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Title page

Soil microbiome biomass, activity, composition and CO_2 emissions in a long term organic and conventional farming systems.

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Running Title: Soil microbiome in ORG vs CON systems

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Abstract

The implementation of environmentally-friendly agricultural policies has increased the need to compare agricultural aspects of conventional (CON) and organic farming (ORG) systems.

The objective of the present work was to compare the effects of an organic and conventional long-term experiment on bacterial and fungal biomass and activity, as well as soil CO_2 emission and readily available nitrogen forms in a soil cultivated with *Helianthus annuus L.* The microbial biomass was more active and abundant in ORG as well as soil CO_2 emission. Despite being less abundant, fungi were more active than bacteria in both ORG and in CON experiments. 16S rRNA gene sequencing showed that the ORG treatment had a significantly greater bacterial richness than CON. *Cyanobacteria, Actinobacteria and Proteobacteria* were the most abundant phyla contributing more than others to the differences between the two systems. Moreover, the soil NH_4^+ and NO_2^- content was not significantly different between ORG and CON, while NO_3^- was less in ORG. ORG sunflower yield was significantly less compared to CON. While much remains to be discovered about the effects of these agricultural practices on soil chemical properties and microbial diversity, our findings may contribute to this type of investigation.

Keywords: *soil metagenome, qPCR, CO_2 emissions, organic and conventional agriculture, microbial biodiversity*

Highlights:

- Compare the effects of an organic and conventional long-term experiment on bacterial and fungal biomass and activity, as well as soil CO_2 emission and readily available nitrogen forms into the soil.
- ORG treatment had a significant increase of bacterial richness and biomass activity and a reduced NO_3^- content.
- Soil nitrate leaching potential in organic farming is lower than conventional farming.

1 INTRODUCTION

Soil quality has been defined as "the capacity of a soil to function within ecosystem and land-use boundaries, to sustain biological productivity, maintain environmental quality and promote plant and animal health" (Doran & Parkin, 1994). Soil microorganisms play a crucial role in maintaining soil quality; they are generally considered the driving force behind litter decomposition processes and play a major role in numerous ecosystem functions, such as organic matter turnover, nitrogen (N) cycling, nutrient mobilization/immobilization, humification, degradation of pollutants and maintenance of the soil structure (Xue et al., 2006). Soil microbiological properties, such as microbial biomass and metabolic activity, are often measured to obtain immediate and accurate information about changes in soil due to land use and agronomic practices. For these reasons the microbial biomass can be taken as a sensitive indicator of changes in soil fertility (Campos et al., 2014).

For a sustainable environment it is important to improve existing land management systems in order to minimize environmental problems.

Conventional agriculture utilizes fertilizers and herbicides to increase crop yields, but also cause a progressive decline in soil organic matter levels, which affect physical, chemical and biological soil properties (Mäder et al., 2002; Pimentel et al., 1995). The use of herbicides can modify the function and structure of soil microbial communities altering the normal ecosystems functionality, which in turn has important implications for soil fertility and quality (Pampulha & Oliveira, 2006). An active soil microflora which provides accessible nutrients for crops, is an important priority in all farming systems.

A sustainable alternative to conventional agriculture is organic farming, now quite well received on a global scale and covering approximately 72.3 million hectares in 2021 (Willer et al., 2021). As reported by Mäder et al. (2002), soil microbial biomass, dehydrogenase, protease, and phosphatase activities were higher in organic systems than in the conventional systems, indicating a higher overall microbial activity.

Nevertheless, the number of long-term field trials comparing organic and conventional systems is limited and still there are only a few investigations about the effects of these two systems on soil microbial properties.

The hypothesis at issue is that there is an urgent need to better understand how soil use and management affect microbial activity and soil quality. Long-term field trials can help to better investigate soil quality since changes in soil quality may only become apparent over the long-term.

Therefore, our analyses were carried out at the Montepaldi long term experiment (MoLTE, San Casciano Val di Pesa, Florence, Tuscany), which is the longest experiment on organic farming anywhere in the Mediterranean area.

The objective of the present work was to compare the effects of an organic and conventional long-term experiment on the bacterial and fungal biomass and activity, as well as soil CO_2 emission and readily available N forms in the soil. Moreover, we investigated the composition of the bacterial community through 16S rDNA sequencing and we focused on ammonia oxidizing bacteria (AOB) by qPCR, as the AOB plays a crucial role in the N cycle being very sensitive to environmental stresses (Ceccherini et al., 2007).

2 MATERIAL AND METHODS

2.1 Site description and experimental design

The trials were located at the experimental farm of the University of Florence (Montepaldi, San Casciano, Val di Pesa, 11°09'08''E, 43°40'16''N) inside the MoLTE (Montepaldi Long Term Experiment) site. The MoLTE has been active since 1991 and covers a slightly sloping surface of about 15 ha. The experimental area is characterized by a typical Mediterranean and Sub-Appennines climate with average annual precipitations of 770 mm. The summer period is characterized by dry conditions with high temperatures and little precipitations (Bedini et al., 2013). Annual mean temperatures during the experimentation were 14.2°C (Fig. 1). Soil, derived from the Pesa river fluvial deposit, is between silty clay loam and clay loam in terms of texture (Tab.1).

The MoLTE includes different agroecosystem management (MAN) systems; for this experiment we considered an organic arable system under organic management (EC reg. 2092/91 and following regulations), where certified organic fertilizers, amendments and green manure were used from 1991 (ORG), and a conventional/high-input arable system where chemical xenobiotics, mineral and synthetic fertilizers have been applied since 1991 (CON), both cultivated with *Helianthus annuus L.*. The agronomic aspects of the experiment are described in Table 2.

Two plots (47 x 132 m each) per management option (ORG and CON) were considered. Within each plot a chronological data collection for each indicator (microbial and chemical analysis, and GHGs emissions) was applied, which corresponded to the most important phenological phases of the sunflower (TIME in days, i.e. t0-seedling, t7 and t18-intermediate time, t52-raising, t83-intermediate time, t104-flowering and t138-harvest).

For the microbial and chemical analyses three soil samples with random coordinates were taken for each MAN*TIME combination. For the monitoring of soil carbon dioxide (CO_2) emissions, the closed static chamber technique was adopted (Verdi et al., 2019a) for each MAN*TIME combination.

2.2 Soil sampling for DNA extraction and soil microbial biomass determination

Soil samples were collected with a core sampler (3 cm diam.) from the top 15 cm in each plot (ORG and CON). Samples were collected from field in sealed plastic bags and transported on ice to the laboratory. Soil samples were sieved at 2 mm and stored at -20°C until DNA extraction. From each soil sample, 0.5 g of soil were used for total DNA extraction by FastDNA Kit for Soil (MPBiomedicals) as described in Ascher et al. (2009). Estimation of soil microbial biomass was carried out on the base of DNA yield, using picodrop-based quantification of double-stranded DNA (dsDNA) and stored at -20 °C (Fornasier et al., 2014; Marstorp & Witter, 1999).

2.3 Quantitative PCR (qPCR)

Quantitative PCR was performed to determine the 16S rRNA gene copy number of bacteria, the 18S rRNA gene copy number of fungi and the functional gene *amo*A copy number of ammonia oxidizers (AOB) in soil, using 40 ng DNA templates for all the samples. Reactions were performed in an iCycler (BioRad, Hercules, CA, USA) and the results were analyzed with the manufacturer's software (Optical System Software v 3.0a). Amplification was carried out in a 25 µl final volume containing: 2.5 pmol of each primer, 12.5 µl of iQ SYBR Green Supermix (2X) and sterile ddH_2O to reach the appropriate volume; three replicates were carried out for each sample. Amplification reactions were performed in 96-well microtiter plates (BioRad, Hercules, CA, USA); with a known amount of *Bacillus subtilis* BD1512 341f/515r 174bp PCR fragment previously amplified and purified (Simmons et al., 2007), *Saccharomyces boulardii* (Zambon Italia) FF390/FR1 390bp PCR fragment (Chemidlin Prévost-Bouré et al., 2011) and *Nitrosolobus multiformis* ATCC 25196 *amo*A1F/2R 490bp PCR fragment (Rotthauwe et al., 1997; Ceccherini et al., 2007) in each plate, were used to develop the standard curve for the

respective qPCRs by plotting the logarithm of known concentrations (from 10^{-1} to 10^{-6} ng in 25 µl reaction for eubacteria and fungi; from 10^{-4} to 10^{-9} ng in 25 µl reaction for ammonia oxidizers) against the threshold Cycle (Ct) values.

The qPCR program for eubacteria had an initial step of denaturation (3 min, 95°C) followed by 40 cycles of 15 sec at 95°C, 30 sec at 63°C, 30 sec at 72°C; for fungi an initial step of denaturation (3 min, 95°C) followed by 40 cycles of 45 sec at 95°C, 30 sec at 50°C, 50 sec at 70°C, 25sec at 90°C and 4min at 72°C; for ammonia oxidizers an initial step of denaturation (3 min, 95°C) followed by 40 cycles of 45 sec at 95°C, 30 sec at 55°C, 50 sec at 72°C. After each cycle, a melting curve program was run for which measurements were made at 0.5°C temperature increments every 10 sec within a range of 60–100°C.

2.4 16S rRNA Gene Sequencing

The V3-V4 region of 16S rRNA gene was amplified using the Illumina barcoded primer pair 341F/ 805R (Klindworth et al., 2013) by using a TProfessional thermal cycler (Biometra, biomedizinische Analytik GmbH). The PCR reaction mix (50µl) contained: 40 ng of template DNA, with KAPA Hifi Hotstart readyMix (Roche). PCR running conditions were: 3 min denaturation at 95 °C, followed by 25 sequential cycles each consisting of 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C, followed by a final extension step at 72 °C for 5 min. PCR products (amplicon size ~550 bp) were purified using a AMPure XP beads (Fisher Scientific) and then quantified by an Invitrogen™ Qubit™ 2.0 Fluorometer (ThermoFisher Scientific). Purified amplicons were used for library preparation and sequencing, according to the Illumina 16S Metagenomic Sequencing Library Preparation guide (downloaded from https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf. Paired-end sequencing (2 × 300 bp) was carried out by using a MiSeq System (Illumina, California, USA). Since the ORG and CON soils belonged to a long-term experiment (28 years), it can reasonably be considered that the established microflora were stabilized. Moreover, since the purpose of the 16S rDNA sequencing was intended only to highlight any possible differences in the soil bacterial community under the two types of management, then, at the outset, DNA replicates of each sampling time were pooled together and considered as a representative sample for each management practices (each of which having seven replicates).

2.5 Sequencing Data Processing

Paired reads were assembled, quality-filtered, and analyzed using the pipeline SEED 2.0.3 with the inclusion criteria of mean quality score ≥32 and length ≥250bp. Briefly, chimeric sequences were detected using the de novo VSEARCH algorithm (Větrovsky & Baldrian, 2013) and removed from the dataset. Sequences were then clustered into operational taxonomic units (OTUs) at a 97% sequence identity threshold using the VSEARCH algorithm; consensus sequences were constructed for all clusters (Rognes et al., 2016). Low abundant sequences (≤5 of total count) were excluded from further analysis. Identification and the taxonomic assignment were done using representative sequences retrieved from RDP database (Wang et al., 2007) and the NCBI using a 10^{-4} E value threshold. Sequences identified other than bacteria were discarded. The remaining sequences were used to create OTU table and then normalized by dividing sequences of individual OTU. Phylogenetic assignment to bacterial phyla and class level was based on best hits, by dividing the number of sequences belonging to each phylogenetic group by the total number of sequences in the given sample.

A Venn diagram was constructed to identify shared and unique OTUs between the two different management practices (ORG *vs* CON). Rarefaction and *alpha* diversity of OTUs were performed on resampled datasets with the same number of sequences randomly selected from all samples (50.000 sequences) using the SEED 2.0.3 software (Větrovsky & Baldrian, 2013). OTUs with > 0.1 % abundance were used to evaluate differences in *beta* diversity.

2.6 Soil CO_2 emissions, fluxes estimation and specific respiration of biomass ($mqCO_2$)

For the monitoring of soil carbon dioxide (CO_2) emissions, the closed static chamber technique was adopted. Chambers were constructed as described by Parkin & Venterea (2010) and Verdi et al., (2019b). Emissions were monitored using a portable gas analyser (Madur, XCGM 400) as described by Verdi et al. (2019a). Gas sampling was carried out inserting a needle, connected to the gas analyser by a polytetrafluoroethylene tube, for one minute. Gas samplings were carried out immediately after chamber closing (t0) and after one hour of gas accumulation (t1) with the chamber closed. Gas samplings were carried out biweekly throughout the growing season, from 20th April (cotyledons emergence) until 5th September (physiological maturity) 2018.

The ratio of soil CO_2 emissions to the microbial biomass, this latter expressed as DNA yield (Fornasier et al., 2014), has been used similarly to the metabolic quotient (Blagodatskaya et al., 2003) here indicated as $mqCO_2$ and expressed as kg CO_2 per kg DNA yield per hectare of soil.

The ratio of soil CO_2 emissions to the bacterial and fungal gene copies (obtained by quantitative PCR) has been considered here as the metabolic activity of these two microbial communities, indicated as Bac qCO_2 and Fun qCO_2.

2.7 Soil NH_4^+, NO_2^-, NO_3^- and readily mineralizable organic N content

Soil samples were analyzed to determine the concentration of readily available forms of N in soil (RA-N): ammonium-N ($NH_4^+ - N$), nitrate-N ($NO_3^- - N$), nitrite-N ($NO_2^- - N$), and readily mineralizable organic N (RMO-N). N forms were determined after extraction with the calcium chloride ($CaCl_2$) procedure by Houba et al. (1995), which has the advantage of extraction uniformity for the considered N forms and it was found being a good extracting solution for organic N readily available for mineralization and plant uptake (Nunan et al., 2001). Thus, 50 g of air-dried soil from each sample were extracted with a 0.01 M $CaCl_2$ solution (Sigma-Aldrich, 97%) at a soil: solution ratio of 1:10. The suspension was shaken for 2 h at 150 rev min^{-1} at room temperature and then filtered through Whatman no. 42 nitrate-free filter paper.

The concentrations of N forms in solution were determined by spectrophotometry using a Lambda 20 spectrometer (PerkinElmer, Waltham, Massachusetts, USA). The $NO_2^- - N$ concentration in solution was determined by means of Griess reaction (EPA, 1993). Aliquots of 10 ml of soil extract solution were treated with the Griess reagent (Sigma-Aldrich) containing 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride solution and a 1% sulphanilamide solution in 5% phosphoric acid. The absorbance of the nitrite-containing sample was measured at 540 nm (value A). Aliquots of soil extract solution (25 ml) were treated according to the nitrate copper-cadmium reduction method (APHA, 2000) to reduce nitrate to nitrite. The resulting solutions were treated by means of Griess reaction and then spectrophotometrically analyzed at 540 nm as previously described to determine the concentration of $NO_2^- - N + NO_3^- - N$ (value B). Finally, $NO_3^- - N$ concentration (value C) was calculated by subtracting nitrite values (value B – value A). Further aliquots (10 ml) of soil extract solution were treated following the Nessler method (ASTM, 2015) and then absorbance analyzed at 420 nm for the determination of $NH_4^+ - N$ concentration (value D). Aliquots (20 ml) of soil extract solution were acid digested for 2 h using 2 ml of H_2SO_4 (Nunan et al., 2001). The resulting digested solutions were then transferred to 50 ml volumetric flasks, pH adjusted to 7 with 1N sodium hydroxide and then brought to volume with deionized water. Then, according to (Nollet et al., 2014) the solutions were treated with

Nessleration as previously described and analyzed at 420 nm for the determination of $NH_4^+ - N$ concentration (value E). RMO-N concentration (value F) was determined by difference between E and D values. Finally, the total RA-N was calculated by summing the N determined after reading A, C, D and F. Quality Control (QC) for N measurements includes triplicate analysis of each sample and 7 of every 50 samples analyzed were known QC samples (distilled water blank, 0.5, 2.5, 5, 25, 50, 250 mg l^{-1}).

2.8 Sunflower yields and morphological parameters

Plant morphological parameters were assessed in order to test the effects of different farming systems on sunflower. Crops were harvested on 5th September 2018 in a sampling area of 500 m^2 for the analysis of plant height, flowers diameter, average number of seeds per plant, average weight of seeds per plant and yields (kg ha^{-1}). Three sampling sites with random coordinates where identified in the sampling area. On each coordinate, a two metres long ruler was used to collect sunflowers plants. Crop samples were collected in field and dried in a lab stove at 80°C for 48 hours until constant weight detection for dry weight determination. Dry matter yield was then calculated by averaging the three replicate samples and by standardizing sunflower seeds to tons ha^{-1}.

2.9 Statistical analyses

The analytical process was as follows. The microbial qPCR, chemical and CO_2 results were analysed by linear mixed-effects (LME) models built by lme() function. Analysis of residuals did not show substantial deviation from normality. To compare the models, we used *Akaike Information Criterion* (AIC) (Sakamoto et al., 1986), choosing the model with the lowest AIC (Pinheiro & Bates, 2000). This analyses were performed using the R statistical software (R Core Team, 2020).

The bacterial sequencing data was analysed by PAST 3.03 (Hammer et al., 2001) and R statistical software (R Core Team, 2020). A*lpha* diversity of OTUs was performed by One-way ANOVA followed by Tukey's post-hoc test at $p < 0.05$ level of significance to analyze the individual significance. A Principal Coordinate Analysis (PCoA) and PERMANOVA test were conducted based on Bray-Curtis similarity distance to determine the distribution of diversity and statistical significance of *beta* diversity, respectively. A SIMPER test to estimate which OTUs are responsible more than others for the differences between the two managements was performed.

3 RESULTS

3.1 DNA extraction, soil microbial biomass, qPCR

DNA yield, taken as a measure of microbial biomass, showed a similar trend among the two different managements during the time of plant growth (Fig. 2). In particular, the amount of DNA was maximum at t18 days and minimum at t104 days for both organic and conventional treatments. However, considering the complete growing season of sunflower, indicated here as t0-138 days, the overall DNA yield was significantly higher in ORG (2.9E+04 ± 4.7E+03 kg DNA ha^{-1} soil) than in CON (2.3E+04 ± 7.0E+03 kg DNA ha^{-1} soil); this latter representing almost 78% of the organic one. The microbial biomass, evaluated as DNA yield, followed the same trend in the two farming systems.

By using qPCR, the 16S rRNA (bacteria), 18S rRNA (fungi) and the functional gene *amo*A (ammonia oxidizing bacteria) copy numbers were evaluated in both the ORG and CON.

Looking at the whole period t0-138, bacterial gene sequences were significantly greater in ORG ($1.4x10^{19} \pm 3.7x10^{18}$ copies ha^{-1}) than in CON ($9x10^{18} \pm 4.1x10^{18}$ copies ha^{-1}) samples and the same was for the fungal sequences ($5.7x10^{17} \pm 1.7x10^{17}$ and $3.1x10^{17} \pm 1.9x10^{17}$ copies ha^{-1}, respectively). In general, bacterial gene copies were more abundant than fungal for the two treatments, the fungi representing the 4.2% and the 3.4% of bacteria in ORG and CON samples, respectively.

The *amo*A gene sequences (ammonia oxidizing bacteria) was the smallest number at t7 and the greatest between t83 and t104 in the CON system; the greatest at t18, the least at t0 in the ORG plot (Tab 3). Moreover, considering the data for the whole growing season, ammonia oxidizers showed an opposite behavior to bacteria and fungi; in fact, *amo*A gene copies were significantly less abundant in the ORG ($1.8x10^{16} \pm 5.3x10^{15}$ copies ha^{-1} corresponding to the 38% of the conventional soil) than in the CON farming system ($4.8x10^{16} \pm 2{,}2x10^{16}$ copies ha^{-1}) and AOB sequences were the 0.1% and 0.5% of the eubacteria in ORG and CON, respectively.

3.2 Soil carbon emissions and mqCO_2

CO_2 from aerobic and anaerobic processes, respiration of soil fauna, dark respiration of plants as well as CO_2 from root respiration, are included to the CO_2 fluxes measured with the static chambers and could be considered as community CO_2 production. Despite the similar emissions trend from the two farming systems, ORG showed significantly greater CO_2 emissions than CON (Tab.4).

Data from the whole growing season (t0-138) showed that the CO_2 evolution was similar between the two farming systems, but it was significantly less in CON (462.97±102.6 $kgCO_2$-C ha^{-1}) than in ORG (1932.68±216.9 $kgCO_2$-C ha^{-1}).

The ratio of soil CO_2 emission to the DNA yield ($mqCO_2$) was not constant but varied with time: it was minimal at the beginning and at the end of the growing season and peaked at t83 days for both farming systems. The time interval t52 to t104 showed the greatest activity of the microbial biomass, a sort of *hot moment* more evident in ORG.

Considering the data as a mean of the entire sunflower growing season, the microbial activity was significantly less in CON ($3.1x10^{-3}$±$1.5x10^{-3}$) than in ORG ($9.4x10^{-3}$± $4.7x10^{-3}$) calculated as kg-C per kg of DNA per soil hectare.

We applied the ratio of soil CO_2 emission to the amount of bacterial and fungal gene copies, Bac qCO_2 and Fun qCO_2, respectively, to distinguish the physiological activity of these two microbial communities, considering the whole growing season of the crop. Again, both the bacterial and fungal activities were significantly less in CON ($4.5x10^{-19}$±$3.5x10^{-19}$ Bac qCO_2 and $1.5x10^{-17}$±$1.4x10^{-17}$ Fun qCO_2) than ORG ($9.5x10^{-19}$±$4.0x10^{-19}$ Bac qCO_2 and $2.4x10^{-17}$ ±$1.0x10^{-17}$ Fun qCO_2). Thus, the bacterial respiration activity in CON samples was 46.7% of the ORG one, while the fungal respiration activity in CON was 64.9% of the ORG one. Anyway, the fungal respiration activity was significantly higher than the bacterial one.

3.3 Bacterial Sequencing data (*Alpha* Diversity)

After quality filtering, chimera cleaning and removal of low abundant sequences (≤ 5 total count), 2,581,403 16S rRNA sequences, and 17,416 OTUs were obtained from a total of 14 samples. The rarefaction curve was reached to saturation for all samples, indicating the sequencing depth was sufficient to cover detectable species in all samples (Fig. 3). The Venn diagram revealed that 16,692 OTUs (95.84%) were shared by soil of ORG and CON management practices, while 272 and 453 were exclusive of CON and ORG samples, respectively (Fig. 4).

Estimated diversity indices, Shannon index, Evenness, Species richness and Chao1 richness are shown in Fig. 5. No significant differences were observed in Shannon index and Evenness, while Species and Chao1 richness were significantly greater under ORG management compared to the CON one.

3.4 Changes in Bacterial Community Structure (*Beta* Diversity)

Beta diversity evaluates how different the population structure is in various environments. PCoA of Bray-Curtis distance was used to analyze the variation in the bacterial community as affected by management practices (Fig. 6). The significance level of variation was checked by PERMANOVA. The first two principal coordinators explain a high percentage of variance (~72%, coordinate 1: 57.11% and coordinate 2: 14.98%) with distinction in community structure associated with management practices. Plots revealed that communities were not completely clustered differently under both management practices. PERMANOVA results also showed that there were not significant differences in community structure of bacteria (F = 2.078, p = 0.061).

3.5 Changes in Bacterial taxonomic composition

To analyze the effect of management practices on soil bacterial composition, we assessed the bacterial relative abundance at two different taxonomic levels, phylum and family; we showed those present >1% (Fig. 7a and b). Overall, the *Proteobacteria* phylum (~21%) with classes *alpha*, *beta*, *gamma* and *delta* was the most abundant followed by *Actinobacteria* (~19%), *Acidobacteria* (~12%), *Planctomycetes*, *Bacteriodetes*, *Chloroflexi*, *Verrucomicrobia*, *Firmicutes*. No significant differences were observed in the relative abundance of bacteria between the two systems except for *Gemmatimonadetes* (*Gemmatimonadaceae*).

A SIMPER test, to estimate which OTUs are responsible more than others for the differences between the two managements was performed (Tab. 5). Results showed 22.02% of dissimilarity between ORG and CON management practices. OTU 3, classified as *Tychonema* CCAP 1459-11B belonging to the phylum *Cyanobacteria*, contributed the most to the differences in the bacterial communities (10.65% of total dissimilarity). Other contributing OTUs were OTU 1 *Pseudarthrobacter* belonging to *Actinobacteria*, OTU 6 *Microvirga* belonging to *Proteobacteria*, OTU 4 belonging to *Planctomycetes*, OTU 63 *Microcoleus* PCC-7113 belonging to *Cyanobacteria*, OTU 10 belonging to *Proteobacteria*.

3.6 Soil NH_4^+, NO_2^-, NO_3^- and readily mineralizable organic N content

The concentration of $NH_4^+ - N$ in soil varied among sampling dates. Over the growing period, the soil $NH_4^+ - N$ concentration ranged from 0.49 to 1.63 mg N kg^{-1} in CON and from 0.28 to 1.35 mg N kg^{-1} in ORG (Fig. 8). The soil $NH_4^+ - N$ concentration in CON being significantly greater than that measured in ORG at t0, t7, t52, and t138, while being significantly less at t18, t83 and t104. The average $NH_4^+ - N$ concentration in soil over all sampling dates was greater in CON (1.43 ± 0.56 mg N kg^{-1}) than in ORG

treatment (1.35 ± 0.78 mg N kg^{-1}); however, this difference was not significant. The average $NH_4^+ - N$ concentration represented 10.1% and 11.6% of the total readily available N forms in CON and ORG, respectively.

The average concentration of $NO_2^- - N$ was 3.54 ± 0.85 mg N kg^{-1} in CON, ranging from 2.09 to 4.76 mg N kg^{-1}, while it was 3.92 ± 0.57 mg N kg^{-1} in ORG, ranging from 3.00 to 4.51 mg N kg^{-1}. The average $NO_2^- - N$ concentration represented 25.5% and 33.3% of the total readily available N forms in CON and ORG, respectively. No significant differences were observed between CON and ORG managements.

The concentration of $NO_3^- - N$ in soil ranged from 6.78 to 10.57 mg N kg^{-1} in CON and from 4.12 to 8.16 mg N kg^{-1} in ORG. When considering the whole period, there was a decreasing trend in the $NO_3^- - N$ concentration in CON, while an increasing trend in ORG was observed. For most sampling dates (t0, t18, t83), the soil $NO_3^- - N$ concentration in CON was significantly greater than ORG. For all sampling dates, it was observed that the concentration of $NO_3^- - N$ in ORG increased as the $NH_4^+ - N$ decreased and *vice versa*. On the contrary, this relationship was not found in CON. Considering the entire growing season period, the average $NO_3^- - N$ concentration in soil was significantly greater in CON (8.58 ± 1.46 mg N kg^{-1}) than in ORG (6.02 ± 1.47 mg N kg^{-1}).

The average $NO_3^- - N$ concentration in soil represented 60.7% and 50.3% of the total readily available N forms in CON and ORG, respectively.

During the growing period, the average concentration of RMO-N was 0.53 ± 0.18 mg N kg^{-1} in CON, ranging from 0.3 to 0.78 mg N kg^{-1}, while it was 0.59 ± 0.31 mg N kg^{-1} in ORG, ranging from 0.22 to 1.04 mg N kg^{-1}. The average RMO-N concentration in soil represented 3.8% and 4.9% of the total readily available N forms in CON and ORG, respectively. Throughout the season, the average RMO-N concentration in ORG was greater than in CON. The soil RMO-N concentration in CON resulted being significantly more than that measured in ORG at t18, t52 and t83, while being significantly less at t0, t7 and t104.

3.7 Sunflower yields and morphological parameters

Sunflower yields were significantly greater in CON than in ORG (Tab. 6). However, in both treatments, yields were less than average for sunflower in Tuscany. According to yields, CON had better performances for all measured morphological parameters, except plant height. In particular, flower

diameter, average number of seeds per plant and average weight of seeds per plant were 56.8%, 56.9% and 54.4% greater in CON than ORG. However, plant height was not affected by the farming systems and no significant differences were observed.

4 DISCUSSION

The objective of the present manuscript was to compare the effects of organic and conventional farming systems on the microbial biomass, activity and composition as well as soil CO_2 emission and readily available nitrogen forms into the soil in a long-term experiment in Tuscany, Italy.

The implementation of environmentally-friendly agricultural policies has increased the need to compare some agricultural aspects of conventional and organic farming systems (García-Ruiz et al., 2008).

This type of study is strongly needed to better understand the role of organic farming to improve soil quality and benefit the environment. A recent study (Zani et al., 2022) reported a significant potential of organic farming to improve soil quality (fertility, biodiversity, C and nutrients stock). However, due to the complexity of the soil system, there is still a lack scientific knowledge to maintain soil productivity and biodiversity in the long term. The novelty of this study lies in the essence of the MoLTE experiment itself; in fact, long-term experiments can give important information to assess soil fertility in a long-term perspective. Backed by these considerations, we have measured different parameters relating to soil microbial community, GHG emissions, N content and sunflower production at various intervals corresponding to the main sunflower phases (Tab. 2).

The greatest microbial biomass expressed as DNA yield, was found at t18, as well as the greatest amounts of bacterial and fungal sequences evaluated by 16S and 18S sequences in qPCR, for both the ORG and CON. Reasonably, this was the consequence of fertilization carried out at the beginning of the experiment (t0) in ORG and CON, respectively. The ammonia oxidizing bacteria, monitored by *amo*A qPCR, increased significantly between t83 and t104 in CON, only. This delay was expected, due to the slow growth rate of the AOB population compared to other fast-growing bacteria and fungi, but also the soil N content and its availability has to be considered. In fact, the amount of *amo*A gene copies was more abundant in CON differently from the bacterial and fungal sequences. As regard to the N content and release, it is known that chemical fertilizers used in conventional farming, such as ammonium nitrate and urea, result in significant accumulation of ammonium, easily available for AOB, and nitrate (Jia &

Conrad, 2009). After all, the ability of many ammonia oxidizers to hydrolyze urea is well known and this fertilizer has been found to stimulate autotrophic nitrification in soil, independently from pH (Burton & Prosser, 2001). Moreover, in CON, ammonia oxidizers during the t52-t104 interval were more abundant than in ORG while, after this time, their copy number decreased to almost the same amount at t0.

CO_2 emissions were not constant during the experiment in either of the two managements. In fact, it was minimal at the beginning and at the end of the full growing season, when presumably the soil microbial communities existed under steady-state like conditions. Interestingly, only in ORG, were emissions greatest between t52 and t104, during which sunflower stem elongation and flowering occurred. This could be a result of the intense metabolic activity occurring during the vegetative phase in the period of intense stimulation and interactions among plant roots and microorganisms (Alami et al., 2000) and we could refer to this period as the *hot moment* of the microflora in the soil systems (Kuzyakov & Blagodatskaya, 2015). We could argue that during this period there were more inputs of labile organics in soil deriving from root exudates and decomposing materials, but also other internal triggering signals as auto inducer molecules secreted by the microbial communities themselves able to wake them up from dormancy to activity (Raffa et al., 2005).

We also considered biochemical and molecular data for the full period of the sunflower cycle (t0-138 days) to provide a global vision of the microflora, its activity, soil N emissions and RMO-N. The soil total DNA yield, corresponding to the microbial biomass, was less in CON than in the ORG. Applying the ratio of soil CO_2 emissions to the total DNA yield and also to bacterial (16S) and fungal (18S) gene copies separately, it was possible to distinguish the physiological activity, indicated here as $mqCO_2$, of the soil microbial biomass as a whole, and of the bacterial and fungal communities distinctly. The latter two parameters constitute a new methodological aspect that we have applied in this work. Results showed that the microbial biomass was more active and abundant in ORG; despite a lower amount, fungi were more active than bacteria, both in ORG and in CON farming. The lower $mqCO_2$ of bacteria may indicate that they could belong more to maintenance strategists than to resource acquisition strategists (Ramin & Allison, 2019).

Still considering the full period, ammonia oxidizers represented 0.1% and 0.5% of the bacterial community in ORG and CON soils, respectively. The positive correlation between soil NO_3^-

concentration and AOB was indicative of ammonia oxidation activity (i.e. end product), supporting the soil mineral-N associations with AOB populations and the easier availability of N content of the mineral fertilizers used in conventional agricultural systems (Tao et al., 2017).

Moreover, we decided to conduct a preliminary study, focusing on the bacterial community, by 16S rRNA gene sequencing, on the basis of the essentiality of microbial diversity for soil. We therefore examined *alpha* and *beta* diversity and related indices comparing the two types of agricultural managements. The Venn diagram showed that a large proportion of bacteria was shared between the two managements and these might be considered a "core microbiome" (Estendorfer et al., 2020) composed of poorly-characterized microbes and presumably present in many soils, although not equally abundant. The presence of unique OTUs in ORG and CON samples may be due to selective soil properties deriving from different managements. The results of Chao1 and species Richness clearly showed that ORG treatment significantly increased the bacterial richness. This may be due also to the green manuring adopted for the ORG management, based on a grass-legume mixture, more easily decomposable and known for greater N mineralization, as well as having a positive influence on the physical and chemical properties of the soil (Fageria, 2007).

The variations in *beta* diversity and relative abundance at phylum and family level were not significantly affected by the management practices, other than for the phylum *Gemmatimonadetes* (family *Gemmatimonadaceae*); in fact, they were significantly greater in CON than in ORG soil samples. This phylum has a wide distribution in soil systems and it is frequently detected in environmental 16S rRNA gene libraries, representing the top nine phyla in soils, comprising 2% of soil bacterial communities. Since such microorganisms have only recently been studied, little is still known about their role in agricultural systems, other than that they are particularly suitable for arid environments. Their constant presence suggests a versatile metabolism that allows to survive well in soil, and perhaps to withstand the impacts of global warming (DeBruyn et al., 2011; Douglas Madison et al., 2021; Orr et al., 2015). *Cyanobacteria*, *Actinobacteria* and *Proteobacteria* were the most abundant phyla, contributing more than others to the differences between 16S rRNA gene pools in the two management systems as shown by the SIMPER test. The presence of these three phyla, to which many generalist bacteria suitable for different environmental conditions belong, was of some importance. The sunflower crop could have influenced the soil microbial community through its root exudates (Tejeda-Agredano et al., 2013), but

this aspect was not taken into consideration in this study. We are aware that these are preliminary results and that further metagenomic studies will have to be done, but, nevertheless, these data could be indicative of the microbial diversity in the considered soil under long term managements.

The transfer of nitrate from cultivated soil to groundwater is another environmental concern linked to agriculture. In this sense, organic farming has come into focus as a possible way to reduce nitrate leaching from arable land (Kirchmann & Bergström, 2001). In this study, the soil NH_4^+ and NO_2^- contents in the t0-138 period were not significantly different between ORG and CON, while NO_3^- was 30% less in ORG. This latter result, although referring to one year only, is in line with literature where NO_3^- concentrations were greater in conventional than in organic plots (Benoit et al., 2015; Kramer et al., 2006). The greater RMO-N concentration in ORG could be caused by the differences in fertilization methods. However, it cannot be excluded that, as soon as the conditions would be favorable for AOB in organic systems, the soil RMO-N content could increase.

Nevertheless, despite greater soil microbial community development and activity, ORG sunflower yield was about 33% less than CON, confirming previous experimental evidence (Mazzoncini et al., 2006; Seufert et al., 2012). However, both CON and ORG produced smaller yields compared to the regional average production and this was mainly due to the dry season that occurred in 2018 (Fig. 1) and the absence of an irrigation system. Yield gap is the main issue of organic farming, and some authors share the concern that organic agriculture may need an increased cultivated area due to reduced yields (Villanueva-Rey et al., 2014; Tuomisto et al., 2012). Indeed, the increase of resource use efficiency per unit area of land is a key point for the improvement of organic farming performance, although research studies have not yet reached a unique answer to this point. For reducing yield gap, a great challenge for future research in organic farming is to deepen the knowledge on weed control, phosphorus (P) availability in soils, stimulation of soil microbial biomass and use of selected crop varieties able to grow on low input farming systems.

Our results also showed that differences in N rate due to different fertilization in ORG and CON, significantly affected morphological parameters such as flower diameter, number of seeds per flower and seed weight per flower, as found by other authors (Abdel-Motagally & Osman, 2010a, 2010b; Tripathi et al., 2003). Nevertheless, plant height seems to be not affected by farming systems and this is

probably due to the physiology of the crop that consumed the main part of soil resources for the vegetative growth and, in ORG, a nutrient lack for grain differentiation.

Sustainability assessment requires a comprehensive perspective that accounts for the interrelationships between the technical, environmental, social, economic and political aspects (Pacini et al., 2003). In this study we did not consider the impacts of ORG and CON on financial and food quality aspects, which instead hold considerable importance in terms of overall sustainability of farming systems. Indeed, Var. Toscana chosen for ORG is a sunflower variety used as seed for human consumption; they are consumed as snacks and obtain considerably higher prices on the market than Var. LG50.525 cropped for CON. Hence, smaller yields in organic farming can nevertheless produce greater revenues than conventional agriculture. From a health perspective, organic products can provide a valid tool for sustainable food consumption (European Commission, 2020). However, on a global scale, the consumer education is crucial to discriminate that there is no low or high price but a fair or unfair price for a healthier food chain production.

5 CONCLUSION

Organic agriculture is a holistic production management system, which promotes and enhances agroecosystem health, biodiversity and biological cycles. Thus, it becomes crucial to compare the effects of long-term organic and conventional systems on soil indicators. Our results showed that, during the year of the study, bacteria and fungi were more abundant, active and diverse in soil under organic farming despite the lesser N inputs, while the sunflower yield was significantly less in organic than in conventional farming. However, the benefits of organic farming should be considered in the overall context of the environmental-friendly production that includes social, economic and environmental aspects. In this sense, the scientific community can have an important role in promoting low-input farming systems to farmers, policy makers and citizens.

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AUTHOR CONTRIBUTIONS

Margherita Santoni: Conceptualization; data curation; formal analysis; investigation; methodology; writing-review & editing.

Leonardo Verdi: Conceptualization; data curation; methodology; writing-review & editing.

Shamina Imran Pathan: sequencing data curation; investigation.

Marco Napoli: data curation; methodology; writing-review & editing.

Anna Dalla Marta: Conceptualization; funding acquisition; project administration; writing-review & editing.

Francesca Romana Dani: Conceptualization; funding acquisition; project administration; writing-review & editing.

Gaio Cesare Pacini: Project Manager of the MoLTE, Montepaldi Long Term Experiment.

Maria Teresa Ceccherini: Conceptualization; funding acquisition; project administration; supervision; writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author (Dr. Verdi). The authors are pleased to share the data upon request.

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Table 1. Soil characteristics of organic (ORG) and conventional (CON) farming systems.

	Units	ORG	CON
Sand	%	20.2	21.0
Silt	%	46.3	44.6
Clay	%	32.9	33.8
Texture		Clay Loam	Clay Loam
pH		8.3	8.3
Gravel	%	6.3	6.1

Table 2. Agronomical details of the MoLTE experiment in 2018. The abbreviations ORG and CON indicate organic and conventional managed plot.

	ORG	**CON**
Previous crop	*Hordeum vulgare,*	*Hordeum vulgare,*
	var. Campagne	*var. Campagne*
Actual crop	*Helianthus annuus L.,*	*Helianthus annuus L.,*
	var. Toscana	*var. LG50.525*
Plant density	55.384 plant/ha	55.384 plant/ha
Date	**ORG**	**CON**
Oct/19/2018	Harrowing	Ploughing
Oct/23/2018	Green manure sowing[a]	-
Oct/24/2018	Subsoiling	-
Apr/20/2018	Green manure incorporation[f]	Harrowing
Apr/26/2018	Sunflower sowing	Sunflower sowing[e]
		Localized fertilization[b]
Apr/27/2018	-	Chemical weeding[d]
Jun/11/2018	-	Localized fertilization[c]
Jun/12/2018	Weed Hoeing	Weed Hoeing
Sep/05/2018	Harvest	Harvest

a: Avena sativa L. (40 kg ha^{-1}) and Vicia faba L. var. minor (80 kg ha^{-1})

b: 20.10.10 (150 kg ha^{-1})

c: urea (150 kg ha^{-1})

d: DUAL GOLD, p.a S-metolachlor (1.15 lt ha^{-1})

e: seeds treated with Apron-xl a.i. metalaxil-m 30.95%

f: harrowing was used for green manure incorporation into the soil

Table 3. 16S rRNA (bacteria), 18S rRNA (fungi) and *amo*A (ammonia oxidizing bacteria) sequences per hectare.

Time	ORG 16S seq ha^{-1} ± sd	CON 16S seq ha^{-1} ± sd	ORG amoA seq ha^{-1} ± sd	CON amoA seq ha^{-1} ± sd
t0	$1.1x10^{19}$ ±$1.3x10^{18}$	$6.8x10^{18}$ ±$5.2x10^{17}$	$9.6x10^{15}$ ±$1.1x10^{15}$	$3.6x10^{16}$ ±$3.0x10^{15}$
t7	$1.3x10^{19}$ ±$1.3x10^{18}$	$7.5x10^{18}$ ±$1.1x10^{18}$	$1.5x10^{16}$ ±$1.7x10^{15}$	$2.9x10^{16}$ ±$3.5x10^{15}$
t18	$1.8x10^{19}$ ±$7.0x10^{18}$	$1.7x10^{19}$ ±$4.5x10^{18}$	$2.4x10^{16}$ ±$5.0x10^{15}$	$3.1x10^{16}$ ±$5.6x10^{15}$
t52	$1.2x10^{19}$ ±$6.5x10^{17}$	$5.8x10^{18}$ ±$1.0x10^{18}$	$1.8x10^{16}$ ±$1.3x10^{15}$	$4.1x10^{16}$ ±$5.9x10^{15}$
t83	$1.6x10^{19}$ ±$3.5x10^{18}$	$7.6x10^{18}$ ±$1.9x10^{18}$	$2.3x10^{16}$ ±$5.2x10^{15}$	$8.2x10^{16}$ ±$7.7x10^{15}$
t104	$1.2x10^{19}$ ±$2.9x10^{18}$	$6.4x10^{18}$ ±$6.8x10^{17}$	$1.9x10^{16}$ ±$1.5x10^{15}$	$8.1x10^{16}$ ±$6.0x10^{15}$
t138	$1.4x10^{19}$ ±$3.4x10^{18}$	$1.2x10^{19}$ ±$9.7x10^{17}$	$1.9x10^{16}$ ±$2.9x10^{15}$	$3.7x10^{16}$ ±$2.7x10^{15}$
	ORG 18S seq ha^{-1} ± sd	**CON 18S seq ha^{-1}** ± sd		
t0	$3.0x10^{17}$ ±$3.6x10^{16}$	$2.0x10^{17}$ ±$7.9x10^{15}$		
t7	$5.8x10^{17}$ ±$7.2x10^{16}$	$1.7x10^{17}$ ±$1.9x10^{16}$		
t18	$6.8x10^{17}$ ±$9.0x10^{16}$	$6.9x10^{17}$ ±$7.8x10^{16}$		
t52	$5.3x10^{17}$ ±$2.9x10^{16}$	$1.5x10^{17}$ ±$7.5x10^{15}$		
t83	$6.0x10^{17}$ ±$8.7x10^{16}$	$2.2x10^{17}$ ±$2.5x10^{16}$		
t104	$8.6x10^{17}$ ±$6.2x10^{16}$	$2.8x10^{17}$ ±$2.7x10^{16}$		
t138	$4.6x10^{17}$ ±$2.6x10^{16}$	$4.7x10^{17}$ ±$2.9x10^{16}$		

Table 4. Daily CO_2 emission rate at each sampling time for organic (ORG) and conventional (CON) farming system.

Time	ORG kg CO_2-C ha^{-1}	CON kg CO_2-C ha^{-1}
t0	8.09 ± 4.6	2.86 ± 2.2
t7	4.34 ± 2.0	1.57 ± 1.3
t18	12.65 ± 7.2	3.10 ± 1.4
t52	24.32 ± 6.1	4.64 ± 5.4
t83	15.67 ± 7.12	0.82 ± 0.7
t104	14.15 ± 8.8	5.13 ± 2.5
t138	6.71 ± 2.6	0.52 ± 0.9

Table 5. Principal OTUs contribute most to change 16S rRNA gene pools under organic (ORG) and conventional (CON) management practices. OTUs with >1% dissimilarity contribution shown in the table.

OTUs	Overall dissimilarity: 22.02 %		Accession	Phylum	Family	Taxon	Mean abundance (%)	
	Av. dissim	Contrib. %					ORG	CON
CL000003	2.349	10.65	KX508362	Cyanobacteria	Phormidiaceae	Tychonema CCAP 1459-11B	0.0154	0.00476
CL000001	1.313	5.952	DQ125870	Actinobacteria	Micrococcaceae	Pseudarthrobacter	0.0166	0.011
CL000006	0.5399	2.448	JF417789	Proteobacteria	Beijerinckiaceae	Microvirga	0.00653	0.00486
CL000004	0.5218	2.366	FJ479536	Planctomycetes	WD2101 soil group	uncultured bacterium	0.00743	0.00983
CL000063	0.4899	2.221	KC463683	Cyanobacteria	Coleofasciculaceae	Microcoleus PCC-7113	0.00296	0.0000665
CL000010	0.4684	2.124	FJ478818	Proteobacteria	Burkholderiaceae	Unknown	0.00391	0.00542
CL000038	0.4549	2.063	JQ712903	Cyanobacteria	Phormidiaceae	Unknown	0.00135	0.00264
CL000002	0.3981	1.805	FJ479500	Actinobacteria	Rubrobacteriaceae	Rubrobacter	0.00999	0.0106
CL000011	0.3864	1.752	EU135114	Planctomycetes	WD2101 soil group	uncultured bacterium	0.00345	0.0055
CL000069	0.3693	1.674	FJ444635	Cyanobacteria	Unknown Family	Leptolyngbya EcFYyyy-00	0.000474	0.00228
CL000019	0.3278	1.486	DQ125870	Actinobacteria	Micrococcaceae	Pseudarthrobacter	0.00428	0.00294
CL000021	0.3263	1.479	KC921182	Planctomycetes	WD2101 soil group	planctomycete WWH14	0.00256	0.00405
CL000031	0.3196	1.449	KX508692	Cyanobacteria	uncultured bacterium	Unknown	0.00195	0.00235
CL000007	0.3153	1.429	EU440691	Proteobacteria	Azospirillaceae	Skermanella	0.0056	0.00529
CL000017	0.2916	1.322	EF688375	Actinobacteria	uncultured	uncultured soil bacterium	0.00276	0.00432
CL000009	0.2695	1.222	GQ249621	Actinobacteria	Rubrobacteriaceae	Rubrobacter	0.00511	0.00487
CL000016	0.2666	1.209	JF045039	Actinobacteria	Streptomycetaceae	Streptomyces	0.00337	0.0039
CL000028	0.2388	1.083	CP011509	Proteobacteria	Archangiaceae	Archangium	0.00269	0.00199
CL000094	0.2328	1.056	JQ979031	Cyanobacteria	Unknown Family	Leptolyngbya EcFYyyy-00	0.00112	0.00121

Table 6. Yields and morphological parameters of sunflower in ORG and CON systems.

	Units	ORG	CON	
Yield	t ha^{-1}	1.41 (± 0.66)	2.10 (± 0.89)	***
Flowers diameter	cm	6.35 (± 3.5)	14.7 (± 6.6)	***
Number of seeds per flower	-	377.1 (± 205.1)	873.1 (± 393.1)	***
Seeds weight per flower	gr	19.9 (± 10.9)	43.6 (± 19.7)	***
Plant height	cm	116.0 (± 46.1)	143.4 (± 41.2)	NS

Standard Deviations of data are in brackets. Statistical difference according to the ANOVA analysis are reported: NS - not significant; *** - significant at probability level $p < 0.001$

FIGURE CAPTIONS

Figure 1. Maximum and minimum temperature and precipitation trends during the experiment period.

Figure 2. DNA yield (kg DNA ha^{-1}) per time of sampling.

Figure 3. Rarefaction curves of soil bacterial communities based on observed OTUs at 3% distance for each MAN*TIME combination.

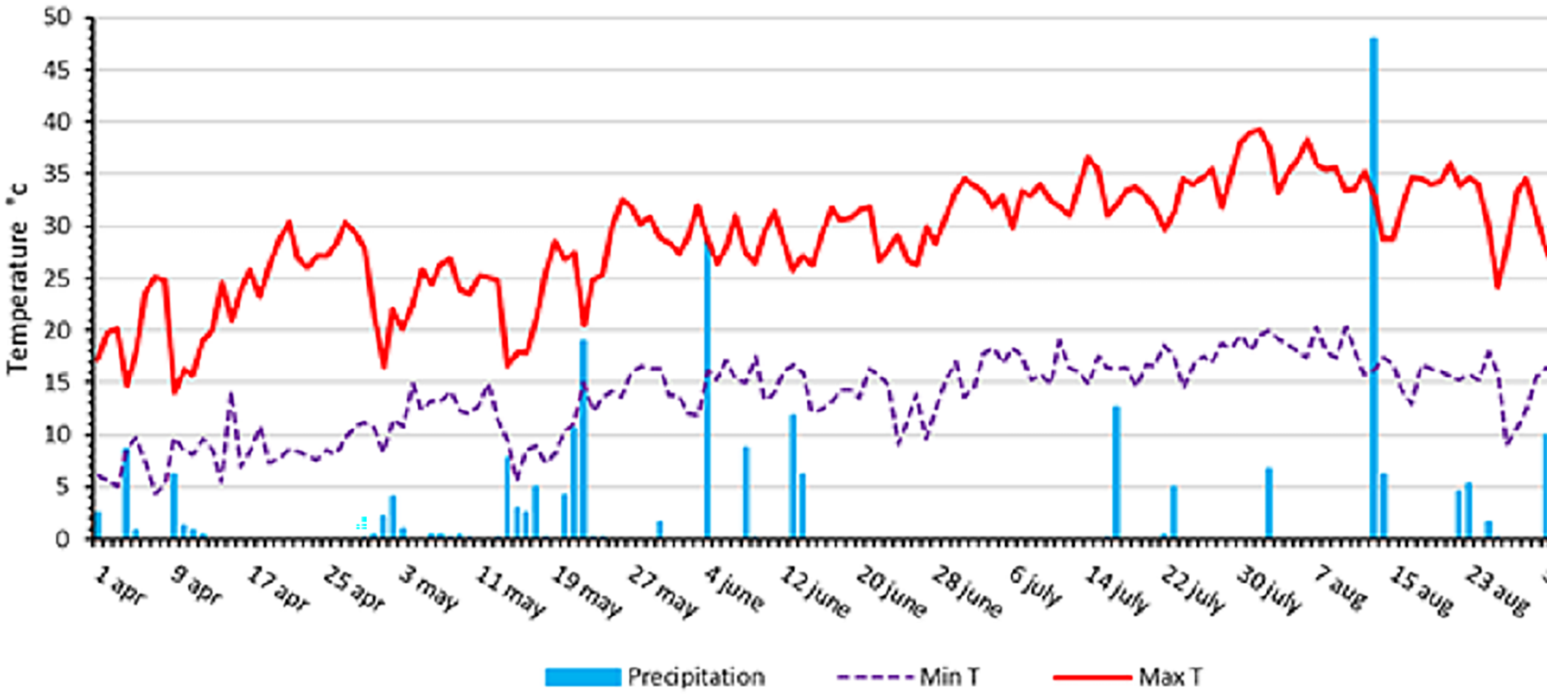
Figure 4. Venn diagram of exclusive and shared bacterial operational taxonomic units (OTUs) (at the 3% of evolutionary distance) under organic (ORG) and conventional (CON) management.

Figure 5. Effect of management practices on variability of *alpha* diversity. Data represent means and errors of three replicates. Significant differences are indicated by different superscript letters (One-way ANOVA followed by Tukey post hoc test, $p < 0.05$).

Figure 6. Principal Coordinate Analysis (PCoA) based on Bray–Curtis similarity distance of OTUs with abundance > 0.1 % of soil bacterial community under organic (ORG) and conventional (CON) management practices. Significant differences detected by permutational ANOVA (PERMANOVA).

Figure 7. The variation in bacterial community composition in soil at phylum and family level under organic (ORG) and conventional (CON) management. Data represent means and errors of three replicates. Significant differences are indicated by different superscript letters (One-way ANOVA followed by Tukey post hoc test, $p < 0.05$).

Figure 8. Variation in soil ammonium-nitrogen ($NH_4^+ - N$), nitrite-nitrogen ($NO_2^- - N$), nitrate-nitrogen ($NO_3^- - N$), and Readily Mineralizable Organic Nitrogen (RMO-N) in organic (black bars) and conventional (grey bars) treatments at different sampling times (t0-t138).



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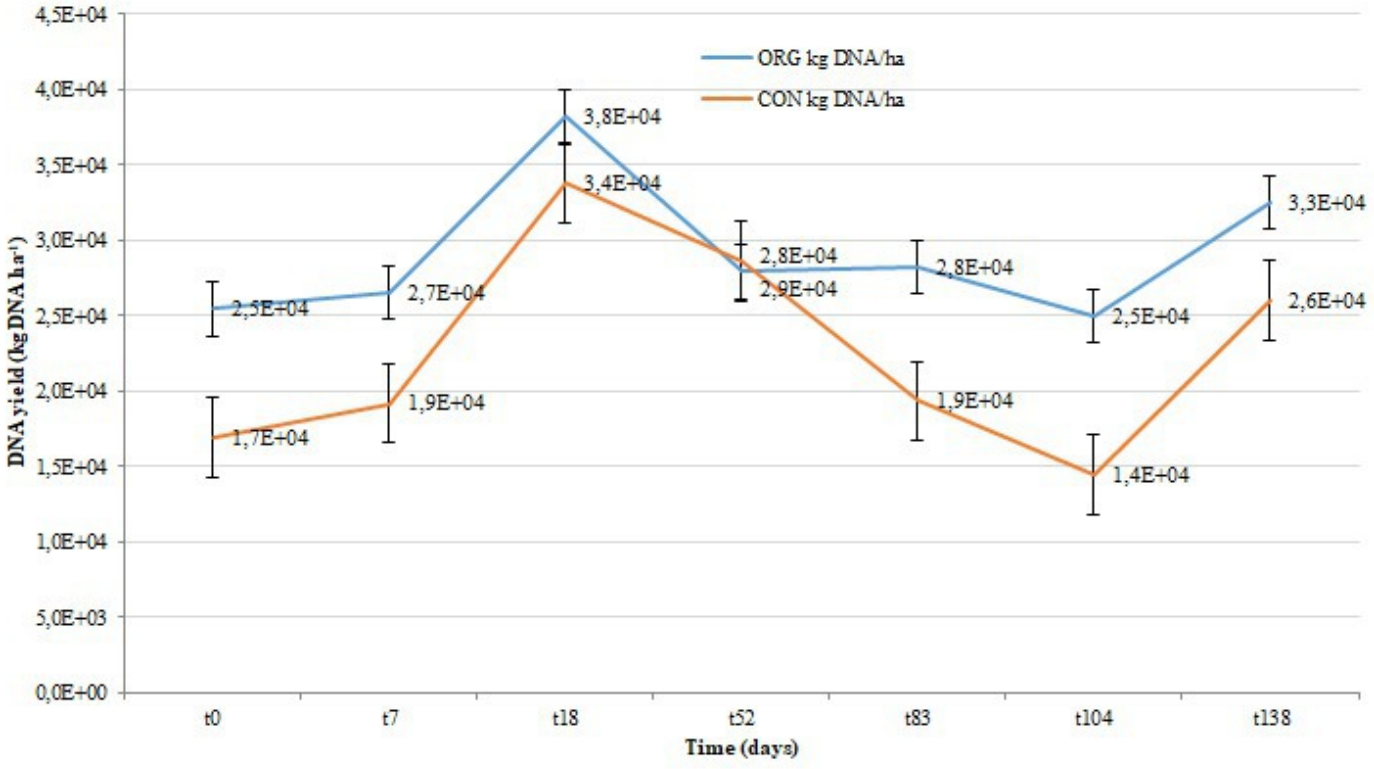

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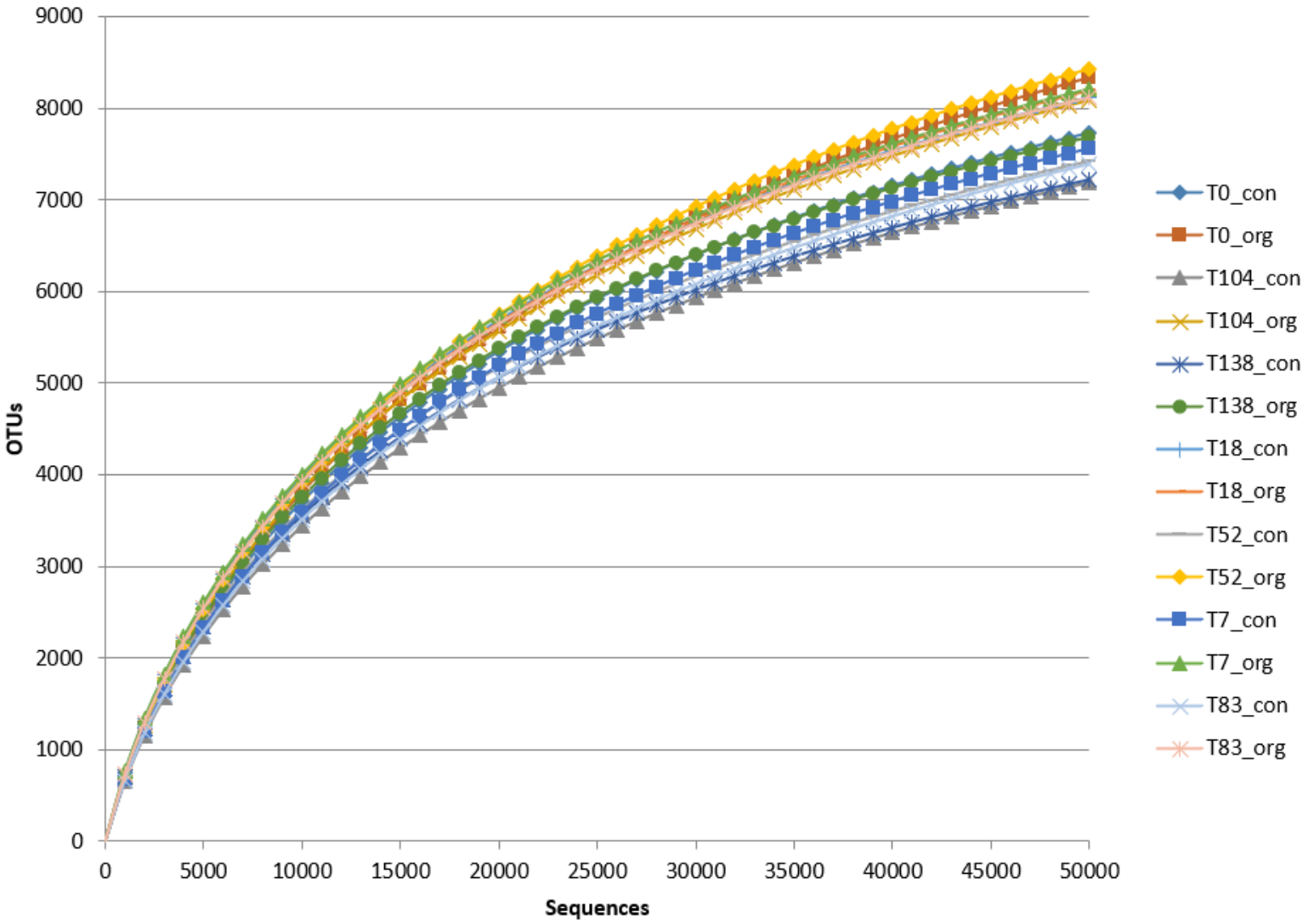

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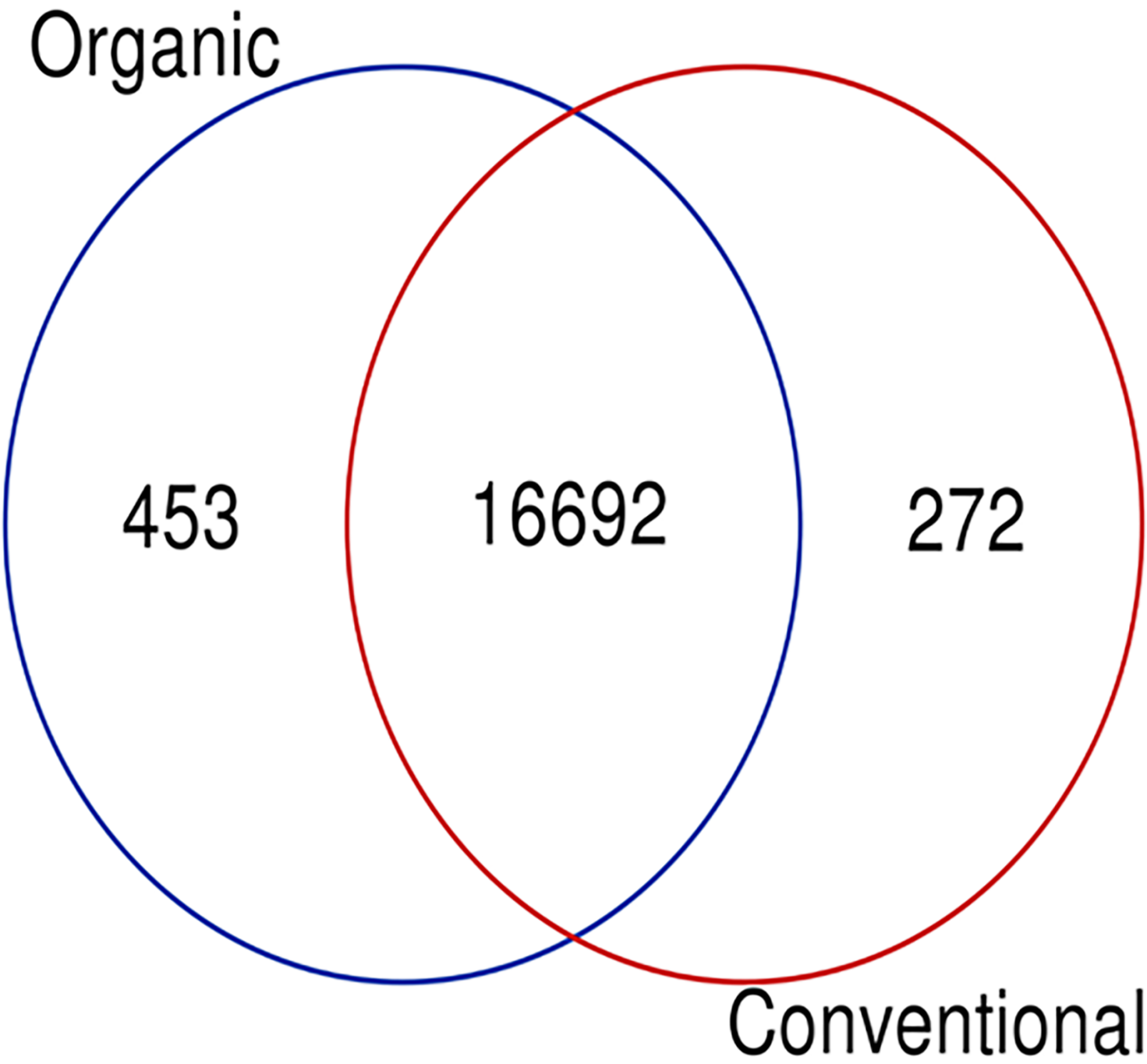

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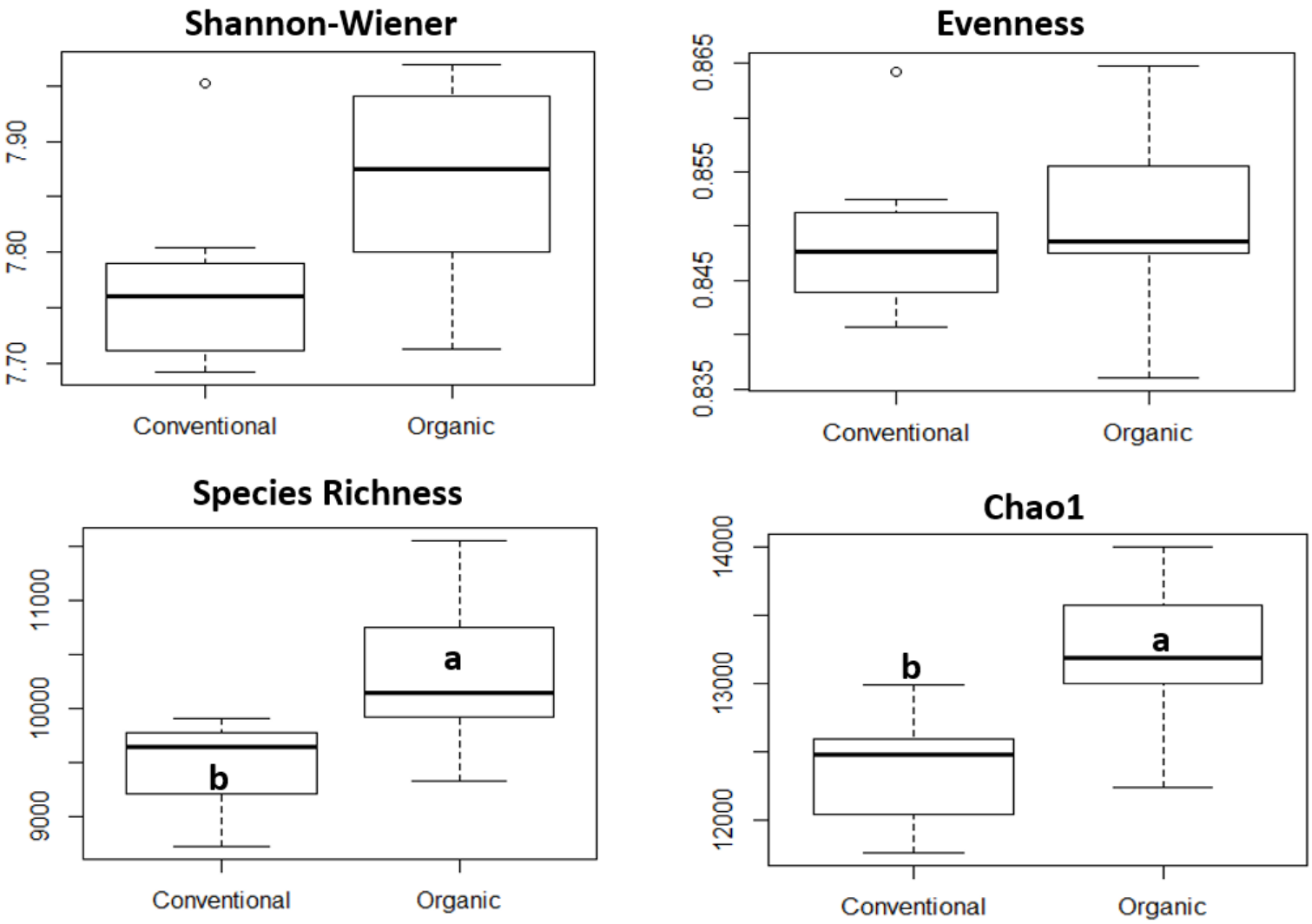

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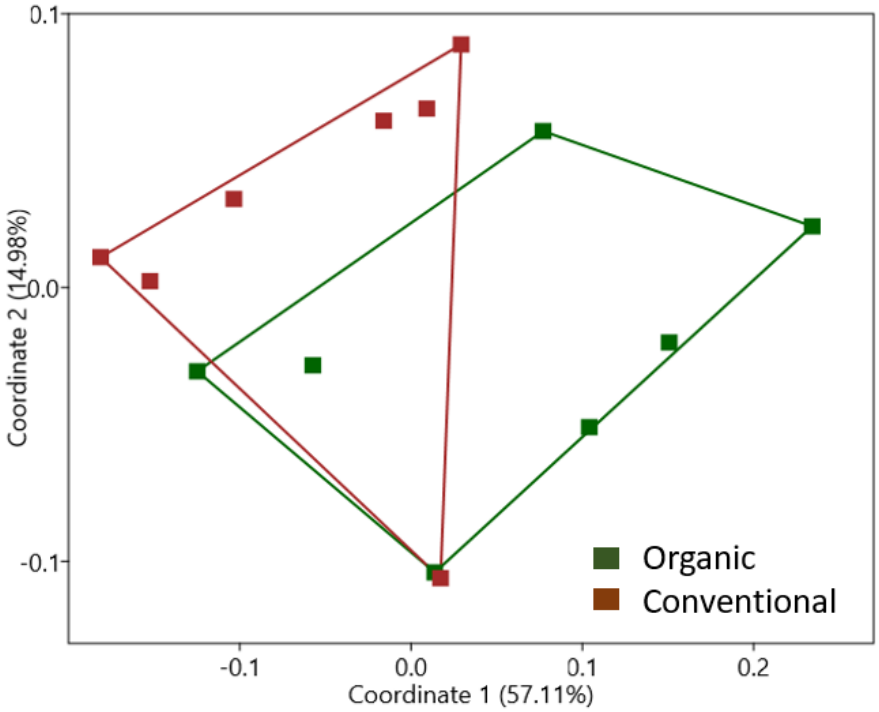

PERMANOVA	
Permutation N	9999
Total sum of squares	0.3176
Within-group sum of squares	0.2592
F	2.078
p	0.061
***p* value**	
	Organic
Conventional	0.061

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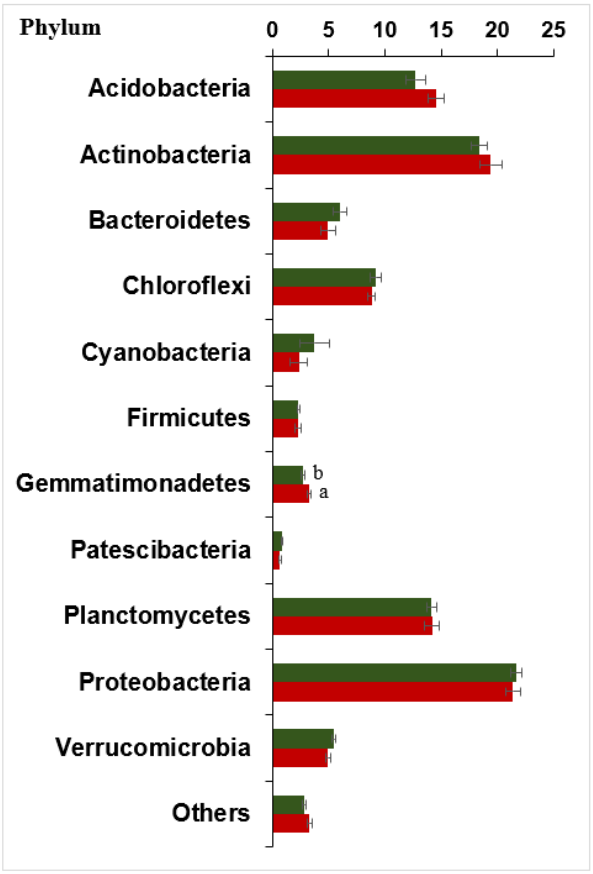

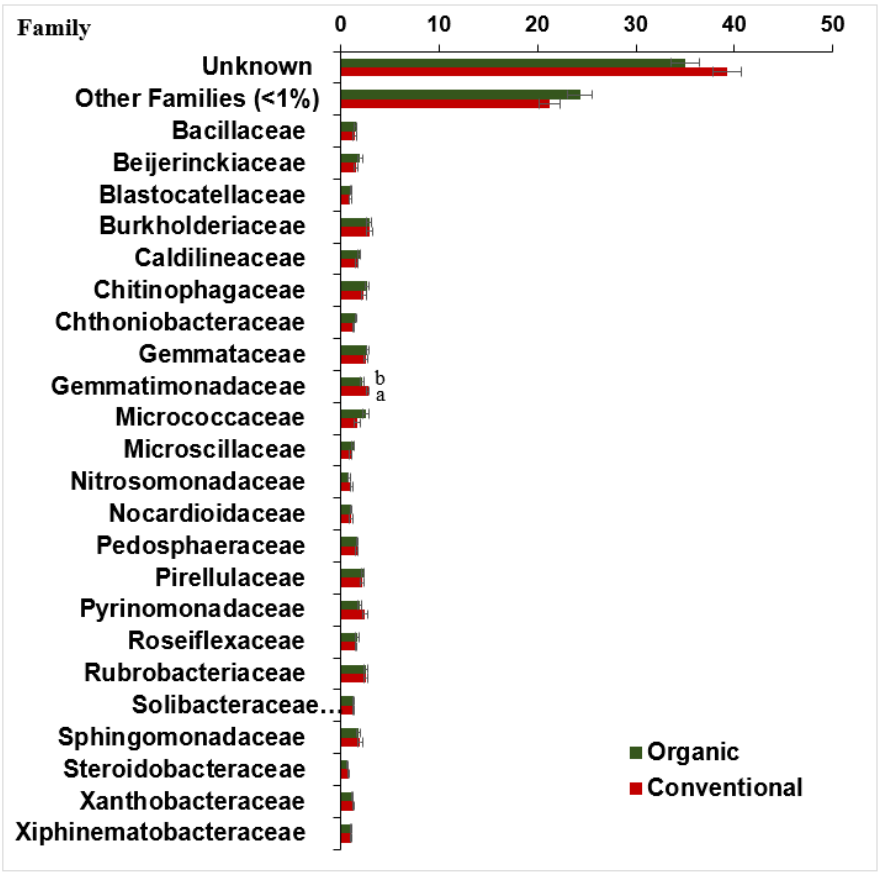

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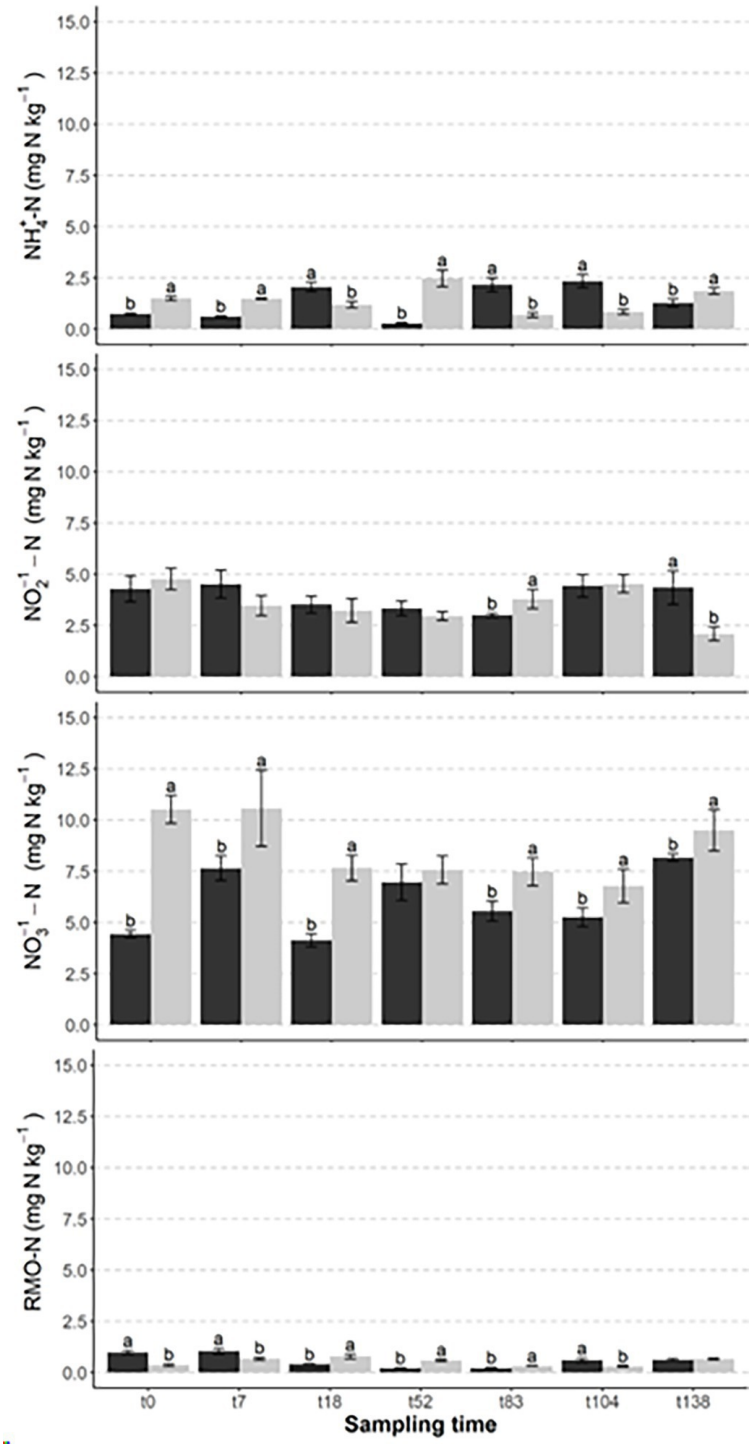

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