding FOTUs showed relative abundances below 1%. Inclusion of confounding effects from row, soil depth and fertilizer treatment through pairwise testing did not result in the detection of additional FOTUs significantly responding to plant stage.

Spatial effects (i.e. row of experimental plots, see Fig. 1) played a major role in shaping the fungal community (Fig. 5A, Table 1). *Papiliotrema* sp. FOTU0001 significantly decreased from 11.3 ± 2.42% in row 1 to 4.4 ± 2.9% in row 4 in the topsoil (0–10 cm) (Pearson; $r^2 = 0.613$; $p = 0.0003$), whereas no differences in the lower soil layers (10–20 cm and 20–30 cm) were established (Supplementary Fig. 6). Although this pattern somehow resembled differences in SOC and N_{tot} at different soil depths (Fig. 3), no correlations between these two factors and the abundance of *Papiliotrema* sp. were found. Other FOTUs responding significantly to the row effect – i.e. *Pilidium concavum* FOTU0035 and *Zopfiella marina* FOTU0045 – showed mean relative abundances <1%.

Sampling depth had a weak influence on the composition of the fungal community (Fig. 5A, Table 1), and only one FOTU was found that significantly responded to upon soil depth: *Stellatospora terricola* FOTU0018 increased from 0.07 ± 0.21 at the top to 1.18 ± 1.02 at the bottom layer (Pearson; $r^2 = 0.244$; $p = 0.0004$). No response of the fungal communities to fertilizer treatments could be identified, even when confounding effects of row and depth were accounted for. Similarly, none of the other environmental properties (SOC, N_{tot}, exchangeable NH_4-N^+ and NO_3^--N) significantly shaped the fungal communities or showed significant correlations with individual FOTUs.

Contrary to the fungal community, the bacterial community as analyzed by CCA did not significantly respond to plant growth stage (Fig. 5B, Table 1). A slight but significant increase in relative abundance of the phylum Gemmatimonadetes was observed from 2.10 ± 0.53% during the vegetative plant stage to 2.48 ± 0.39 during the reproductive plant stage (LDA = 3.37; $p < 0.01$). All other taxa with significant differences between plant stages reached an average relative abundance relative abundances <0.5%. Spatial effects of plot row position did not influence the bacterial communities ($p > 0.05$; Monte Carlo permutation test).

Soil depth, on the other hand, caused a shift in bacterial community composition (Fig. 5B, Table 1), and shifts for single taxa were more pronounced at the vegetative than at the reproductive plant stage (Supplementary Fig. 7A). During the vegetative stage, the class Actinobacteria decreased in relative abundance from 5.46 ± 0.57% at the top layer to 2.85 ± 0.73% at the bottom layer (Pearson; $r^2 = 0.713$; $p < 0.0001$). Although most of the families of Actinobacteria (class) had a similar tendency to decrease from top to bottom, four mostly aerobic families were mainly responsible for the observed effect: Intrasporangiaceae, Geodermatophilaceae, Micrococcaceae, and Nocardioidaceae (Supplementary Fig. 7B). The candidate classes MB-A2–108 and NC10, on the other hand, increased in relative abundance with soil depth at the vegetative plant stage, from 2.37 ± 0.14% to 3.80 ± 0.39% ($r^2 = 0.610$; $p < 0.0001$) and from 0.89 ± 0.27% to 1.69 ± 0.16% ($r^2 = 0.648$; $p < 0.0001$), respectively. Consequently, statistically significant Pearson correlations were found between SOC and relative abundances of Actinobacteria ($r^2 = 0.3197$; $p < 0.01$), MB-A2–108 ($r^2 = 0.5413$; $p < 0.0001$), and NC10 ($r^2 = 0.3390$; $p < 0.01$) at the vegetative but not at the reproductive plant stage. Strictly anaerobic bacteria from the orders Clostridia and Anaerolineae were present in all samples, but no increase in abundance with soil depth was observed.

4. Discussion

4.1. Fertilizer effects on plant and soil properties

Total rates of N application were similar in the two fertilization treatments (manure vs. mineral fertilizer) but differed in the timing of applications. While a total N application of 75 kg ha^{-1} is prevalent in sub-Saharan Africa (SSA) farming systems, it is well below the amount used for rice cultivation in other parts of the world (e.g. Ahn et al., 2016). Mineral fertilization in three doses of 25 kg N ha^{-1} each had a clear positive impact on all measured plant properties in our study, including a substantial grain yield increase. This increase is of utmost importance in countries such as Kenya that heavily depend on rice imports to meet the population's demand. Nevertheless, farmyard manure is widely used in crop agriculture in SSA due to its general availability and as a cheaper alternative to mineral fertilizer. Overall, rice grain yield in Kenya's experimental field is substantially lower than in other countries like China, Japan, or Egypt, but typical for SSA (mean estimated rice yield for irrigated rice in SSA is 4.2 ± 0.2 t ha^{-1} (Rodenburg and Demont, 2009). Increased rates of manure applications combined with an optimized timing and placement of mineral fertilization (integrated soil-fertility management, ISFM) and improved manure management practices that aim at preserving nutrients during manure storage could potentially increase yields and nutrient use efficiency by replenishing nutrient deficient soils and restoring SOC stocks for improved water retention (Sánchez, 2010). In the present study, type of fertilization did not influence any of the measured soil properties. Manure addition did not increase SOC or the measured N-pools and chemical fertilizer application did not lead to soil acidification. This could, however, not be expected in a short-term field trial.

4.2. Microbial responses to fertilization and environmental factors

The pronounced effect of farmyard manure on fungal and bacterial communities in agricultural soils identified in other studies might be attributable to long-term applications (Hartmann et al., 2015), whereas in this investigation, manure was applied only once in 2018. Short-term applications to paddy fields of manure and NPK at high doses can have pronounced effects on both fungal and bacterial communities (Tang et al., 2020). Long-term manure application to a paddy rice field at doses comparable to the amounts used herein only caused minor shifts in the bacterial community, whereas higher doses induced more pronounced changes (Liu et al., 2020). Effects of both short-term and long-term manure applications were mostly explained by associated increases in soil nutrients and pH (Liu et al., 2020; Tang et al., 2020).

In general, we found gene copy numbers of fungi and bacteria in this study to be similar to abundances detected in other rice fields and greenhouse experiments performed with different rice varieties (Lee et al., 2011; Wang et al., 2016; Li et al., 2018), but nearly one order of magnitude lower than those reported by Ahn et al. (2012) for rice soils in Korea. SOC has been identified as the most important factor affecting microbial biomass and activity across a wide range of climatic zones, continents, farming systems and soil types (Lori et al., 2017). SOC contents were, however, similar in the Korean rice fields (Lee et al., 2011; Ahn et al., 2012) the Chinese glasshouse experiment (Li et al., 2018) and in the current Kenyan rice field study. SOC content can therefore explain similar gene copy numbers by most studies but is an unlikely driver of higher gene copy numbers in the study by Ahn et al. (2012).

Concerning the effects of environmental parameters on microbial communities, we observed a decrease of fungal and bacterial absolute gene abundances (copy numbers) with soil depth at the vegetative plant stage, together with positive correlations with SOC, N_{tot} and extractable NH_4^+-N. Correlations with soil properties were stronger for fungal compared to bacterial gene abundances. Thus, nutrient availability seems to be an important determinant for the microbial gene abundance in general and especially the fungal gene abundance at the plant vegetative plant stage in this rice paddy field. During the plant reproductive stage, additional factors that were not measured in this study might have been involved, which potentially have confounded the influence of C and N on fungi and bacteria in the studied soil. During growth, roots from rice plants change soil properties and exudate easily available organic C into the rhizosphere, where it is rapidly taken up by microbes (Yuan et al., 2016). It is thus likely that at the reproductive stage the depth gradient of easily available C is less pronounced than for SOC. Soluble and thus relatively mobile C would explain similar microbial gene abundances at different soil depths during the reproductive stage.

In fungal communities of paddy rice fields, a strong dominance of Ascomycota as seen in our experimental field is generally observed (Pili et al., 2016; Li et al., 2018; Long and Yao, 2020), and this is usually more pronounced than in other agricultural systems including wheat, barley, maize and grassland (e.g. for agroecosystems in temperate climate Klaubauf et al., 2010; Hartmann et al., 2015; Moll et al., 2016; Keiblinger et al., 2018; Deltedesco et al., 2020). Accordingly, Basidiomycota are of lower abundance in rice fields compared to other agroecosystems. Glomeromycota, i.e. arbuscular mycorrhizal fungi, are generally of low abundance in soil, and are more easily detected in roots (e.g. Moll et al., 2016). Similarly, Pleosporales are regularly found among the dominant orders in rice fields, but they are normally not dominant in other agroecosystems. Among Pleosporales, *Westerdykella purpurea* FOTU0003 was the dominant taxon in our study, and this genus, together with other taxa from the Sporomiaceae, is routinely found in other surveys of fungal communities in rice fields (Long and Yao, 2020; Maguire et al., 2020), and it has even been described as a rice endophyte in Kenya (Pili et al., 2016). *Westerdykella* spp. have, however, also been described as coprophilous, i.e. dung-loving fungi (Zhang et al., 2012). It is thus possible that *Westerdykella* spp. can live as plant endophytes, and after being consumed by herbivores and passed through the digestive tract, can propagate as coprophilous fungi, similar to what has been observed for members of the Lasiosphaericeae (Miranda et al., 2020). Both groups of fungi – *Westerdykella* spp. and Lasiosphaeriaceae – are prevalent in the Kenyan rice field soil in this study but in contrast to our expectations (hypothesis ii) we found no increase of coprophilous fungi in plots fertilized with manure compared to the mineral fertilizer plots. The expectations have derived from studies in Europe, where an increase in coprophilous fungi was found in fields that received slurry (Hartmann et al., 2015) or in grassland plots with increased prevalence of macrofauna (Deltedesco et al., 2020). We thus conclude that the high relative abundance of coprophilous fungi in both treatments of this Kenyan rice field rather derive from faecal-contaminated irrigation water or wildlife macrofauna faeces – e.g. from rats (Onyango, 2014) – and not from the one-time manure input in our fertilization study.

The hypocrealean genus *Emericellopsis*, which contains the most abundant FOTU from our study (*Emericellopsis* cf. *persica* FOTU0002), is well known to prefer periodically waterlogged soils (see Zuccaro et al., 2004 and references therein). Further prominent members of the Hypocreales in the Kenyan rice field are potentially plant-pathogenic fungi in Nectriaceae, especially from the genus *Fusarium*. These fungi are often found in high numbers in rice field soil (Long and Yao, 2020) and as endophytes in rice roots (Pili et al., 2016). Three of the four FOTUs in the genus *Fusarium* are closely affiliated to the *F. fujikuroi* species complex, which can together with other pathogens cause rice sheath rot (Bigirimana et al., 2015), a disease frequently encountered at the experimental site. In addition, *Fusarium* spp. can contribute to N_2O production through fungal denitrification under anaerobic conditions (Maeda et al., 2015; Keuschnig et al., 2020).

The main environmental influence shaping the fungal community was plant development stage, with an increase of *Cladosporium cladosporioides* in the reproductive plant stage. *Cladosporium cladosporioides* is a widespread secondary invader on necrotic parts of many different plants and is frequently isolated from litter (Bensch et al., 2010). It has recently been proposed as an agent for plant growth promotion and yield

increase, and for biocontrol of rice blast (Chaibub et al., 2020). Among the Basidiomycota, only one taxon was present in relatively high levels in our study, *Papiliotrema* sp. FOTU0001 (0.7–14.6%), a genus which is mainly known as colonizer of the phylloplane (including the rice phylloplane; Into et al., 2020), although it is also found in soil samples (Li et al., 2020). Interestingly, in the first two rows of the field plot, its abundance was higher in the upper soil layer than in the middle or bottom layer, but this difference was not observed in the other two rows. *Papiliotrema* sp. FOTU0001 was mainly responsible for the significant row effect on fungal community composition as revealed by CCA. Although spatial effects of differences between rows independent of the fertilizer treatments have been observed for selected soil parameters and for the fungal community, no strong correlations between SOC and specific fungal taxa could be found. The reason for the row effect is currently unknown and does not correspond to the main wind direction (SSW) or water inflow from the canal (see Fig. 1). Row effects could have masked more subtle microbial community responses to other environmental parameters. No strong effects of soil depth or fertilizer treatment were established for the fungal community. Soil pH that was repeatedly described as a major driver of fungal community composition (Tedersoo et al., 2014) did not change at the herein studied Kenyan rice paddy field, neither with depth nor with fertilizer treatment. SOC, on the other hand, decreased with soil depth but mainly influenced the fungal abundance but not fungal community composition.

The composition of the soil bacterial community in the permanently flooded Kenyan rice field generally resembles what has been described for Asian rice fields even though fertilization rates in the Kenyan field were lower than typically found in intensively flooded rice fields in Asia. Overall and similar to the fungal community, no influence of fertilizer type could be established for the prokaryotic community composition. Even though a higher persistence of faecal indicator bacteria in the environment has been suggested for the tropics (Rochelle-Newall et al., 2015), the relative abundances of bacteria potentially harmful for humans or livestock did not increase with the application of manure in this study. Relative abundance of an enterobacterial BOTU affiliated to *Escherichia/Shigella* was, however, on average above 3%, but independent of manure application. Faecal pollution from contaminated irrigation water or wildlife macrofauna together with higher persistence at higher temperatures might explain these high abundances. Manure application for longer time periods and in higher rates can, however, have distinct effects on the bacterial community in rice cropping systems (Liu et al., 2020).

The overall composition of the bacterial communities in our study was similar to paddy rice fields from other countries and continents (Lee et al., 2011; Ahn et al., 2012; Xuan et al., 2012; Li et al., 2018; Ezeokoli et al., 2021). Several predominantly aerobic taxa from Actinobacteria decreased in relative gene abundance with soil depth during the vegetative plant stage, but not during the reproductive stage. This decrease was compensated for by increasing the relative gene abundance of the candidate classes MB-A2-108 (phylum Actinobacteria) and NC10 (phylum Rokubacteria) with soil depths in the vegetative plant stage. The class MB-A2-108, which was originally found in deep marine sediments (Reed et al., 2002), seems to prefer deeper soil layers in flooded ecosystems (Steger et al., 2019), but little is known about the ecology of this group. The class NC10 couples methane oxidation under anaerobic conditions to the reduction of nitrite and is typically found in flooded soils (Ettwig et al., 2010) including Italian paddy rice fields, where a similar increase in relative gene abundance of NC10 with soil depth has been observed (Vaksmaa et al., 2017). Likely NC10 bacteria could successfully compete for nitrite from the initial step of nitrification, as ammonium-oxidizing bacteria from Nitrosomonadaceae had higher gene abundances at our experimental site than nitrite-oxidizing bacteria from *Nitrospira* spp. and Nitrospinae. This is in agreement with the very low amounts of NO_3^-, the final product of nitrification, at our experimental site. Low oxygen levels in the flooded soil potentially limit the nitrification process, although nitrite-oxidizing bacteria from Nitrospinae are present, a nitrifier group adapted to reduced oxygen levels (Spieck et al., 2014). Complete anoxia is not expected at the site, even not at deeper soil layers, because oxygen is transported through the aerenchyma tissue formed in the rice roots. In support of this, high numbers of mainly aerobic bacteria from Bacilli and Gaiellales and the presence of nitrifying bacteria point to a certain level of oxygen availability in the investigated rice fields. Accordingly, depth effects on soil properties and selected bacterial taxa were more pronounced at the vegetative plant stage, when plants were still growing and not yet fully developed, than at the reproductive stage. Roots were, however, not detected in the deepest soil layer (20–30 cm) at either plant stage.

5. Conclusions

A deeper understanding of the processes occurring in the soil-microorganism-plant system is crucial for developing sustainable agricultural management practices and helps to meet the challenges of food production. Here we present the first in-depth analysis of fungal and bacterial communities in paddy rice soil on the African continent as affected by two different fertilizer types (i.e. manure vs mineral fertilizer). Research hypothesis 1 stating that application of livestock manure would result in similar rice yields as application of mineral fertilizer at the same N dose was rejected because fertilization strategy strongly influenced plant parameters including rice grain yields. Research hypothesis 2 stating that fertilizer would have direct and indirect effects on fungal and bacterial communities in the soil, depending on rice growth stage and soil depth was also rejected. No effects of fertilizer type were observed for the abundance and composition of fungal and bacterial communities or on any of the soil chemical parameters considered. Plant stage was the main influencing factor for fungal communities, whereas soil depth shaped the bacterial communities. To ensure a better provision with rice from local production while preserving soil health and feeding soil microorganisms with organic carbon, an integrated formulation of mineral and organic fertilizers might be a solution for this specific Kenyan situation. This could compensate for the low fertilizer quality of local farmyard manure while accounting for prohibitive prices of mineral fertilizer in Kenya. However, although minimal, this action is still a form of agricultural intensification whose negative effects (e.g. enhanced methane emissions or N leaching) urge to be carefully monitored to ensure environmental sustainability in Kenya. Future studies should investigate long term effects of different fertilizer regimes – including mixed application of manure and mineral fertilizer – on soil, plant and microbial community properties in SSA rice cultivation systems. Additionally, research on the emissions of the potent greenhouse gases methane and nitrous oxide is necessary to minimize negative environmental impacts – a study that is currently ongoing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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