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Research Article

Organic farming systems improve soil quality and shape microbial communities across a cotton-based crop rotation in an Indian Vertisol

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Abstract

The adverse effects of intensified cropland practices on soil quality and biodiversity become especially evident in India, where nearly 60% of land is dedicated to cultivation and almost 30% of soil is already degraded. Intensive agricultural practice significantly contributes to soil degradation, highlighting the crucial need for effective countermeasures to support sustainable development goals. A long-term experiment, established in the semi-arid Nimar Valley (India) in 2007, monitors the effect of organic and conventional management on the plant-soil system in a Vertisol. The focus of our study was to assess how organic and conventional farming systems affect biological and chemical soil quality indicators. Additionally, we followed the community structure of the soil microbiome throughout the vegetation phase under soya or cotton cultivation in the year 2019. We found that organic farming enhanced soil organic carbon and nitrogen content, increased microbial abundance and activity, and fostered distinct microbial communities associated with traits in nutrient mineralization. In contrast, conventional farming enhanced the abundance of bacteria involved in ammonium oxidation suggesting high nitrification and subsequent nitrogen losses with regular mineral fertilization. Our findings underscore the value of adopting organic farming approaches in semi-arid subtropical regions to rectify soil quality and minimize nitrogen losses.

Keywords: amplicon sequencing; arable cropping; diversity; nitrogen; sub-tropical

Introduction

Cropland expansions, intensifications, and land degradation have exerted significant pressures on biodiversity, biosphere integrity, and biogeochemical cycles, pushing them beyond planetary boundaries (Richardson et al. 2023). However, recognizing the pivotal role of healthy soils in regulating nutrient cycles within natural and agroecosystems, and as essential habitats for belowground biodiversity is imperative for proper stewardship of soils and the attainment of sustainable development goals (Lal et al. 2021).

In India, ∼60% of the land area is dedicated to agricultural activities (data.worldbank.org). However, also in India, the agricultural use of soils is accompanied by land degradation and deterioration of soil health (Basak et al. 2021, Das et al. 2022) with the total geographic area affected by degradation reaching 29% (SAC 2016). Particularly, these effects are evident in Vertisols, widespread clayey soils that occupy ∼22% of the country's total geographical area (Mandal et al. 2013). These soils are considered highly productive due to their high clay content, but they are also at high risk of degradation when poorly and intensively managed (Schweizer et al. 2022). Recently, Das et al. (2022) reported that Indian soils often exhibit low soil organic carbon (SOC) stocks, which, coupled with low cation exchange capacity and low water-holding capacities, results in poor soil quality. A decrease in soil quality, defined as the capacity of soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health (Doran and Parkin 1994, Bünemann et al. 2018), can severely affect agroecosystem functioning. To address this concern, various strategies have been proposed, encompassing the use of farmyard manure (FYM) or green manures, adoption of legume-based cropping systems, cultivation of horticultural crops, adoption of zero tillage practices, and incorporation of crop residues.

One effective approach to sustainable land management, encompassing the measures described above, is organic farming. Organic farming aims at closing nutrient cycles by reintegrating plant residues and livestock manure back into the field, placing a particular focus on soil health (Gattinger et al. 2012, Seufert et al. 2012). Additionally, nutrient management in organic farming relies on the cultivation of leguminous plants for biological nitrogen fixation, thereby improving crop nutrient supply.

Overall, the concept of organic farming centres around the vital role of an abundant and active microbial community, enabling crucial soil processes such as organic matter decomposition and the promotion of carbon and nitrogen cycling, among others. Several studies in temperate regions have demonstrated that

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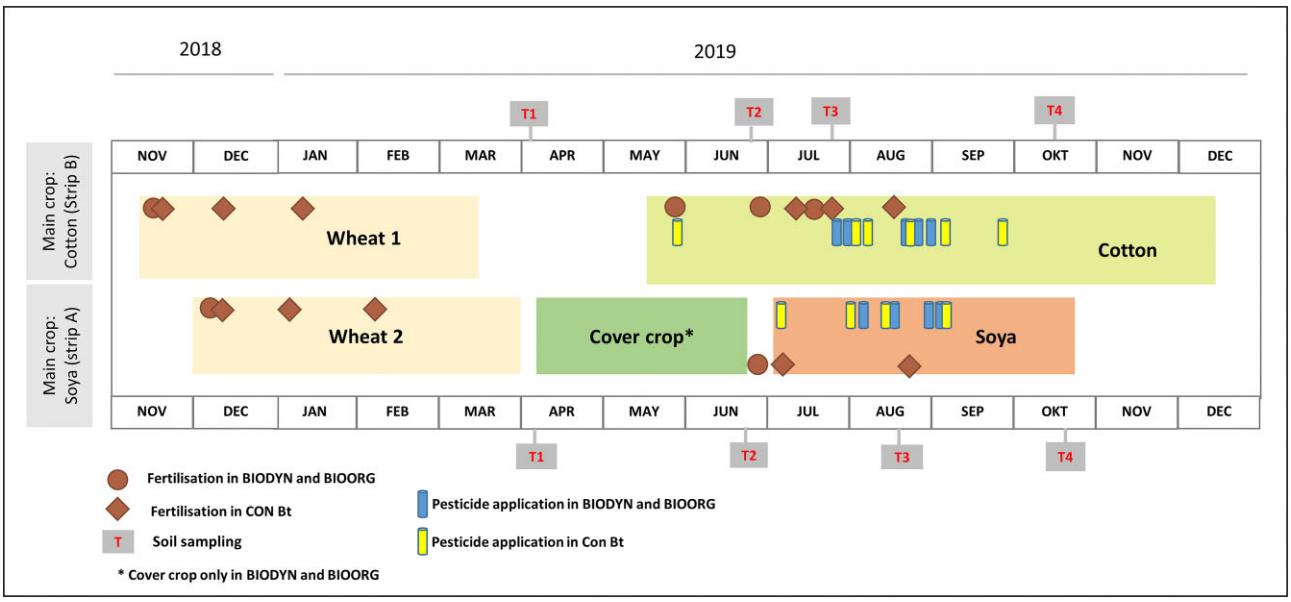

Figure 1. Schematic overview of the crop cultivation phases for the two strips with the main crop being either cotton or soya in 2019. Fertilization events within 2018–2019 (organic farming systems: circles; conventional farming systems: diamonds), and sampling dates (T1–T4) conducted in 2019 are indicated. Pesticide application events are indicated in yellow for conventional and in blue for organic and biodynamic farming systems. BIODYN: biodynamic farming system; BIOORG: bio-organic farming system; CON Bt: conventional farming system with Bt cotton cultivation in the crop rotation.

organic farming also induces a distinct microbial community structure in the soil (e.g. Hartmann et al. 2015, Francioli et al. 2016, Lin et al. 2016, Lupatini et al. 2017). The changes in microbial communities induced by distinct management practices might change microbial capacity for organic matter mineralization and nitrogen cycling in organic and conventional farming systems. However, the majority of research in this area has been conducted in temperate arable systems, while studies in (sub-)tropical climates are still relatively scarce (Pajares et al. 2016, Lori et al. 2017). Further research is essential to comprehend the potential benefits and challenges of implementing organic farming in tropical agroecosystems, considering the close link between the soil microbiome and soil nutrient cycling (Delgado-Baquerizo et al. 2016, Wagg et al. 2019).

The objective of the current study is to advance our yet still poor understanding of the influence of various farming systems on soil quality and the soil microbiome in the subtropics using a long-term field experiment established in 2007 in Madhya Pradesh, India. Understanding the different functional impacts that organic and conventional practices have on soil quality, including microbial communities in Vertisols in India, can assist in targeted management of these soils to enhance their health.

We collected soil samples from field plots subjected to >10 years of continuous organic or conventional management practices in cotton-based farming systems on a Vertisol (Forster et al. 2013)) throughout the vegetation period of cotton and soya. Our analyses focused on assessing soil bacterial and fungal alpha- and beta-diversity, abundance of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), as well as geochemical soil quality indicators. The assessment of AOB and AOA abundance was conducted based on their crucial involvement in regulating the nitrogen cycle by facilitating the initial step of nitrification (Prosser and Nicol 2008, 2012, Lu et al. 2020) and thus mineral N-cycling. As key contributors to nitrogen cycling processes, including plant nitrogen provisioning and potential nitrogen losses, understanding AOB and AOA abundance can help optimizing nitrogen dynamics in agricultural systems.

Due to system-specific management practices such as organic fertilization and non-chemical plant protection measures and existing knowledge we have from temperate regions, we first hypothesize for the herein assessed subtropical Vertisols to observe elevated soil quality in organic compared to conventional systems. We second hypothesize that organic and conventional farming leads to distinct soil bacterial and fungal communities, which additionally shift throughout the crop vegetation period due to variations in nutrient availability. Third, we hypothesize that microbes known for their capability to degrade complex carbon sources are enriched in organically managed systems. Conversely, indicative microbes with the ability to metabolize mineral nitrogen and a higher abundance of AOA and AOB are likely to be associated with the conventional system, influencing the soil's nitrifying potential.

Materials and methods

Study site description and management practices

The long-term farming system comparison experiment was established in 2007 and is located in the Nimar Valley in Madhya Pradesh (India). The site is situated at 250 m a.s.l. (22°8′30.28″N; 75°37′48.97″E) in a subtropical (semi-arid) climate with an average annual precipitation of 800 mm. The soil is a 'black cotton soil', with a mean clay content of ∼60% and is classified as Vertisol. The field experiment compares biodynamic (BIODYN), bioorganic (BIOORG), and conventional agricultural practices, including Bt-cotton cultivation (CON Bt), in a randomized block design with four replicated plots (plot size: 16 × 16 m) per farming system. The BIOORG and BIODYN treatments are hereafter also summarized as organic systems.

The field experiment is composed of two adjacent strips (strips A and B) representing alternating stages in the crop rotation cycle, as described in Forster et al. (2013). The crop rotation in all farming systems follows a two-year cycle with wheat and soya in the first year (main crop: soya) followed by wheat and cotton in the second year (main crop: cotton), as shown in Fig. 1. In the two organic

Table 1. Overview of fertilizer and nutrient inputs in the two strips during 2018–2019 season with either main crop cotton (Strip B) or main crop soya (Strip A).

Strip	Crop	System	Compost	Rock phosphate	Neem oil cake	Urea	SSP	MOP	N	P	K
			t/ha			kg/ha				kg/ha	
Strip A	Wheat 1	BIODYN	9.25	-	-	-	-	-	47.5	16.6	64.4
		BIOORG	9.25	-	-	-	-	-	47.5	16.6	64.4
		CON BT		-	-	325	375	70	149.5	25.7	32.5
	Cotton	BIODYN	12.5	-	390	-	-	-	123.8	49.9	31.1
		BIOORG	12.5	-	390	-	-	-	123.8	49.9	31.1
		CON BT		-	-	317.5	515	90	145.5	34.9	41.5
Strip B	Wheat 2	BIODYN	9.25	-	-	-	-	-	47.5	16.6	64.4
		BIOORG	9.25	-	-	-	-	-	47.5	16.6	64.4
		CON BT		-	-	325	375	70	149.5	25.7	32.5
	Soya	BIODYN	2.5	175	-	-	-	-	25.0	26.4	8.3
		BIOORG	2.5	175	-	-	-	-	21.3	24.3	6.2
		CON BT		-	-	62.5	500	105	28.8	34.3	49.8

BIODYN: biodynamic farming system, BIOORG: bio-organic farming system, CON Bt: conventional farming system with Bt cotton cultivation in the crop rotation. SSP: Single Super Phosphate fertilizer, MOP: Muriate of Potash. Details on averaged annual fertilizer and nutrient inputs across 2007–2019 are given in Supplementary Table S2.

input systems, a cover crop (cowpea) is cultivated for two months before the sowing of soya. Cotton was sown on the 21st of May and harvested on the 12th of December 2019. The two organic farming systems received biodynamic and bioorganic compost, respectively (applied on the 27th of May, 26th of June, and 17th of July). For the CON Bt system, single superphosphate, urea, and muriate of potash were provided on the 2nd of July as initial fertilizers, followed by two more urea applications during the growing season (18th of July and 13th of August). In the strip with main crop soya, green manure was sown in the BIODYN and BIOORG farming systems on the 20th of April, and compost was applied on the 25th of June. In the CON Bt system, a single dose of superphosphate and muriate of potash was applied on the 2nd of July, followed by urea application on the 22nd of August. Soya cultivation started on the 10th of July and crops were harvested on the 17th of October. Weed and pest management were followed as per standard norms recommended for organic and conventional systems, and a list of applied products can be found in Supplementary Table S1, and application time points are illustrated in Fig. 1.

From 2007 to 2019, conventionally managed cotton plots received an average annual application of 183 kg N ha^{-1}, 44 kg P ha^{-1}, and 86 kg K ha^{-1} from synthetic mineral fertilizers. Additionally, an average of 5.4 t ha^{-1} of FYM was applied every alternate year per cotton crop. In comparison, conventionally managed wheat fields received an average annual application of 147 kg N ha^{-1}, 27 kg P ha^{-1}, and 33 kg K ha^{-1}, while soybean fields received 30 kg N ha^{-1}, 34 kg P ha^{-1}, and 55 kg K ha^{-1} annually. In contrast, organically managed cotton plots received an average annual application of 107 kg N ha^{-1}, 30 kg P ha^{-1}, and 113 kg K ha^{-1}, with organically managed wheat fields receiving 53 kg N ha^{-1}, 20 kg P ha^{-1}, and 77 kg K ha^{-1} annually. Organically managed soybean fields were fertilized with 26 kg N ha^{-1}, 18 kg P ha^{-1}, and 35 kg K ha^{-1} on average per year. Detailed breakdown of fertilizer inputs between BIODYN and BIOORG, as well as distinctions between wheat 1 and wheat 2, can be found in Supplementary Table S2. Specific fertilizer and nutrient inputs for the year 2019 are presented in Table 1, while Fig. 1 visually represents fertilization events throughout the year 2019 in the respective strips.

Soil sampling and processing

Four soil sampling campaigns took place throughout the crop cultivation phases. A basic soil sampling campaign (T1) was conducted on the 4th and 5th of April 2019 during bare fallow with soil samples taken from the topsoil (0–20 cm) in both strips. The soil was sieved to 5 mm directly after sampling, a subsample was taken to determine gravimetric water content by oven-drying at 105°C for 24 h. For molecular analyses, a subsample of 10 g was oven-dried at ~80°C for 2 h (Pfeiffer et al. 2017) at the field site infrastructure, before transport to the laboratory in Switzerland. By quickly removing soil moisture, we aimed to preserve soil microbial community structure during transport from the field site infrastructure to the laboratory in Switzerland, as an uninterrupted cold chain could not be guaranteed. After transport samples were stored at −20°C. For the assessment of geochemical and biological soil quality indicators, a subsample of 200 g was air-dried at the field site infrastructure and transported to Switzerland, where it was stored at 4°C until laboratory analyses.

During the crop cultivation phase of cotton in strip A, the sampling procedure for molecular biological analysis was repeated before the second fertilization on the 24th of June (T2), before the 4th fertilization on the 24th of July (T3), and during cotton ripening on the 24th of October (T4) in 2019. Soil sampling in cotton was done on soil ridges. In strip B, soil samples were taken after the green manure incorporation on the 19th of June (T2), before the second fertilization on the 20th of August (T3), and before the final harvest of soya on the 15th of October (T4) in 2019. All sampling dates, crop cultivation periods, and fertilization events are shown in Fig. 1. At all samplings, a subsample of fresh soil was taken to the field site infrastructure to assess the soil mineral nitrogen content.

Physical and chemical soil quality indicators

Soil bulk density was determined from undisturbed ring samples (100 cm^3) in each experimental plot ($n = 4$) on the 4th–6th of September. Soil pH was measured in air-dried soil samples collected at T1 in an aqueous suspension (1:2.5 mass/volume) with a pH meter (WTW InoLab pH 7110, Xylem Analytics Germany GmbH, Weilheim, Germany). Organic carbon and total nitrogen were determined in 1 g of milled subsample by dry combustion at 900°C on a CN Analyzer (Elementar Analysensysteme GmbH, Vario MAX Cube, Hanau, Germany) as described in Krause et al. (2022). Permanganate oxidizable carbon (POXC), POXC serving as a proxy for labile carbon and closely associated with soil biological processes (Bongiorno et al. 2019, Bongiorno 2020), was extracted

using a 0.2 M K_2MnO_4 solution and quantified spectrometrically using a GENESYS 10S UV–VIS Spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA) following the principles of Weil et al. (2003) with modifications described in Bongiorno et al. (2019).

Mineral nitrogen (Nmin) was extracted frozen soil using 0.5 M K2SO4 and nitrate (NO3-) and ammonium (NH4+) were quantified using the Spectroquant® Nitrate Test and Spectroquant® Amonium Test (Merck, Darmstadt, Germany).

Microbial soil quality indicators

Microbial biomass carbon and nitrogen contents were determined by the chloroform fumigation approach (Vance et al. 1987) in analytical duplicates. To determine soil basal respiration and nitrogen mineralization potential small incubation experiments were conducted in hermetically sealed microcosms as described in Lori et al. (2022).

DNA extraction

To characterize the soil microbial community, DNA was extracted from 400 mg soil using the MP Biomedicals FastDNA™ SPIN Kit for (MP Biomedicals, California, USA) following the manufacturer's instructions. DNA quantity was assessed using Qubit system reagents (ThermoFisher Scientific, Waltham, USA) and relative fluorescent units on a CFX96Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Before any downstream applications, DNA was diluted 1:16 in sterile water to reduce inhibitory effects.

Functional gene quantification

To assess the abundance of AOB and AOA involved in ammonia oxidation, quantitative polymerase chain reactions (qPCR) were performed targeting the archaeal *ammonia monooxygenase (amoA)* (Leininger et al. 2006) and bacterial *amoA* genes (Rotthauwe et al. 1997). Furthermore also the abundances of *alkaline (apr)* and *neutral (npr) metallopeptidases* (Bach et al. 2001) as well *as urease subunit C (ureC)* (Gresham et al. 2007) were assessed as their involvement is crucial for the regulation of organic nitrogen mineralization. Cycling conditions and PCR chemistry were optimized for each primer pair to reach standard curve R^2s higher than 0.95 and amplification efficiencies between 80 and 100%. Reactions were performed in technical duplicates using an SYBR-green approach (Kapa SYBR Fast qPCR Kit Master Mix (2×) Universal; Kapa Biosystems, Wilmington, MA, USA) on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Primer sequences and cycling conditions for functional gene quantifications are provided in Supplementary Table S3. Quality control of generated amplicons, as well as non-template controls and serial dilutions of plasmids carrying the respective target genes, were assessed by melting curve analyses for each reaction and by gel electrophoresis for a random selection of samples.

Amplicon sequencing and bioinformatic processing

A two-step PCR approach using CS1/CS2-tagged (Fluidigm, South San Francisco, CA, USA) primer targeting the V3-V4 region of the 16S rRNA gene (341F and 806R as modified in Frey et al. (2016); Supplementary Table S4), and primers targeting the fungal internal transcribed spacer region ITS2 (ITS3ngsmix1-5 and ITS4ngsUni, Tedersoo and Lindahl 2016; Supplementary Table S4). A negative control was included in all PCR runs, containing double-distilled water instead of DNA, and positive controls containing ZymoBIOMICS Microbial Community DNA Standard (Zymo Research Corporation, Irvine, CA, USA) (Supplementary Fig. S1). The first PCR was performed in technical triplicates, which were pooled afterwards and purified using magnetic beads (https://openwetware.org/wiki/SPRI_bead_mix). To visualize and validate the size and quality of the amplicons, subsamples of the purified DNA were inspected by gel electrophoresis (1.25% agarose gel). The second PCR, library preparation, and paired-end sequencing on an Illumina MiSeq sequencing platform (Illumina, San Diego, CA, USA) using MiSeq v3 chemistry were performed at the Genome Quebec Innovation Centre (https://www.genomecanada.ca/en, Montreal, Canada). The resulting sequences are deposited on NCBI (PRJNA841470).

Bioinformatic processing of amplicon sequences was done as described in Lori et al. (2023). Briefly, USEARCH v11.0.667 (Edgar 2010) was used for the pre-processing of raw reads, including the removal of primer sequences and low-complexity reads. Quality filtering was done using PRINSEQ v0.20.4 (Morgulis et al. 2006). Pre-processing resulted in 4 367 590 and 5 005 262 high-quality reads for the 16S rRNA gene and ITS2 gene region, respectively. UNOISE3 (Edgar 2016b) was used to denoise reads into zero-radius OTUs (ZOTUs), which afterwards were clustered at 97% sequence similarity (Edgar 2013). Then, taxonomy for 16S and ITS data was assigned using SINTAX (Edgar 2016a)based on the references SILVA_128_16S_utax_work.fa (Quast et al. 2013) and UNITE_v82_Fungi_04.02.2020.fasta, respectively (Abarenkov et al. 2010). ITSx (Bengtsson-Palme et al. 2013) was used to ensure that the taxonomic annotation is precise and reliable. Finally, 2 945 757 bacterial reads clustered into 2549 ZOTUs, and 3 706 451 fungal reads into 1089 ZOTUs. Before downstream analysis, non-bacterial (archaea, mitochondria, and cyanobacteria) were removed, resulting in 2 884 836 bacterial sequences assigned to 2501 ZOTUS (Supplementary Table S5).

Statistical analysis

All analyses were conducted in RStudio (version 2023.3.0.386, Posit team 2023), a development environment for R (version 4.3.0, R Core Team 2023) graphs were created using the R package ggplot2 (Wickham 2016). Univariate data was analysed with linear mixed-effect models using the nlme:: lme function (Pinheiro et al. 2020). To assess farming system effects on soil quality indicators data from T1 was analysed jointly for the two crops (strips A and B). To account for the experimental setup, field pairs were nested in blocks and set as a random factor. Additionally, we modelled farming system-specific variances, recognizing that management practices can influence both the mean and the variation. Due to distinct management practices in the two strips, data collected across the growing season (T1–T4), were analysed for each strip individually. We assessed the effects of the farming system, crop cultivation phase, and the two-way interaction between factors on each response variable and accounted for the spatial set-up of the experiment by including the field blocks as random effect. Furthermore, we modelled farming system as well as crop cultivation specific variations too. The nlme:: anovoa_lme function (Pinheiro et al. 2020) was used to retrieve the statistical significance of the main effects. If statistically significant main effects for farming system or the interaction were found, Sidak post hoc tests (correcting for multiple comparisons) were performed to identify differences between the distinct farming systems. Estimated marginal means and 95% confidence intervals for each farming system within the different time points were obtained using the emmeans:: emmeans function (Lenth et al. 2023) and plotted along with the raw data. Data were transformed using log, square-root, inverse, or arc sin functions to satisfy the assumption of normal distribution and variance homogeneity of model residuals.

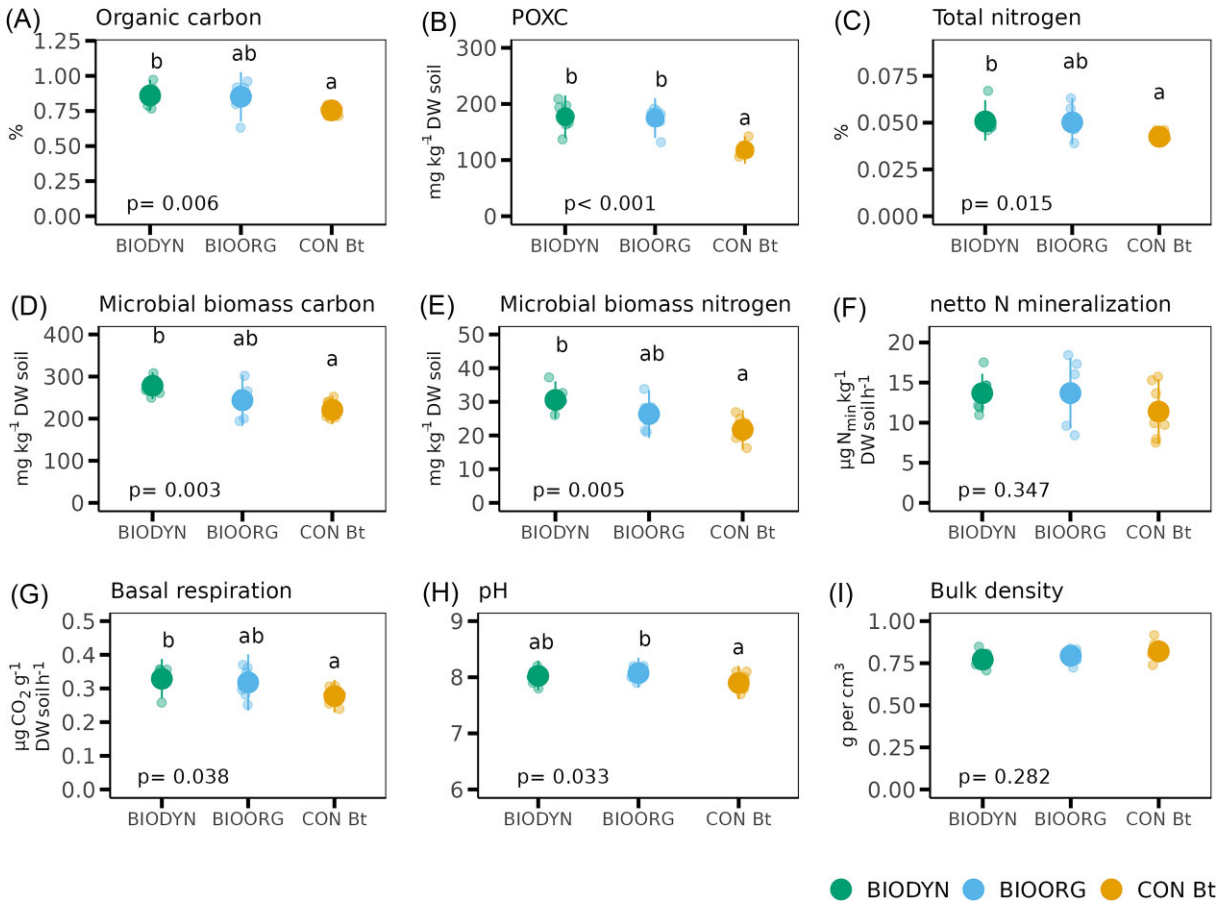

Figure 2. Farming system effects on chemical and biological soil quality indicators. Graphs show raw data and estimated marginal means with 95% confidence intervals of linear mixed effect models assessing the farming system (system) effect on soil quality indicators. *P*-values are presented within each graph. Sidak post hoc tests are indicated as letters for significant ($P < 0.05$) main effects. BIODYN: biodynamic farming system; BIOORG: bio-organic farming system; CON Bt: conventional farming system with Bt cotton cultivation in the crop rotation. POXC: permanganate oxidizable carbon. N= nitrogen.

Sequencing data were inspected using the PHYLOSEQ package (McMurdie and Holmes 2013). Before any downstream analyses, data were rarefied to even depth (Schloss 2023) using rrarefy:: vegan resulting in 2501 bacterial and 1089 fungal ZOTUs (Supplementary Table S5) to remove the impact of different sequencing depths in the downstream analyses. Rarefaction plots were created using phyloseq.extended:: ggrare (Supplementary Fig. S2). Observed ZOTU richness, as the number of unique ZOTUs, was then analysed with linear mixed effect models as described above. For β-diversity analyses, the dataset was filtered to remove ZOTUs with fewer than 20 reads occurring in less than four samples for bacteria and fungi (Supplementary Table S5) to reduce the extreme sparsity of microbiome data (Cao et al. 2021). After filtering, the data contained 1 840 954 bacterial and 1 603 972 fungal sequences clustering into 2488 unique bacterial and 874 unique fungal ZOTUs. The obtained sequence counts were transformed into relative abundances by total sum scaling and the effects of the farming system, crop cultivation phase and the interaction thereof on the community composition were assessed by permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) with the function vegan:: adonis2 (Oksanen et al. 2022) using the Bray–Curtis dissimilarity matrix with permutations being restricted on the field blocks. Considering that PERMANOVA results can be confounded by differences in multivariate dispersion, PERMDISP (Anderson et al. 2006) implemented through the betadisper function in VEGAN (Oksanen et al. 2022) was used to characterize the multivariate spread in the treatment groups (Supplementary Table S6). To graphically present bacterial and fungal community structure, a canonical analysis of principal coordinates (Anderson and Willis 2003) was performed using the CAPdiscrim function implemented in the BiodiversityR package (Kindt and Coe 2005) constraining for farming systems and crop cultivation phases. Finally, the indicspecies:: multipatt function ('r.g' function to correct for unequal group sizes) (De Cáceres and Legendre 2009) was used to identify ZOTUs associated with different farming systems or time points with 10^4 permutations (Dufrêne and Legendre 1997, De Cáceres et al. 2012). For this test, we removed sequences with fewer than 150 reads in <15% of the samples for bacteria and fewer than 100 reads in <15% of the samples for fungi. The multiple testing correction was performed by calculating q-values using the QVALUE package (Storey et al. 2021). Venn diagrams have been created using the ps_venn function form the MicEco package (Russel 2024).

Results

Soil quality indicators as affected by farming systems

Basic soil sampling (T1) at bare soil showed differences in soil quality indicators as affected by the farming system for all response variables measured, except for netto nitrogen mineralization and bulk density (Fig. 2F and H). Most notably, SOC was

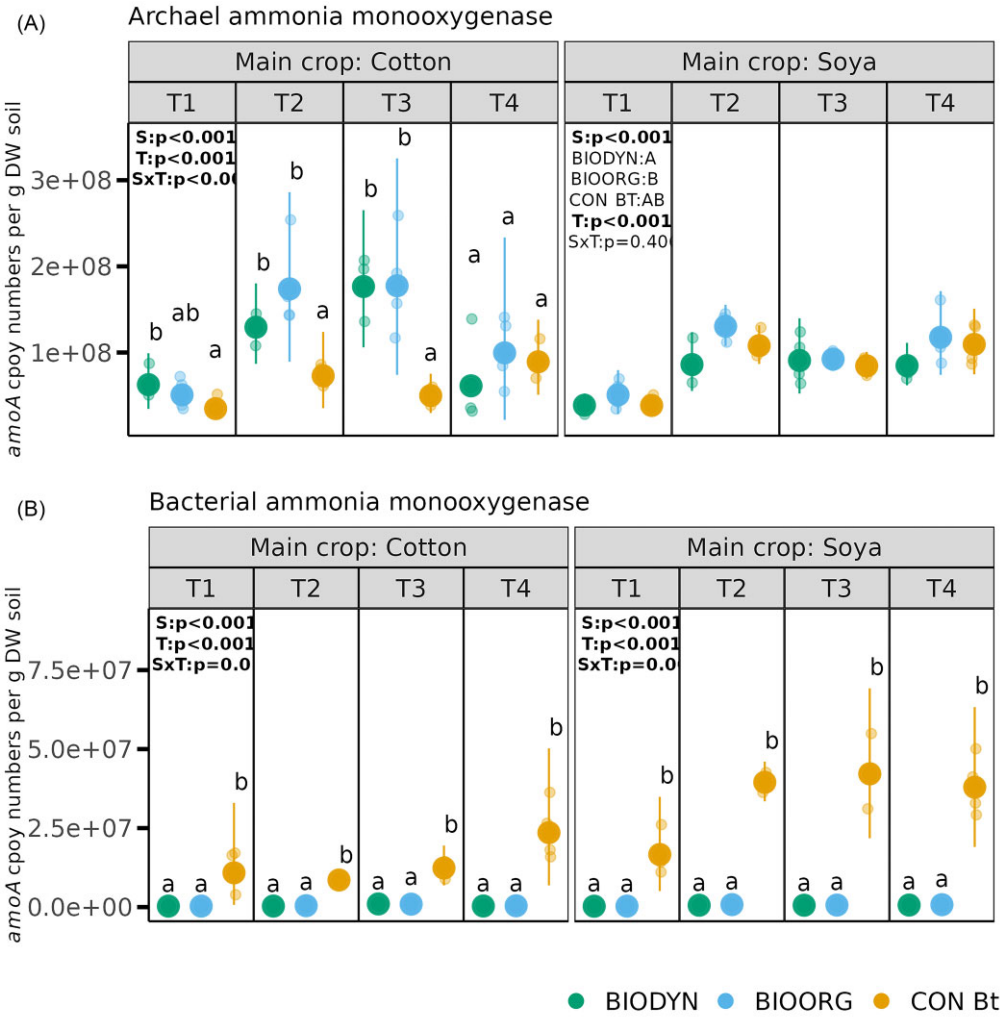

Figure 3. Treatment effects on bacterial and archaeal *amoA* abundance as assessed with quantitative real-time polymerase chain reaction (qPCR). Plots show raw data and estimated marginal means with 95% confidence intervals of linear mixed effect models assessing farming system (S) and crop cultivation phase (T) and the interaction effect (SxT) on bacterial and archaeal *amoA* abundance during cotton and soya cultivation phase. *P*-values of the main effects are presented within each plot in bold. Results of Sidak post hoc tests are indicated as small letters when a significant interaction effect was found. Without a significant interaction effect, Sidak posthoc tests were performed for systems across sampling dates, and results are given below the main effects in capital letters. BIODYN: biodynamic farming system; BIOORG: bio-organic farming system; CON Bt: conventional farming system with Bt cotton cultivation in the crop rotation.

significantly higher in BIODYN compared to CON Bt (+13.1%; Fig. 2A). POXC was comparable in the two organic systems with significantly higher values than CON Bt (BIODYN vs CON Bt: +40.2%; BIOORG vs CON Bt: +38.9%, Fig. 2B). As for SOC, significantly higher values in BIODYN compared to CON Bt were also detected for total soil nitrogen (+17.6%; Fig. 2C), and for the biological soil quality indicators microbial biomass carbon (+23.0%; Fig. 2D), microbial biomass nitrogen (+33.7%; Fig. 2E), and basal respiration (+17.0%; Fig. 2G). Data on $NH4^+$ was affected only by time and exhibiting considerable variability among biological replicates. For $NO3^-$, both the time point for cotton and the system-time interaction under soy cultivation were significant, with higher values observed in conventional systems compared to organic systems at T2 in the strip with main crop soya (Supplementary Fig. S3).

Functional gene abundances as affected by farming system and crop cultivation phase

AOA abundance in strip cotton was affected by the farming system, yet this effect was dependent on the crop cultivation phase (Fig. 3A): Differences between the systems were most pronounced at T2 and T3, where values in the CON Bt system were significantly lower when compared to the BIODYN system (T2: −35.8%, T3: −52.8%, Fig. 3A) and BIOORG system (T2: −45.0%, T3: −52.8%, Fig. 3A). In the strip growing soya, distinctions arose exclusively from the farming systems, with significant differences existing only between the two organic systems (BIOORG vs BIODYN: 25.9%, Fig. 3A). AOB abundance in both strips, was affected by the farming system. More specifically, AOB abundance was strongly enhanced in CON Bt compared to both organic farming systems, with the magnitude of the effect being dependent on the crop cultivation phase (Fig. 3B). Overall, AOB abundance was highest in the strip with soya (Fig. 3B). The abundance of functional genes involved in the regulation of organic nitrogen mineralization showed a variable pattern. While *npr* remained below the detection limit, *apr* was significantly higher under the organic compared to the conventional system in the strip growing soya as the main crop. Under cotton cultivation, however, a significant interaction between time and system was observed, with the highest abundance in CONBt except at T3 (Supplementary Fig. S4).

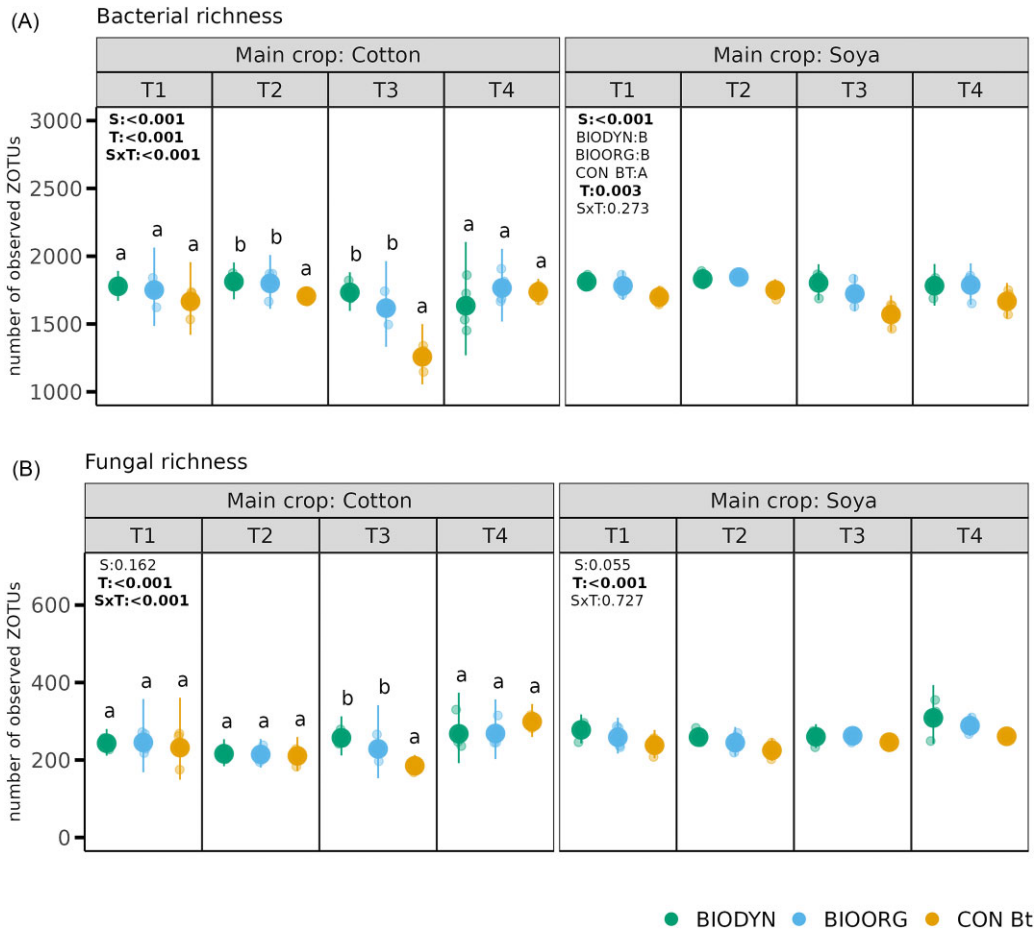

Figure 4. Treatment effects on bacterial and fungal observed richness. Plots show raw data and estimated marginal means with 95% confidence intervals of linear mixed effect models assessing the effects of farming system (S) and crop cultivation phase (T) and their interaction (SxT) on bacterial and fungal richness during the cotton and soya cultivation phase. *P*-values of the main effects are presented within each plot in bold. Results of Sidak post hoc tests are indicated as small letters when a significant interaction effect was found. Without a significant interaction effect, Sidak posthoc tests were performed for systems across sampling dates, and results are given below the main effects in capital letters. BIODYN: biodynamic farming system; BIOORG: bio-organic farming system; CON Bt: conventional farming system with Bt cotton cultivation in the crop rotation.

Soil microbial richness as affected by farming system and crop cultivation phase

In total, we detected 22 bacterial phyla with the five most abundant phyla being assigned to *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, and *Firmicutes* (Supplementary Fig. S5A); 1.4% of the sequences remained unassigned on the phylum level. Fungal communities were composed of 10 phyla with the five most abundant phyla being *Ascomycota*, *Basidiomycota*, *Glomeromycota*, *Chytridiomycota*, and *Mucoromycota* (Supplementary Fig. S5B); 22% of the fungal sequences remained unassigned on the phylum level.

In the cotton strip, the farming system and the crop cultivation phase interactively affected bacterial richness with no statistically significant differences between the farming system at T1 and T4, but with significantly higher ZOTU richness, both, in the BIODYN and BIOORG compared to the CON Bt system at the time points T2 and T3 (Fig. 4A, left panel). No interaction effect was found in the strip with soya, where the two organic systems constantly showed significantly higher values across the crop cultivation phase compared to CON Bt (Fig. 4A, right panel). In the cotton strip, fungal richness was interactively affected by the farming system and the crop cultivation phase with comparable ZOTU richness between the three systems at all time points except for T3, where BIODYN and BIOORG showed higher values compared to CON Bt (Fig. 4B, left panel). In the soya strip, fungal richness was marginally affected by the farming system (Fig. 4B, right panel).

Soil microbial community structure as affected by farming system and crop cultivation phase

PERMANOVA analysis revealed that the bacterial and fungal community structures were significantly and interactively influenced by farming system and crop cultivation phase, across all kingdoms and crops except for bacteria under soya cultivation. In the latter case, we only found significant main effects for the farming system and the crop cultivation phase (Table 2).

CAP ordinations of bacterial and fungal community structures revealed further insight into the structural alteration of bacterial and fungal communities across farming systems and along the crop cultivation phase (Fig. 5). Bacterial communities in the cotton strip differed between sampling dates along the first CAP axis with T1 and T2 most dissimilar to each other. The organic and conventional farming systems were separated along the second CAP axis with BIOORG and BIODYN being very similar (Fig. 5A) and clearly separated from CON Bt. The interaction effect was visible in T2 and T3, where microbial community structure in CON Bt remain rather similar, while in the organic systems a shift in community composition was observed along the second CAP axis.

Table 2. Treatment effects on bacterial and fungal community structure (ß-diversity).

Kingdom	Main crop	Source of variation	Df	Sum Of Sqs	R2	P-value
Bacteria	Cotton	System	2	0.19	0.09	0.005
		Time	3	0.75	0.35	0.005
		System:Time	6	0.23	0.11	0.015
		Residual	36	0.95	0.46	
		Total	47	2.12	1.00	
	Soya	System	2	0.17	0.12	0.005
		Time	3	0.37	0.25	0.005
		System:Time	6	0.15	0.10	0.195
		Residual	35	0.80	0.53	
		Total	46	1.50	1.00	
Fungi	Cotton	System	2	1.82	0.23	0.005
		Time	3	1.46	0.18	0.005
		System:Time	6	0.83	0.11	0.045
		Residual	36	3.80	0.48	
		Total	47	7.90	1.00	
	Soya	System	2	2.32	0.27	0.005
		Time	3	2.27	0.27	0.005
		System:Time	6	0.80	0.09	0.020
		Residual	36	3.10	0.36	
		Total	47	8.48	1.00	

Results of PERMANOVA assessing the effects of farming system (System), crop cultivation phase (Time), and their two-way interactions on bacterial and fungal community structure in the two strips 'main crop: cotton' and 'main crop: soya'. Factors printed in bold explain a statistically significant amount of variation in bacterial and fungal community structure. Df: degrees of freedom, Sum Of Sqs: sum of squares

Comparing the bacterial community structure in the soya strip across the cultivation phase, we found that, regardless of the farming system, the bacterial community remained stable at T2, T3, and T4. However, at T1, it was notably distinct when compared with the communities observed at the subsequent sampling dates (Fig. 5B). Congruent with PERMANOVA results, which showed no interaction effect between sampling date and farming system, the clustering of the farming systems was consistent across the crop cultivation phases with organic and conventional systems being separated along the first CAP axis.

For fungal community structure in cotton, a clear separation between organic and conventional systems was observable as well between T1 compared to T2, T3, and T4 (Fig. 5C). The interaction effect is evident in the more distinct separation of the sampling dates T2, T3, and T4 under conventional management, while there is no clear distinction between those dates of sampling observable under organic farming. Fungal community structure in soya similarly shifted over the crop cultivation phase and clustered in organic versus conventional; however, effects of sampling dates during the crop cultivation phase were more pronounced in the organic than the conventional system (Fig. 5D), which was already indicated by the interaction effect of the PERMANOVA (Table 2).

Analyses for multivariate homogeneity of group dispersion indicate that the main effect's significance was influenced by differences in both location (centroid) and spread (multivariate dispersion) for bacteria under cotton and fungi under soya (Supplementary Table S6). However, the ordination pattern in Fig. 5A and D clearly illustrates that any observed effect primarily derives from differences in location rather than from pure variations in dispersion.

Indicative species associated with farming systems

In total and across the crop cultivation phase, we identified ten bacterial ZOTUs and three fungal ZOTUs in strip cotton and nine bacterial ZOTUs and eight fungal ZOTUs in strip soya (Table 3) as indicator ZOTUs ($q < 0.05$, $r2 > 0.7$) for one or two farming systems. All hereafter listed taxonomic names enclosed in brackets are assignments to the lowest possible taxonomic rank. More detailed, ZOTU9 (*Chaetomiaceae*), ZOTU6606 (*Rhodospirillales*), ZOTU1272 (*Acidimicrobiales*), and ZOTU113 (*Chloroflexi*) were identified as indicator ZOTUs for the two organic systems in both strips. In contrast, ZOTU2812 (*Nitrosospira*) and ZOTU25 (*Sordariomycetes*) were identified as indicator species for the CON Bt system in both strips.

Additionally, six bacterial and one fungal indicator ZOTU for the farming systems were found in cotton but not in soya. More specifically, ZOTU649 (*Peptostreptococcaceae*), ZOTU 1290 (*Actinobacteria*), and ZOTU4357 (*Gaiellaceae*) were identified as indicators for the two organic systems and bZOTU1871 (*Thermomicrobia*), ZOTU 761 (*Nitrolancea*), ZOTU1875 (*Gaiellales*), and ZOTU988 (*Fusarium acutatum*) were found to be indicative for the CON Bt system.

Another five bacterial and six fungal indicator ZOTUs for the farming systems were found in soya but not in cotton. More specifically, ZOTU5440 (*Xanthomonadales*), ZOTU2474 (*Planococcaceae*), ZOTU6604 (*Solirubrobacterales*), and ZOTU2 (*Ascomycota*) were all identified as indicators for BIODYN and BIOORG. Contrary, ZOTU2742 (*Nitrospira*), bZOTU147 (*Gemmatimonadaceae*), ZOTU633 (*Eurotiomycetes*), ZOTU57 (*Neotestudina*), ZOTU50 (*Fusarium algeriense*), ZOTU261 (*Penicillium*), ZOTU25 (*Sordariomycetes*), and ZOTU15 (*Acrophialophora*) were identified as indicator ZOTUs for the CON Bt system.

As already indicated by CAP ordination, there were little differences in BIODYN and BIOORG microbial communities and no indicator ZOTUs distinguishing these systems could be identified.

Discussion

Higher soil quality in organic compared to conventional farming system

The first hypothesis of this study was supported by generally elevated soil quality indicators in organic compared to conventional

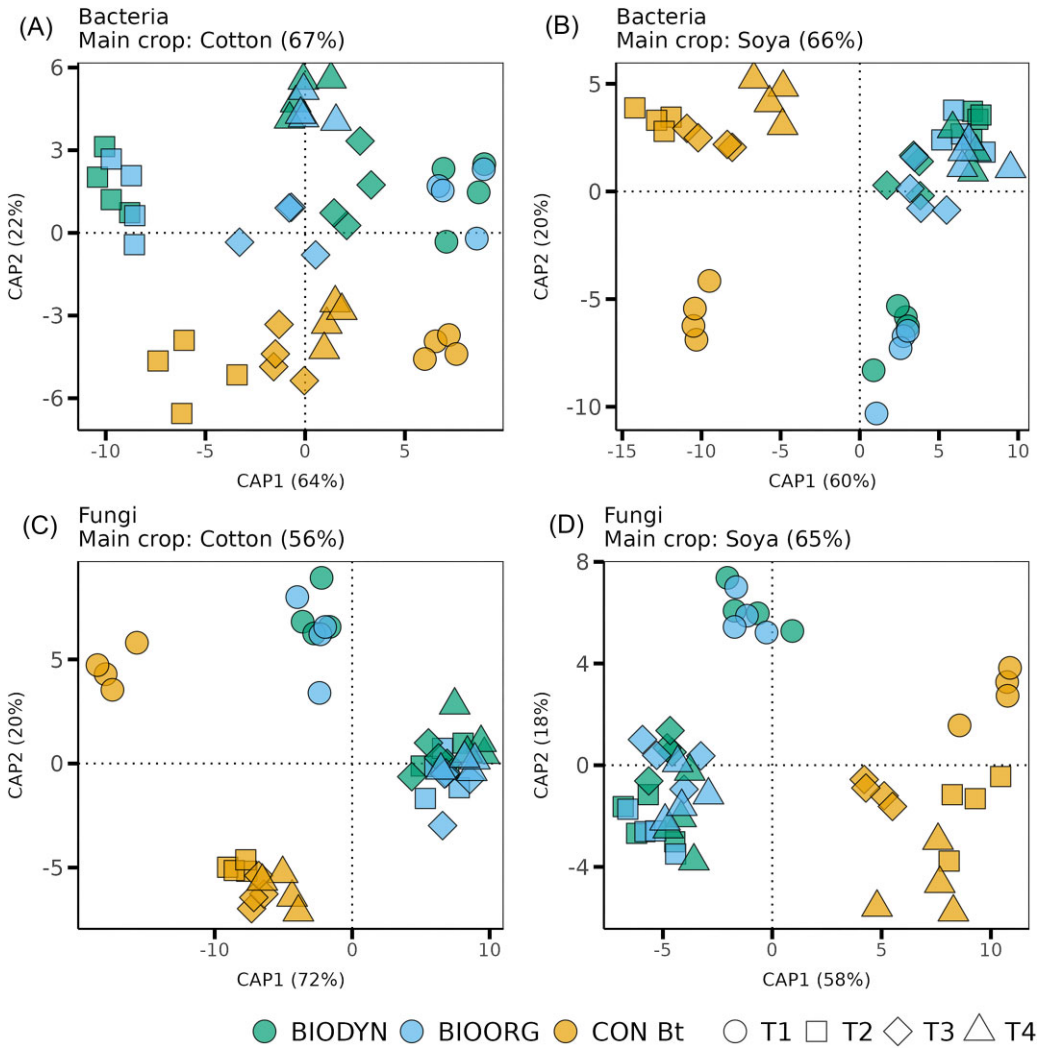

Figure 5. Canonical analyses of principal coordinates (CAP) of bacterial and fungal communities during cotton and soya vegetation period. CAP ordinations of bacterial (A and B) and fungal (C and D) communities based on relative abundances and Bray–Curtis distances, constrained by farming system and crop cultivation phase. The overall reclassification success rate is given in the title for each CAP ordination. Shapes represent the different crop stages (sampling dates) and colours represent farming systems: BIODYN: biodynamic farming system, BIOORG: bio-organic farming system, CON Bt: conventional farming system with Bt cotton cultivation in the crop rotation.

systems. This finding is consistent with existing knowledge from other geographical regions, as organic inputs like FYM, compost, and slurry are recognized for their ability to increase SOC concentration, thereby stimulating biological soil quality indicators such as microbial biomass and activity, especially in carbon-depleted agricultural soils. However, it is noteworthy that even in conventional plots, on average 5.4 t ha^{-1} manure was applied once a year before sowing of cotton at until 2018 (i.e. every second year to a particular strip). Nevertheless, the higher SOC and nitrogen contents in BIODYN compared to CON Bt derives from nutrient inputs through compost (Table 1). The enhanced SOC contents provide the substrate and habitat for microbial growth, thereby promoting higher microbial biomass and basic activity while additionally stabilizing soil pH, as observed especially between BIODYN and CON Bt (Fig. 2).

The long-term impact of farming systems on soil quality extends over multiple vegetation periods and it is important to acknowledge that measurable changes might take more than a decade to manifest (Krause et al. 2022). Therefore, it is not surprising that in the same field experiment, after seven years of establishment, Bhat et al. (2017) investigated soil biological parameters and found no difference between organic and conventional systems in terms of soil microbial biomass and soil respiration using the same methodology. Similarly, the study by Schweizer et al. (2022) could not identify differences in organic carbon contents between organically and conventionally managed systems after seven years of establishment. Still, it appears that the sensitivity of the employed methods was adequate to resolve differences between organic and conventional systems for most soil quality indicators twelve years after the establishment of the experiment. Particularly, POXC serving as a proxy for labile carbon was able to distinguishing organic and conventional systems. This sensitivity of POXC reflects the availability of carbon for the soil microbiome and was identified as a predictive indicator for soil quality, closely associated with soil biological processes (Bongiorno et al. 2019, Bongiorno 2020). Thus, in our study, we speculate that the difference in the labile SOC fraction between the organic and conventional systems may be a key driver of the distinct microbial community structures observed in these systems.

Table 3. List of indicators ZOTUs associated with different farming systems or farming system combinations.

Main crop	Association	ZOTU	r_pb	q-value	RA [%]	*Kingdom*	*Phylum*	*Class*	*Order*	*Family*	*Genus*
Cotton	BIODYN, BIOORG	ZOTU649	0.78	0.004	0.035	Bacteria	*Firmicutes*	*Clostridia*	*Clostridiales*	*Peptostreptococcaceae*	
	BIODYN, BIOORG	ZOTU1290	0.71	0.004	0.037	Bacteria	*Actinobacteria*	*MB-A2-108*			
	BIODYN, BIOORG	**ZOTU1272**	0.75	0.004	0.055	Bacteria	*Actinobacteria*	*Acidimicrobiia*	*Acidimicrobiales*	*OM1_clade*	
	BIODYN, BIOORG	**ZOTU6606**	0.80	0.004	0.075	Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Rhodospirillales*	*MSB-1E8*	
	BIODYN, BIOORG	ZOTU4357	0.70	0.004	0.110	Bacteria	*Actinobacteria*	*Thermoleophilia*	*Gaiellales*	*Gaiellaceae*	
	BIODYN, BIOORG	**ZOTU113**	0.75	0.004	0.360	Bacteria	*Chloroflexi*	*KD4-96*			
	BIODYN, BIOORG	**ZOTU9**	0.72	0.006	9.036	Fungi	*Ascomycota*	*Sordariomycetes*	*Sordariales*	*Chaetomiaceae*	
	CON Bt	ZOTU1817	0.70	0.004	0.011	Bacteria	*Chloroflexi*	*Thermomicrobia*	*JG30-KF-CM45*		
	CON Bt	ZOTU761	0.72	0.004	0.027	Bacteria	*Chloroflexi*	*Thermomicrobia*	*Sphaerobacterales*	*Sphaerobacteraceae*	*Nitrolancea*
	CON Bt	**ZOTU2812**	0.88	0.004	0.046	Bacteria	*Proteobacteria*	*Betaproteobacteria*	*Nitrosomonadales*	*Nitrosomonadaceae*	*Nitrosospira*
	CON Bt	ZOTU1875	0.73	0.004	0.091	Bacteria	*Actinobacteria*	*Thermoleophilia*	*Gaiellales*		
	CON Bt	ZOTU988	0.73	0.006	0.543	Fungi	*Ascomycota*	*Sordariomycetes*	*Hypocreales*	*Nectriaceae*	*Fusarium*
	CON Bt	**ZOTU25**	0.72	0.006	0.712	Fungi	*Ascomycota*	*Sordariomycetes*			
Soya	BIODYN, BIOORG	ZOTU6604	0.70	0.003	0.020	Bacteria	*Actinobacteria*	*Thermoleophilia*	*Solirubrobacterales*	*Elev-16S-1332*	
	BIODYN, BIOORG	**ZOTU1272**	0.71	0.003	0.055	Bacteria	*Actinobacteria*	*Acidimicrobiia*	*Acidimicrobiales*	*OM1_clade*	
	BIODYN, BIOORG	ZOTU2474	0.70	0.003	0.069	Bacteria	*Firmicutes*	*Bacilli*	*Bacillales*	*Planococcaceae*	
	BIODYN, BIOORG	**ZOTU6606**	0.79	0.003	0.075	Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Rhodospirillales*	*MSB-1E8*	
	BIODYN, BIOORG	ZOTU5440	0.75	0.003	0.180	Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*		
	BIODYN, BIOORG	**ZOTU113**	0.74	0.003	0.360	Bacteria	*Chloroflexi*	*KD4-96*			
	BIODYN, BIOORG	**ZOTU9**	0.71	0.001	9.036	Fungi	*Ascomycota*	*Sordariomycetes*	*Sordariales*	*Chaetomiaceae*	
	BIODYN, BIOORG	ZOTU2	0.78	0.001	9.235	Fungi	*Ascomycota*				
	CON Bt	**ZOTU2812**	0.93	0.003	0.046	Bacteria	*Proteobacteria*	*Betaproteobacteria*	*Nitrosomonadales*	*Nitrosomonadaceae*	*Nitrosospira*
	CON Bt	ZOTU2742	0.75	0.003	0.083	Bacteria	*Nitrospirae*	*Nitrospira*	*Nitrospirales*	*Nitrospiraceae*	*Nitrospira*
	CON Bt	ZOTU147	0.72	0.003	0.466	Bacteria	*Gemmatimonadetes*	*Gemmatimonadetes*	*Gemmatimonadales*	*Gemmatimonadaceae*	
	CON Bt	ZOTU261	0.73	0.001	0.083	Fungi	*Ascomycota*	*Eurotiomycetes*	*Eurotiales*	*Aspergillaceae*	*Penicillium*
	CON Bt	ZOTU633	0.81	0.001	0.243	Fungi	*Ascomycota*	*Eurotiomycetes*			
	CON Bt	ZOTU50	0.73	0.001	0.339	Fungi	*Ascomycota*	*Sordariomycetes*	*Hypocreales*	*Nectriaceae*	*Fusarium*
	CON Bt	**ZOTU25**	0.70	0.001	0.712	Fungi	*Ascomycota*	*Sordariomycetes*			
	CON Bt	ZOTU57	0.77	0.001	2.425	Fungi	*Ascomycota*	*Dothideomycetes*	*Pleosporales*	*Testudinaceae*	
	CON Bt	ZOTU15	0.71	0.001	6.153	Fungi	*Ascomycota*	*Sordariomycetes*	*Sordariales*	*Chaetomiaceae*	

Fungal and bacterial indicators ZOTUs were identified separately for each main crop with associations to one or two farming systems allowed. ZOTUs with $q < 0.05$ and $r^2 > 0.7$ are presented. Bold highlighted ZOTUs were found indicative for a specific farming system in both crops. RA = relative abundance. r_pb = point biserial correlation coefficient.

The absence of significant differences among the two organic systems appears to be attributed to their nearly similar management practices targeted to achieve the same nutrient inputs. In addition to compost, minute quantities of 'clay pot dung', 'vermicompost', and 'Jeev Amrut' were used in BIOORG from 2007 to 2010 years (Forster et al. 2013). Despite a tendency towards higher soil quality in BIODYN compared to BIOORG, we were unable to resolve the potential effects of biodynamic management on soil quality twelve years after the establishment of the experiment. This finding aligns with another long-term field experiment from a temperate region that investigated the impacts of biodynamic preparations in organic farming under reduced tillage and plough and found no significant effect on key indicators including SOC, microbial biomass carbon, soil respiration, and soil pH (Krauss et al. 2020). In summary, we found higher soil quality in organic systems compared to conventional farming systems, presumably driven by organic input management. However, we could not resolve differences between bioorganic and biodynamic management on geochemical soil quality indicators.

Higher soil quality, however, did not directly translate into higher plant biomass or nutrient contents. For soya, plant biomass and nitrogen, phosphorus, and potassium contents were not significantly different across the three systems (Supplementary Fig. S6), even though the conventional system received slightly higher amounts of nitrogen, phosphorus, and potassium fertilization (Table 1). This finding partially aligns with previous meta-analyses comparing the yield gap between organic and conventional farming, showing soya to have in general a rather small one, comparable to other crops (Seufert et al. 2012, Knapp and van der Heijden 2018). Thus we conclude that organic soybean production is a viable option under the semi-arid conditions in India as it improves soil quality and leads to similar yields (Forster et al. 2013) compared to conventional. However, for organic cotton, we observed that the plant nitrogen content was 17% lower in BIODYN and 35% lower in BIOORG compared to CONBt, while the organic systems received 17% less nitrogen fertilizer.

Distinct soil microbial communities in organically compared to conventionally managed systems

The study confirmed our second hypothesis, showing clear differences in microbial communities between organic and conventional farming. These communities also varied during crop cultivation phases, highlighting the influence of management practices on microbial community structure across the vegetation period. Soil life is intricately linked to carbon availability and soil pH, which serve as key determinants influencing the structure and composition of the soil microbiome (Lammel et al. 2018, Garcia-Palacios and Ji 2022). Fungi, in particular, being primarily classified as heterotrophs with a narrow range of nutritional strategies, are strongly affected by carbon availability. In comparison to a prior investigation in tropical arable systems (Choudhary et al. 2022), we observed a relatively low fungal richness despite adequate sequencing coverage (Supplementary Fig. S2). While low richness does not necessarily imply low fungal biomass, it is necessary to recognize that the chloroform fumigation extraction method employed in this study does not allow us to differentiate fungal from bacterial biomass. To establish a link between richness and biomass alternative methodological approaches such as qPCR/ddPCR (Wang et al. 2022b) or Ergosterol (Mille-lindblom et al. 2004) may be required. Yet, it is plausible that relatively low fungal richness reflects generally harsh conditions in Vertisols characterized by periodically extreme alteration in soil conditions compared to other studies in arable tropic systems (Choudhary et al. 2022, Krause et al. 2023). Consistent with a recent regional study (Choudhary et al. 2022), the fungal community structure in our study was dominated by *Ascomycota* (Supplementary Fig. S3), which is ubiquitously in soil environments (Egidi et al. 2019). The majority of *Ascomycota* are saprophytic and serve as the primary decomposers of plant residues in the soil. The bacterial phyla identified in our study exhibit close similarities with the findings of Khatri et al. (2023), who investigated microbial communities in arable systems in India and found *Plancotymcetes* and *Bacteroidetes* rather than *Actinobacteria*, in addition to *Proteobacteria, Chloroflexi,* and *Acidobacteria* to be the most dominant phyla. On the phylum level, both fungal and bacterial community structures changed throughout the crop cultivation phase in our study, with more pronounced shifts observed during cotton compared to soya cultivation (Supplementary Fig. S5). Potentially, these observations might be explained by higher fertilizer inputs during the cultivation of cotton compared to soya. Additionally also soil water content is known to have a profound impact on soil bacterial and fungal community structure (Yan et al. 2015), and our samples from the different dates of sampling varied in their water content (Supplementary Fig. S7). The first date of sampling took place before sowing of cotton or cover crop and the end of the dry season was approaching. Thus, very low soil moisture combined with bare soil likely was the main determinant for microbial community structure. Despite strong temporal effects on bacterial and fungal community structure, we found consistent and strong farming system effects across both crop cultivation phases (Table 2). The application of organic fertilizer has previously been shown to be a major driver in shaping microbial community structure of organic and conventional systems (Hartmann et al. 2015) and is likely also a major driver of distinct microbial communities in the organically and conventionally managed systems in this study. Interestingly, the two organically managed systems consistently showed to be very similar to each other and dissimilar to CON Bt. Interestingly, bacterial richness dropped during cotton cultivation phase in conventional but not in organic systems indicating a possible response of microbes to mineral fertilization events (Fig. 4). Our results are in line with a recent global meta-analysis assessing the effect of organic and mineral fertilizer on soil microbial diversity and showing that bacterial taxonomic diversity is about 2.9% higher in organically compared to minerally fertilized systems (Bebber and Richards 2022). However, using a system comparison approach, we cannot disentangle whether the observed differences between organically and conventionally managed systems are driven by fertilizer only or whether pest and disease control as well contributed.

In summary, our data demonstrate that 12 years of distinct management practices implemented in the different farming systems in the semi-arid subtropics exert a discernible influence on the soil microbial community structure throughout the vegetation periods of cotton and soya. Nevertheless, notable shifts in microbial community structure occur during the crop cultivation phases, likely attributable to fertilization events and abiotic factors, such as soil moisture.

Distinct indicative taxa in conventional and organic farming systems

Lastly, our final hypothesis was validated, as we indeed identified specific taxa with distinct potential functions in both the organically and conventionally managed systems. Although the vast

majority of ZOTUs were shared among all treatments (99.0% for bacteria, 85% for fungi, Supplementary Fig. S8), indicator species analyses showing the association of specific indicator ZOTUs with either organic or conventional systems may suggest drivers of shifts in community structure. In conjunction with the very similar beta-diversity observed in both organically managed systems and the indicator species analysis highlighting numerous ZOTUs indicative of either both organic systems collectively or exclusively associated with the conventional system (Table 3). These findings further underscore the substantial resemblance between the organically managed systems likely attributed to the application of organic fertilizers as a potential driving force behind their microbial communities' similarity.

This observation is further supported by the finding of the fungal ZOTU9, taxonomically classified as *Chaetomiaceae,* which was consistently identified as an indicator of the organically managed systems in both crops. A study conducted by Dang et al. (2021) showed that *Chaetomiaceae,* being saprophytic ascomycetes, is one of the most significant groups increasing in relative abundance in response to compost addition. Also, ZOTU6606, taxonomically assigned to *Rhodospirillales* and known to be a diverse group of bacteria with a range of ecological functions in soil environments, was one of three bacterial ZOTUs associated with the organically managed systems for both crops. *Rhodospirillales* are phototrophic, meaning they are capable of using light as an energy source, and they are also facultative anaerobes (Baldani 2014), highlighting their diverse metabolic lifestyle. The second bacterial ZOTU associating with organically managed systems in both crops is taxonomically assigned to *Chloroflexi*, which also exhibit diverse metabolic lifestyles as facultative anaerobic phylum, including autotrophic, heterotrophic, and mixotrophic taxa (Speirs et al. 2019). The last bacterial indicator identified for the two organic farming systems in both crops was ZOTU1272, taxonomically assigned to the *Acidimicrobiales*. Interestingly, a study by Randall et al. (2019) showed *Acidomicrobiales* to decrease upon P fertilization, highlighting their potential role in P mobilization when soil P concentrations are low. These findings may serve as a basis for understanding the diverse energy metabolism mechanisms required in microbial communities under organic farming, which can efficiently degrade organic carbon sources and obtain energy and nutrients through various metabolic strategies, and are consistent with observations by Bhat et al. (2017), who reported higher activity of alkaline phosphatase activity in the organically managed systems of the herein analysed soils.

Further evidence of enhanced P-mineralization under organic systems was found under cotton cultivation with enriched abundances of ZOTU4357 in organic systems. ZOTU4357 assigns to the family of *Gaiellaceae,* which has been previously linked to available phosphorus content and phosphatase activity (Wang et al. 2022b). Their study revealed that organically managed soils could attain equivalent or higher P availability compared to conventionally managed soils receiving regular inputs of mineral P fertilizers and might be driven by the elevated microbial activity associated with organic farming practices.

In turn, conventional systems resulted in enhanced abundance of the bacterial ZOTU 2812, taxonomically classified to the family of *Nitrosospira* in both crops. *Nitrosospira* are AOB playing a pivotal role in facilitating the initial step of the aerobic nitrification process (Hayatsu et al. 2021), having significant implications for nitrogen cycling (Kowalchuk and Stephen 2001). Generally, ammonia-oxidation is performed by AOB belonging to two monophyletic groups within the beta- and gamma-proteobacteria (Purkhold et al. 2000), and by AOA belonging to *Thaumarchaeota* phylum (Brochier-Armanet et al. 2008). In the present experiment, a divergent response of AOA and AOB abundance to organic and conventional farming systems was observed indicating a niche differentiation, which has also recently been reviewed by Prosser et al. (2020). AOB demonstrated higher abundance in conventionally managed systems as opposed to organically managed systems but the extent of the difference varied throughout the crop cultivation phases (interaction effect) (Fig. 3). However, the consistently elevated abundance of AOB in the conventional system might be directly linked to regular application of mineral nitrogen (Taylor et al. 2012). The strong enrichment of AOB in conventionally managed systems might enhance nitrification and subsequently potential losses of nitrogen fertilizers via nitrate leaching or emissions of nitrous oxide from soils (Prosser et al. 2020). Our data on soil mineral nitrogen exhibits considerable variability. However, we observed significantly higher $NO3^-$ levels in conventional systems compared to organic systems at T2 only in the strip growing soya as main crop. This finding may be explained by the fact that, prior to T2, the soil had been bare since the harvest of winter wheat 2 (see Fig. 1) in the conventional plots. The higher abundance of AOB in the conventional system might have led to elevated NO_3 levels, which were not absorbed by plants due to the lack of plant cover. The effects of farming systems on AOA highly depend on the crop and vegetative phase. Enriched AOA gene were especially present in organic systems during cultivation of cotton, when soil water and temperature conditions facilitate decomposition of organic inputs. Our results of AOA and AOB abundance are in line with previous studies showing that AOB growth is favoured in soils fertilized by single additions of high levels of inorganic NH_3, while AOA grows preferentially when NH_3 is produced through mineralization of organic nitrogen (Verhamme et al. 2011, Hink et al. 2018, Prosser et al. 2020).

Additionally, during soya cultivation, enhanced abundances of ZOTU2742 in conventional systems likely linked to potentially enhanced nitrification activity, as this taxon is assigned to the genus of *Nitrospira,* which is considered to be strictly nitrite-oxidizing. However, recent studies have revealed the presence of several *Nitrospira* species that possess the ability to perform complete ammonia oxidation (comammox) in a singular cell (Daims et al. 2016). The increased abundance of *Nitrospira* in conventionally managed soil can likely be attributed to the application of mineral nitrogen as well. This finding is in line with previous discussions around AOB and is likely associated with enhanced risks for nitrogen losses. Our study provides important insights into the genetic nitrification potential in a semi-arid suptropical agroecosystem and we strongly recommend to verify putative enhanced N-leaching and N_2O emissions under conventional management using follow-up experiments.

For organic cotton farming, we found an indicative ZOTU assigned to the *Peptostreptococcaceae* belonging to the phylum of *Firmicutes,* which has been previously shown to be more abundant in organically compared to conventionally managed soil (Harkes et al. 2019). Interestingly, we found an association of putatively pathogenic fungi with the conventional systems under soya and cotton cultivation. More precisely, a ZOTU annotated as *Fusarium acutatum*, which is known to cause root rot on legumes (Gautam et al. 2016) and is a major pathogen contributing to basal rot of onion in India (Bhat et al. 2023)) was indicative of conventional cotton. Whereas under soya cultivation, we identified two indicative ZOTUs with putative pathogenic traits. More precisely, ZOTU assigned to *Fusarium algeriense* is a novel toxigenic crown rot pathogen of durum wheat (Laraba et al. 2017) and winter wheat (Özer et al. 2018) and is associated with CON Bt. Another

potentially toxigenic ZOTU assigned to *Neotestudina* was indicative of CON Bt under cotton cultivation as well. Indicative taxa annotated as potentially pathogenic fungi might be linked to diminished disease suppression under conventional farming due to reliance on external synthetic chemicals. A disease suppression test would be an interesting task to verify this hypothesis.

In summary, the taxa identified as indicative of organic farming systems are putatively characterized by diverse metabolic lifestyles associated with the degradation of organic compost and phosphorus mineralization. On the other hand, the taxa indicative of conventional systems were more often associated with nitrification and may possess pathogenic properties. However, it is important to acknowledge that the ecological functions of the identified indicator ZOTUs are based on putative lifestyles only. To gain an even deeper understanding of soil multi-functionality under distinct farming practices in subtropical regions, further comprehensive analyses such as profiling microbial metabolic capacity (Creamer et al. 2016), proteomics (Qian and Hettich 2017), shotgun metagenomics (Vogel et al. 2009), and combinations of these techniques (Martinez-Alonso et al. 2019) would be beneficial.

Conclusion

In summary, our study indicates that twelve years of organic farming have improved soil quality and exerted a notable influence on microbial communities, fostering taxa with diverse metabolic lifestyles associated with organic compost degradation and phosphorus mineralization when compared to conventional farming in semi-arid subtropics. Conversely, conventional farming promotes the genetic potential for ammonium oxidation, which may increase the risk of nitrogen losses, and encourages the presence of indicator ZOTUs associated with pathogenic traits more frequently than organic farming. Given these findings, the adoption of organic farming practices in semi-arid subtropical areas can help improving distorted soil quality and likely mitigate the risk of nitrogen losses.

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Supplementary data

Supplementary data is available at *FEMSEC Journal* online.

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