Variations in the diversity of soil bacterial and archaeal communities in response to different long-term fertilization regimes in maize fields

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ABSTRACT

Understanding the effect of different agricultural practices on soil microbiome is very important since it has a decisive role in the multifunctionality of soils. Our research aimed to reveal and compare the changes in the composition of soil bacterial and archaeal communities in response to cultivation and fertilization regimes. Soil samples were collected from a long-term (60-year-old) field experiment with maize (*Zea mays* L.) monoculture under inorganic fertilization, combined fertilization (inorganic and farmyard manure) and without fertilization, and a nearby fallow land five times over a crop cycle. The bacterial and archaeal community compositions were revealed by amplicon sequencing of the 16S rRNA genes on Illumina MiSeq platform. Significant differences were observed between cultivated soils and fallow land, as well as among cultivated soils under different fertilization regimes in both bacterial and archaeal community structures. Many taxa (e.g. *Gaiella, Burkholderiaceae, Saprospiraceae, Rhodoplanes*, MB-A2-108 and *Cand*. Nitrososphaera) were found to be more abundant in the fallow land than in the cultivated soils. The exclusive use of inorganic fertilizer resulted in a significantly different bacterial and archaeal community structure with higher relative abundance of sequences affiliated with mostly acid-otolerant taxa (e.g. *Bryobacter, Cand*. Solibacter, Acidibacteriales, WPS-2 and *Cand*. Nitrosotalea). These taxa were not enriched in the combined fertilizer treatment, even though the manure was applied only once every four years. The results demonstrated a strong effect of inorganic fertilizers on the soil bacterial and archaeal communities, however, these effects can be decreased by the application of farmyard manure.

1. Introduction

Microorganisms are fundamental living components of soil ecosystems, as their activity can be linked directly or indirectly to several important processes such as biogeochemical cycles of elements (Murphy et al., 2007), regulation of water holding capacity, formation of soil structure (Bossuyt et al., 2001) and promotion of soil fertility (Miltner et al., 2012). Moreover, microbes can assist the nutrient uptake of plants (Oliveira et al., 2009), suppress plant diseases and stress (Feng et al., 2019).

Many studies have been published on the impact of intensive agricultural practices on soil quality, microbial biomass, diversity and activity in soils. Application of fertilizers is one of the most critical issue in sustainable agriculture, therefore, the response of soil parameters and microbes to the type, dose and combinations of fertilizers has received more and more attention. As nitrogen is the primary growth-limiting nutrient for plants, the increased available N level improves crop productivity but it has severe environmental consequences through soil acidification (Jenkinson, 1991; Tian and Niu, 2015), loss of organic matter content (Fließbach et al., 2007) and exchangeable base cations (Lucas et al., 2011) from soils at the same time. Soil microorganisms are sensitive to the changes in soil physico-chemical parameters due to agricultural practices. There are evidences showing that mineral N addition can lead to a decrease in microbial diversity (Sun et al., 2019; Zhen et al., 2014) and, furthermore, significant changes in the composition of soil microbial communities (Fierer et al., 2012; Sun et al., 2019). Studies have found that the inorganic N input can cause a shift in the metabolic profiles also. For instance, microbial communities in soils receiving mineral N have lower respiration rates (Ramirez et al., 2010), decompose less recalcitrant C compounds (Ramirez et al., 2012), but a positive correlation has been found between N fertilizers and nitrification, denitrification rates (Zhu et al., 2016).

There is a growing need for realistic, economically viable alternative agricultural practices that can prevent or reduce the undesired effects of

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conventional farming while maintaining soil productivity. For example, farmyard manure along with mineral fertilizers can significantly improve soil quality and crop productivity even after a few years (Hou et al., 2012; Masood et al., 2014). Regarding the soil microbial communities, both the exclusive and additional applications of manure were proven to increase the soil microbial diversity, biomass, basal respiration and N mineralization activity (Bongiorno et al., 2020; Dambreville et al., 2006; Fließbach et al., 2007; Sun et al., 2015; Zhen et al., 2014).

Maize (*Zea mays* L.) is an important and widely cultivated crop; its production reached 1.14 Gt in 2018, surpassed that of rice and wheat (Food and Agriculture Organization of the United Nations, 2018). It is a valuable source of human food, green forage, biofuel and alcoholic beverages, as well (Ranum et al., 2014). Like other plants, maize can also alter the biomass and composition of soil microbial communities by producing root exudates, e.g. polysaccharides (Benizri et al., 2007) and aromatic compounds (Hu et al., 2018).

Concerns about the possible adverse effects of conventional and intensive agricultural management on the crop yields and soil quality inspired the establishment of long-term field experiments worldwide (Debreczeni and Körschens, 2003). In Hungary, a considerable number of maize field experiments were started decades ago, in order to study different hybrids, culture types (monoculture, crop rotation), use of organic and mineral fertilizers and fertilizer dosage (Debreczeni and Körschens, 2003). These agricultural practices also provide an excellent opportunity to reveal the long-term changes in the composition of soil microbial communities. Nevertheless, the first systematic and detailed reports on the bacterial and fungal communities were published only in the last few years (Csitári et al., 2014; Gazdag et al., 2018; Magurno et al., 2014; Mayer et al., 2019; Sasvári et al., 2012; Ujvári et al., 2020). All of these highlight the importance of monitoring the impact of different crop production systems on the soil microbial communities. We intended to characterize the soil bacterial and archaeal community of a maize monoculture and to test whether microbial shifts occur under different fertilization regimes and without agricultural disturbance. Therefore, the present research aimed to reveal and compare the composition of soil bacterial and archaeal communities of a 60-year-old field experiment of maize monoculture and a control fallow land by monitoring it over a growing season. We assumed that the bacterial and archaeal communities of a fallow land has been changed compared to the cultivated soil due to abandonment in 60 years and that the different types of fertilization influence the bacterial and archaeal community structures in different ways.

2. Materials and methods

2.1. Description of the field experiment and treatments

The studied long-term field experiment located near Martonvásár, Hungary (47.331597 N; 18.789412 E) was initiated in 1961 by the legal predecessor of the Centre for Agricultural Research. Since then, the trial has been maintained under the same controlled conditions. The field experiment is in a two factorial (crop sequences and fertilizer treatments), split-plot design with four replications, and consists of a total of 140 plots (each 7 × 7 m). In this study, continuous maize (*Zea mays* L.) monoculture (crop "1") under three different fertilization regimes and a nearby uncultivated fallow land were sampled (detailed in Table 1). The control plots ("A") were not fertilized since the start of the experiment. The inorganic fertilizer applied in the experiment consists of N (as ammonium nitrate, NH_4NO_3), P_2O_5 (as superphosphate, $Ca(H_2PO_4)_2$), and K_2O (as potassium chloride, KCl). Between 1961 and 1984, the nitrogen, phosphorous and potassium doses were 100, 50, 50 kg ha^{-1} year^{-1}, respectively, but they were increased to a dose of 180, 90, 150 kg ha^{-1} year^{-1}, respectively, from 1985 (fertilization regime "D"). The dosage of NPK was the same in fertilization regime "B" as in "D", but farmyard manure (cattle) has been added every four years as a supplementary (30 t ha^{-1} until 1980, 60 t ha^{-1} from 1984), the last application

Table 1

Summary of samples. Sampling sign abbreviations: A1: unfertilized maize monoculture; B1: maize monoculture fertilized with both inorganic and organic fertilizers; D1: maize monoculture fertilized with inorganic fertilizer; FL: fallow land; M, maize monoculture; sampling date (yy.mm); NPK, inorganic fertilizer (nitrogen, phosphorous, potassium); FYM, farmyard manure every 4 years.

	Sample	Fertilization	Cultivation	Sampling date	Growth stage
●	A1-M-1803	None	Maize monoculture	2018. March	Ploughed
●	A1-M-1805	None	Maize monoculture	2018. May	BBCH 14–15
●	A1-M-1806	None	Maize monoculture	2018. June	BBCH 65
●	A1-M-1808	None	Maize monoculture	2018. August	BBCH 87
●	A1-M-1810	None	Maize monoculture	2018. October	Harvested
●	B1-M-1803	NPK + FYM	Maize monoculture	2018. March	Ploughed
●	B1-M-1805	NPK + FYM	Maize monoculture	2018. May	BBCH 14–15
●	B1-M-1806	NPK + FYM	Maize monoculture	2018. June	BBCH 65
●	B1-M-1808	NPK + FYM	Maize monoculture	2018. August	BBCH 87
●	B1-M-1810	NPK + FYM	Maize monoculture	2018. October	Harvested
●	D1-M-1803	NPK	Maize monoculture	2018. March	Ploughed
●	D1-M-1805	NPK	Maize monoculture	2018. May	BBCH 14–15
●	D1-M-1806	NPK	Maize monoculture	2018. June	BBCH 65
●	D1-M-1808	NPK	Maize monoculture	2018. August	BBCH 87
●	D1-M-1810	NPK	Maize monoculture	2018. October	Harvested
▲	FL-1803	None	Fallow	2018. March	Natural
▲	FL-1805	None	Fallow	2018. May	Natural
▲	FL-1806	None	Fallow	2018. June	Natural
▲	FL-1808	None	Fallow	2018. August	Natural
▲	FL-1810	None	Fallow	2018. October	Natural

before the sampling was in 2016. The average annual yields of maize for different fertilizer treatments were the following between 1961 and 2018: A: 4.86 ± t ha^{-1}, B: 6.97 t ha^{-1}, D: 7.16 t ha^{-1}. The soil is ploughed in autumn to a depth of 28–30 cm, incorporating field residues and fertilizers. Pre-sowing soil preparation involves mechanized soil compaction. The plots were free from weeds and pests throughout the growing season. Weed control includes the use of chemical (pre-emergent and if necessary post-emergent herbicides) and mechanical methods. Targeted pesticides are applied only when pests appear.

The currently uncultivated fallow land is located approximately 50 m from the experiment. This grassland has been abandoned for more than 50 years; therefore, it was considered as a local control area.

The soil type of the studied arable land can be described as an Eutric Regosol (Loamic) (Ujvári et al., 2020) according to IUSS Working Group WRB2014 (Schad et al., 2015). Local climate and soil chemical data can be found in Supplementary Tables 1 and 2, respectively.

2.2. Sampling and processing

The three fertilizer treatments (A, B, D) of maize monoculture and the fallow land (FL) were sampled 5 times between March and October 2018, adjusted in time to the characteristic growth stages of maize plant. Three soil cores of horizon A (upper 10–15 cm of topsoil) were collected at a distance of about 15 cm from the stem of the maize plants in each plot into single-use plastic bags and stored at 6–8 °C until laboratory processing. After a thorough mixing and removal of plant residues, soil

samples were used to immediate DNA isolation. Soil physico-chemical data were provided by Institute for Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research. Measurements were conducted according to Hungarian standards MSZ-08-0206-2:1978, MSZ-08-0210-2:1977 and MSZ 20135:1999 4.2. The $CaCO_3$ content was determined by gas volumetric method using a Scheibler type calcimeter. Humus amount was determined following wet digestion with sulfuric acid and potassium dichromate.

2.3. Bacterial and archaeal diversity analysis by high-throughput amplicon sequencing

Community DNA was isolated using DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The isolated DNA of four biological replicates was pooled in equal proportions before amplification in triplicate using Bacteria-specific (Bakt_341F: 5′-CCTACGGGNGGCWGCAG −3′ and Bakt_805R: 5′-GACTACNVGGG-TATCTAATCC-3′) (Herlemann et al., 2011) and Archaea-specific (A519F: 5′-CAGCMGCCGCGGTAA-3′ and Arch855R: 5′-TCCCCCGCCAATTCCTTTAA-3′) (Klindworth et al., 2013) primers with tags on the 5′ ends targeting the V3-V4 region of 16S rRNA gene. The PCR reaction mix contained 9.8 μl nuclease free water, 0.2 μl Phusion Hot Start II High-Fidelity DNA Polymerase (2 U/μL), 4 μl 5× Phusion HF Buffer (Thermo Scientific), 4 μl dNTP mixture (10 μM), 0.4 μl BSA (20 mg/ml, Thermo Scientific), 0.3 μl of each bacterial or archaeal primer (40 μM) and 1 μl template DNA. The target region was amplified by PCR with the following protocol: primer denaturation at 98 °C for 5 min, 25 cycles of 95 °C for 30 s, 55 °C and 60 °C for 30 s (for Bacteria and Archaea, respectively), 72 °C for 30 s, final extension at 72 °C for 5 min. Replicate PCR products were pooled and their concentration was measured with Qubit dsDNA HS Assay Kit (Thermo Scientific, Waltham, USA). To normalize DNA concentration, amplicons from each sample were pooled in equal proportions. The barcoded libraries were sequenced on an Illumina MiSeq platform (Illumina Inc., San Diego, USA) at the Genomics Core, Research Technology Support Facility (Michigan State University, Trowbridge, USA) using a MiSeq Reagent Kit v2 (500 cycle).

Raw sequence reads were deposited in NCBI's Sequence Read Archive database under the BioProject accession number PRJNA663467. Sequence reads were processed using mothur v1.42.3 (Schloss et al., 2009) following the MiSeq SOP (www.mothur.org/wiki/MiSeq_SOP downloaded at 01/08/2019) (Kozich et al., 2013) with some modifications: deltaq value was adjusted to 10 in the make.contigs command, UCHIME (Edgar et al., 2011) was used for chimera detection. Singletons were removed prior to clustering (Kunin et al., 2010). After generation of operational taxonomic units (OTUs) at 0.15 cutoff level in mothur's dist.seqs command and using 97% 16S rRNA gene sequence similarity as species-level threshold (Tindall et al., 2010), taxonomy was assigned to each OTU using a minimum bootstrap confidence score of 80% calculated after 1000 iterations and based on the ARB-SILVA SSU Ref NR 132 database (Quast et al., 2012). All OTUs not classified as Bacteria or Archaea were excluded from further analysis. Richness and diversity indices (i.e. ACE, Chao1, inverse Simpson) were estimated using mothur on datasets rarefied to the same sequence depth ($n = 9 327$ for Bacteria, $n = 1 476$ for Archaea).

2.4. Statistical analyses

Downstream statistical analyses were performed using R 3.5.1. Bray-Curtis dissimilarity matrix of OTU counts was ordinated using metaMDS in vegan package (Oksanen et al., 2018). Permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations was carried out on Bray-Curtis distance matrix of rarefied ($n = 9327$ for Bacteria, $n = 1476$ for Archaea), double square-root transformed OTU tables using adonis function of vegan package to test if OTU compositions of sample groups (FL, A1, B1, D1) were significantly different. After that a pairwise multilevel comparison was performed using pairwiseadonis function of pairwise Adonis package (Martinez Arbizu, 2020) in order to identify which particular differences between treatments were significant. Differentially abundant genera were identified using edgeR package (Robinson et al., 2010). Generalized linear models were fitted to filtered (filterByExpr function) and normalized (TMM, Trimmed Mean of M-values) abundance data assuming a negative binomial distribution. After that, standard scaled (row Z-score) abundance data of genera that were differentially abundant (Benjamini and Hochberg adjusted p-value, e.g. false discovery rate, <0.05) in at least one comparison were visualized on heatmap generated with pheatmap package (Kolde, 2015). Euclidean distance metric and average linking clustering method were applied for hierarchical clustering. Differences in alpha diversity between sample groups were compared using one-way ANOVA with the aov function, and Levene's test (leveneTest function) was used to check the homogeneity of variances in R. Tukey test (TukeyHSD function) was performed for multiple comparisons.

3. Results

The amplicon sequencing provided deep insight into the taxonomic composition of bacterial and archaeal communities inhabiting the agricultural soils with different fertilization and a fallow land. After the processing of the raw data, a total of 364,017 bacterial and 117,653 archaeal sequences were assigned to 13,253 and 219 OTUs, respectively. The number of sequences per sample ranged between 9327 and 28,954 in the case of Bacteria, 1476 and 16,207 in the case of Archaea, with an average of 18,200 and 5882, respectively. As shown in Supplementary Fig. 1, the bacterial rarefaction curves indicated that the sequencing depth was adequate, but not all curves approached the asymptote in the case of Archaea. However, 99% of all archaeal OTUs calculated at 0.03 dissimilarity was recovered in every sample, as estimated by Good's coverage.

3.1. Taxonomic composition of soil bacterial and archaeal communities

Altogether 37 bacterial phyla were detected in the soil samples. The core of each community composed of five phyla: Proteobacteria (25.7% ± 2.3%), Acidobacteria (18.7% ± 3.9%), Bacteroidetes (13.9% ± 3.4%), Actinobacteria (9.4% ± 5.5%) and Verrucomicrobia (9.6% ± 1.7%) (Supplementary Fig. 2). The phylum Proteobacteria had a constant presence with the highest relative abundance in each sample. The soil bacterial communities were found highly diverse at order level as the most abundant 20 orders (above 3% relative abundance) accounted for 60.1–72.6% of the total sequences per samples (Fig. 1). The dominant orders were Chitinophagales (7.3% ± 1.6%), Betaproteobacteriales (6.2% ± 1.0%), Pedosphaerales (5.5% ± 1.4%), Pyrimonadales (5.1% ± 1.9%), an acidobacterial Subgroup 6 (4.9% ±1.4%) and Gemmatimonadales (4.7% ± 1.7%).

The archaeal sequences belonged to phyla Euryarchaeota, Nano-archaeota, Thaumarchaeota and an unclassified phylum (Supplementary Fig. 3). Thaumarchaeota was the most abundant archaea in every sample (90.9% ±3.6%), while Euryarchaeota (5.4% ± 2.4%) and Nanoarchaeota (2.8% ± 1.6%) together made up the minority of the communities. An uncultured Nitrososphaeraceae genus (60.8% ± 6.4%) of Thaumarchaeota dominated all the samples regardless of the fertilization type, the cultivation and sampling time (Fig. 2). Candidatus Nitrososphaera and another unclassified Nitrosophaeraceae genus accounted for 20.6% ± 6.4% and 8.2% ± 4.5% of total sequences, respectively. Candidatus Nitrosotalea was detected only in the NPK fertilized soil samples (0.06–12.4%). An unclassified Woesearchaeia genus was a constant minor component of the archaeal communities during the year (3.1% ± 2.6%).
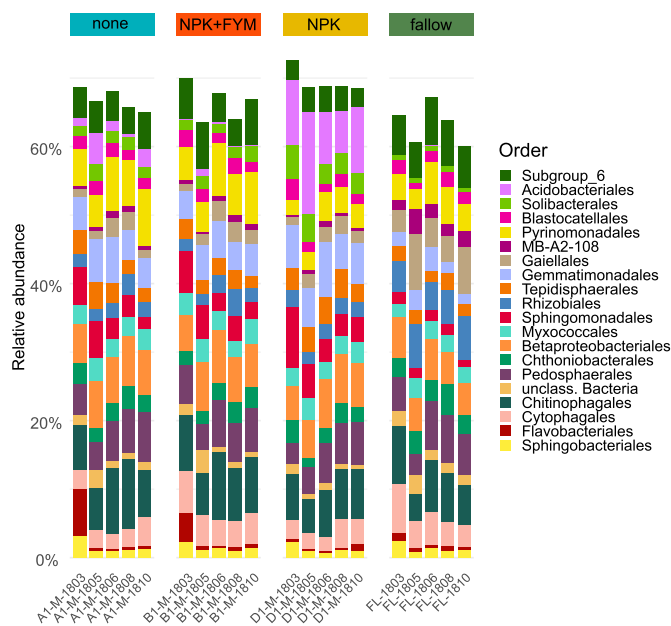
Fig. 1. Distribution of the predominant bacteria at order level. Relative abundance is expressed as the percentage of total sequences, only taxa with a percentage above 3% in at least one sample are shown.
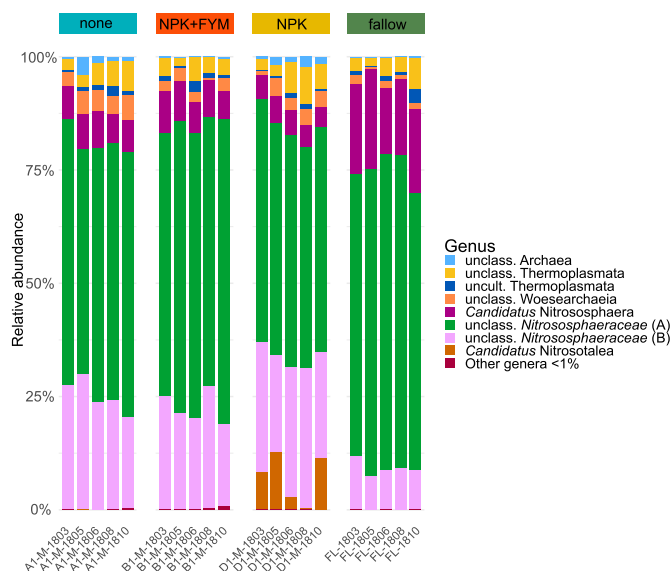

Fig. 2. Distribution of the dominant archaea at genus level. Relative abundance is expressed as the percentage of total sequences, genera with a percentage under 1% are combined and displayed as "Other genera".

3.2. Differences in bacterial and archaeal diversity and richness

Diversity and richness indices calculated from a rarified dataset are presented in Supplementary Fig. 4. According to analysis of variance, there were no statistically significant ($p > 0.05$) differences between group means (e.g. fallow land, non-fertilized, NPK-fertilized and NPK + FYM fertilized maize monoculture) in any of the bacterial diversity indices (inverse Simpson, Chao1 and ACE). In comparison with the bacterial communities, the diversity and richness indices of the archaeal communities were two orders of magnitude lower. Archaeal richness values did not showed significant differences among the four groups. On the contrary, we found that the inverse Simpson index for Archaea was significantly ($p < 0.05$) higher in the NPK-fertilized soil than in any

other samples.

3.3. Effects of fertilization and cultivation on bacterial and archaeal communities

Non-metric multidimensional scaling (NMDS) analysis was conducted on the sequencing data to explore the dissimilarities of bacterial communities and to assess the effects of the fertilization and cultivation on the β-diversity (Fig. 3). The OTU matrix was reduced into two dimensions based on Bray-Curtis distance (stress = 0.06) to illustrate the differences among the samples. The arrangement of objects on the ordination plot formed four clusters. The samples taken in the fallow land (FL) clearly separated from the cultivated soil samples. The other sample separation could be observed according to the fertilization regimes rather than sampling dates. The bacterial communities of the soils treated exclusively with mineral fertilizers were clearly distinct from the other fertilizer treatments. After that, we performed PERMANOVA significance test for group-level differences. We found a significant effect of cultivation on bacterial communities at $p < 0.05$ level for the four conditions ($F(3,16) = 2.47$, $p = 0.001$). All the pairwise comparisons showed significant differences (p values ≤0.012) between groups except for non-fertilized (A1-M) vs. NPK + FYM fertilized (B1-M) soils.

The results of Archaea-specific NGS was subjected to NMDS analysis (Fig. 4), too. The fallow and cultivated samples could also be distinguished based on the observed OTUs (stress = 0.05). Cultivated soils exhibited clustering according to fertilization, but they slightly overlapped. The PERMANOVA test indicated significant differences among the four groups ($F(3,16) = 4.58$, p = 0.001). The results were the same for the archaeal communities as for the bacterial: the OTU distribution was significantly different (p values ≤0.014) among sample groups, but not in the comparison of non-fertilized (A1-M) and NPK + FYM fertilized (B1-M) soils.

Given the results of the PERMANOVA significance test, we identified the genera driving the changes in the soil bacterial and archaeal communities between the fallow land and the non-fertilized maize monoculture, and among the maize monocultures under different fertilization regimes. Differential abundances at genus level were calculated using edgeR. The results showed (adjusted p < 0.05) that a total of 106 bacterial and 6 archaeal genera were differentially abundant in the comparisons (Figs. 5 and 6). While several genera accounted for the differences in the bacterial community structure between the fallow land and the non-fertilized cultivated soil, the type of fertilization regime influenced only a few bacterial genera in the maize monocultures significantly. Heatmap plots (Supplementary Figs. 5 and 6) visualize the abundance distribution of the significant bacterial and archaeal genera in details, and hierarchically group the samples and genera, as well.

As shown in Supplementary Fig. 5, genera of the first separated cluster belonged mainly to Actinobacteria (e.g. *Gaiella*, uncult. Gaiellales, MB-A2-108), Proteobacteria (uncult. *Burkholderiaceae*, *Rhodoplanes*, *Phyllobacterium*) and Bacteroidetes (e.g. *Terrimonas*, unclass. *Microscillaceae*, uncult. *Saprospiraceae*). They were responsible for the distinct bacterial community composition of the fallow land soil samples. The second large cluster consisted of genera belonging mainly to Proteobacteria (e.g. unclass. *Acetobacteraceae*, SC-I-84, unclass. *Burkholderiaceae*, unclass. *Micropepsaceae*) and Acidobacteria (e.g. *Bryobacter*, *Cand*. Solibacter, unclass. *Acidobacteriaceae*, uncult. Acidobacteriales, uncult. *Blastocatellaceae*) which were more abundant in the NPK fertilized maize monoculture. The third taxonomically diverse group of genera (e.g. RB41, *Nitrospira*, *Haliangium*, uncult. *Gemmatimonadaceae*, MND1, unclass. *Steroidobacteraceae*, uncult. *Latescibacteraceae*) was present with a higher relative abundance than average in the NPK + FYM and the non-fertilized maize monocultures.

Compared to bacterial communities, the archaeal communities of soil samples consisted of much fewer genera. The archaeal taxa were reduced to eight relevant genera by filtering and normalization before analysis, out of which only six had significantly different abundances in
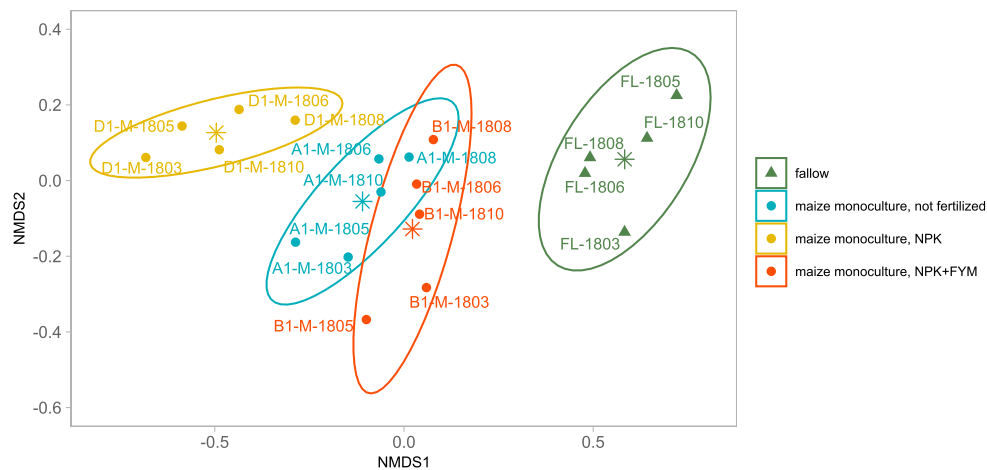
Fig. 3. Non-metric multidimensional scaling (NMDS) of bacterial communities. Fertilization and cultivation are indicated by color and symbol, respectively, sample designation is detailed in Table 1. Asterisks show the centroids of sample groups, ellipses represent a 90% confidence level. Stress = 0.06.
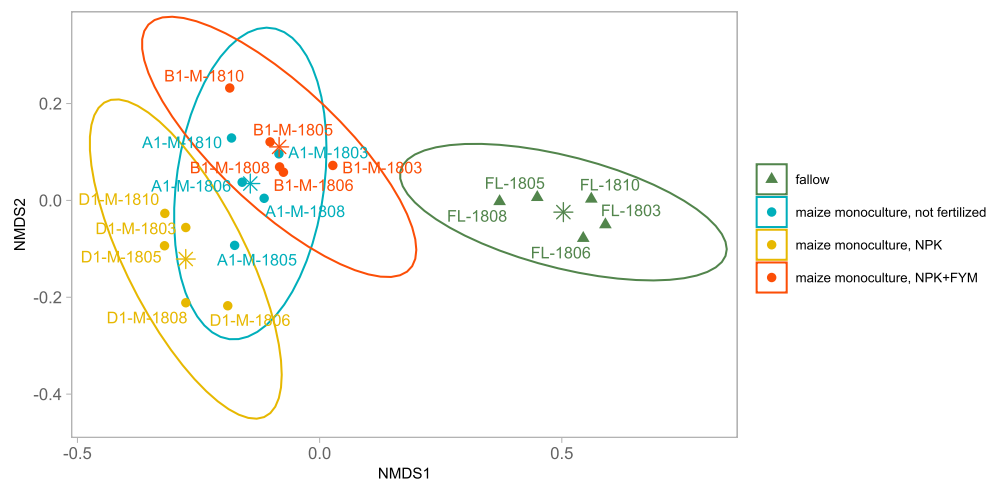

Fig. 4. Non-metric multidimensional scaling (NMDS) of archaeal communities. Fertilization and cultivation is indicated by color and symbol, respectively, sample designation is detailed in Table 1. Asterisks show the centroids of sample groups, ellipses represent a 90% confidence level. Stress = 0.05.

the sample groups (Fig. 6 and Supplementary Fig. 6). *Candidatus* Nitrososphaera and an unclassified *Nitrosophaeraceae* genus were significantly more abundant in the fallow land than in the maize monoculture; the opposite was true for another unclass. *Nitrosophaeraceae* genus, an unclassified Woesearchaeia and unclassified Archaea genus. The occurrence of *Candidatus* Nitrosotalea was specific to the NPK fertilized soil samples. An unclassified Archaea genus had significantly lower relative abundance in the NPK + FYM fertilized maize monoculture than in the other cultivated soils.

4. Discussion

There have been growing number of studies examining the impact of maize cultivation on the composition and activity of soil microbial communities regarding the application of fertilizers and crop rotation systems (Cheng et al., 2019; Maarastawi et al., 2018; Sun et al., 2019; Zhen et al., 2014). In this study, the 16S rRNA gene amplicon sequencing revealed the prevalence of bacterial phyla Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia, Gemmatimonadetes, Planctomycetes and Chloroflexi in all soil samples (Supplementary Fig. 2), that is consistent with previous next-generation sequencing studies on maize cultivated soils (Cui et al., 2018; Johnston-Monje et al., 2016; Li et al., 2014; Sun et al., 2019; Wen et al., 2017). The

archaeal communities were dominated by the phylum Thaumarchaeota in every sample with a relative abundance over 79% (Supplementary Fig. 3). The majority of archaeal OTUs were closely related to the family *Nitrososphaeraceae* (Group I.1b).

Land management can have a prolonged effect on microbial communities (Jangid et al., 2011), microbial biomass, soil C and N stocks (Rosenzweig et al., 2016) even after decades of conversion. Our data indicates that the composition of both the bacterial and archaeal communities of the fallow land differed significantly from those of the cultivated soil after 60 years. The long pause of previous disturbance originating from agricultural practice (e.g. tillage, fertilization, plant residue removal) caused a shift in the proportions of phyla Actinobacteria and Acidobacteria. Sequences related to Actinobacteria were found to be more abundant in the fallow land than in the cultivated soils. Similar phenomena were previously detected in grasslands (Ghimire et al., 2019; Nacke et al., 2011) and restored farmlands (Xu et al., 2020; Zhang et al., 2016) compared to cultivated soils. As reported by Zhang et al. (2016), the bacterial communities of an abandoned farmland transitioned from the Acidobacteria-dominated to a Proteobacteria- and Actinobacteria-dominated communities, because the increase of soil organic matter and N after decades of natural succession favored the r-selected copiotrophs against slow-growing oligotrophs preferring nutrient-poor environments. Differentially abundant bacteria that might
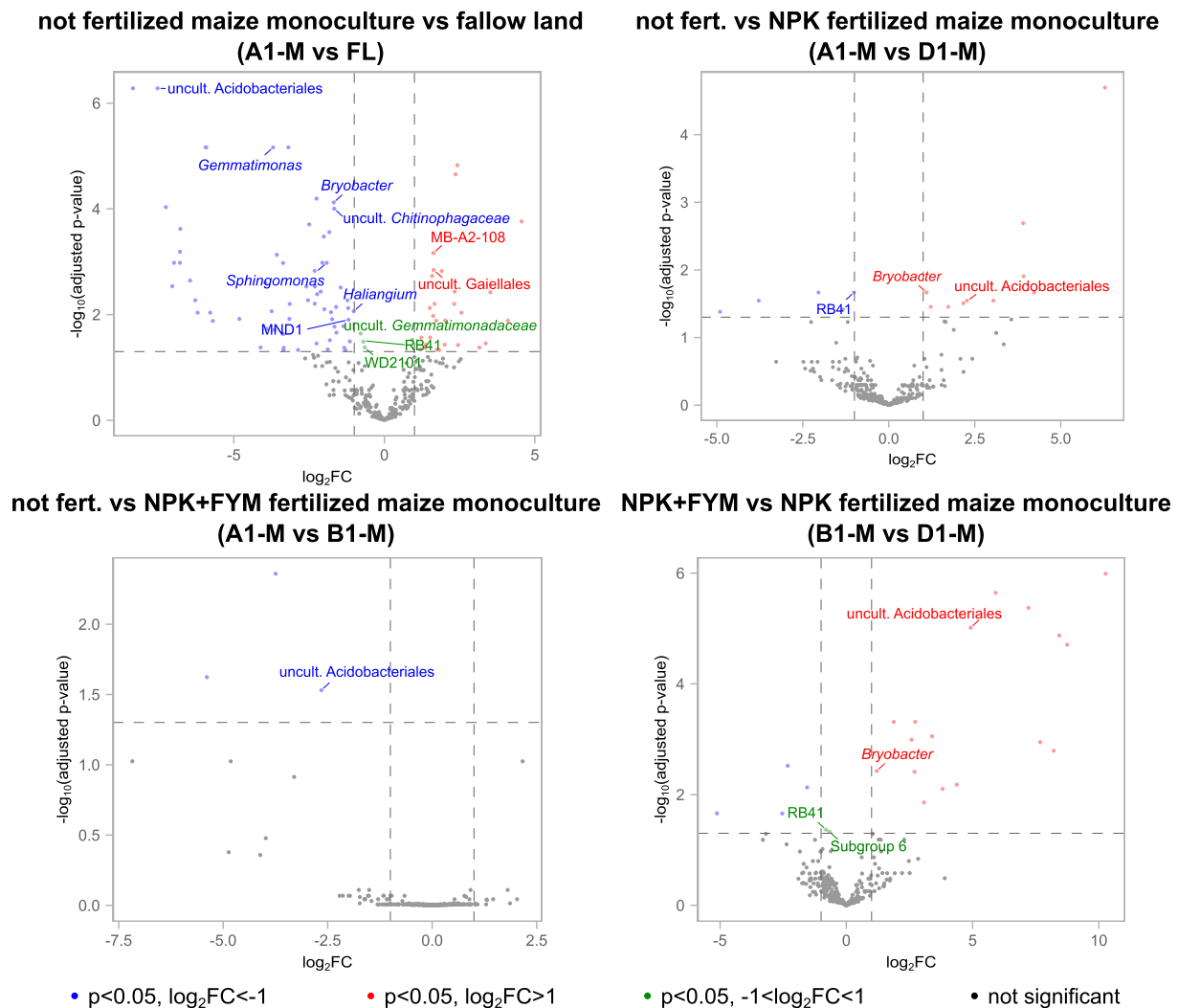
Fig. 5. Results of the differential abundance analyzes of each pair of groups summarized as volcano plots. Points are bacterial genera involved in the study after filtering and normalization. FC: fold-change. Genera with greater than a 2-fold significant increase or decrease are red and blue, respectively; significant genera with only a small increase or decrease are green. Genus name is shown if the genus had a minimum of 10,000 CPM (count per million) in the normalized library. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

also contribute to the restoration processes include the family *Saprospiraceae* capable of protein hydrolysis and degradation of complex organic compounds, the saprophytic *Agromyces* commonly found in rhizospheres, the root-nodulating *Microvirga*, the denitrifying *Rhodoplanes*, the plant growth promoting and N_2-fixing *Phyllobacterium*, the ammonia-oxidizing *Cand.* Nitrososphaera and an unclassified *Nitrososphaeraceae* genus that might provide the possibility for bacterial nitrate reduction in the fallow land.

The use, type and dosage of fertilizers have proven to effect microbial communities substantially (Sun et al., 2019; Yang et al., 2020). Comparing the maize monocultures under different fertilization regimes, the exclusive application of NPK resulted in significantly distinct bacterial and archaeal community structures. The lowest pH value was measured in the NPK treatment, probably due to the long-term sole application of the inorganic fertilizer. Many Acidobacteria genera responded positively to soil acidification, e.g. *Bryobacter*, *Cand.* Solibacter, unclassified genera of Acidobacteriales and an uncultured genus from *Acidobacteriaceae* Subgroup 1. Members of Acidobacteria are ubiquitous in agricultural soils where they can utilize a wide variety of carbon substrates including plant polymers (Eichorst et al., 2011) and survive low-nutrient conditions (Ward et al., 2009). Other genera (e.g. two unclass. *Micropepsaceae* genera, an uncult. *Ktedonobacteraceae*

genus, *Acidothermus*, WPS-2) were also found in significantly higher relative abundances in the NPK samples. Another important finding of the present results was that the uncultured phylum WPS-2 (or alternate name *Cand.* Eremiobacterota) was detected only in the NPK-fertilized maize soil samples. This phylum has been associated with bare, acidic soils with low organic matter content (Sheremet et al., 2020). The members of the acidophilic *Cand.* Nitrosotalea (Gubry-Rangin et al., 2011; Lehtovirta-Morley et al., 2011) were also observed only in the NPK-fertilized samples. As reported previously by Hartmann et al. (2015), these findings also suggest that the long-term mineral fertilized agricultural soils are preferred by bacteria and archaea adapted to low pH and nutrient-limited conditions. The nitrogen, in this long-term field experiment, was supplied as ammonium-nitrate both of which can be taken up by plant roots. However, nitrate is the prevalent form of nitrogen available for plants in aerobic soils, because ammonium is rapidly converted to nitrate in a two-step process by nitrifiers. Although ammonia oxidation can be performed by both ammonia-oxidizing archaea (AOA, all belonging to Thaumarchaeota) and bacteria (AOB), there is a growing evidence that AOA outnumber AOB in various type of soil ecosystems, e.g. in cultivated soils (He et al., 2007; Leininger et al., 2006; Nelson et al., 2010; Segal et al., 2017), therefore, their ecological importance in the nitrogen cycle is indisputable. Some reports stated
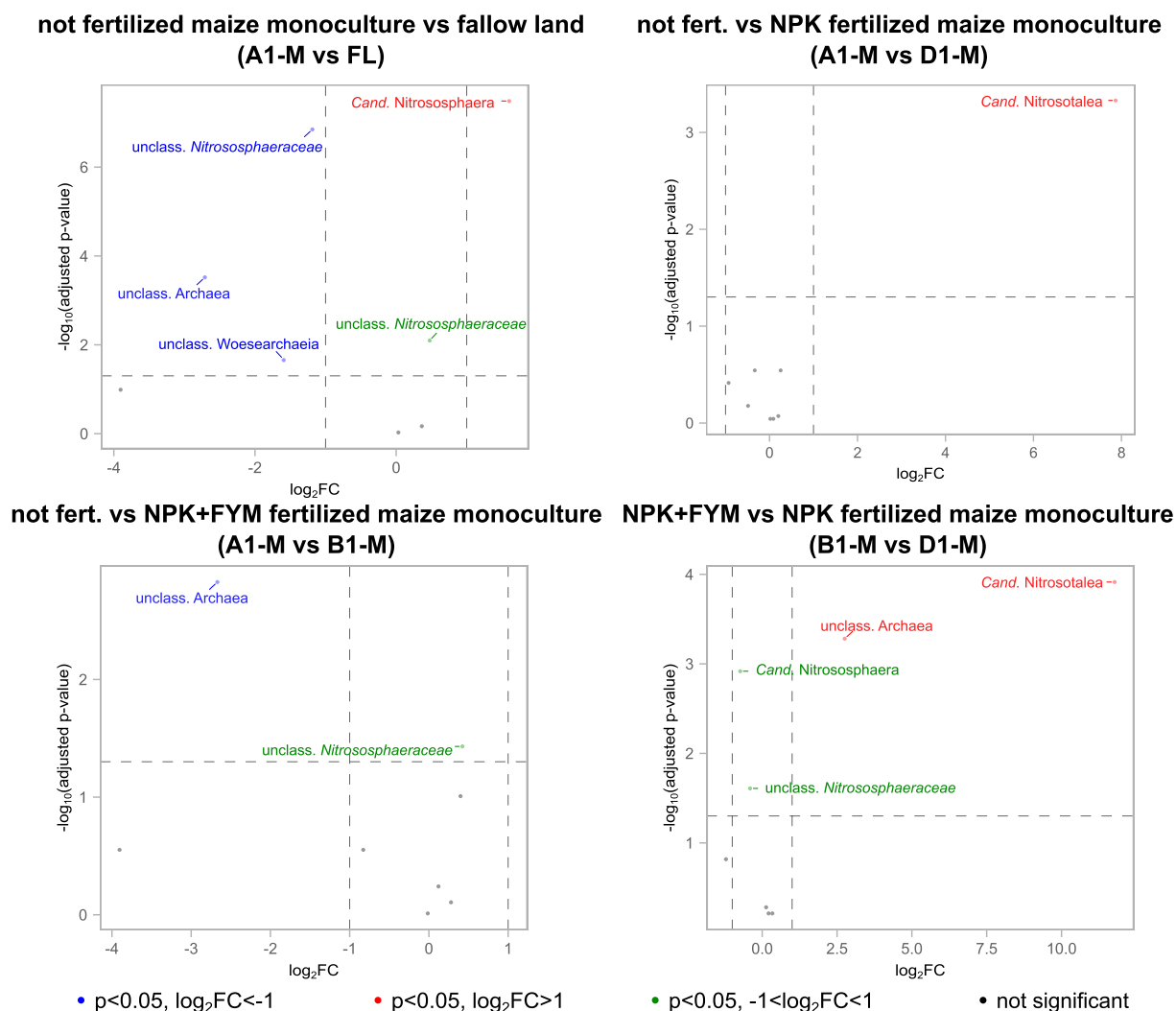
Fig. 6. Results of the differential abundance analyzes of each pair of groups summarized as volcano plots. Points are archaeal genera involved in the study after filtering and normalization. FC: fold-change. Genera with greater than a 2-fold significant increase or decrease are red and blue, respectively; significant genera with only a small increase or decrease are green. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that AOB are more effected by the N rate and the combination of mineral and organic fertilizers than AOA (Florio et al., 2016; Segal et al., 2017). As the pH decreases ammonia ionizes to ammonium and its availability decreases for the mostly neutrophilic ammonia-oxidizers (e.g. *Cand. Nitrososphaera*). However, nitrification still occurs in acidic soils by acidophilic AOA such as *Cand.* Nitrosotalea, in fact, there is a slightly significant negative correlation between soil pH and nitrification rate (Booth et al., 2005). Nitrifiers gradually release the positively charged ammonium ions captured on soil particles and oxidize it to water-soluble nitrate. Therefore, ammonium-nitrate fertilizers can provide a long-lasting nitrogen supply for plants. On the other hand, nitrification can lead to nitrate leaching and soil acidification due to proton accumulation. Minimizing the loss of nitrogen from fertilizers due to microbially-catalyzed processes would be an important step towards improving the efficiency of sustainability of agriculture (Cameron et al., 2013).

The additional organic fertilizer in the combined treatment significantly increased the relative abundance of a few genera (RB41, *Pedomicrobium*, *Phyllobacterium*, uncult. *Latescibacteriaceae*, unclass. *Steroidobacteraceae*, Acidobacteria Subgroup 6, *Cand.* Nitrososphaera and unclass. *Nitrososphaeraceae*) in contrast to the NPK fertilized maize monoculture soils. In the case of the RB41 these results correlate positively with the findings of Ai et al. (2018). Only three genera belonging to Acidobacterales and an unclassified Archaea genus were significantly

overrepresented in the NPK + FYM treatment compared to the non-fertilized soil. Consequently, the composition and structure of bacterial and archaeal communities of NPK + FYM treated soil were more similar to the non-fertilized soil than the soil fertilized exclusively with NPK. Even though these results differ from some earlier studies (Hartmann et al., 2015), they are consistent with those of Sun et al. (2015). A plausible explanation is that we did not detect the immediate influence and the specific taxa associated with manure, but its prolonged benign effect two years after the last application.

Based on the analysis of time series of several decades from the Martonvásár experiments, various conclusions can be made regarding the effects of fertilization and management on maize yield. According to Micskei et al. (2012) the combined treatment with farmyard manure and mineral fertilizer resulted in higher maize yield in wet years than the manure alone, but the highest yield was achieved with the exclusive application of NPK. Although in unfavorable, dry years, the yield loss was severest in the NPK fertilizer treatment, while the yield stability of FYM treatments was higher. The average annual maize yield of the combined NPK + FYM treatment (6.97 t ha^{-1}) exceeded almost one and a half times the non-fertilized soil (4.86 t ha^{-1}) and approached the solely NPK treatment soil (7.16 t ha^{-1}). Jenkinson (1991) also pointed out that plots receiving inorganic fertilizers contain half as much organic matter as the ones given FYM but gave higher yield in the classical

Broadbalk experiment (Rothamsted) due to the beneficial effects of manure such as providing microelements, increasing porosity and improvement of water management.

5. Conclusions

The sustainability of agriculture is a compromise between economic advantages and long-term impacts on the soil parameters and microbiome. Our results showed that the lack of disturbance originating from agriculture enriched several genera mainly belonging to Actinobacteria and *Nitrososphaeraceae*, compared to the cultivated soils. The exclusive use of inorganic fertilizer had the greatest effects on the bacterial and archaeal diversity in the maize monoculture soils, while the communities of non-fertilized and combined fertilized soils were similar to each other. Consequently, our data suggest that the use of manure can moderate the effects of inorganic fertilizer on the soil bacterial and archaeal communities and provide a stable, competitively high yield, even if it is applied only once every four years.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2021.104120.

References

Ai, C., Zhang, S., Zhang, X., Guo, D., Zhou, W., Huang, S., 2018. Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation. Geoderma 319, 156–166. https://doi.org/10.1016/j.geoderma.2018.01.010.

Benizri, E., Nguyen, C., Piutti, S., Slezack-Deschaumes, S., Philippot, L., 2007. Additions of maize root mucilage to soil changed the structure of the bacterial community. Soil Biol. Biochem. 39, 1230–1233. https://doi.org/10.1016/j.soilbio.2006.12.026.

Bongiorno, G., Bünemann, E.K., Brussaard, L., Mäder, P., Oguejiofor, C.U., de Goede, R. G.M., 2020. Soil management intensity shifts microbial catabolic profiles across a range of European long-term field experiments. Appl. Soil Ecol. 154, 103596 https://doi.org/10.1016/j.apsoil.2020.103596.

Booth, M.S., Stark, J.M., Rastetter, E., 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. Ecol. Monogr. 75, 139–157. https://doi.org/10.1890/04-0988.

Bossuyt, H., Denef, K., Six, J., Frey, S., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. Appl. Soil Ecol. 16, 195–208. https://doi.org/10.1016/S0929-1393(00)00116-5.

Cameron, K.C., Di, H.J., Moir, J.L., 2013. Nitrogen losses from the soil/plant system: a review. Ann. Appl. Biol. 162, 145–173. https://doi.org/10.1111/aab.12014.

Cheng, Z., Chen, Y., Zhang, F., 2019. Effect of cropping systems after abandoned salinized farmland reclamation on soil bacterial communities in arid northwest China. Soil Tillage Res. 187, 204–213. https://doi.org/10.1016/j.still.2018.12.015.

Csitári, G., Horváth, Z., Dunai, A., Tóth, Z., 2014. Trágyaadagok, művelési módok és az időjárás hatása a talaj mikrobiális biomassza tömegére és a növényi termésre. [Effects of agricultural practices, fertilization levels and weather on soil microbial biomass and crop yield] Georg. Agric 19, 227–233.

Cui, X., Zhang, Y., Gao, J., Peng, F., Gao, P., 2018. Long-term combined application of manure and chemical fertilizer sustained higher nutrient status and rhizospheric

bacterial diversity in reddish paddy soil of Central South China. Sci. Rep. 8, 16554 https://doi.org/10.1038/s41598-018-34685-0.

Dambreville, C., Hallet, S., Nguyen, C., Morvan, T., Germon, J.-C., Philippot, L., 2006. Structure and activity of the denitrifying community in a maize-cropped field fertilized with composted pig manure or ammonium nitrate. FEMS Microbiol. Ecol. 56, 119–131. https://doi.org/10.1111/j.1574-6941.2006.00064.x.

Debreczeni, K., Körschens, M., 2003. Long-term field experiments of the world. Arch. Agron. Soil Sci. 49, 465–483. https://doi.org/10.1080/03650340310001594754.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.

Eichorst, S.A., Kuske, C.R., Schmidt, T.M., 2011. Influence of plant polymers on the distribution and cultivation of bacteria in the phylum Acidobacteria. Appl. Environ. Microbiol. 77, 586–596. https://doi.org/10.1128/AEM.01080-10.

Feng, K., Cai, Z., Ding, T., Yan, H., Liu, X., Zhang, Z., 2019. Effects of potassium-solubilizing and photosynthetic bacteria on tolerance to salt stress in maize. J. Appl. Microbiol. 126, 1530–1540. https://doi.org/10.1111/jam.14220.

Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME J. 6, 1007–1017. https://doi.org/10.1038/ismej.2011.159.

Fließbach, A., Oberholzer, H.-R., Gunst, L., Mäder, P., 2007. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agric. Ecosyst. Environ. 118, 273–284. https://doi.org/10.1016/j.agee.2006.05.022.

Florio, A., Felici, B., Migliore, M., Dell'Abate, M.T., Benedetti, A., 2016. Nitrogen losses, uptake and abundance of ammonia oxidizers in soil under mineral and organo-mineral fertilization regimes. J. Sci. Food Agric. 96, 2440–2450. https://doi.org/10.1002/jsfa.7364.

Food and Agriculture Organization of the United Nations, 2018. FAOSTAT statistical database [WWW document]. http://www.fao.org/faostat/en/#data/QC.

Gazdag, O., Takács, T., Ködöböcz, L., Mucsi, M., Szili-Kovács, T., 2018. Soil metabolic activity profiles of the organic and conventional land use at Martonvásár. Columella. J. Agric. Environ. Sci. 5, 27–35. https://doi.org/10.18380/SZIE.COLUM.2018.5.1.27.

Ghimire, R., Thapa, V.R., Cano, A., Acosta-Martinez, V., 2019. Soil organic matter and microbial community responses to semiarid croplands and grasslands management. Appl. Soil Ecol. 141, 30–37. https://doi.org/10.1016/j.apsoil.2019.05.002.

Gubry-Rangin, C., Hai, B., Quince, C., Engel, M., Thomson, B.C., James, P., Schloter, M., Griffiths, R.I., Prosser, J.I., Nicol, G.W., 2011. Niche specialization of terrestrial archaeal ammonia oxidizers. Proc. Natl. Acad. Sci. 108, 21206–21211. https://doi.org/10.1073/pnas.1109000108.

Hartmann, M., Frey, B., Mayer, J., Mäder, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. ISME J. 9, 1177–1194. https://doi.org/10.1038/ismej.2014.210.

He, J., Shen, J., Zhang, L., Zhu, Y., Zheng, Y., Xu, M., Di, H., 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. Environ. Microbiol. 9, 2364–2374. https://doi.org/10.1111/j.1462-2920.2007.01358.x.

Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. 5, 1571–1579. https://doi.org/10.1038/ismej.2011.41.

Hou, X., Wang, X., Li, R., Jia, Z., Liang, L., Wang, J., Nie, J., Chen, X., Wang, Z., 2012. Effects of different manure application rates on soil properties, nutrient use, and crop yield during dryland maize farming. Soil Res. 50, 507. https://doi.org/10.1071/SR11339.

Hu, L., Robert, C.A.M., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., Van Der Heijden, M.G.A., Schlaeppi, K., Erb, M., 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nat. Commun 9, 1–13. https://doi.org/10.1038/s41467-018-05122-7.

Jangid, K., Williams, M.A., Franzluebbers, A.J., Schmidt, T.M., Coleman, D.C., Whitman, W.B., 2011. Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biol. Biochem. 43, 2184–2193. https://doi.org/10.1016/j.soilbio.2011.06.022.

Jenkinson, D.S., 1991. The Rothamsted long-term experiments: are they still of use? Agron. J. 83, 2–10. https://doi.org/10.2134/agronj1991.00021962008300010008x.

Johnston-Monje, D., Lundberg, D.S., Lazarovits, G., Reis, V.M., Raizada, M.N., 2016. Bacterial populations in juvenile maize rhizospheres originate from both seed and soil. Plant Soil 405, 337–355. https://doi.org/10.1007/s11104-016-2826-0.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, e1. https://doi.org/10.1093/nar/gks808.

Kolde, R., 2015. pheatmap: Pretty heatmaps [Software].

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79, 5112–5120. https://doi.org/10.1128/AEM.01043-13.

Kunin, V., Engelbrektson, A., Ochman, H., Hugenholtz, P., 2010. Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. Environ. Microbiol. 12, 118–123. https://doi.org/10.1111/j.1462-2920.2009.02051.x.

Lehtovirta-Morley, L.E., Stoecker, K., Vilcinskas, A., Prosser, J.I., Nicol, G.W., 2011. Cultivation of an obligate acidophilic ammonia oxidizer from a nitrifying acid soil. Proc. Natl. Acad. Sci. U. S. A. 108, 15892–15897. https://doi.org/10.1073/pnas.1107196108.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806–809. https://doi.org/10.1038/nature04983.

Li, X., Rui, J., Mao, Y., Yannarell, A., Mackie, R., 2014. Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. Soil Biol. Biochem. 68, 392–401. https://doi.org/10.1016/j.soilbio.2013.10.017.

Lucas, R.W., Klaminder, J., Futter, M.N., Bishop, K.H., Egnell, G., Laudon, H., Högberg, P., 2011. A meta-analysis of the effects of nitrogen additions on base cations: implications for plants, soils, and streams. For. Ecol. Manag. 262, 95–104. https://doi.org/10.1016/j.foreco.2011.03.018.

Maarastawi, S.A., Frindte, K., Linnartz, M., Knief, C., 2018. Crop rotation and straw application impact microbial communities in Italian and Philippine soils and the rhizosphere of Zea mays. Front. Microbiol. 9, 1–17. https://doi.org/10.3389/fmicb.2018.01295.

Magurno, F., Sasvári, Z., Barchi, L., Posta, K., 2014. From monoculture to Norfolk system: how the number of crops in rotation can influence the biodiversity of arbuscular mycorrhiza assemblages in the soil. Open J. Ecol. 04, 1080–1088. https://doi.org/10.4236/oje.2014.417088.

Martinez Arbizu, P., 2020. pairwiseAdonis: Pairwise Multilevel Comparison Using Adonis. R Package Version 0.4.

Masood, S., Naz, T., Javed, M.T., Ahmed, I., Ullah, H., Iqbal, M., 2014. Effect of short-term supply of farmyard manure on maize growth and soil parameters in pot culture. Arch. Agron. Soil Sci. 60, 337–347. https://doi.org/10.1080/03650340.2013.792990.

Mayer, Z., Sasvári, Z., Szentpéteri, V., Pethőné Rétháti, B., Vajna, B., Posta, K., 2019. Effect of long-term cropping systems on the diversity of the soil bacterial communities. Agronomy 9, 878. https://doi.org/10.3390/agronomy9120878.

Micskei, G., Árendás, T., Berzsenyi, Z., 2012. Relationships between maize yield and growth parameters in a long-term fertilization experiment. Acta Agron. Hungarica 60, 209–219. https://doi.org/10.1556/AAgr.60.2012.3.4.

Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111, 41–55. https://doi.org/10.1007/s10533-011-9658-z.

Murphy, D., Stockdale, E.A., Brookes, P.C., Goulding, K.W.T., 2007. Impact of microorganisms on chemical transformations in soil. In: Abbott, L.K., Murphy, D.V. (Eds.), Soil Biological Fertility. Springer, Netherlands, Dordrecht, pp. 37–59.

Nacke, H., Thürmer, A., Wollherr, A., Will, C., Hodac, L., Herold, N., Schöning, I., Schrumpf, M., Daniel, R., 2011. Pyrosequencing-based assessment of bacterial community structure along different management types in German forest and grassland soils. PLoS One 6, e17000. https://doi.org/10.1371/journal.pone.0017000.

Nelson, D.M., Cann, I.K.O., Mackie, R.I., 2010. Response of archaeal communities in the rhizosphere of maize and soybean to elevated atmospheric CO$_2$ concentrations. PLoS One 5, e15897. https://doi.org/10.1371/journal.pone.0015897.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., 2018. Wagner, H. Community ecology package, Package "vegan".

Oliveira, C.A., Alves, V.M.C., Marriel, I.E., Gomes, E.A., Scotti, M.R., Carneiro, N.P., Guimarães, C.T., Schaffert, R.E., Sá, N.M.H., 2009. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. Soil Biol. Biochem. 41, 1782–1787. https://doi.org/10.1016/j.soilbio.2008.01.012.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.

Ramirez, K.S., Craine, J.M., Fierer, N., 2010. Nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied. Soil Biol. Biochem. 42, 2336–2338. https://doi.org/10.1016/j.soilbio.2010.08.032.

Ramirez, K.S., Craine, J.M., Fierer, N., 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Glob. Chang. Biol. 18, 1918–1927. https://doi.org/10.1111/j.1365-2486.2012.02639.x.

Ranum, P., Peña-Rosas, J.P., Garcia-Casal, M.N., 2014. Global maize production, utilization, and consumption. Ann. N. Y. Acad. Sci. 1312, 105–112. https://doi.org/10.1111/nyas.12396.

Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140. https://doi.org/10.1093/bioinformatics/btp616.

Rosenzweig, S.T., Carson, M.A., Baer, S.G., Blair, J.M., 2016. Changes in soil properties, microbial biomass, and fluxes of C and N in soil following post-agricultural grassland restoration. Appl. Soil Ecol. 100, 186–194. https://doi.org/10.1016/j.apsoil.2016.01.001.

Sasvári, Z., Magurno, F., Posta, K., 2012. Hosszú időtartamú monokultúrás termesztésből és különböző vetésforgó rendszerekből származó növények arbuszkuláris mikorrhiza (AM) gomba-közösségeinek vizsgálata. [Investigation of arbuscular mycorrhizal (AM) fungal communities of plants from long-term monoculture cultivation and different crop rotation systems]. Tájökológiai Lapok 10, 351–360.

Schad, P., van Huyssten, C., Michéli, E. (Eds.), 2015. World Reference Base for Soil Resources 2014, International Soil Classification System for Naming Soils and Creating Legends for Soil Maps, 16th ed. FAO, Rome.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.

Segal, L.M., Miller, D.N., McGhee, R.P., Loecke, T.D., Cook, K.L., Shapiro, C.A., Drijber, R.A., 2017. Bacterial and archaeal ammonia oxidizers respond differently to long-term tillage and fertilizer management at a continuous maize site. Soil Tillage Res. 168, 110–117. https://doi.org/10.1016/j.still.2016.12.014.

Sheremet, A., Jones, G.M., Jarett, J., Bowers, R.M., Bedard, I., Culham, C., Eloe-Fadrosh, E.A., Ivanova, N., Malmstrom, R.R., Grasby, S.E., Woyke, T., Dunfield, P.F., 2020. Ecological and genomic analyses of candidate phylum WPS-2 bacteria in an unvegetated soil. Environ. Microbiol 15054, 1462–2920. https://doi.org/10.1111/1462-2920.15054.

Sun, R., Zhang, X.-X., Guo, X., Wang, D., Chu, H., 2015. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. Soil Biol. Biochem 88, 9–18. https://doi.org/10.1016/j.soilbio.2015.05.007.

Sun, R., Zhang, P., Riggins, C.W., Zabaloy, M.C., Rodríguez-Zas, S., Villamil, M.B., 2019. Long-term N fertilization decreased diversity and altered the composition of soil bacterial and archaeal communities. Agronomy 9, 574. https://doi.org/10.3390/agronomy9100574.

Tian, D., Niu, S., 2015. A global analysis of soil acidification caused by nitrogen addition. Environ. Res. Lett. 10, 024019 https://doi.org/10.1088/1748-9326/10/2/024019.

Tindall, B.J., Rosselló-Móra, R., Busse, H.J., Ludwig, W., Kämpfer, P., 2010. Notes on the characterization of prokaryote strains for taxonomic purposes. Int. J. Syst. Evol. Microbiol. 60, 249–266. https://doi.org/10.1099/ijs.0.016949-0.

Ujvári, G., Borsodi, A.K., Megyes, M., Mucsi, M., Szili-Kovács, T., Szabó, A., Szalai, Z., Jakab, G., Márialigeti, K., 2020. Comparison of soil bacterial communities from juvenile maize plants of a long-term monoculture and a natural grassland. Agronomy 10, 341. https://doi.org/10.3390/agronomy10030341.

Ward, N.L., Challacombe, J.F., Janssen, P.H., Henrissat, B., Coutinho, P.M., Wu, M., Xie, G., Haft, D.H., Sait, M., Badger, J., Barabote, R.D., Bradley, B., Brettin, T.S., Brinkac, L.M., Bruce, D., Creasy, T., Daugherty, S.C., Davidsen, T.M., DeBoy, R.T., Detter, J.C., Dodson, R.J., Durkin, A.S., Ganapathy, A., Gwinn-Giglio, M., Han, C.S., Khouri, H., Kiss, H., Kothari, S.P., Madupu, R., Nelson, K.E., Nelson, W.C., Paulsen, I., Penn, K., Ren, Q., Rosovitz, M.J., Selengut, J.D., Shrivastava, S., Sullivan, S.A., Tapia, R., Thompson, L.S., Watkins, K.L., Yang, Q., Yu, C., Zafar, N., Zhou, L., Kuske, C.R., 2009. Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. Appl. Environ. Microbiol 75, 2046–2056. https://doi.org/10.1128/AEM.02294-08.

Wen, X., Wang, M., Ti, J., Wu, Y., Chen, F., 2017. Bacterial community composition in the rhizosphere of maize cultivars widely grown in different decades. Biol. Fertil. Soils 53, 221–229. https://doi.org/10.1007/s00374-016-1169-6.

Xu, M., Gao, D., Fu, S., Lu, X., Wu, S., Han, X., Yang, G., Feng, Y., 2020. Long-term effects of vegetation and soil on the microbial communities following afforestation of farmland with *Robinia pseudoacacia* plantations. Geoderma 367, 114263. https://doi.org/10.1016/j.geoderma.2020.114263.

Yang, Q., Zheng, F., Jia, X., Liu, P., Dong, S., Zhang, J., Zhao, B., 2020. The combined application of organic and inorganic fertilizers increases soil organic matter and improves soil microenvironment in wheat-maize field. J. Soils Sediments 20, 2395–2404. https://doi.org/10.1007/s11368-020-02606-2.

Zhang, C., Liu, G., Xue, S., Wang, G., 2016. Soil bacterial community dynamics reflect changes in plant community and soil properties during the secondary succession of abandoned farmland in the Loess Plateau. Soil Biol. Biochem. 97, 40–49. https://doi.org/10.1016/j.soilbio.2016.02.013.

Zhen, Z., Liu, H., Wang, N., Guo, L., Meng, J., Ding, N., Wu, G., Jiang, G., 2014. Effects of manure compost application on soil microbial community diversity and soil microenvironments in a temperate cropland in China. PLoS One 9, e108555. https://doi.org/10.1371/journal.pone.0108555.

Zhu, S., Vivanco, J.M., Manter, D.K., 2016. Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize. Appl. Soil Ecol. 107, 324–333. https://doi.org/10.1016/j.apsoil.2016.07.009.