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Organic management enhances soil quality and drives microbial community diversity in cocoa production systems

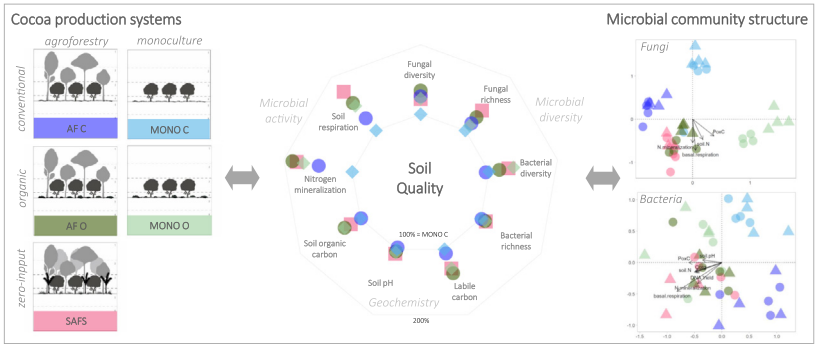
Martina Lori [a], Laura Armengot [b], Monika Schneider [b], Ulf Schneidewind [c], Natacha Bodenhausen [a], Paul Mäder [a], Hans-Martin Krause [a,*]

[a] Department of Soil Sciences, Research Institute of Organic Agriculture (FiBL), 5070 Frick, Switzerland
[b] Department of International Cooperation, Research Institute of Organic Agriculture (FiBL), 5070 Frick, Switzerland
[c] Georg-August University, Department of Physical Geography, 37077 Göttingen, Germany

HIGHLIGHTS

- Organic soil management increased soil quality in agroforestry systems
- Zero-input and organic agroforestry systems enhanced soil microbial activity compared to conventional monocropping.
- Fungal community composition changes in response to organic management practice and agroforestry
- Distinct soil bacterial community composition especially between organic and conventional systems
- Taxonomically diverse indicator species are associated with organically managed systems

GRAPHICAL ABSTRACT



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ABSTRACT

Maintaining soil quality for agricultural production is a critical challenge, especially in the tropics. Due to the focus on environmental performance and the provision of soil ecosystem services, organic farming and agroforestry systems are proposed as alternative options to conventional monoculture farming. Soil processes underlying ecosystem services are strongly mediated by microbes; thus, increased understanding of the soil microbiome is crucial for the development of sustainable agricultural practices. Therefore, we measured and related soil quality indicators to bacterial and fungal community structures in five cocoa production systems, managed either organically or conventionally for 12 years, with varying crop diversity, from monoculture to agroforestry. In addition, a successional agroforestry system was included, which uses exclusively on-site pruning residues as soil inputs. Organic management increased soil organic carbon, nitrogen and labile carbon contents compared to conventional. Soil basal respiration and nitrogen mineralisation rates were highest in the successional agroforestry system. Across the field sites, fungal richness exceeded bacterial richness and fungal community composition was distinct between organic and conventional management, as well as between agroforestry and monoculture. Bacterial community composition differed mainly between organic and conventional management. Indicator species associated with organic management were taxonomically more diverse compared to taxa associated with conventionally managed systems. In conclusion, our results highlight the importance of organic management for maintaining soil quality in agroforestry systems for cocoa production.

* Corresponding author.
E-mail address: hans-martin.krause@fibl.org (H.-M. Krause).

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1. Introduction

Within the last century, agricultural production was intensified to sustain the global population growth, and today agricultural activity has transformed ~40% of the earth's land surface (FAO, 2021; Kopittke et al., 2019). Consequently, biosphere integrity and biochemical flows have exceeded the planetary boundaries, and severe impacts on ecosystem functioning have been predicted (Steffen et al., 2015). Therefore, it is a critical challenge to develop agricultural practices that reduce their toll on biodiversity (Brondizio et al., 2019) and maintain ecosystem services provided by agricultural landscapes, especially soils (Adhikari and Hartemink, 2016).

In this regard, Lehmann et al. (2020) emphasise the role of "soil health as overarching principle that contributes to sustainability goals". The concept of soil health perceives soils as a living entity and is closely related to the concept of soil quality, which is defined as the continued capacity of soils to provide ecosystem services, such as sustaining biological productivity and regulation of elemental and water cycles (Lehmann et al., 2020). Within the last decades, soil quality assessment was mainly based on chemical and physical indicators (Lehmann et al., 2020). Thanks to a growing focus on environmental health, biological soil indicators such as soil respiration and nitrogen mineralisation (Bünemann et al., 2018) have gained increasing attention. In recent years, the number of studies investigating soil microbial communities via high throughput sequencing has grown, and characterization of microbial community structure evolved as emerging tool to complement biological assessments of soil quality (Fierer et al., 2021). Although the relationship between soil microbial diversity and ecosystem functioning is still not well understood, there is growing evidence that soil microbial diversity is an important biological resource at the base of ecosystem functioning and must be considered in agricultural management decisions (Wagg et al., 2021).

Organic farming is discussed as one possible option for more sustainable food production and aims at making optimal use of internal natural resources and processes to secure productivity while minimising environmental impacts such as loss of biodiversity, nutrient leakage and soil degradation (Seufert and Ramankutty, 2017). Despite lower yields, organic farming increasingly gains shares in food production (Seufert et al., 2012; Willer and Lernoud, 2017). Since synthetic fertilisers and pesticides are not allowed in organic farming systems, crop production heavily relies on soil fertility management via organic inputs and the inclusion of legumes in the crop rotation (Mäder et al., 2002). Consequently, organic management enhances topsoil carbon contents (García-Palacios et al., 2018; Gattinger et al., 2012) and soil microbial abundance and activity (Lori et al., 2017). While in temperate climates, the effect of organic farming on soil microbial community structure has been demonstrated (e.g. Hartmann et al., 2015), studies from the tropical region are still scarce (Lori et al., 2017; Pajares et al., 2016).

The diversification of food production systems, e.g. through the inclusion of perennial crops in agroforestry systems, aims to enhance environmental and economic performance of agricultural systems and is proposed as a multifunctional land-use strategy especially for the tropical climates (Niether et al., 2020; Ramachandran Nair et al., 2010). Agroforestry systems are supposed to enhance soil quality through root exudates and enhanced root structures (Dollinger and Jose, 2018), but the accurate determination of soil quality across agroforestry systems is inherently challenging due to spatial soil heterogeneities introduced by root structures of tree crops and/or shade trees (Lorenz and Lal, 2014). Therefore, soil analysis in agroforestry systems is often based on multiple sampling locations dependent on the distance to the tree crop (Cardinael et al., 2020).

While insights on the impact of organic farming on soil quality and soil microbial diversity are accumulating under temperate climates, few studies have addressed tropical climates. Moreover, it is still unclear whether organic farming in diversified agroforestry systems has a beneficial impact on soil quality. Therefore, we used a long-term field trial investigating organic and conventional management of monocultures and diversified cocoa production systems to assess soil quality and the underlying soil microbial community structure. We focused on biological soil quality indicators associated with organic matter mineralisation and hypothesised that agroforestry and organic management in cocoa production systems would: 1) enhance chemical and biological soil quality indicators, 2) result in higher microbial diversity with distinct community structure, and 3) enhance the diversity of soil bacterial and fungal indicator species.

2. Materials and methods

2.1. Study site and experimental set up of the field trial

The system comparison trial in Bolivia is located in Sara Ana (15°27′36.60″S and 67°28′20.65″W), in the Alto Beni region at an altitude of 380 m.a.s.l. at the eastern foothills of the Bolivian Andes. The site covers ~9 ha and soil types vary between lixisols and luvisols classes. Clay content across the field site ranges from 17 to 35%, and the average initial organic carbon (Corg) content of the field site was 1.5% (Schneidewind et al., 2019). The climate is tropical humid with dry winters (1535 mm) and a mean annual temperature of 26 °C. In 2007, a secondary forest was cleared and the field trial was set up in a complete randomised block design with four repetitions comparing five cocoa production systems. The cocoa production systems include two monoculture systems, under organic (MONO O) and conventional (MONO C) management. Furthermore, two agroforestry systems under organic (AF O) and conventional management (AF C) are included. Lastly, a highly diverse successional agroforestry system without external input use (SAFS) is included. The plot size is 48 m × 48 m, with a net plot for data collection of 24 m × 24 m located in the center of each plot (Fig. S1).

Cacao trees grow at a distance of 4 m × 4 m (36 cocoa trees in the net plot, 625 trees ha^{-1}). In the agroforestry systems bananas, fruit (e.g. *Theobroma grandiflorum*), timber (e.g. *Swietenia macrophylla*), and leguminous (e.g. *Erythrina* spp. and *Inga* spp.) trees grow in between cacao tree lines. A complete list of trees grown in the AF system can be reviewed in Schneider et al. (2016). In the SAFS, the same planting scheme for cocoa trees was followed but trees from the natural succession were kept, and additional seeds of trees and other crops were dispersed or planted. The total density of shade trees was 243 trees ha^{-1} for AF O and AF C and about 1181 trees ha^{-1} for SAFS, respectively (Niether et al., 2018).

In conventionally managed plots, weeds were controlled using brushcutters and herbicides (mixed with adherents), with 4–5 applications per year. AF C and MONO C received 150 and 300 kg synthetic fertiliser (Blaukorn BASF, 12-8-16-3 N-P_2O_5-K_2O-MgO) per ha and year, which was applied around each cacao tree by spreading the fertilisers at a distance of 0.25–1 m from each tree stem. In the organically managed plots, cacao trees were fertilised with compost prepared with biomass of the surrounding area (e.g. banana stems and pruning residues), purchased sawdust and chicken manure. Each tree received 21 l of compost in MONO O, which corresponds to ~8 kg of compost ha^{-1} and $year^{-1}$. Until 2016 the AF O systems received half the compost dose used in MONO O and was left unfertilized thereafter. Additionally, in the organically managed plots, a leguminous perennial cover crop (*Neonotonia wigthii*) was planted at the beginning of the trial to maintain soil cover and suppress weeds. In 2019, *Neonotonia wigthii* was still present in MONO O but almost completely vanished in the AF O system due to the development of the shade trees. The successional agroforestry system did not receive any external fertiliser input, but intensive pruning was regularly performed. In all production systems, pruning and crop residues were deposited at around 0.25–1 m distance to the stem of cacao trees together with the fertiliser and left for decomposition. Plant protection in the conventional plots is based on synthetic pesticides, while indirect measures and manual work are employed in the organic systems. Detailed management of the field trial can be reviewed in Schneider et al. (2016) and Pérez-Neira et al. (2020).

2.2. Soil sampling procedure

Soil sampling took place during the dry season, the peak cacao harvest, from the 28th until 31st of July 2019. After removing course organic

residues from the soil surface, soil cores were taken with an auger to 0–10 cm depth. Accounting for the possible impact of fertilisers addition around cacao trees and the impact of root structures of the shade trees in the agroforestry systems, separate soil samples were taken at 0.5 m ("under cacao") and 2 m ("between row") distance to the cacao tree. Across the field plot, composite soil samples from 20 and 16 soil cores were collected at 0.5 m and 2 m distance to cacao trees, respectively. Soil samples were sieved to 5 mm and homogenised. For molecular biological analysis, a subsample of around 50 g was oven-dried at 80 °C for 2 h as described in Pfeiffer et al. (2017). By quickly removing soil moisture we aimed to preserve soil microbial community structure during transport, as an uninterrupted cold chain was not guaranteed. Soil samples for geochemical analysis and quantification of microbial activities were air-dried for 48 h. Packs of silica beads were added during transport to Switzerland to avoid humidification of soil samples. After arrival and before analysis, samples for molecular biological and geochemical analyses were stored at −20 °C, and subsamples for biological activity analyses were stored at 4 °C.

2.3. Geochemical analysis

To determine Corg and total nitrogen (Ntot) contents, subsamples of the air-dried soil were ground and homogenised. For each sample two times 1 g was analysed via dry combustion method on a CN analyser (Elementar Analysensysteme GmbH, Vario MAX Cube, Hanau, Germany) at 500 °C and 950 °C to quantify inorganic and total C, respectively. Since inorganic C was negligible across the field site, total C was assumed to be similar to Corg. Soil pH was determined with a pH-Meter in an aqueous suspension 1:2,5 (w/v). Permanganate oxidisable carbon (PoxC) was extracted and analysed following the principles described in Weil et al. (2003) with slight modifications. Briefly, 2.5 g air-dried soil was weighed into a polyethylene tube, amended with 2 ml of a 0.2 M K_2MnO_4 solution and shaken for 2 min at 120 rpm. After an undisturbed reaction time of 8 min, 0.5 ml of the suspension was transferred to 49.5 ml of demineralised water and absorbance at 550 nm was measured using a GENESYS 10S UV–VIS Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) as described in Bongiorno et al. (2019).

2.4. Quantification of microbial activity

Before microbial activity measurements, air-dried soil samples were rewetted to 40% water holding capacity and incubated at constant 25 °C for 7 days. Aerobic C mineralisation was determined by incubation of soil samples for 28 days in hermetically sealed microcosms and the capture of CO_2 in alkali acid trap (0.025 M NaOH) as described in von Arb et al. (2020). Alkali traps were replaced after 1, 4, 7, 14 and 28 days. Soil basal respiration was defined as the average C mineralisation rate during the second week of incubation (Haney et al., 2001). Aerobic N mineralisation was determined by quantifying mineral N contents (NH_4^+-N and NO_3^--N) before and after the incubation period via automated flow injection analysis (Smartchem 450 Discrete Analyser, AMS France, Frepillon, FR). All incubations were performed with 15 g dry soil equivalents and technically replicated four times.

2.5. Molecular biology

DNA was extracted with the "NucleoSpin 96 Soil" kit (Machery-Nagel, Germany) according to the manufacturer's instructions. For each biological replicate, two technical replicates (400 mg dried soil each) were individually extracted and pooled afterwards. DNA quantity was assessed using Qubit system reagents (ThermoFisher Scientific, Waltham, USA), and relative fluorescent units were measured on a CFX96Touch Real-Time PCR Detection System (Bio-Rad, Switzerland). Dilution series of DNA extracts were tested for inhibitor presence and, consequently, DNA extracts were diluted 1:10 before further downstream application.

2.5.1. Amplicon sequencing

DNA extracts were processed in a two-step polymerase chain reaction (PCR) approach using fluidigm tagged primers targeting the 16S rRNA (314F - CCTAYGGGDBGCWSCAG and 806R - GGACTACNVGGGTHTCTAAT, modified by Frey et al. (2016)) and ITS2 genes (TS3ngs - CANCGATGAAGAACGYRG and ITS4ngs - CCTSCSCTTANTDATATGC (Tedersoo and Lindahl, 2016)). The first PCR was performed in triplicates using a 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, Switzerland) (Table S1). PCR triplicates of each sample were subsequently pooled and purified using a magnetic bead solution (https://openwetware.org/wiki/SPRI_bead_mix). A subsample of the purified DNA was loaded on agarose gels (1.25%) for visualisation and validation. The subsequent second PCR, library preparation and sequencing on an Illumina MiSeq sequencing platform (Illumina, San Diego, CA, USA), was performed at the Genome Quebec Innovation Center (Montreal, Canada) according to the amplicon guidelines provided by Illumina. PE300 bp Reagent Kit was used for the ITS2 and 16S rRNA amplicon libraries. Raw sequences are deposited on NCBI (PRJNA747886). The bioinformatics pipeline was conducted at the Scientific Computer Cluster Euler at ETH Zurich. USEARCH v11.0.667 (Edgar, 2010) was used to remove phiX and merge read pairs with a minimum overlap of 30 bp and minimum merge length of 100 bp. Primer sequences were removed, and paired reads were size selected, quality filtered and denoised using USEARCH v11.0.667 (Edgar, 2010). Removal of chimaera and clustering into zero radius operational taxonomic units (ZOTUs) was done via UNOISE (Edgar, 2016a). Additionally, clustering at 97% sequence identity was done by UPARSE (Edgar, 2013). Taxonomy was assigned via SINTAX (Edgar, 2016b) using SILVA v128 (Quast et al., 2013) and UNITE_v82 (Bengtsson-Palme et al., 2013) as reference for the 16S rRNA and ITS2 dataset at 0.85 and 0.5 tax filter identity threshold, respectively. After removal of chloroplast, mitochondrial and archaeal sequences, 1366 bacterial and 3806 fungal ZOTUs were found across the field trial.

2.5.2. Functional gene quantification

The abundance of *alkaline* (*apr*) and *neutral* (*npr*) *metallopeptidase* genes (Bach et al., 2001) was assessed by quantitative PCR (qPCR) using primer sequences and cycling conditions listed in Supplementary Table S2 and as described in Lori et al. (2018).

2.6. Statistical analysis

Statistical analysis was conducted in R version 4.0.2 (R Core Team, 2017) and R STUDIO (RStudio Team, 2020). The effect of experimental factors on soil quality indicators and microbial alpha-diversity was assessed by linear mixed effect models. Lme1 targeted the experimental factors production system (MONO O, MONO C, AF O, AF C and SAFS), location ("between row" and "under cacao") and their interaction while lme2 assessed the effect of management (organic versus conventional), crop diversity (agroforest versus monoculture) and their interaction. For lme2, SAFS was excluded to maintain a balanced experimental design. Lme1 and lme2 were run with "plot" nested in "block" as a random effect (Pinheiro et al., 2020). The anova_lme function was used to retrieve the statistical significance of factors tested in linear mixed effect models and the emmeans function of the EMMEANS package (Russell et al., 2020) was used to calculate estimated marginal means. Tukey post-hoc tests were calculated for lme1 and can be reviewed in Supplementary Tables S3–S5. To satisfy the assumption of normal distribution and variance homogeneity of model residuals, raw data was log or sqrt transformed.

A total of 823,560 bacterial (1366 ZOTUs) and 2,262,979 fungal sequences (3806 ZOTUs) were used to calculate α-diversity indices (Shannon diversity (H) and observed richness (S)) using the 'estimate_diversity' command within the PHYLOSEQ package (McMurdie and Holmes, 2013). Evenness was calculated with H/log(S). For assessment of β-diversity, ZOTUs with fewer than 20 reads and occurring in fewer than 5% of the samples were removed. The filtered dataset contained 818,197 bacterial (1194

ZOTUs) and 2,224,724 fungal sequences (2285 ZOTUs). Rarefaction plots are shown in Supplementary Fig. S2. For microbial community composition analysis, zeros in bacterial and fungal ZOTU tables were replaced by a Bayesian-multiplicative replacement strategy implemented as the cmultRepl function in the zCompositions package (Palarea Albaladejo et al., 2015), followed by centered log-ratio (clr) transformation as suggested by Gloor et al. (2017). Permutational multivariate analyses of variance (PERMANOVA) based on Euclidean distance metrics were conducted using the adonis command of the VEGAN package (Oksanen et al., 2019). The effect of production system and location (PERMANOVA1) and the effect of management and crop diversity (PERMANOVA2, omitting SAFS) were tested with 10^4 permutations, and block set as strata. Fdr p-value correction was used for pairwise PERMANOVA, to test for distinct community structure between production systems (Arbizu, 2017). For visualisation of microbial community structure, unconstrained ordination by principal component analyses (PCA) based on clr transformed ZOTU tables (Euclidean distance metrics) was performed, followed by distance-based redundancy analyses (db-RDA) constraining for statistically significant factors identified in PERMANOVA1 and conditioning for block (Oksanen et al., 2019). Correlations of environmental variables with the projections of the db-RDA ordination were assessed using the envfit function of the VEGAN package (Oksanen et al., 2019), and the graphical display was limited to a threshold of p-value > 0.001. Due to the complex experimental design, indicator ZOTUs associated to one but also multiple groups (production systems) were identified using multilevel pattern analysis of the INDICSPECIES package (De Cáceres et al., 2012; Dufrêne and Legendre, 1997). Therefore, we run the multipatt function with 10^4 permutations and the "r.g" function to correct for unequal group sizes on untransformed data filtered to a minimum abundance of 300. p-value distribution of identified indicator ZOTUs was visualised via histograms (Fig. S3) before correction for multiple testing was performed using the QVALUE package (Storey et al., 2020). A bipartite network was generated via Cytoscape 3.8.2 with ZOTUs as target nodes, treatments as source nodes and association strength as connecting edges. Fungal and bacterial indicator ZOTUs were merged, and an edge-weighted spring-embedded layout algorithm was used for visualisation of treatment associations with $p < 0.01$ and a minimum relative abundance of 0.05%, as described in Hartmann et al. (2015). Herein performed indicator species analysis could not take in account the compositional nature of sequencing data (Gloor and Reid, 2016), and is thus more sensitive to sparsity and false-positive identification of indicator species (Thorsen et al., 2016).

3. Results

3.1. Chemical soil quality indicators

The chemical soil quality indicators Corg, Ntot, PoxC and soil pH were enhanced in production systems under organic management (Fig. 1, Table S3). Across the field site, chemical soil quality indicators were similar between sampling locations, except for higher Corg contents close to cacao trees in MONO O and higher soil pH close to cacao trees in SAFS compared to between the rows. In detail, between the rows we identified highest Corg content in AF O soils, followed by SAFS, MONO O and MONO C, and lowest values in AF C. Close to cacao trees, highest Corg content was found in MONO O, followed by AF O, SAFS, and AF C, while lowest amounts were found in MONO C (Fig. 1, Table S3). Soil pH, Ntot and PoxC contents followed the pattern of Corg (Fig. 1, Table S3). Consequently, chemical soil quality was higher under organic management, while increased crop diversity through the implementation of agroforestry system could not enhance key indicators for chemical soil quality.

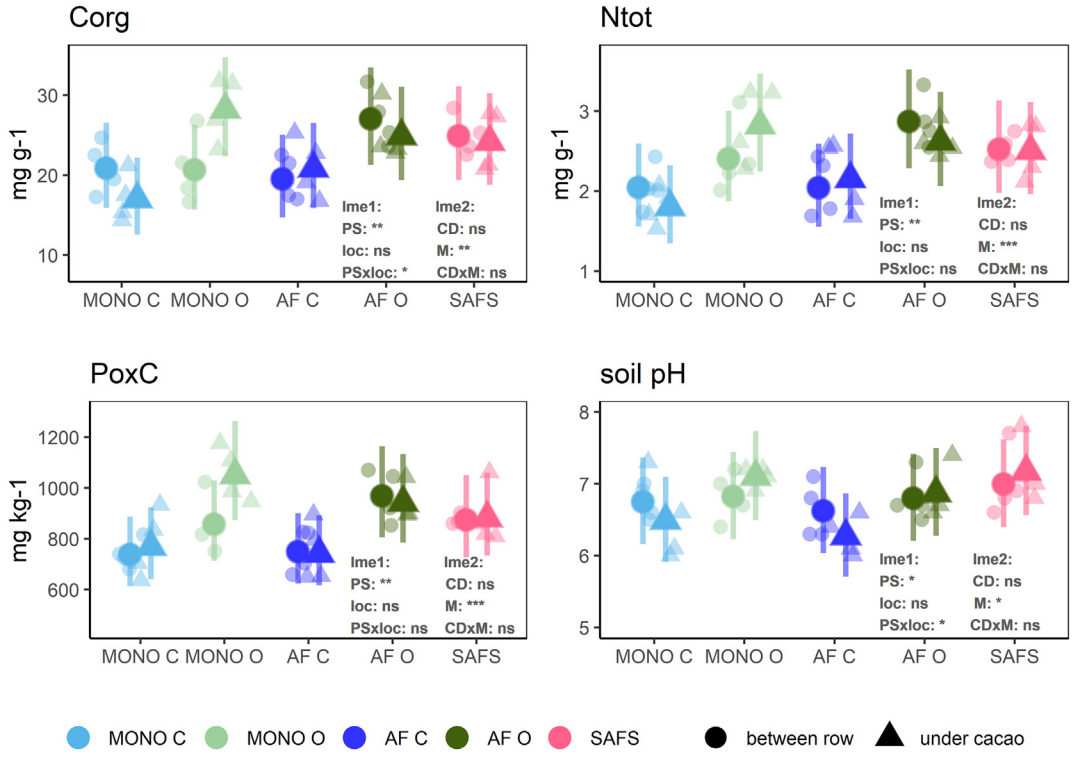

Fig. 1. Chemical soil quality indicators in cocoa production systems. Estimated marginal means, confidence intervals (95%) and raw data (n = 4) of a linear mixed effect model assessing effect of production systems and location (lme1) on chemical soil quality indicators are shown. The production systems are abbreviated as followed: MONO C = conventionally managed monoculture, MONO O = organically managed monoculture, AF C = conventionally managed agroforest, AF O = organically managed agroforest, SAFS = successional agroforest sequence. The main effects of lme1 assessing production system (PS) and location (loc) effects and lme 2 assessing management (M) and crop diversity (CD) effects are given as well and significances abbreviated as followed: ns = non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Extended statistical detail is listed in SI Table 3.

3.2. Biological soil quality indicators

Similar to chemical soil quality indicators, the biological indicators basal respiration, nitrogen mineralisation, DNA yield and *apr* abundance were enhanced in production systems under organic management (Fig. 2, Table S4). The sampling location had no effect on biological indicators. Soil from the SAFS system showed highest basal respiration, nitrogen mineralisation rates and DNA yields, followed by AF O, MONO O, AF C and MONO C (Table S4). Interestingly, MONO O outperformed AF C in terms of soil basal respiration, nitrogen mineralisation, DNA yields and *apr* abundance. MONO C soils mostly showed the lowest values for biological soil quality indicators. Consequently, biological soil quality was higher under organic compared to conventional management, despite enhanced crop diversity in conventional agroforestry systems.

3.3. Microbial α-diversity indices

The three most abundant bacterial phyla were *Firmicutes*, *Proteobacteria* and *Actinobacteria*, while *Nitrospira*, *Planctomycetes*, *Chloroflexi*, *Verrucomicrobia* and *Gemmatimonadetes* were less abundant (Fig. S4). Higher bacterial richness and Shannon diversity and lower community evenness was found in production systems under organic compared to conventional management (Table S5). Close to cacao trunks, higher Shannon index was found in SAFS and MONO O compared to MONO C, while between the rows, higher richness was found in MONO O compared to AF C and MONO C (Table S5). Across the field site, total fungal richness was higher compared to total bacterial richness (Fig. S2). On the phylum level, fungal communities were dominated by *Ascomycota*, *Basidiomycota* and sequences which remained "not assigned" (Fig. S4). Higher fungal richness was found in agroforestry compared to monoculture systems, especially when sampled close to cocoa trunks (Fig. S5). Overall, we identified highest fungal richness in SAFS and lowest in MONO C (Fig. S5).

3.4. Microbial community structure

PREMANOVA1 and PERMANOVA2 identified distinct fungal communities in different production systems and sampling location locations. Differing soil fungal community structure was found between organic and conventional management ($p < 0.001$), as well as between agroforestry and monoculture systems ($p < 0.001$) (Table 1). Pairwise PERMANOVA revealed distinct fungal communities in all production systems, except when comparing AF O with SAFS (Table 1). In line with this, constrained (db-RDA) and unconstrained (PCA) ordinations showed an apparent clustering of fungal communities for the distinct production systems, while the effect of sampling location was especially present in MONO O (Fig. 3A and C).

Similarly to fungi, PERMANOVA1 and PERMANOVA2 identified bacterial communities to differ between organic and conventional management ($p < 0.001$), as well as between agroforestry and monoculture ($p = 0.48$) (Table 1). Yet, pairwise PERMANOVA only revealed statistically significant dissimilarities in bacterial community structure between conventional (MONO C and AF C) and organic (AF O, SAFS and MONO O) systems (Table 1). Unconstrained ordination (PCA) of bacterial community composition mainly separated organic and conventional systems (Fig. 3B), whereas the constrained ordination (db-RDA) shows clustering of management and crop diversity (Fig. 3D).

3.5. Linking microbial community structure and soil quality indicators

Correlation of soil chemical and biological indicators with projections of the ordination revealed four and five associative indicators at $p < 0.001$ for the bacterial and fungal communities, respectively (Fig. 3C and

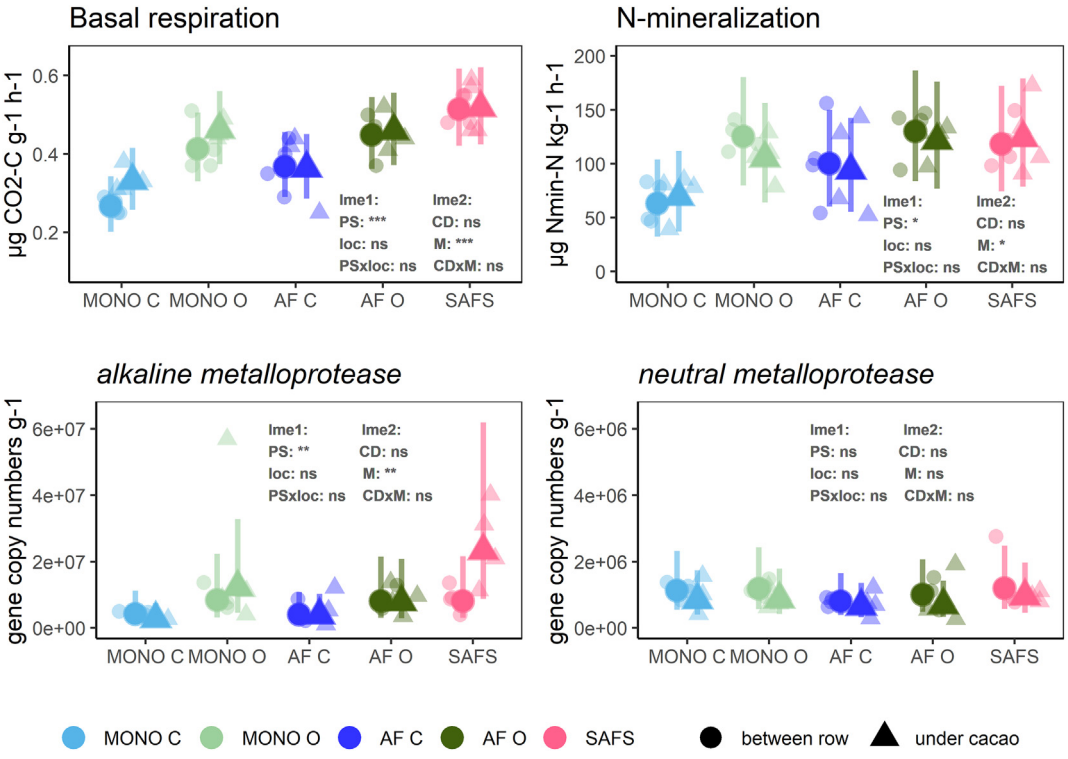

Fig. 2. Biological soil quality indicators in cocoa production systems. Estimated marginal means, confidence intervals (95%) and raw data (n = 4) of a linear mixed effect model assessing effect of production systems and location (lme1) on biological parameters are shown. The production systems are abbreviated as followed: MONO C = conventionally managed monoculture, MONO O = organically managed monoculture, AF C = conventionally managed agroforest, AF O = organically managed agroforest, SAFS = successional agroforest sequence. The main effects of lme1 assessing production system (PS) and location (loc) effects and lme 2 assessing management (M) and crop diversity (CD) effects are given as well and significances abbreviated as followed: ns = non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Extended statistical detail is listed in SI Table 4.

Table 1
Microbial beta-diversity in cocoa production systems. Effects of production system, sampling location, crop diversity and management on fungal and bacterial community composition were assessed by PERMANOVA (9999 permutations) based on filtered and center log-ratio transformed operational taxonomic units (ZOTUs) (ITS2 for fungi and 16S rRNA for bacteria) using Euclidean distances. Pairwise PERMANOVA assessed differences between each production system with false discovery rate p-value corrections.

	Permanova	Fungi					Bacteria				
		Df	SumOfSqs	R^2	F-value	p-value	Df	SumOfSqs	R^2	F-value	p-value
PERMANOVA1	**Production system**	4	166,090	0.19	2.04	**>0.001**	4	21,077	0.18	1.92	**>0.001**
	Location	1	32,197	0.04	1.58	**0.005**	1	3534	0.03	1.29	0.083
	Production system × Location	4	82,758	0.09	1.02	0.306	4	11,044	0.10	1.01	0.333
PERMANOVA2[a]	**Crop diversity**	1	49,518	0.07	2.40	**>0.001**	1	4040	0.04	1.44	**0.048**
	Management	1	45,244	0.06	2.19	**>0.001**	1	10,486	0.11	3.74	**>0.001**
	Crop diversity × Management	1	25,583	0.04	1.24	0.052	1	3021	0.03	1.08	0.241
Pairwise PERMANOVA		**MONO C**	**MONO O**	**AF C**	**AF O**	**SAFFS**	**MONO C**	**MONO O**	**AF C**	**AF O**	**SAFFS**
	MONO C	–	**>0.001**	**0.0017**	**>0.001**	**>0.001**	–	**0.004**	0.065	**0.006**	**0.011**
	MONO O			**>0.001**	**>0.001**	**>0.001**			**0.001**	0.103	0.233
	AF C				**>0.001**	**>0.001**				**>0.001**	**0.001**
	AF O					**0.011**					0.522

The production systems are abbreviated as followed: MONO C = conventionally managed monoculture, MONO O = organically managed monoculture, AF C = conventionally managed agroforest, AF O = organically managed agroforest, SAFS = successional agroforest sequence. P-values < 0.05 are expressed in bold.
[a] PERMANOVA2 assessing factors crop diversity (agroforest versus monoculture) and management (organic versus conventional), omits the SAFS production system to ensure a balanced and full factorial design.

D). C mineralising capacity showed the strongest correlation with bacterial (r^2 = 0.66) and fungal (r^2 = 0.53) community structure and was associated to SAFS and AF O. Also, N mineralizing capacity was associated with the bacterial (r^2 = 0.40) and fungal (r^2 = 0.39) community structure of AF O and SAFS. High PoxC concentrations were linked to organic management and strongly correlated with bacterial (r^2 = 0.45) and fungal (r^2 = 0.47) community structure. Corg (r^2 = 0.39) and Ntot (r^2 = 0.41) correlated with fungal community composition, while soil pH (r^2 = 0.24) was linked

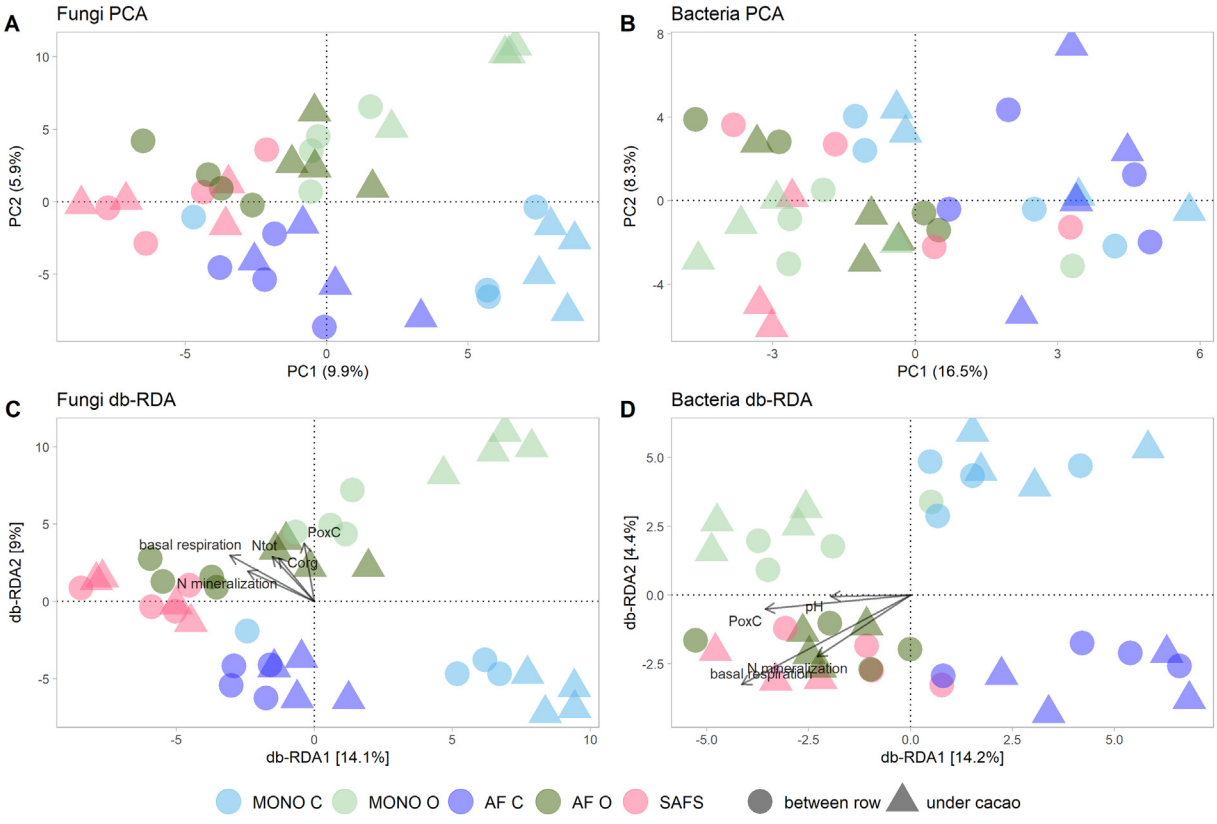

Fig. 3. Bacterial and fungal community composition based on zero radius operational taxonomic units (ZOTUs) of the 16S rRNA and ITS2 genes. Principle component analyses (PCA) and constrained distance-based redundancy analysis (db-RDA) on fungal (A,C) and bacterial (B,D) community composition based on centered log-ratio (clr) transformed data and Euclidean distance. Constraining factors were selected based on their significance in PERMANOVA1 (Table 4: Fungi constrained for production system and location, bacteria constrained for production system only) while Block was set as conditioning term. Arrows represent correlations of biological and geochemical parameters with the ordination scores. Only correlations with p < 0.001 are shown and arrow length is scaled according to correlation strength. Details on environmental variables and ordination axis are shown in Supplementary Tables S6 and S7. The production systems are abbreviated as followed: MONO C = conventionally managed monoculture, MONO O = organically managed monoculture, AF C = conventionally managed agroforest, AF O = organically managed agroforest, SAFS = successional agroforest sequence.

with bacterial community composition in organically managed plots. R^2 and p-values for all environmental parameters are listed in Supplementary Table S7.

3.6. Indicative fungal and bacterial taxa

The bi-partite network revealed distinct clusters associated with single production systems to be mainly dominated by fungal ZOTUs (fZOTU) (Fig. 4). We did not identify a distinct cluster for AF O as this treatment shared most indicative ZOTUs with either MONO O or SAFS. The three bacterial ZOTUs (bZOTU) indicative for MONO C made up 2.08% of all bacterial sequences, while the twelve fZOTUs associated with MONO C made up 1.86% of all fungal sequences. Especially in the MONO O cluster, fungi played a distinct role, as 6.82% of the total fungal sequences clustered to ZOTUs indicative for this treatment. The majority of indicative bZOTUs were either associated with organic or conventional production systems. Consequently, two bacteria-driven clusters were identified distinguishing between organic (O-cluster: MONO O, AF O and SAFS) and conventional (C-cluster: MONO C and AF C) management. The C-cluster did not contain any indicative fZOTUs and made up 11.95% of the overall bacterial sequences (Fig. 4). The O-cluster showed a greater taxonomic diversity and phylum richness of indicative bZOTUs compared to the C-cluster.

The relative abundance of the six most abundant ZOTUs are shown in Fig. 5. Most prominently, bZOTU 255, associated to the C-cluster, showed a relative abundance up to ~17% in MONO C and AF C systems and was assigned to the genus of *Bacillus*. Three out of the six most abundant indicative taxa were associated with the O-cluster, namely bZOTU 1825, bZOTU 115 and bZOTU 3 (Fig. 5). The representative sequence for bZOTU 1825 assigned to *Nitrospirales* (Family 0319-6A21). bZOTU 1825 was especially present in SAFS close to cacao trees, but elevated abundances under organic management were found across the field trial. Abundance of bZOTU 115 (genus *Pedomicrobium*) and bZOTU 3 (family *Rhodobiaceae*) were elevated under organic management compared to conventional management. fZOTU 99 assigned to *Bisifusarium dimerum,* and reached up to 4% of the fungal community in MONO O soils between the rows. fZOTU 245 assigned to the genus of *Stachybotrus limonispora* and highest values were found between the rows of the SAFS (Fig. 5). Details on the most abundant indicator species are listed in Supplementary Table S8.

Summarizing, our results show that chemical and biological quality indicators differ between cocoa production systems with beneficial effects mainly under organic management. Soil quality was highest in organic and successional agroforestry. In line with this, we found an overall highly diverse fungal community and specific fungal community structure for each production system. Distinct bacterial community structure was found between organic and conventionally managed systems and indicator species associated with organic management were taxonomically more diverse.

4. Discussion

4.1. Enhanced soil quality in production systems under organic farming

Investigating soil quality in agroforestry systems is inherently difficult due to the variable spatial impact of crop and/or shade trees and fertilisation strategy on local soil processes. Consequently, soil quality indicators were assessed at different distances to cacao trunks. Interaction effect between sampling location and production system on Corg and soil pH

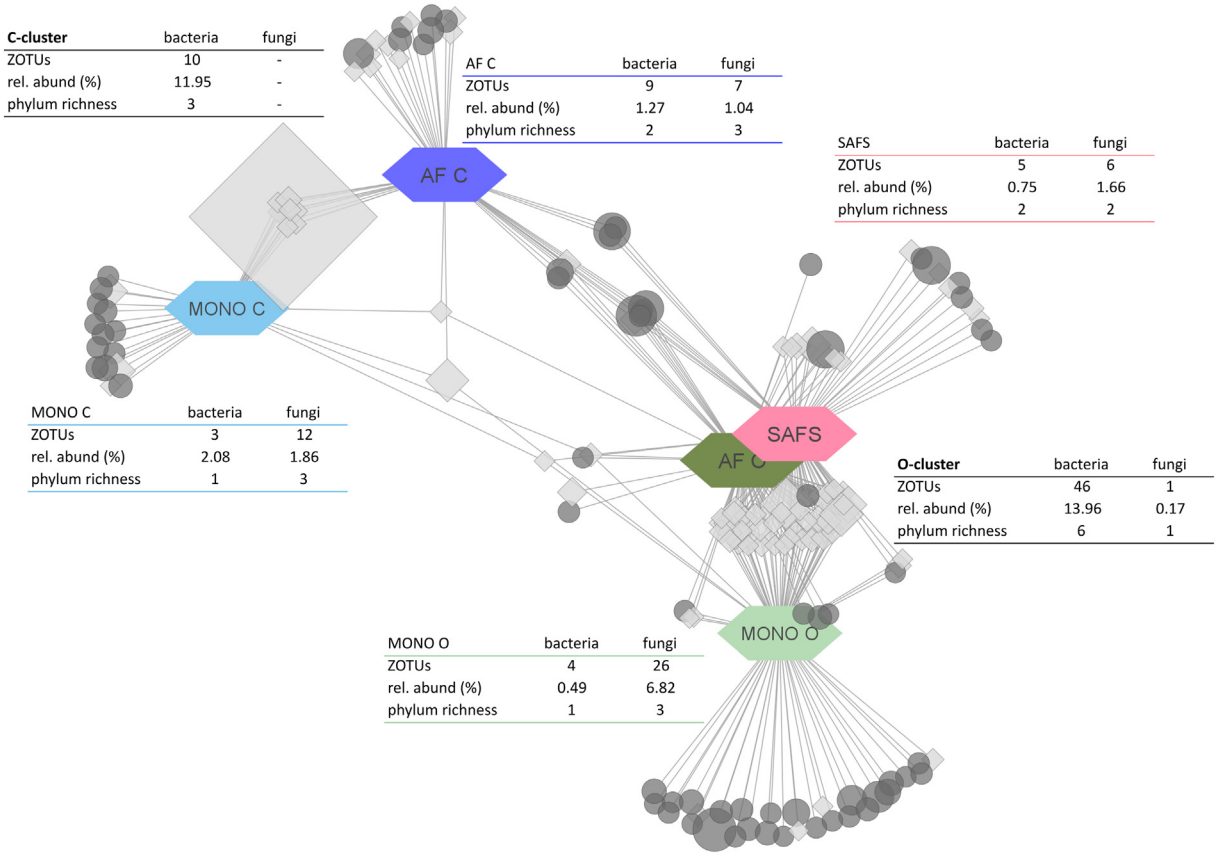

Fig. 4. Bipartite network showing statistically significant and positive associations ($p < 0.01$) between production systems and fungal (circle) and bacterial (diamond) zero radius operational taxonomic units (ZOTUs) with a relative abundance > 0.05 across the field trial. An edge-weighted spring-embedded algorithm was used to cluster ZOTUs with similar associations. Node size reflects relative abundance of indicative ZOTUs across all samples. Full list of statistically significant associated ZOTUs are provided in Supplementary Table S8. Relative abundance (rel.abund %) represents the abundance of given ZOTUS across all samples. The production systems are abbreviated as followed: MONO C = conventionally managed monoculture, MONO O = organically managed monoculture, AF C = conventionally managed agroforest, AF O = organically managed agroforest, SAFS = successional agroforest sequence. C-cluster represents AF C and MONO C while O-cluster represents MONO O and AF O.

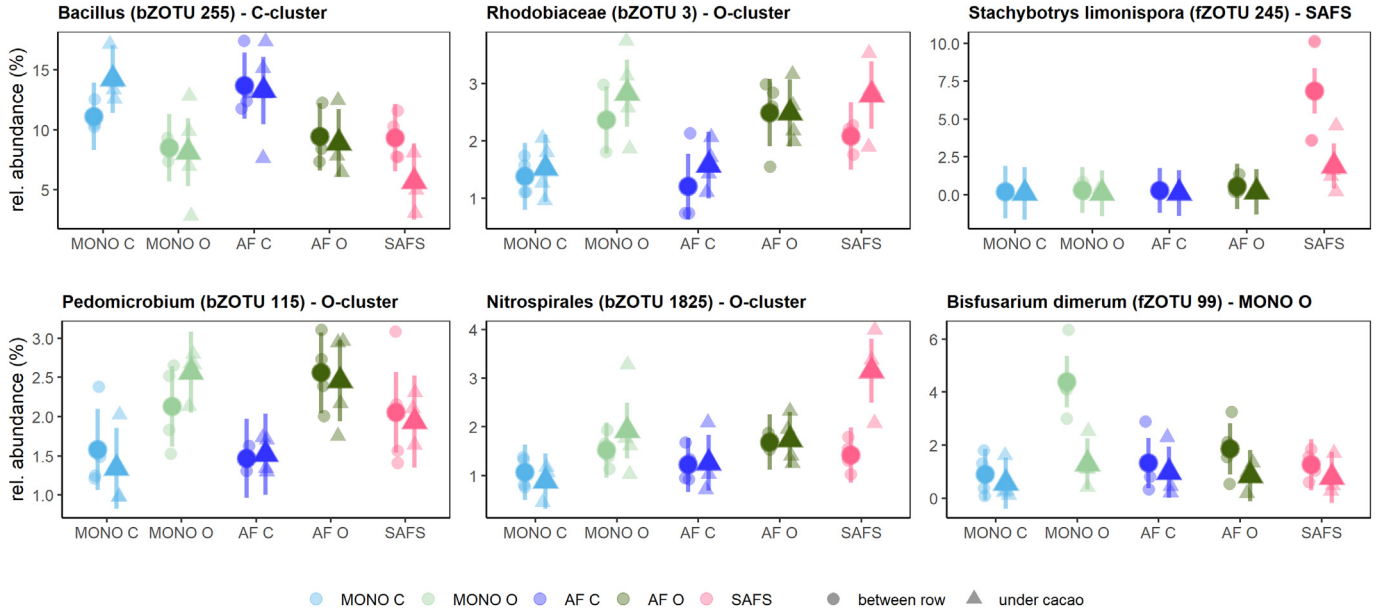

Fig. 5. Relative abundance of the six most abundant indicative fungal and bacterial zero radius operational taxonomic units as affected by cocoa production system and sampling location (ZOTU). Graphs show raw data (n = 4), as well as estimated marginal means and confidence intervals (95%) of a linear mixed effect model assessing production system and location effects. The production systems are abbreviated as followed: MONO C = conventionally managed monoculture, MONO O = organically managed monoculture, AF C = conventionally managed agroforest, AF O = organically managed agroforest, SAFS = successional agroforest. C-cluster represents AF C and MONO C while O-cluster represents MONO O and AF O.

(Fig. 1, Table S3) can be explained by organic matter inputs around the cacao trees in the organically managed plots. Cocoa trees in AF O received only half the amount of composted inputs compared to MONO O and similar Corg contents and soil pH was found at both sampling locations. Enhanced Corg contents were found under organic compared to conventional management, with highest Corg contents in MONO O around the cocoa trees (28.22 ± 2.01 mg g^{-1}). In line with our results, a global meta-analysis showed organic management in arable systems to enhance Corg contents (Gattinger et al., 2012). Given the strong link of Corg to other geochemical soil quality indicators, it is not surprising that organic management also enhances soil pH, soil nitrogen and PoxC contents (Fig. 1, Table S3). Apart from management, agroforestry's potential as a measure for Corg buildup is increasingly gaining attention (Lorenz and Lal, 2018; Nair et al., 2010), but highly variable effects have been observed (Lorenz and Lal, 2014). Also in this study, Corg contents between the cocoa rows was highly variable and ranged from 19.53 ± 1.57 to 27.04 ± 1.85 mg g^{-1} in AF C and AF O respectively (Table S3). A recent meta-analysis concluded that increased Corg contents in agroforestry systems mainly occur in silvoarable systems and are highly dependent on soil type and climatic conditions (Hübner et al., 2021). With the present field trial we could show that organic production systems can further promote soil Corg contents in tropical agroforestry systems.

Soil basal respiration rates can describe the activity of the soil microbiome and the soil capacity for mineralising organic input materials (Fliessbach et al., 2007). Especially the SFAS system, which showed the highest respiration rates (Fig. 2, Table S4), soil capacity to mineralize nutrients from pruning residues is the base for plant nutrition (Schneider et al., 2016). Basal respiration, N-mineralisation rates and *apr* abundance followed a similar pattern of lowest values in MONO C and highest in organically managed systems (Fig. 2, Table S4). This indicates that the aim of organic management to promote a biologically active soil also translates into enhanced nutrient provisioning from organic sources through nitrogen mineralising bacteria. Similar to this study, enhanced biological soil quality has regularly been reported in organically managed soils under temperate and tropical climates (Fliessbach et al., 2007; Lori et al., 2018; von Arb et al., 2020).

Since AF O and SAFS mostly present the highest chemical and biological soil quality, lower soil quality in AF C compared to MONO O systems should not be interpreted as poor performance of agroforestry systems. Soil quality in MONO O instead reflects the benefits of compost application around cacao trunks and maintenance of a leguminous cover crop as a typical organic management practice. While our data show that organic management enhances soil chemical and biological quality in cocoa production systems effectively, it needs to be noted that we only investigated topsoil in this study. Positive effects from enhanced root structures in agroforestry compared to monoculture systems might improve the quality of the soil below 10 cm depth and needs further investigation (Niether et al., 2019).

4.2. Distinct microbial community structure in cocoa production systems

Across the field site, total fungal exceeded total bacterial richness (Fig. S2). This is in contrast to what is known for soil microbial communities in temperate climates (Zhang et al., 2021) and highlights the importance of fungal communities in tropical soils (Brinkmann et al., 2019; Thedersoo et al., 2014). Additionally, 28% of the identified fungal ZOTUs could not be annotated on the phylum level emphasising the need to further study soil fungal communities in tropical soils.

Especially in perennial systems, plant-fungal symbiosis might affect soil biodiversity. Indeed we observed enhanced fungal richness in agroforestry compared to monoculture systems (Table S5), which is in line with previous observations showing plant diversity in agroecosystems promote soil fungal diversity (Shen et al., 2021). Bacterial richness, however, was less sensitive to crop diversity and more to management (Table S5).

The strong management effect on bacterial community composition occurred at both sampling locations and suggests that apart from fertilisation of cocoa trees, management-specific plant protection strategy might shape bacterial community composition (Table 1) as it has previously been shown (Meena et al., 2020). Unfortunately, the system comparison approach of the field trials does not allow us to disentangle the effects of fertilisation and agrochemicals on the microbial community composition.

The strong correlation between fungal and bacterial community compositions of organically managed systems and soil respiration,

N-mineralisation and PoxC (Fig. 3C and D) underpins the crucial role of heterotrophic soil activity for degradation processes and the provision of nutrients under organic management. Even though the role of microbial diversity in promoting multifunctionality (Delgado-Baquerizo et al., 2016) and stability (Wagg et al., 2021) of terrestrial ecosystems was demonstrated, it is a complex undertaking to quantify the contribution of biological soil activity to crop productivity. In 2015 cocoa yields were higher in monocropped systems than agroforestry, with higher yields in MONO C than MONO O but similar yields in AF O and AF C (Niether et al., 2019). This suggests that agroforestry systems can benefit from organic management with comparable yields and enhanced soil quality. Concerning total systems yield including crops from non-cocoa trees, agroforestry outperformed monocropping (Niether et al., 2019). SAFS showed the lowest cocoa yields but highly diversified total system yields similar to AF O and AF C (Niether et al., 2019). Although it is inherently difficult to directly link the interplay between soil quality and soil microbial diversity to specific ecosystems services, such as crop productivity or nutrient regulation, there is growing evidence that the biological base of soil quality especially benefits from organic management.

4.3. Potential role of identified indicator species

Stachybotrus limonispora (fZOTU 245) was most abundant in the SAFS (Fig. 5) and is known for its decomposing activity and association to cellulose-rich habitats (Seifert and Gams, 2011). The high pruning activity in SAFS might thus favour the abundance of *Stachybotrus limonispora,* possibly through increased litterfall and/or root decomposition after pruning events (Peter and Lehmann, 2000). *Bisifusarium dimerum* (fZOTU 99), indicative for the MONO O system, reached up to 4% in relative abundance between the rows. A characteristic trait of this specific sampling location was a thick leguminous cover crop, yet the functional linkage to the high abundance of *Bisifusarium dimerum* remains speculative. So far, this taxon has been found as an inhibitory agent for nematodes and is regarded as a cosmopolitan saprotroph in soils (Schroers et al., 2009). The genus *Bacillus* (e.g. bZOTU 255) describes a common, gram-positive soil bacterium with an aerobic lifestyle, which forms dormant endospores in response to nutritional or environmental stress (Alcaraz et al., 2010; Nicholson, 2002; Nicholson et al., 2000). The high relative abundance of bZOTU 255 in conventionally managed plots (Fig. 5), together with low bacterial diversity (Table S8) indicates an impoverished bacterial community under conventional management. In contrast, bacterial taxa indicative for organic systems are related to various soil processes. Members of the *Rhodobiaceae* family (bZOTU 3) are known to form symbiotic relationships with legumes for nitrogen-fixation and might be a response to the maintenance of a leguminous cover crop in the organic systems. A high abundance of *Nitrospirae* (bZOTU 1825) in soils around cacao trees in SAFS indicates enhanced nitrogen-cycling activity, and is consistent with elevated abundance of *apr* genes in SAFS (Fig. 2). A high abundance of *Nitrospirae* was previously shown to be associated with elevated soil nitrogen contents (Banerjee et al., 2016; Zhang et al., 2021) and organic arable cropping systems (Zhang et al., 2019). The genus *Pedomicrobium* (bZOTU 115) is known for its chemo-organotrophic lifestyle using acetate as a carbon source under aerobic conditions (Hirsch and Mauchline, 2015). Since *Pedomicrobium* mainly uses organic substrates, it occupies an intermediate role for nutrient cycling in agroecosystems and complements the metabolic capacity of the soil microbiome under organic soil management.

In summary, we found indicator ZOTUs of versatile metabolic functions associated with organic management, whereas less metabolic diversity was associated with the conventional systems. However, we can only speculate about possible ecological functions of identified ZOTUs. Extended analyses such as the profiling of microbial metabolic capacity via substrate-induced respiration assays (Creamer et al., 2016), proteomics (Qian and Hettich, 2017), whole shotgun sequencing metagenomics (Vogel et al., 2009) or its combination (Martinez-Alonso et al., 2019) could further reveal distinct effects of management and crop diversity on soils' ecosystem multifunctionality.

We finally note that culture-independent techniques offer many advantages but results have to be handled carefully due to manifold reasons (Hugerth and Andersson, 2017; Semenov, 2021; Schirmer et al., 2015). Suitable sampling strategies and storage conditions for soil microbial ecology were discussed (Schroeder et al., 2021; Nannipieri et al., 2019; Vestergaard et al., 2017) and in our case soil was oven-dried before DNA extraction due to logistical reasons. Thus, comparison to other studies should be treated with caution.

5. Conclusions

Our results show that organic management in monoculture and agroforestry systems enhances chemical and biological soil quality and harbors distinct soil fungal and bacterial communities. Crop diversity had a minor effect on soil microbial communities, and although highest soil quality was found in organic agroforestry, organic monoculture outcompeted conventional agroforestry. Consequently, we conclude that organic soil management facilitates soil quality also in diversified agroforestry systems.

CRediT authorship contribution statement

Martina Lori: Methodology, Formal analysis, Visualization, Writing – original draft. **Laura Armengot:** Conceptualization, Writing – review & editing. **Monika Schneider:** Resources, Writing – review & editing. **Ulf Schneidewind:** Methodology, Writing – review & editing. **Natacha Bodenhausen:** Conceptualization, Data curation, Writing – review & editing. **Paul Mäder:** Conceptualization, Resources, Funding acquisition, Writing – review & editing. **Hans-Martin Krause:** Conceptualization, Methodology, Funding acquisition, Project administration, Writing – original draft.

Declaration of competing interest

The authors declare that they have no 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.org/10.1016/j.scitotenv.2022.155223.

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