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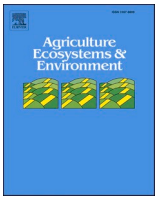

Effects of solarisation combined with compost on soil pathogens and the microbial community in a spinach cropping system

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ABSTRACT

This study compares the effects of solarization combined with compost (72% vineyard prunings and 28% leek residues) (CAS) versus solarization (NAS) in soils used for intensive spinach cultivation in the Mediterranean area. The objective was to study the beneficial effects of the compost on the soil microbial community, soil fertility and soil functionality related to C and N cycling during solarization, during the spinach crop growth period and after harvesting. CAS did increase soil fertility and microbial activity, in addition, CAS increased the microbial alpha diversity in the soil to a greater extent than NAS, and the Principal Coordinate Analysis (PCoA) of the beta diversity in the soil revealed changes in the bacterial and fungal community at the different sampling times, except after the plastic was lifted. During solarization and after harvesting, different beneficial bacteria and fungi related to C and N cycling were more abundant in CAS than in NAS, in the same way as the genera involved in plant defense and plant growth (*Pseudomonas, Sphingomonas, Bacillus, Thermomyces, Streptomyces, NMD1 or Nitrospira)*. CAS also had a notable effect on the abundance of predictive genes involved in the C and N cycles. The functional genes showed their lowest activity level a week after covering the soil with plastic, but they increased after lifting the plastic and after harvesting. Compared to NAS, CAS also showed higher N_2-fixation and greater conversion of N_2O to N_2. Moreover, the abundance of several predictive genes involved in hemicellulose, lignin and cellulose degradation suggested that CAS produced an increase in nutritional availability. From this study, it can be concluded that the combination of solarization and compost increased soil fertility, microbial activity, microbial diversity and functionality. Compost could provide added value by stimulating the microbiological community in the soil until harvest.

1. Introduction

Spinach (*Spinacia oleracea L.*) is an economically important leafy vegetable crop in many countries. As with most agricultural commodities, diseases impose significant production constraints affecting both yield and overall quality of spinach. Many diseases have been reported on spinach, *Fusarium oxysporum* (McDonald et al., 2021; Mitsuboshi et al., 2022), *Alternaria spp*. (Kipkogei et al., 2019), *Stemphylium botryosum* (Koike et al., 2001), *Pythium ultimum* (Magnée et al., 2022) or *Olpidium spp*. that indirectly affect spinach as they are carriers of viruses (Gratsia et al., 2012). Thus, an integrated disease management approach is often necessary to produce a high quality product (Correll et al., 1994).

Solarization—a chemical-free way to control pathogens and weeds—entails covering the soil with a clear plastic film, to trap solar radiation and accumulate heat and moisture in the soil (Kanaan et al., 2018), alters the bacterial microbial community in the soil, causing a biological vacuum that it should diminish soil pathogens (Kanaan et al., 2018). The incorporation of an organic amendment (fresh or composted) during solarization may produce a synergistic effect due to the accumulation of biopesticide compounds—volatile compounds such as alcohols, aldehydes, sulfides and isothiocyanates— caused by organic matter decomposition that produce CO_2 water and heat (Kanaan et al., 2017; Fernández-Bayo et al., 2019) and the addition of beneficial microorganisms that they can produce some other beneficial effects such as biopesticide, biostimulant or biofertilizer (Bonanomi et al., 2008).

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Microorganisms are key in the formation of soil structure, in the degradation of organic matter, elimination of toxins, in the cycling of nutrients and suppression of soil pathogens (Cuartero et al., 2022). It might be expected that the soil microbial community would vary after solarization combined with compost amendment (Simmons et al., 2013). Kanaan et al. (2017) observed that thermo-tolerant microorganisms survived during solarization and could play a role in disease resistance. Moreover, Simmons et al. (2016) showed that taxa such as Firmicutes and *Geobacillus*, were present when the use of compost was combined with solarization, and they were able to degrade the lignocellulose of the different compost raw materials (Fernández-Bayo et al., 2019). Compost is rich in organic matter that can alter the microbial communities in the soil and the abundance of microorganisms related to the C and N cycle through changes in the physicochemical properties of the soil (Yanardağ et al., 2017).

Metagenomic sequencing is a novel molecular tool used to analyse mixed genomic materials extracted from different samples, providing detailed information on the diversity, abundance and structure of microbial communities in the population. In addition, the phylogenetic relationship or functional genes PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) is a powerful approach to understanding the functional diversity of the bacterial community in the soil (Alami et al., 2020), including the abundance of different microbial functional genes involved in N-transformation and C processes (Xiao et al., 2019). FUNGuild found that the taxonomic groups characteristic of each habitat (i.e., saprotrophic and arbuscular mycorrhizal fungi in grassland soils, saprotrophic, saprotrophic and ectomycorrhizal fungi, wood decomposers, and plant pathogenic fungi) were well represented (Nguyen et al., 2016).

A deeper understanding of the restructuring of the soil microbiota (taxa and functionality of bacterial and fungal community) of combined compost addition to soil solarization will be able to answer a) How significant are the changes in the bacterial and fungal community related to compost amendment during the solarization process?; b) How significant are the changes in the functional genes related to C and N-cycling related to compost amendment during the solarization process? and c) Do bacterial and fungal taxonomic and functional changed through compost addition to solarization are remained after a spinach crop?

2. Materials and methods

2.1. Field experiment

The experiment was carried out in an agricultural field in Cartagena (SE Spain), between July 2020 and February 2021. According to the USDA the soil is silty loam and its characteristics are in Table 1. This area had a semi-arid Mediterranean climate (the average temperatures, rainfall and annual sunshine hours were; 18.23º C, 418 mm and 3000 h, respectively. In the solarization-only treatment, the soil was covered with transparent polyethylene plastic (NAS); in the treatment combining solarization and compost, the soil was covered with transparent polyethylene plastic after incorporating the compost (CAS). The polyethylene plastic was 30-μm thick (Serplasa 120 gr). The compost (72% vineyard prunings and 28% leek residues as raw materials) had the following characteristics: pH 8.93; EC 4.86 mS cm^{-1}; TOC 324 g kg^{-1}; TN: 25.8 g kg^{-1}; K: 20.9 g kg^{-1}; and P: 3.8 g kg^{-1}. The compost was added to the soil at a rate of 1.5 kg per m^2 and mixed using a rotovator. The design of the experiment was completely randomized. It involved six randomized plots with two treatments (NAS and CAS) and three replications of each treatment. Each plot had an area of 40 m^2, and the plots were 2 m apart. The soils were moistened at 60–70% water holding capacity (WHC) using a drip irrigation system. Ambient and soil (0–10 cm) during the experiment, temperatures were monitored by four thermometers data logger (HOBO-Data Logger U12–006), located close to where the samples were taken, in each plot, connected to a data logger. After four months, the plastic film was removed and the spinach plants (*Spinacia oleracea* L, variety Nembus) were sown. The spinach seeds were sown at a ratio of 800–900 seeds per m^2 and weregrown under commercial conditions and according to the requirements of the crop and the farmer.

2.2. Sampling

Soil samples were taken at a depth of 10 cm from each of the three field replicates of the two treatments (NAS and CAS), three soil subsamples were collected at random from each replicate. There were four samplings throughout the assay: just after adding compost and before adding the plastic cover (July 24, 2020) (T1); a week after placing the plastic cover (August 14, 2020) (T2); before removing the plastic cover (November 18, 2020) (T3); and after harvesting the spinach crop (February 15, 2021) (T4). Each soil sample was separated two parts: one was 4º C for determining chemical properties and other was stored at − 80º C for microbiological analysis.

2.3. Physico-chemical and properties

The pH of the compost samples was measured after making a water-soluble extract (1:10 w/v) using a pH meter, the electrical conductivity (EC) was measured in the same way but a conductivity meter was used. Total nitrogen (TN) and total organic carbon (TOC) were determined using an elemental CHNS-O analyzer (Truspec CN, Leco, St. Joseph, Mich., USA).

Table 1
Soil physico-chemical, chemical and microbial activity properties along the experiment.

Soil Properties	Treatment	T1	T2	T3	T4	Treatment	Time	Treatment*Time
pH	NAS	8.61 ± 0.08	8.41 ± 0.09	8.67 ± 0.21	8.62 ± 0.01	* *	NS	*
	CAS	8.25 ± 0.03	8.39 ± 0.12	8.35 ± 0.12	8.23 ± 0.07			
EC	NAS	0.43 ± 0.04	0.62 ± 0.05	0.47 ± 0.07	0.38 ± 0.05	* **	* **	* **
(dS m^{-1})	CAS	0.78 ± 0.01	0.58 ± 0.05	0.87 ± 0.01	0.64 ± 0.04			
TOC	NAS	36.28 ± 0.11	37.27 ± 0.65	34.73 ± 0.06	35.43 ± 1.21	* *	*	NS
(g Kg^{-1})	CAS	42.67 ± 2.49	40.13 ± 2.60	37.27 ± 1.63	40.23 ± 2.08			
Total N	NAS	0.92 ± 0.02	0.93 ± 0.06	0.73 ± 0.06	0.77 ± 0.12b	* *	* *	NS
(g Kg^{-1})	CAS	1.55 ± 0.30	1.17 ± 0.23	0.83 ± 0.15	1.10 ± 0.20a			
C/N	NAS	39.13 ± 1.17	37.27 ± 0.65	47.55 ± 3.50	46.73 ± 5.07	*	* **	*
	CAS	28.17 ± 4.33	40.17 ± 2.60	45.47 ± 3.51	37.20 ± 5.26			
DHA	NAS	14.93 ± 1.37	11.63 ± 1.08	11.79 ± 0.79	15.36 ± 0.15	*	* **	* *
(μmol INTF g^{-1} h^{-1})	CAS	14.52 ± 0.29	15.89 ± 1.34	13.43 ± 0.47	18.38 ± 1.79			

NAS: non-amended soil; CAS: compost amended soil. DHA: dehydrogenase activity. T1: time 1, T2: time 2, T3: time 3, T4: time 4. Data are mean ± standard error: n = 3. A mixed ANOVA was performed to test the significant differences between treatment and time. Statistically significant differences by treatment and time * , * *, * ** : significant at $p < 0.05$, 0.01 and 0.001, respectively; NS.: not significant.

2.4. DNA extraction, amplification and sequencing

DNA was extracted with the DNeasy PowerSoil kit (Quiagen, Germany) from 0.5 g of soil. DNA extraction was performed in triplicate. The DNA was purified with the QIAquick Gel kit (Qiagen). To measure the quality of the DNA, electrophoresis was performed on a 1.5% agarose gel. In addition, a NanoDrop 2000 fluorospectrometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to quantify the DNA in the samples.

2.5. Soil-borne pathogens

The presence of different pathogens (*Alternaria spp., Fusarium oxysporum, Fusarium Solani, Olpidium brassicae, Stemphylium botryosum, Pythium ultimum, Olpidium bornavanus* and *Monosporascus cannonballus*) estimated in the different samples in triplicate. The quantification was carried out from the extracted soil DNA in a real-time quantitative PCR (qPCR) by using 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA) following the PCR protocol by Santísima-Trinidad et al. (2018).

2.6. Bacteria and fungi amplification and sequencing

DNA sequencing was performed at the Institute of Microbiology of the Czech Academy of Sciences (Prague, Czech Republic) using the Illumina MiSeq platform. Barcode primers 515 F and 806 R were used to amplify the V4 region of bacterial 16 S rRNA (Argonne National Laboratory) (Caporaso et al., 2012), and barcode primers gITS7 and ITS4 were used to amplify the ITS2 region of the fungus (Ihrmark et al., 2012). Sequencing was also performed in triplicate. PCR products were purified using a MinElute PCR purification kit (QIAgen) according to the manufacturer's instructions. A TruSeq PCR-Free kit (Illumina) was used for library preparation. The sequencing of fungal and bacterial amplicons was performed in an Illumina MiSeq C4SYS facility at the Institute of Microbiology of the Czech Academy of Sciences (Prague, Czech Republic).

2.7. Sequencing data processing

Raw sequence data from sequencer were processed by QIIME2 v 2020.2.0 (Caporaso et al., 2010) using the Oracle Virtualbox v 6.1. Before that, dada quality was checked using fastq, then the data was imported into QIIME2 usinf 'single end method', and denoised using dada2 (Callahan et al., 2016). The sequences were truncated to an average length of $Q > 30$. The Amplicon Sequence Variants (ASVs) obtained were taxonomically classified against the UNITE v.8.0 (Abarenkov et al., 2010) or SILVA 132 (Quast et al., 2012) database. Singletons, eukaryota, archea, mitochondria, chloroplasts and unassigned sequences were removed before performing the statistical analysis. Functional analysis of the bacterial community was carried out using the PICRUSt2 (Phylogenetic investigation of communities by reconstruction of unobserved states) algorithm (Douglas et al., 2020), whereas FUNGuild v.1.0 (Nguyen et al., 2016) was used for the identification of functional fungal groups (guilds). Prior to statistical analysis, the data were rarefied at the depth of the sample with the fewest reads: 2522.000 sequences for each sample in bacteria and 2146.000 sequences for fungi.

Sequences have been deposited in the European Nucleotide Archive (ENA) with the study accession code PRJEB50624.

2.8. Statistical test

All tests and visualizations were performed using R language (R Core Team, 2020) v 3.6.3 and v 4.1.1. To test significant differences among disinfectation systems over time, a mixed ANOVA was performed using the 'rstatix' package v 0.7.0 (Kassambara and Kassambara, 2020). Prior to that, outliers were removed and normality, homogeneity of variance and homogeneity of covariance were tested using a Shapiro-Wilk, Levene's and box test, respectively. When assumptions were not met, the nparLD function from the 'nparLD' package v 2.1 (Noguchi et al., 2012) was performed using an f1.ld. f1 design. The vegan package v 2.5–7 and HillR package v 0.5.1 (Li et al., 2014) were used to calculate the alpha diversity (Hill index $Q = 2$).

To evaluate differences in beta-diversity between disinfestation systems at different times, a Permutation Multivariate Analysis of Variance (PERMANOVA) using 'adonis' with 999 permutations from the vegan package as a function, previously checking for homogeneity of variance using the 'betadister' function, when homoscedasticity was not fulfilled, an Analysis of Similarities (ANOSIM) was performed. The visualization of the variation in the composition of the community was carried out by means of a principal coordinate analysis (PcoA) as a function of the Bray-Curtis distance.

The 'aldex' function from the ALDEx2 v 1.24.0 package was used as an ANOVA-like univariate comparison tool incorporating the Bayesian estimate of taxon abundance into a compositional framework, useful for the differentiation of ASV abundances between two conditions at the genus level to identify taxa that are significantly associated with each condition (Fernandes et al., 2013). A repeated measures ANOVA was performed to evaluate changes in microbial abundance at the genus level over time; when assumptions were not met, a non-parametric Friedman test was performed instead.

Temporal autocorrelation within and among samplings was adjusted by principal response curves (PRC) to analyze the effects of compost on the community composition among disinfestation systems. The vegan package v 2.5–7 was used to analyze PRC. Time was expressed as four samplings in a linear sequence to provide a standardized scale and easy comparison among samplings. Functional data was visualized through Non-Metric Multidimensional Scaling (NMDS) and a heatmap. A Spearman's correlation was performed to study the correlation of microbial genus at the genus level with soil properties and functionality. A ggplot2 package v 3.3.5 (Wickham and Chang, 2016) was used to perform the graphics.

3. Results

3.1. Effects of compost application and solarization on soil properties and microbial activity

Changes in soil chemical properties are shown in Table 1. Statistical analysis showed a significant interaction between treatment and time for pH, EC, C/N and DHA (Table 1). Compost-amended soils (CAS) showed significantly lower pH and higher EC values than the non-amended soils (NAS), and the EC values in the NAS treatment increased significantly at T2 and decreased at T3 and T4. However, in the CAS treatment, the EC value decreased at T2 and T4 and increased at T3. CAS showed a significantly lower C/N ratio than NAS, except at T2. Moreover, CAS showed significantly higher TOC and total N levels than NAS and these levels diminished significantly over time in both treatments (Table 1). The DHA activity, a parameter of overall microbial activity (García et al., 1997), was significantly higher in the compost-amended soil (CAS) at T2 and T3, and even after harvesting (T4) (Table 1).

The average ambient temperature (T^a) during the experiment was 26ºC in July and August, 23ºC in September and 18.5 ºC in October and November. During the process, the average T^a of the soil at a depth of 10 cm did not vary between CAS and NAS.

The initial soil T^a was 32ºC and reached a maximum of 40ºC a week after placing the plastic cover (T2). The T^a dropped, reaching 30 ºC, 25 ºC and 22 ºC by the end of September, October, and the first of November, respectively. On this last date, the plastic was removed (T3). The average soil T^a reached 17ºC, 12 ºC and 11ºC by the end of November, December and January, respectively. The T^a was 14ºC when the spinach crop was harvested on February 15, 2021 (T4).

3.2. The impact of compost application and solarization on fungal pathogens

The effects of the treatments, time and their interaction were significantly different for the studied spinach pathogens (Table 2). *Alternaria spp*. and *Olpidium bornavanus* diminished over time, although after the spinach harvest (T4) they increased again with respect to the end of the hygienization process (T3), but they did not show significant differences between treatments (Table 2). *Olpidium brassicae* and *Monosporascus cannonballus*, in the same way, they decreased until the plastic was removed (T3). At the end of the spinach harvest (T4), both pathogens showed higher log copy number in CAS than in NAS (Table 2). *Stemphylium botryosum* was initially present but it disappeared over time in both treatments (Table 2).

3.3. The impact of compost application and solarization on microbial diversity

A total of 297.687 high-quality bacterial and 313.467 fungal reads were obtained after quality filtering. In total, 2.361 bacterial ASV and 325 fungal ASV were identified, with 97% similarity. The bacterial and fungal alpha diversity indexes (Hill_2) showed significant interaction between treatment and time, and values were significantly higher in CAS than in NAS, except in T1 and in T3 in the fungal community (Fig. 1, Table S1). In addition, both indices significantly increased over time, even after harvesting. The principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity revealed differences in both the bacterial and fungal communities between CAS and NAS at T1 and T2, but not after removing the plastic (T3) (Fig. 2). However, after harvesting (T4), the bacterial community again showed differences between CAS and NAS (Fig. 2).

3.4. Impact of compost application and solarization on soil microbial communities

3.4.1. Bacterial community

At the phylum level, 10 bacterial phyla (>7%) were observed in both treatments (Fig. S1A, Table S2). The dominant phylum was Proteobacteria (27.62–35.40%), followed by Actinobacteria (21.92–30.34%). Changes were observed in different phyla according to the treatment and sampling time (Fig. S1A, Table S2). Fig. 3A shows the significant bacterial genus differences (>3%) for each sampling time between treatments; only the genera showing significant differences between treatments are represented (Fig. 3A, Table S4). At T1, of the 14 bacterial genera found, only *Photobacterium*, *Pseudomomas, Nocardioides*, *Glycomyces* and *Blastococcus* showed higher abundance in CAS than in NAS (Fig. 3A). At T2, the significant number of genera reached 22 representative genera, among which *Sphingomonas*, *Woeseia*, *Chryseolinea*, *MND1*, *Luteimonas*, *Pelagibius*, *Pseudonocardia*, *Flavisolibacter*, *Glycomices*, *Mycobacterium,* and *Thermomonas* were more abundant in CAS than in NAS. At T3 (after removing the plastic) and T4 (after harvesting) the significant genera diminished (Table S4). *Nitrospira, NMD1, Luteimonas and Acidibacter* were more abundant in CAS than in NAS in T3; and finally, Bacillus, *Streptomyces, Chryseolinea*, *Micromonospora*, *Massilia* and *Mesorhizobium* were also more abundant in CAS than in NAS at T4.

The PRC (Principal Response Curves) diagram showed different trends in the bacterial community composition changes over time among the different treatments, considering that the compost-amended soil (CAS) differences explained the variation in bacterial community through different genera such as *RB41, Skermanella, Woesiea, Sphingomonas, Blastococcus* and *Nitrospira* (Fig. 4A). This variation was greater in T3, when the plastic was removed from the soil.

3.4.2. Fungal community

At the phylum level, five fungal phyla were observed in the two treatments (Fig. S1B, Table S3). The Ascomycota phylum strongly dominated in both treatments throughout the different sampling times (92.43–98.95%). Fig. S1B depicts the minority fungal phyla (<90%), among which Basidiomycota were more abundant in CAS than in NAS. Chytridiomycota, which was only present at the end of the harvest (T4), was also more abundant in CAS than in NAS.

Fig. 3B shows the significant fungal genus differences (>1%) for each sampling time. As in the bacterial genera, only the genera showing significant differences between both treatments are represented. At T1, the dominant genera in CAS that were not present in NAS were *Acremonium, Preussia* and *Thermomyces*, while *Alternaria, Ascobolus* and *Cladosporium* were present in both treatments. At T2, the only representative genus in the CAS treatment was *Thermomyces*. At T3, *Ascobolus* was present in both treatments. Finally, at T4, *Thermomyces* was again the only genus in the CAS treatment (Fig. 3B and Table S5).

As occurred with the bacterial community, the differences in the CAS explained the significant variation that the fungal community had compared to NAS. This variation was greater after addition of compost and before adding the plastic cover (T1), and a week after placing the plastic cover (T2). Genera such as *Aspergillus, Penicillium* and *Thermomyces* were more abundant in CAS (Fig. 4B).

Table 2
Abundance of pathogens along the experiment.

(Log copy number g^{-1} soil)	Treatment	T1	T2	T3	T4	Treatment	Time	Treatment*Time
Alternaria spp.	NAS	6.17 ± 0.15	5.22 ± 0.08	4.46 ± 0.25	4.64 ± 0.06	NS	* **	* *
	CAS	6.01 ± 0.11	5.45 ± 0.06	4.05 ± 0.27	5.41 ± 0.09			
Fusarium oxysporum	NAS	3.36 ± 0.16	2.08 ± 0.21	2.14 ± 0.12	2.11 ± 0.22	NS	* **	NS
	CAS	3.05 ± 0.19	2.24 ± 0.23	2.26 ± 0.23	2.44 ± 0.05			
Fusarium	NAS	6.33 ± 0.19	6.18 ± 0.20	6.10 ± 0.23	6.20 ± 0.22	NS	NS	NS
Solani	CAS	6.14 ± 0.24	6.14 ± 0.25	6.15 ± 0.24	6.25 ± 0.02			
Olpidium brassicae	NAS	5.00 ± 0.01	4.90 ± 0.19	4.84 ± 0.19	5.20 ± 0.23	*	* **	* *
	CAS	4.98 ± 0.10	4.95 ± 0.17	5.23 ± 0.24	5.86 ± 0.16			
Stemphylium botryosum	NAS	4.78 ± 0.19	4.10 ± 0.20	0.00 ± 0.00	0.00 ± 0.00	*	* **	* **
	CAS	4.99 ± 0.23	3.08 ± 0.23	0.00 ± 0.00	0.00 ± 0.00			
Pythium	NAS	5.65 ± 0.06	5.21 ± 0.23	5.29 ± 0.11	4.91 ± 0.31	NS	*	NS
ultimum	CAS	5.71 ± 0.18	5.50 ± 0.14	5.07 ± 0.15	5.17 ± 0.09			
Olpidium bornavanus	NAS	5.79 ± 0.25	5.48 ± 0.21	4.83 ± 0.27	4.96 ± 0.06	NS	* **	* **
	CAS	5.79 ± 0.31	5.54 ± 0.16	5.25 ± 0.19	5.87 ± 0.25			
Monosporascus cannonballus	NAS	4.28 ± 0.14	4.26 ± 0.09	4.11 ± 0.13	4.87 ± 0.30	*	* **	* **
	CAS	5.29 ± 0.27	4.27 ± 0.24	4.04 ± 0.21	5.73 ± 0.16			

NAS: non-amended soil; CAS: compost amended soil. T1: time 1, T2: time 2, T3: time 3, T4: time 4. Data are mean ± standard error: n = 3. A mixed ANOVA was performed to test the significant differences between treatment and time. Statistically significant differences between each treatment at each time * , * *, * ** : significant at $p < 0.05$, 0.01 and 0.001, respectively; NS.: not significant.

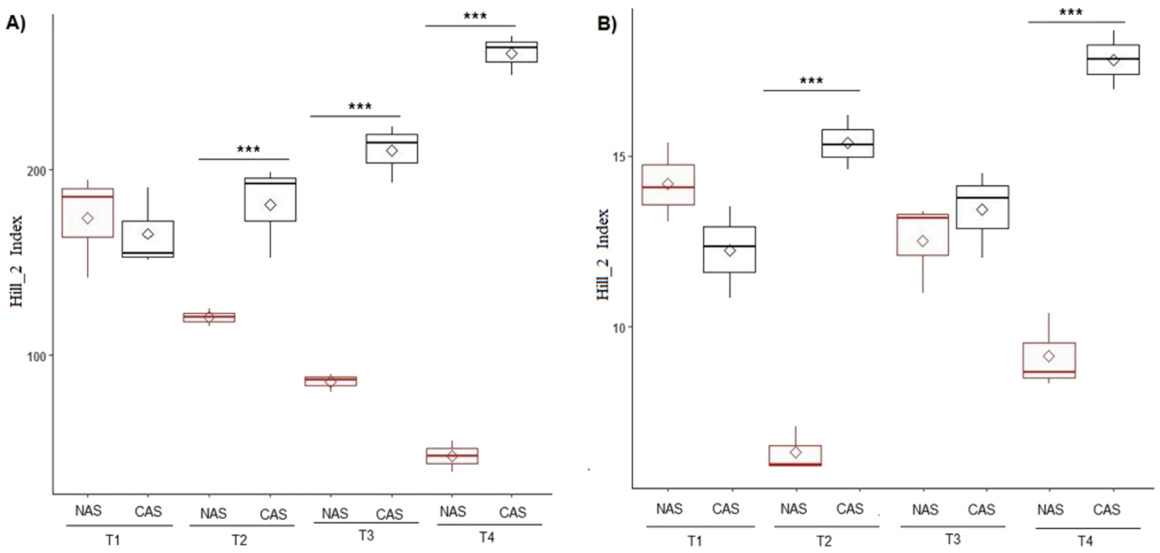

Fig. 1. Diversity index Hill_2 on (A) bacteria community and (B) fungal community during the experiment. T1: time 1, T2: time 2, T3: time 3, T4: time 4. The red and black color correspond to NAS and CAS treatment, respectively. NAS: non-amended soil and CAS: compost amended soil. Boxes represent interquartile range (IQR) between first and third quartiles (which correspond with 25th and 75th percentiles, respectively). Horizontal line in the box defines the median and diamond the mean (n = 3). Whiskers correspond to the lowest and higher values.

3.5. Estimation of microbial community functionality

3.5.1. Predictive genes involved in the N-cycling pathways

All of the six predicted genes encoding N metabolism showed significant interaction between the sampling time and treatment (Fig. 5A, Table S6). The relative abundance of the N_2-fixing functional gene (nifD) increased significantly with time, and CAS showed the highest abundance at T4 (Fig. 5A). In the nitrification process, CAS showed a significantly higher abundance of ammonia oxidation-predicted genes (amoA and amoB) than NAS, but the level did not change along the experiment. Predicted denitrification genes, such as the denitrifying nitrous oxide reductase gene (nosZ), nitrate reductase (narG) and nitrite reductase (nirK), showed significantly higher values in CAS. At T2, both treatments showed the lowest values compared to the other sampling times (Fig. 5A).

3.5.2. Predictive genes involved in the C-cycling pathways

The five genes whose expression has been linked to the degradation of cellulose, hemicellulose, lignin and the carbon pathways were influenced by the interaction between treatment and time (Fig. 5B, Table S6). Moreover, CAS showed a higher abundance than NAS, and the genes had a significantly ($p < 0.05$) higher expression at the end of the process (T3) and after spinach harvesting (T4) (Fig. 5B). At T2, the degradation of cellulose (beta-glucosidase (bglX) and alpha-glucosidase (malZ) showed lower values in both the CAS and NAS treatments (Fig. 5B). The genes that degrade lignin [catalase and glutathione peroxidase (gpx)] and hemicellulose (FUCA) showed significantly higher values in CAS than in NAS at T1, T3 and T4 (Fig. 5B). At T2, both the CAS and NAS treatments showed the lowest significant values (Table S6).

3.5.3. Predictive genes involved in fungal functions

For fungi, only three functional category groups were classified: pathotrophs, saprotrophs, and symbiotrophs (Fig. 5C, Table S7). Saprotrophs were the dominant group in both CAS and NAS, followed by pathotrophs, while the symbiotroph group was only present in NAS at the T1 sampling time (Fig. 5C).

4. Discussion

In our study, both the NAS and CAS treatments showed a decrease in some potential spinach pathogens, such as *Alternaria spp., F. oxysporum, O. brassicae, S. botryosum, P. ultimum, O. bornavanus* and *M. cannonballus*. This fact is mainly attributed to the high temperature that the soil reached during the sanitization process, due to the accumulation of heat by incident short-wave solar radiation and the decrease in evaporation provided by the plastic cover (Marshall et al., 2013), as other authors have previously observed (Tuell-Todd et al., 2009). This is due to the pathogens would compete with the other microorganisms for nutrients and space (Fuchs et al., 2010) by selecting available substrate during the process and producing antagonist metabolites (Hewavitharana et al., 2019). It is clear that the efficacy of disinfection depends on type and availability of soil amendment, volatilization losses, soil properties and the pathogen (Besri, 2021). The incorporation of compost in the solarization process (CAS) did not increase the reduction of pathogens in the soil, contrary to other authors who have found that combining solarization with different organic amendments suppresses pathogens such as *Alternaria* (Mehta et al., 2012; Achmon et al., 2020), *F. oxysporum* (Klein et al., 2011) and *Pythium spp.* (Gamliel and Stapleton, 1997; Shennan et al., 2018) probably due to the type of C source (Goss et al., 2013; Madejón et al., 2016), and the enhance and accumulation of volatile fatty acids (VFAs) (acetic acid, propionic acid or butyric acid) or different cyanates (biocide compounds) (Hestmark et al., 2019). It could be that the accumulation of biocides in CAS was not enough to weaken and inactivate different soilborne plant pathogens, and make them defenseless against different microorganisms (Stapleton and DeVay, 1986). However, the biocides can directly likely responsible for the observed decrease in the pH an increase in EC values in CAS compared to NAS. Although, the decrease in EC after T3 and mainly after spinach harvesting (T4) was possibly due to the absorption of nutrients from the crop, the leaching of ions from the soil and the immobilization of inorganic nitrogen (Medina et al., 2012; Pérez-Murcia et al., 2021).

CAS showed a greater increase in TOC, total nitrogen and microbial activity than NAS, and this activity was maintained throughout the hygienization process (T1, T2 and T3) and even until the end of the crop spinach cycle (T4). This was probably due to the slow mineralization of the compost and to the N consumed by microorganisms (Hestmark et al., 2019) contributing to improve soil microbial activity, increaseing soil sustainability and quality (Bastida et al., 2008; Liu et al., 2019; Ros et al., 2011; Sánchez-Navarro et al., 2022).

Changes in the microbial diversity and composition of the soil have a

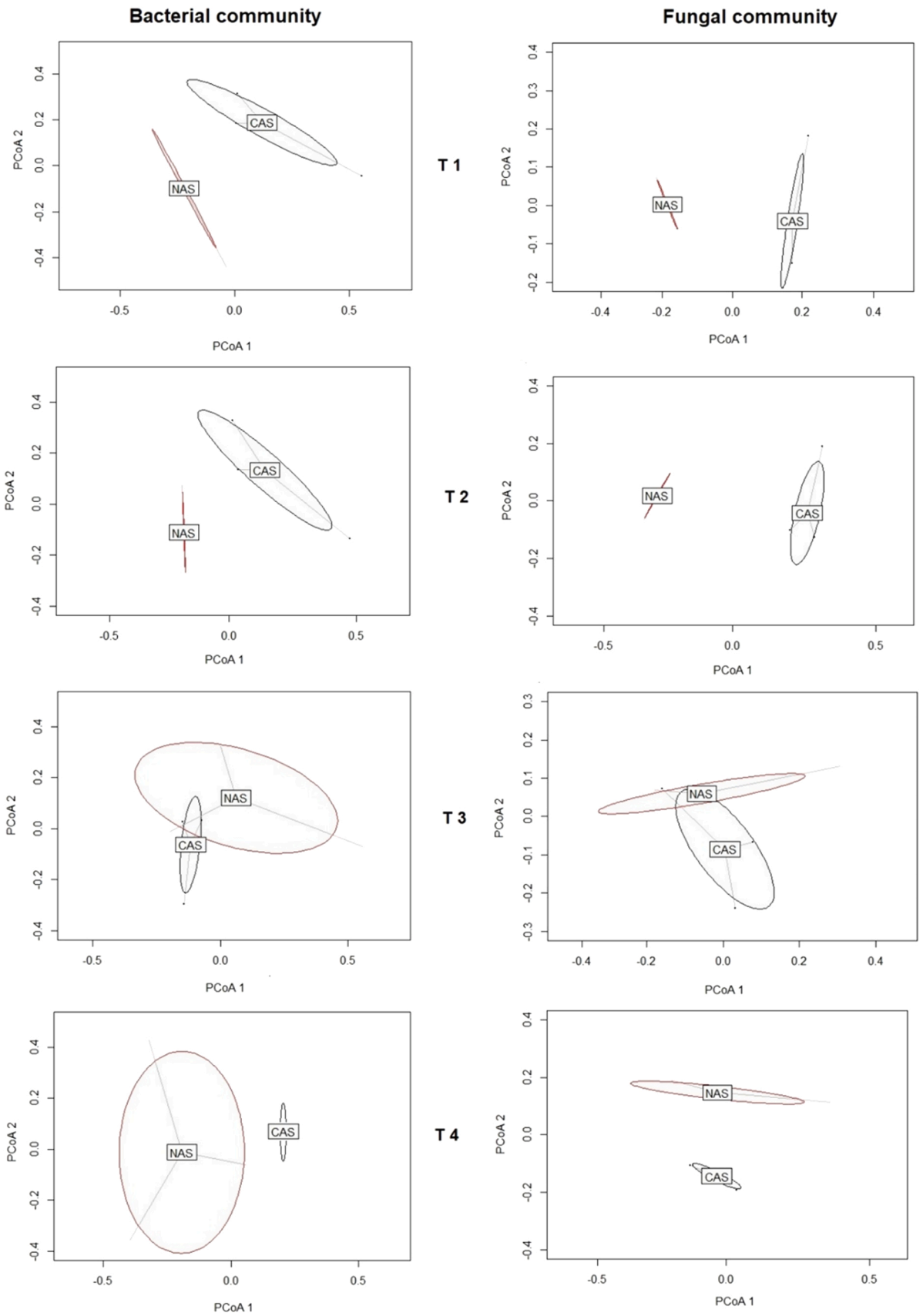

Fig. 2. Principal Coordinate Analysis (PCoA) during the experiment at T1: time 1, T2: time 2, T3: time 3, T4: time 4 of bacterial community (left) fungal community (right). NAS: non-amended soil and CAS: compost amended soil.

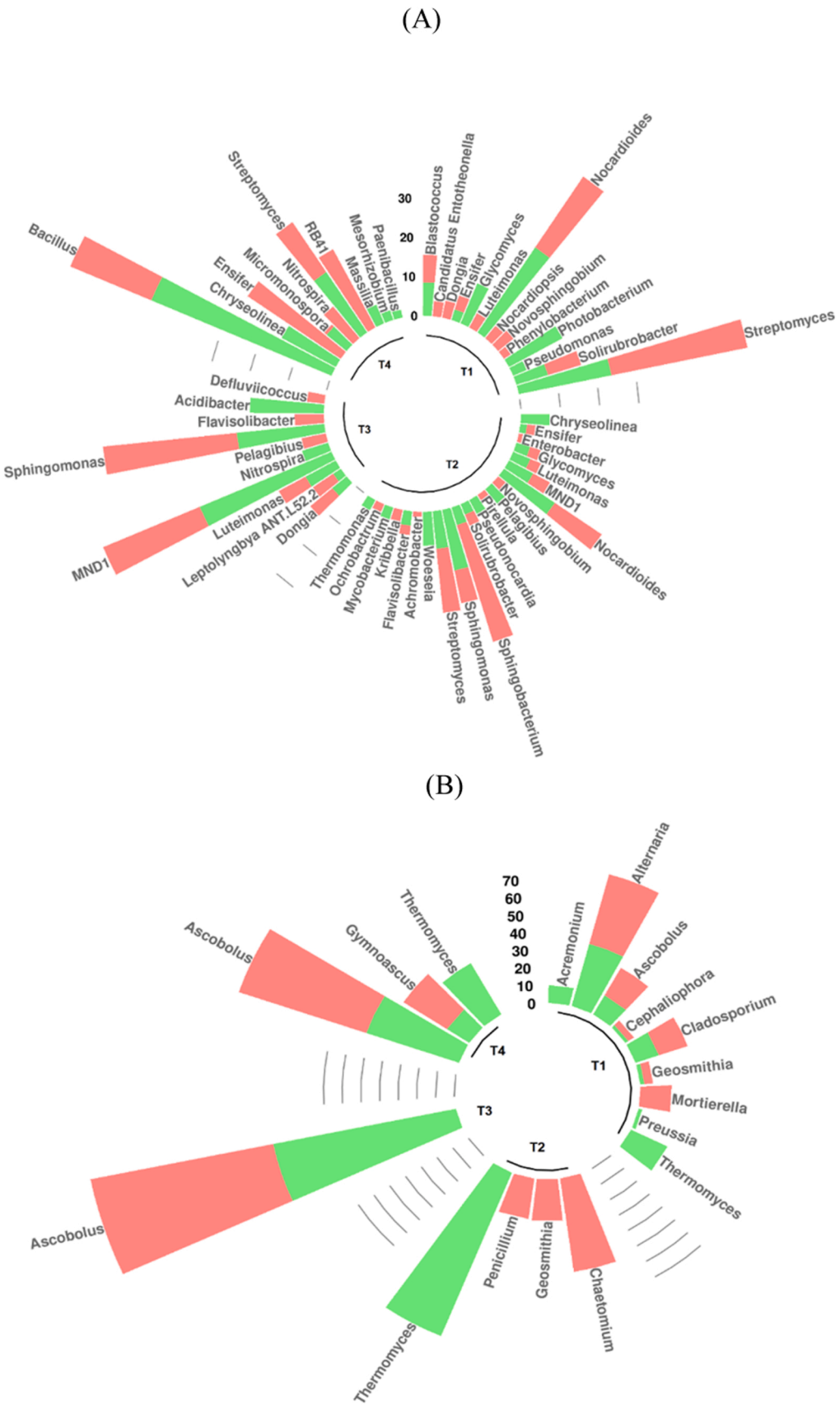

Fig. 3. Circular stacked bar-chart showing the significant bacterial genera (A) and fungal genera (B) along the experiment. T1: time 1, T2: time 2, T3: time 3, T4: time 4. The red color corresponds to NAS treatment and the green color corresponds to CAS. The representation was made using relative abundance (expressed as percentage). NAS: non-amended soil and CAS: compost amended soil.

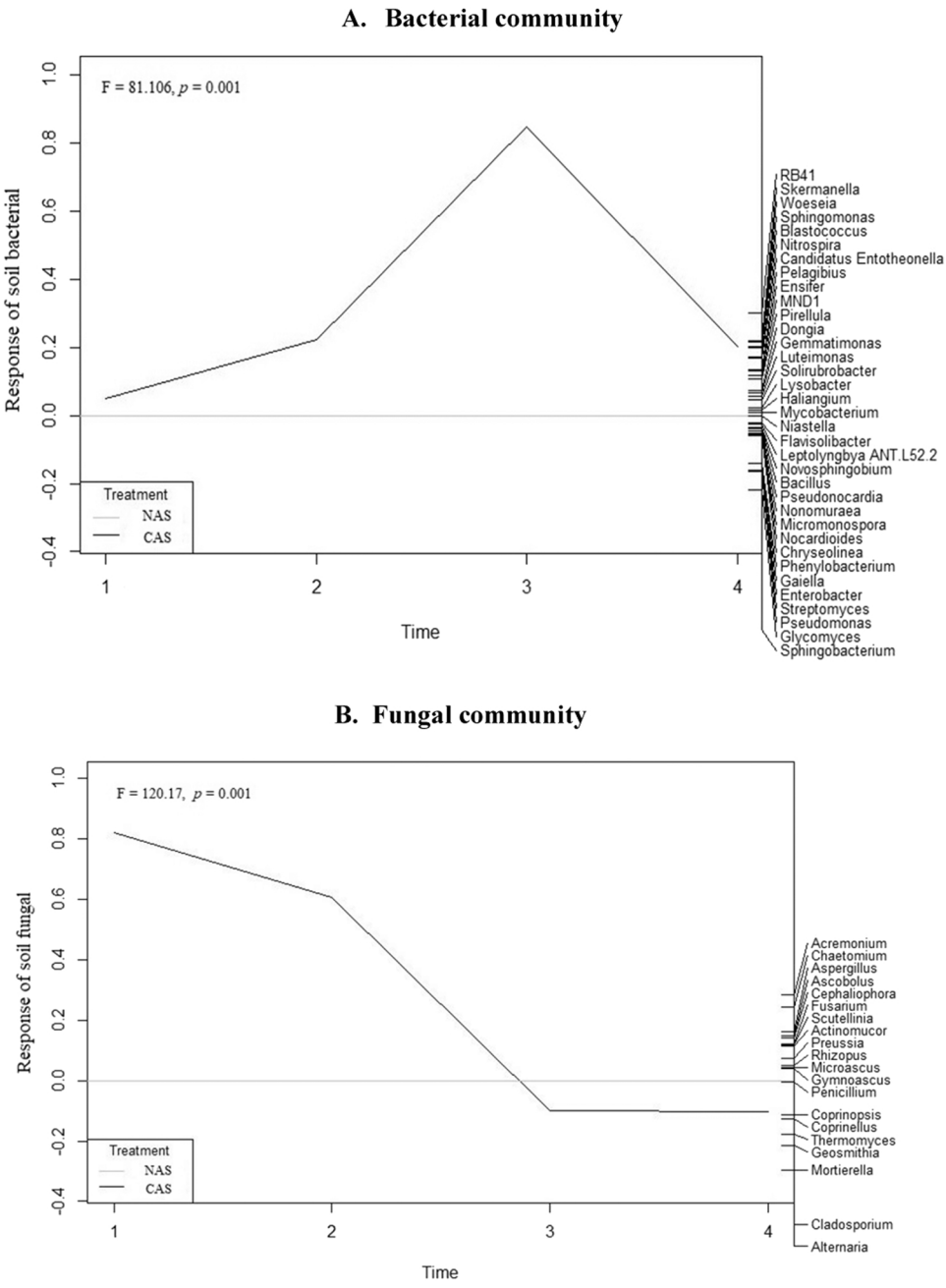

Fig. 4. Principal response curve (PRC) diagram showing the response along the experiment for bacterial (A) and fungal (B) community. Curves represent the deviation between CAS from NAS, as a function of time, with 1 as day of compost amendment until the end of the process. The weight indicates the relative contribution of genera to the community response curve and were shown on the right axis. The genera that showed significant differences between the two treatments at the different times were represented. Monte Carlo permutation test permuting whole time series were applied to compute the statistical significance.). NAS: non-amended soil and CAS: compost amended soil.

considerable influence on the soil health and fertility (Simmons et al., 2016; Hestmark et al., 2019; Nakayasu et al., 2021). The CAS produced a change in soil microbial dynamics considering that the compost can produce a shift on the soil microbiota and strongly influence the microbial use of C, which may in turn affect the preferential development of the microbe groups better adapted to the conditions. The amount, diversity and community structure of soil microbes are important indicators of soil quality and play key roles in plant growth and defense (Nakayasu et al., 2021). In our study, and in contrast to other authors, such as Sosa et al. (2021), bacterial and fungal diversity were greater in CAS than in NAS (Hartmann et al., 2015; Wang et al., 2017), probably due to the C compounds and microorganisms provided by the compost (Hernández et al., 2015). Microorganisms from compost are adapted to high temperatures occurred during composting process, and could be survive to the increase of high temperatures during solarization (Hestmark et al., 2019), while the increase of temperature in NAS forced to decrease microorganisms that were not recovered at the end of the process (Dang et al., 2021; Kanaan et al., 2018). Simmons et al. (2014) provided evidence that solarization, with or without organic amendments, leads to short- or long-term shifts in microbial communities, as we observed in our experiment. Both treatments were dominated by Proteobacteria and Actinobacteria, which are key drivers in the nutrient cycling (Luo et al., 2022), and by Ascomycota and Basidiomycota. These are the most dominant fungal phyla in soil (Llimós et al., 2021) and compost (Hernández-Lara et al., 2022) due to their capacity to resist high temperatures and their capability to use multiple carbon sources (including lignocellulose polymers) (Llimós et al., 2021).

At T1 and T2, differences in the microbial community between CAS and NAS were observed by PCoAs. Different bacteria and fungi were significantly higher in CAS than in NAS due to due to the nutrients, microorganisms and colonizable soil substrates present in the compost (Ros et al., 2011). CAS contained bacteria, that were not present on NAS,

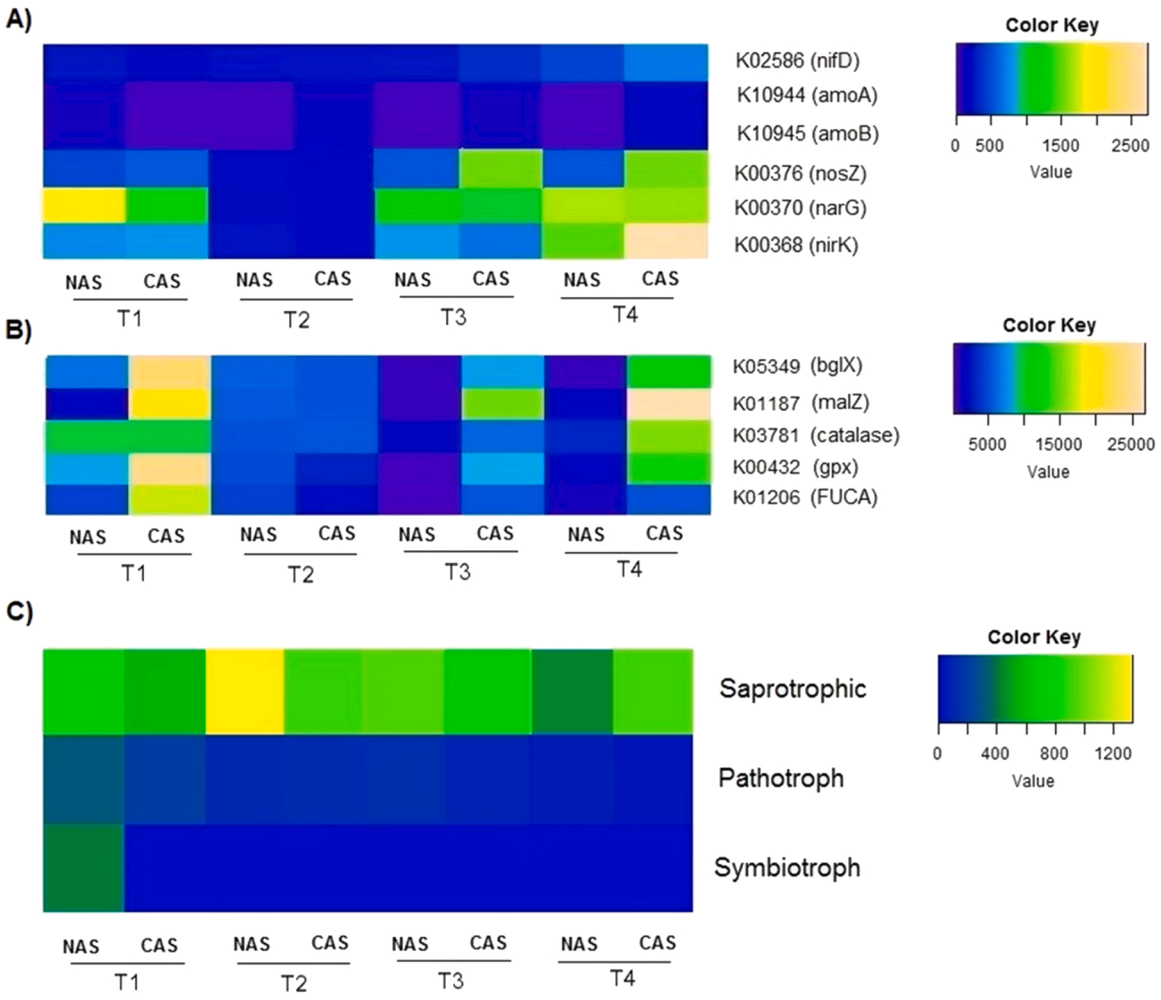

Fig. 5. Heatmap of bacterial gene expression (A) nitrogen genes, (B) carbon genes and (C) pathotroph, saprotrophic and symbiotroph fungi along the experiment. T1: time 1, T2: time 2, T3: time 3, T4: time 4). NAS: non-amended soil and CAS: compost amended soil.

related to biogeochemical functions that has previously been observed in agricultural soils: *Woeseia,* which carry out diverse ecological functions like denitrification (Zhang et al., 2020a); *Glycomyces*, which is involved in organic matter degradation (Xie et al., 2021); *Photobacterium, Pseudomonas* and *Sphingomonas,* which are promoters of plant growth and suppress plant disease (Fu et al., 2021); and NMD1, which belongs to the nitrite-oxidizing bacterial group and is involved in plant nutrition (Xue et al., 2022). As for fungi, CAS contained *Acremonium*, considered as a dominant non-pathogenic fungi in soils and previously observed in soils treated with organic residues (Swer and Dkhar, 2014), and *Thermomyces*, used to boost plant defense against pathogens through the production of chitinase. The latter is also involved in the C cycle due to its capability of degrading cellulose (Samet et al., 2019). *Thermomyces* has also been observed in mature compost made of agro-industrial waste (Hernández-Lara et al., 2022). Furthermore, those latter microorganisms have been shown to have tolerance to high temperatures and can thus survive the hygienization process (Zhang et al., 2020b).

At the end of the hygienization process, when the plastic was lifted (T3), there was a microbial vacuum, where soil was thus able to be recolonized by natural microbiota and no differences were observed between CAS and NAS (Fernandez-Bayo et al., 2017). However, certain beneficial microorganisms were more abundant in CAS than in NAS, such as *Nitrospira, Luteimonas* and *NMD1*, belonging to the nitrite-oxidizing bacterial group and involved in plant nutrition (Xue et al., 2022), or *Acidibacter*, considered an indicator of the good nutritional status of the soil (Fu et al., 2021). Furthermore, the genera *Luteimonas*, *Sphingomonas* and *Ascobolus* can be considered suppressors of soil-borne diseases in compost (Innerebner et al., 2011; He et al., 2021; Hernández-Lara et al., 2022).

After harvesting the spinach crop (T4), CAS and NAS showed differences in the bacterial and fungal communities, indicating that compost can stimulate microbial communities in the soil and those communities were maintained after the spinach harvest. *Bacillus, Streptomyces, Chryseolinea* and *Mesorhizobium* were more abundant in CAS than in NAS and frequently stimulated after harvesting (Okon Levy et al., 2015; Zhao et al., 2019; Readyhough et al., 2021). *Streptomyces* is a well-known growth-promoting bacterium in the rhizosphere of plants (Wu et al., 2021) that also produces antibiotics for biological control (Hassanisaadi et al., 2021). *Bacillus* is also known as a genera where some beneficial species belongs to that improves soil nutrition and suppresses plant diseases (He et al., 2021) and is highly abundant in compost (Saxena et al., 2020). In addition, fungi such as *Thermomyces* are capable of boosting plant defense against different soil pathogens (Samet et al., 2019). The PRC (Principal Response Curves) showed that different bacteria related to compost and involved in biogeochemical cycles—*Woeseia, Sphingomonas, Blatococcus, Nitrospira, MND1, Luteimonas* and *Lysobacter* (Hargreaves et al., 2015; Zhang et al., 2020a; Fu et al., 2021; Zhu et al., 2021; Xue et al., 2022)—and beneficial fungi for the soil—*Acremonium, Chaetomium, Aspergillus* and *Ascobolus* (Samet el at, 2019; Swer and Dkhar, 2014)—can be considered biomarkers of the process, playing a key role in the process even if not abundant.

Microbial community and its gene expression have been affected

through organic soil amendment (Zhaoxiang et al., 2020). However, fewer studies have investigated the impacts of combining compost addition with solarization on N- and C-cycling during disinfection and after spinach harvest. We found that compost had a notable effect on the abundance of such genes involved in the C and N cycles at T1, T3 and T4, but not at T2. It is interesting to highlight the fact that at T2, all the functional genes related to C and N cycling showed the lowest activity, probably due to the temperature increase (Guo et al., 2021).

The nitrogenase gene (nifD) that catalyzes the process of fixing atmospheric nitrogen into the soil, increased slightly in CAS after the plastic was lifted (T3), but above all, after growing spinach (T4), which could explain the increase of microorganisms such as *Thermomyces* (nitrogen-fixing genus) (Llimós et al., 2021) Different microorganisms such as *NMD1*, *Nitrospira* and *Woeseia* (Ammonia oxidation genus) were observed in both treatments. AmoA and amoB genes, which contribute significantly to ammonia oxidation (Xiao et al., 2019), showed lower values in both treatments due to a decrease in the nitrification process. However, an increase of nosZ abundance in CAS at T3 and T4 could potentially indicate a greater conversion of N_2O to N_2, which could decrease the greenhouse effect (Krause et al., 2017). Wolf et al. (2018) observed that higher levels of soil organic carbon may contribute to an increase in nosZ, as we observed in CAS along the experiment. The greater increase of nirk in CAS than in NAS favoured the higher rates of dissimilatory nitrate reduction to ammonium and the lower rates of denitrification (Putz et al., 2018).

The predicted gene-encoding enzyme involved in lignocellulose degradation for the CAS treatment in T3 and T4 resulted in an increase that could be due to a number of factors stimulating microbial activity, including water addition, higher temperatures and organic amendment incorporation (Lisetskii et al., 2019). A similar trend was observed by some genera such as *Nocardioides* and *Thermomyces* that are involved in the C cycle (Yim et al., 2015; Samet el at, 2019) and were present throughout the process and at the end of the harvest. Soil microbes perform critical roles in organic carbon cycling and its fixation (Liang and Balser, 2012). The abundance of several predictive genes involved in cellulose, hemicellulose and lignin degradation suggests that improved nutritional availability in the compost-amended soil increased the potential of the community to metabolize complex polysaccharides, thus improving the microbial activity—as indicated by an increase in dehydrogenase activity—to a greater extent in CAS than in NAS at T3 and T4. Predictive genes encoding cellulolytic activity, beta-glucosidase (bglX) and alpha-glucosidase (malZ) and also hemicellulose hydrolysis, Alpha-L-fucosidase (FUCA)—an important enzyme that catalyzes the hydrolysis of residues in agricultural waste (Jiménez et al., 2014; Yu et al., 2017; Fernández-Bayo et al., 2019)—showed the highest abundance in CAS at T4. This agrees with authors such as (Yu et al., 2017) and Fernández-Blayo et al., (2019), who have shown that organic exudates from the rhizosphere can increase C-degrading pathways. The increase in glutathione peroxidase (gpx) and catalase probably had to do with the high amounts of lignin in the amended compost due to the origin of its raw materials (Fernández-Bayo et al., 2019).

Regarding the functionality of the fungi, the saprotroph microorganism—which plays an essential role in the degradation of cellulosic waste—indicates adaptation to stressors, such as the wide range of temperatures that occurs in the solarization (Marano et al., 2011). Symbiotrophs were not observed in the process, while the pathotropic fungi decreased. The latter generally derive nutrients by attacking host cells and are thus considered to cause disease or have negative effects on plant performance (Anthony et al., 2017).

5. Conclusions

It can be concluded that the combination of compost and solarization (CAS) added value to the soil by increasing soil fertility and microbial activity. In addition, the compost produced significant changes in the soil bacterial and fungal communities compared to NAS during the solarization process and after harvesting. Different beneficial bacteria and fungi were more abundant in CAS than in NAS and also had a notable effect on the abundance of predictive genes involved in the C and N cycles. CAS also showed higher N_2-fixation and greater conversion of N_2O to N_2 after harvesting. Moreover, the abundance of several predictive genes involved in hemicellulose, lignin and cellulose degradation suggested that CAS produced an increase in nutritional availability. Therefore, compost could have induced a priming effect, which benefits the soil after spinach harvesting.

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.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2023.108359.

References

Abarenkov, K., Nilsson, R.H., Larsson, K.-H., Alexander, I.J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., 2010. The UNITE database for molecular identification of fungi–recent updates and future perspectives. N. Phytol. 186, 281–285.

Achmon, Y., Claypool, J.T., Fernández-Bayo, J.D., Hernandez, K., McCurry, D.G., Harrold, D.R., Su, J., Simmons, B.A., Singer, S.W., Dahlquist-Willard, R.M., 2020. Structural changes in bacterial and fungal soil microbiome components during biosolarization as related to volatile fatty acid accumulation. Appl. Soil Ecol. 153, 103602.

Alami, M.M., Xue, J., Ma, Y., Zhu, D., Abbas, A., Gong, Z., Wang, X., 2020. Structure, function, diversity, and composition of fungal communities in rhizospheric soil of Coptis chinensis Franch under a successive cropping system. Plants 9, 244.

Anthony, M.A., Frey, S.D., Stinson, K.A., 2017. Fungal community homogenization, shift in dominant trophic guild, and appearance of novel taxa with biotic invasion. Ecosphere 8, e01951.

Bastida, F., Kandeler, E., Moreno, J.L., Ros, M., García, C., Hernández, T., 2008. Application of fresh and composted organic wastes modifies structure, size and activity of soil microbial community under semiarid climate. Appl. Soil Ecol. 40, 318–329.

Besri, M., 2021. Biofumigation for the control of vegetables soilborne pathogens in some non-temperate climate countries. Glob. J. AGric. Innov. Res. Dev. 8, 140–152.

Bonanomi, G., Chiurazzi, M., Caporaso, S., Del Sorbo, G., Moschetti, G., Felice, S., 2008. Soil solarization with biodegradable materials and its impact on soil microbial communities. Soil Biol. Biochem. 40, 1989–1998.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624.

Correll, J.C., Morelock, T.E., Black, M.C., Koike, S.T., Brandenberger, L.P., Dainello, F.J., 1994. Economically important diseases of spinach. Plant Dis. 78, 653–660.

Cuartero, J., Pascual, J.A., Vivo, J.-M., Özbolat, O., Sánchez-Navarro, V., Egea-Cortines, M., Zornoza, R., Mena, M.M., Garcia, E., Ros, M., 2022. A first-year melon/cowpea intercropping system improves soil nutrients and changes the soil microbial community. Agric. Ecosyst. Environ. 328, 107856.
Dang, Q., Wang, Y., Xiong, S., Yu, H., Zhao, X., Tan, W., Cui, D., Xi, B., 2021. Untangling the response of fungal community structure, composition and function in soil aggregate fractions to food waste compost addition. Sci. Total Environ. 769, 145248.
Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M., Huttenhower, C., Langille, M.G.I., 2020. PICRUSt2 for prediction of metagenome functions. Nat. Biotechnol. 38, 685–688.
Fernandes, A.D., Macklaim, J.M., Linn, T.G., Reid, G., Gloor, G.B., 2013. ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-Seq. PLoS One 8, e67019.
Fernandez-Bayo, J.D., Randall, T.E., Achmon, Y., Hestmark, K., Harrold, D.R., Su, J., Dahlquist-Willard, R., Gordon, T., Stapleton, J., Simmons, C., 2017. Effect of partially stabilized organic amendments on volatile acids production and pest inactivation using soil biosolarization, in: 2017 ASABE Annual International Meeting. American Society of Agricultural and Biological Engineers, p. 1.
Fernández-Bayo, J.D., Hestmark, K.V., Claypool, J.T., Harrold, D.R., Randall, T.E., Achmon, Y., Stapleton, J.J., Simmons, C.W., VanderGheynst, J.S., 2019. The initial soil microbiota impacts the potential for lignocellulose degradation during soil solarization. J. Appl. Microbiol 126, 1729–1741.
Fu, H., Li, H., Yin, P., Mei, H., Li, J., Zhou, P., Wang, Y., Ma, Q., Jeyaraj, A., Thangaraj, K., 2021. Integrated application of rapeseed cake and green manure enhances soil nutrients and microbial communities in tea garden soil. Sustainability 13, 2967.
Fuchs, B.B., O'Brien, E., El Khoury, J.B., Mylonakis, E., 2010. Methods for using Galleria mellonella as a model host to study fungal pathogenesis. Virulence 1, 475–482.
Gamliel, A., Stapleton, J.J., 1997. Improvement of soil solarization with volatile compounds generated from organic amendments. Phytoparasitica 25, S31–S38.
García, C., Hernandez, T., Costa, F., 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun. Soil Sci. Plant Anal. 28, 123–134.
Goss, M.J., Tubeileh, A., Goorahoo, D., 2013. A review of the use of organic amendments and the risk to human health. Adv. Agron. 120, 275–379.
Gratsia, M.E., Kyriakopoulou, P.E., Voloudakis, A.E., Fasseas, C., Tzanetakis, I.E., 2012. First report of Olive mild mosaic virus and Sowbane mosaic virus in spinach in Greece. Plant Dis. 96, 1230.
Guo, H., Gu, J., Wang, X., Song, Z., Nasir, M., Tuo, X., 2021. Elucidating the microbiological characteristics of cyromazine affecting the nitrogen cycle during aerobic composting of pig manure. Sci. Total Environ. 764, 142812.
Hargreaves, S.K., Williams, R.J., Hofmockel, K.S., 2015. Environmental filtering of microbial communities in agricultural soil shifts with crop growth. PLoS One 10, e0134345.
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.
Hassanisaadi, M., Shahidi Bonjar, G.H., Hosseinipour, A., Abdolshahi, R., Ait Barka, E., Saadoun, I., 2021. Biological control of Pythium aphanidermatum, the causal agent of tomato root rot by two Streptomyces root symbionts. Agronomy 11, 846.
He, H., Zhang, S., Shen, W., Zhu, W., Noor, I., Liu, J., Li, G., 2021. Benzoic acid plays a part in rhizosphere microbial composition of peach seedlings grown in replanted soil. Rhizosphere 19, 100364.
Hernández, T., Garcia, E., García, C., 2015. A strategy for marginal semiarid degraded soil restoration: A sole addition of compost at a high rate. A five-year field experiment. Soil Biol. Biochem. 89, 61–71.
Hernández-Lara, A., Ros, M., Cuartero, J., Bustamante, M.Á., Moral, R., Andreu-Rodríguez, F.J., Fernández, J.A., Egea-Gilabert, C., Pascual, J.A., 2022. Bacterial and fungal community dynamics during different stages of agro-industrial waste composting and its relationship with compost suppressiveness. Sci. Total Environ. 805, 150330.
Hestmark, K.V., Fernández-Bayo, J.D., Harrold, D.R., Randall, T.E., Achmon, Y., Stapleton, J.J., Simmons, C.W., VanderGheynst, J.S., 2019. Compost induces the accumulation of biopesticidal organic acids during soil biosolarization. Resour. Conserv. Recycl. 143, 27–35.
Hewavitharana, S.S., Klarer, E., Reed, A.J., Leisso, R., Poirier, B., Honaas, L., Rudell, D. R., Mazzola, M., 2019. Temporal dynamics of the soil metabolome and microbiome during simulated anaerobic soil disinfestation. Front. Microbiol. 2365.
Ihrmark, K., Bödeker, I., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., 2012. New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82, 666–677.
Innerebner, G., Knief, C., Vorholt, J.A., 2011. Protection of Arabidopsis thaliana against leaf-pathogenic Pseudomonas syringae by Sphingomonas strains in a controlled model system. Appl. Environ. Microbiol. 77, 3202–3210.
Jiménez, D.J., Dini-Andreote, F., Van Elsas, J.D., 2014. Metataxonomic profiling and prediction of functional behaviour of wheat straw degrading microbial consortia. Biotechnol. Biofuels 7, 1–18.
Kanaan, H., Medina, S., Raviv, M., 2017. The effects of soil solarization and compost on soil suppressiveness against Fusarium oxysporum f. sp. melonis. Compost Sci. Util. 25, 206–210.
Kanaan, H., Frenk, S., Raviv, M., Medina, S., Minz, D., 2018. Long and short term effects of solarization on soil microbiome and agricultural production. Appl. Soil Ecol. 124, 54–61.
Kassambara, A., Kassambara, M.A., 2020. Package 'ggpubr.' R Packag. version 0.1 6.
Kipkogei, K., Kiptui, K., Kiprop, E., 2019. Antifungal Potential of Curcuma longa (Tumeric) and Zingiber officinale (Ginger) against Alternaria alternata Infecting Spinach in Kenya.
Klein, E., Katan, J., Gamliel, A., 2011. Soil suppressiveness to Fusarium disease following organic amendments and solarization. Plant Dis. 95, 1116–1123.
Koike, S.T., Henderson, D.M., Butler, E.E., 2001. Leaf spot disease of spinach in California caused by Stemphylium botryosum. Plant Dis. 85, 126–130.
Krause, H.-M., Thonar, C., Eschenbach, W., Well, R., Mäder, P., Behrens, S., Kappler, A., Gattinger, A., 2017. Long term farming systems affect soils potential for N2O production and reduction processes under denitrifying conditions. Soil Biol. Biochem. 114, 31–41.
Li, D., Li, M.D., LazyData, T., Imports, F.D., 2014. Package 'hillR. Annu. Rev. Ecol. Evol. Syst. 45, 297–324.
Liang, C., Balser, T.C., 2012. Warming and nitrogen deposition lessen microbial residue contribution to soil carbon pool. Nat. Commun. 3, 1–4.
Lisetskii, F.N., Buryak, Z.A., Marinina, O.A., 2019. Assessment of biogeochemical soil variability using a geostatistical approach: a case study in the steppe Crimea.
Liu, Y., Miao, H., Chang, X., Wu, G., 2019. Higher species diversity improves soil water infiltration capacity by increasing soil organic matter content in semiarid grasslands. L. Degrad. Dev. 30, 1599–1606.
Llimós, M., Segarra, G., Sancho-Adamson, M., Trillas, M.I., Romanyà, J., 2021. Impact of olive saplings and organic amendments on soil microbial communities and effects of mineral fertilization. Front. Microbiol. 12.
Luo, Y., van Veelen, H.P.J., Chen, S., Sechi, V., ter Heijne, A., Veeken, A., Buisman, C.J. N., Bezemer, T.M., 2022. Effects of sterilization and maturity of compost on soil bacterial and fungal communities and wheat growth. Geoderma 409, 115598.
Madejón, P., Alaejos, J., García-Álbala, J., Fernández, M., Madejón, E., 2016. Three-year study of fast-growing trees in degraded soils amended with composts: effects on soil fertility and productivity. J. Environ. Manag. 169, 18–26.
Magnée, K.J.H., Postma, J., Groot, S.P.C., Gort, G., Lammerts van Bueren, E.T., Scholten, O.E., 2022. Evaluation of damping-off tolerance in spinach cultivars in field soils and in a standardized lab assay with Pythium ultimum. Plant Heal. Prog. PHP-07.
Marano, A.V., Gleason, F.H., Barrera, M.D., Steciow, M.M., Daynes, C.N., McGee, P.A., 2011. Rhizophlyctis rosea (Rhizophlyctidales, Chytridiomycota) in soil: frequency, abundance and density of colonization of lens paper baits. Nov. Hedwigia 93, 73.
Marshall, M.N., Rumsey, T.R., Stapleton, J.J., VanderGheynst, J.S., 2013. A predictive model for soil temperature during solarization and model validation at two California field sites. Trans. ASABE 56, 117–133.
McDonald, M.R., Collins, B., Adusei-Fosu, K., 2021. Soil amendments and fumigation for the management of Fusarium wilt of bunching spinach in Ontario, Canada. Crop Prot. 145, 105646.
Medina, E., Paredes, C., Bustamante, M.A., Moral, R., Moreno-Caselles, J., 2012. Relationships between soil physico-chemical, chemical and biological properties in a soil amended with spent mushroom substrate. Geoderma 173, 152–161.
Mehta, C.M., Gupta, V., Singh, S., Srivastava, R., Sen, E., Romantschuk, M., Sharma, A.K., 2012. Role of microbiologically rich compost in reducing biotic and abiotic stresses. In: Microorganisms in Environmental Management. Springer, pp. 113–134.
Mitsuboshi, M., Kioka, Y., Noguchi, K., Asakawa, S., 2022. Evaluation of disease suppressiveness of soils in croplands by co-cultivation of pathogenic fusarium oxysporum and indigenous soil microorganisms. Microbes Environ. 37, ME21063.
Nakayasu, M., Ikeda, K., Yamazaki, S., Aoki, Y., Yazaki, K., Washida, H., Sugiyama, A., 2021. Two distinct soil disinfestations differently modify the bacterial communities in a tomato Field. Agronomy 11, 1375.
Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248.
Noguchi, K., Gel, Y.R., Brunner, E., Konietschke, F., 2012. nparLD: an R software package for the nonparametric analysis of longitudinal data in factorial experiments. J. Stat. Softw. 50, 1–23.
Okon Levy, N., Meller Harel, Y., Haile, Z.M., Elad, Y., Rav-David, E., Jurkevitch, E., Katan, J., 2015. Induced resistance to foliar diseases by soil solarization and T richoderma harzianum. Plant Pathol. 64, 365–374.
Pérez-Murcia, M.D., Martínez-Sabater, E., Domene, M.A., González-Céspedes, A., Bustamante, M.A., Marhuenda-Egea, F.C., Barber, X., López-Lluch, D.B., Moral, R., 2021. Role of proteins and soluble peptides as limiting components during the co-composting of agro-industrial wastes. J. Environ. Manag. 300, 113701.
Putz, M., Schleusner, P., Rütting, T., Hallin, S., 2018. Relative abundance of denitrifying and DNRA bacteria and their activity determine nitrogen retention or loss in agricultural soil. Soil Biol. Biochem. 123, 97–104.
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.
Readyhough, T., Neher, D.A., Andrews, T., 2021. Organic amendments alter soil hydrology and belowground microbiome of tomato (Solanum lycopersicum). Microorganisms 9, 1561.
Ros, M., Knapp, B.A., Peintner, U., Insam, H., 2011. Response of bacterial community composition to long-term applications of different composts in agricultural soils. Span. J. Agric. Res. 9, 338–343.
Samet, M., Karray, F., Mhiri, N., Kamoun, L., Sayadi, S., Gargouri-Bouzid, R., 2019. Effect of phosphogypsum addition in the composting process on the physico-chemical proprieties and the microbial diversity of the resulting compost tea. Environ. Sci. Pollut. Res. 26, 21404–21415.
Sánchez-Navarro, A., Jiménez-Ballesta, R., Girona-Ruiz, A., Alarcón-Vera, I., Delgado-Iniesta, M.J., 2022. Rapid response indicators for predicting changes in soil properties due to solarization or biosolarization on an intensive horticultural crop in semiarid regions. Land 11, 64.

Santísima-Trinidad, A.B.L., del Mar Montiel-Rozas, M., Diéz-Rojo, M.Á., Pascual, J.A., Ros, M., 2018. Impact of foliar fungicides on target and non-target soil microbial communities in cucumber crops. Ecotoxicol. Environ. Saf. 166, 78–85.
Saxena, A.K., Kumar, M., Chakdar, H., Anuroopa, N., Bagyaraj, D.J., 2020. Bacillus species in soil as a natural resource for plant health and nutrition. J. Appl. Microbiol 128, 1583–1594.
Shennan, C., Muramoto, J., Koike, S., Baird, G., Fennimore, S., Samtani, J., Bolda, M., Dara, S., Daugovish, O., Lazarovits, G., 2018. Anaerobic soil disinfestation is an alternative to soil fumigation for control of some soilborne pathogens in strawberry production. Plant Pathol. 67, 51–66.
Simmons, C.W., Guo, H., Claypool, J.T., Marshall, M.N., Perano, K.M., Stapleton, J.J., VanderGheynst, J.S., 2013. Managing compost stability and amendment to soil to enhance soil heating during soil solarization. Waste Manag 33, 1090–1096.
Simmons, C.W., Claypool, J.T., Marshall, M.N., Jabusch, L.K., Reddy, A.P., Simmons, B. A., Singer, S.W., Stapleton, J.J., VanderGheynst, J.S., 2014. Characterization of bacterial communities in solarized soil amended with lignocellulosic organic matter. Appl. Soil Ecol. 73, 97–104.
Simmons, C.W., Higgins, B., Staley, S., Joh, L.D., Simmons, B.A., Singer, S.W., Stapleton, J.J., VanderGheynst, J.S., 2016. The role of organic matter amendment level on soil heating, organic acid accumulation, and development of bacterial communities in solarized soil. Appl. Soil Ecol. 106, 37–46.
Sosa, L.L., de, Moreno, B., Herrera, R.A., Panettieri, M., Madejón, E., Benítez, E., 2021. Organic amendments and sampling date influences on soil bacterial community composition and their predictive functional profiles in an olive grove ecosystem. Agriculture 11, 1178.
Stapleton, J.J., DeVay, J.E., 1986. Soil solarization: a non-chemical approach for management of plant pathogens and pests. Crop Prot. 5, 190–198.
Swer, H., Dkhar, M.S., 2014. Influence of crop rotation and intercropping on microbial populations in cultivated fields under different organic amendments. In: Microbial Diversity and Biotechnology in Food Security. Springer, pp. 571–580.
Tuell-Todd, C.C., Dahlquist, R.M., Kunz, M., Stapleton, J.J., 2009. Time and temperature requirements for thermal death of seeds of yellow starthistle (Centaurea solstistealis L.) and black mustard (Brassica nigra L.). Proceedings. Annu. Calif. Weed Sci. Soc.
Wang, Y., Hu, N., Ge, T., Kuzyakov, Y., Wang, Z.-L., Li, Z., Tang, Z., Chen, Y., Wu, C., Lou, Y., 2017. Soil aggregation regulates distributions of carbon, microbial community and enzyme activities after 23-year manure amendment. Appl. Soil Ecol. 111, 65–72.
Wickham, H., Chang, W., 2016. An implementation of the grammar of graphics. WWW http//ggplot2. org.
Wolf, K.M., Torbert, E.E., Bryant, D., Burger, M., Denison, R.F., Herrera, I., Hopmans, J., Horwath, W., Kaffka, S., Kong, A.Y.Y., 2018. The century experiment: the first twenty years of UC Davis' Mediterranean agroecological experiment.
Wu, C., Wang, F., Ge, A., Zhang, H., Chen, G., Deng, Y., Yang, J., Chen, J., Ge, T., 2021. Enrichment of microbial taxa after the onset of wheat yellow mosaic disease. Agric. Ecosyst. Environ. 322, 107651.
Xiao, Z., Rasmann, S., Yue, L., Lian, F., Zou, H., Wang, Z., 2019. The effect of biochar amendment on N-cycling genes in soils: a meta-analysis. Sci. Total Environ. 696, 133984.
Xie, G., Kong, X., Kang, J., Su, N., Luo, G., Fei, J., 2021. Community-level dormancy potential regulates bacterial beta-diversity succession during the co-composting of manure and crop residues. Sci. Total Environ. 772, 145506.
Xue, Y., Jin, T., Gao, C., Li, C., Zhou, T., Wan, D., Yang, M., 2022. Effects of biodegradable film mulching on bacterial diversity in soils. Arch. Microbiol. 204, 1–13.
Yanardağ, I.H., Zornoza, R., Bastida, F., Büyükkiliç-Yanardağ, A., García, C., Faz, A., Mermut, A.R., 2017. Native soil organic matter conditions the response of microbial communities to organic inputs with different stability. Geoderma 295, 1–9.
Yim, B., Winkelmann, T., Ding, G.-C., Smalla, K., 2015. Different bacterial communities in heat and gamma irradiation treated replant disease soils revealed by 16S rRNA gene analysis–contribution to improved aboveground apple plant growth? Front. Microbiol. 6, 1224.
Yu, C., Harrold, D.R., Claypool, J.T., Simmons, B.A., Singer, S.W., Simmons, C.W., VanderGheynst, J.S., 2017. Nitrogen amendment of green waste impacts microbial community, enzyme secretion and potential for lignocellulose decomposition. Process Biochem 52, 214–222.
Zhang, D., Yan, D., Cheng, H., Fang, W., Huang, B., Wang, Xianli, Wang, Xiaoning, Yan, Y., Ouyang, C., Li, Y., 2020a. Effects of multi-year biofumigation on soil bacterial and fungal communities and strawberry yield. Environ. Pollut. 256, 113415.
Zhang, X., Ji, Z., Shao, Y., Guo, C., Zhou, H., Liu, L., Qu, Y., 2020b. Seasonal variations of soil bacterial communities in Suaeda wetland of Shuangtaizi River estuary, Northeast China. J. Environ. Sci. 97, 45–53.
Zhao, Z., Ge, T., Gunina, A., Li, Y., Zhu, Z., Peng, P., Wu, J., Kuzyakov, Y., 2019. Carbon and nitrogen availability in paddy soil affects rice photosynthate allocation, microbial community composition, and priming: combining continuous 13C labeling with PLFA analysis. Plant Soil 445, 137–152.
Zhaoxiang, W., Huihu, L., Qiaoli, L., Changyan, Y., Faxin, Y., 2020. Application of bio-organic fertilizer, not biochar, in degraded red soil improves soil nutrients and plant growth. Rhizosphere 16, 100264.
Zhu, J., Cao, A., Wu, J., Fang, W., Huang, B., Yan, D., Wang, Q., Li, Y., 2021. Effects of chloropicrin fumigation combined with biochar on soil bacterial and fungal communities and Fusarium oxysporum. Ecotoxicol. Environ. Saf. 220, 112414.