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Influence of organic matter management on the activity and structure of soil microbial community in intensive tomato (*Solanum lycopersicum* L.) greenhouse farming

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Abstract

Aim of study: Intensive agriculture impacts physical, chemical, and biological characteristics of soil; therefore, the addition of organic matter (OM) to soil can have significant implications for crop production. This study investigated the impact of three crop management systems on tomato production and soil microbial communities in intensive greenhouse farming.

Area of study: Province of Almería (Spain).

Material and methods: The three crop management systems included: (1) conventional management, using synthetic chemical fertilizers without OM application (CM); (2) conventional management, using synthetic chemical fertilizers with at least one OM application in the last three years (CMOM); and (3) fully organic management, featuring yearly OM applications and no use of synthetic chemical fertilizers (ORG).

Main results: Compared to CM soils, OM addition in CMOM and ORG led to higher soil NO_3^- and NH_4^+ content, which in turn increased nitrogen (N) availability, leading to an increase in soil respiration. The addition of OM also altered the composition of prokaryotic and fungal soil communities. Besides, the addition of OM reduced the presence and abundance of potential fungal pathogenic organisms, like *Sclerotinia* sp. and *Plectosphaerella cucumerina*. OM addition to conventionally managed greenhouses (CMOM) led to higher crop yields compared to CM greenhouses, resulting in an overall increase of 880 g m^{-2}. Production under fully organic management (ORG) was lowest, possibly due to the nutrient and pest management practices used.

Research highlights: Our data show the importance of organic matter management in shaping microbial communities in intensive greenhouse systems, which can be a key factor in developing a more sustainable agriculture to feed a growing human population.

Additional key words: amplicon sequencing; intensive agriculture; manure; tomato production; respiration.

Abbreviation used: ASV (Amplicon Sequence Variant); CM (Conventional Management); CMOM (Conventional Management with Organic Matter addition); LMM (Linear Mixed-effects Models); OM (Organic Matter); ORG (Organic Management); qPCR (Real-time Polymerase Chain Reaction); SOM (Soil Organic Matter).

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Introduction

There are approximately 170,000 ha of intensively farmed greenhouses in the Mediterranean Basin (Pardossi et al., 2004); the largest concentration of approximately 32,000 ha is in the province of Almería in southeast Spain (Cajamar, 2019). The different management techniques used in this intensive production system can affect the physical, chemical, and microbiological characteristics of soils. One of the major impacts of crop production on soil properties is how organic matter (OM) addition affects soil organic matter (SOM) (Scotti et al., 2015). Many aspects of how OM management influences crop production, in intensive agriculture, are well understood (De Ponti et al., 2012), particularly nutritional aspects (Ewulo et al., 2008). However, the effects of OM management on soil microbial community structure and function, as well as its relation to nutrient availability and plant production, in Mediterranean intensive greenhouse systems remain largely unknown.

Traditionally, the soil in SE Spain greenhouses is covered with a sand mulch to maintain soil moisture and reduce diel temperature fluctuations (Pardossi et al., 2004). The sand mulch makes it difficult to add OM because of the cost of pushing sand aside (Valera et al., 2017). Consequently, in many greenhouses in SE Spain, OM is applied every 3-4 years instead of annually (Cesarano et al., 2017). Reduced OM application can increase the requirement for synthetic chemical fertilizers to ensure that crop nutrient requirements are met (Barberis et al., 1995). This can result in excess N being applied which can cause environmental problems (Soto et al., 2015), such as nitrate leaching into groundwater (Gallardo et al., 2006).

Indirectly, low levels of OM in soil can contribute to an increased incidence of plant pathogens (Savary et al., 2012), and a decrease of soil microbial (Wu et al., 2013) and mesofauna abundance and diversity (Battigelli et al., 2004). Soil chemical disinfectants are now banned, which increases the possibility of higher populations of soil-borne plant pathogen pathogens (Stapleton & DeVay, 1986). Therefore, knowledge concerning microbial population dynamics and activity in greenhouse soils is a pressing need, particularly in the Mediterranean basin, given the global significance of this region to feed an increasing global population.

The diversity of soil microbial communities is directly linked to the amount of SOM (Bausenwein et al., 2008). Soil microbial community composition and structure have a key role in soil function (Gupta et al., 2022) and crop production. This is because they are the major drivers of SOM mineralization (Duchicela et al., 2012) This SOM mineralization lead to higher rates of soil respiration (Mbuthia et al., 2015). Microbial communities are also critical for plant health, as they control soil-borne pathogens (Senechkin et al., 2010; van Bruggen et al., 2015), and are also involved in soil responses after disturbance (Lehman et al., 2015). Diverse soil microbial communities may also contribute to alleviating soil salinity and other stressful conditions (Kumar et al., 2020). Finally, many studies show that soil microbial communities are more diverse, and crops are healthier and more productive when OM is applied regularly and added to conventional inorganic fertilization (Bever et al., 2010).

Soil respiration is linked to OM decomposition and depends on environmental conditions, particularly soil temperature and water availability. Soil respiration results from the activity of several biotic components, such as roots, mesofauna, and the soil microbial community, all of which contribute to the overall respiration (Estruch et al., 2020). The interactions between soil organisms are also important drivers of soil respiration as well. For example, high soil respiration is commonly related to high plant photosynthesis and release of root exudates, which can lead to higher microbial diversity and activity (Baldocchi et al., 2006). Soil respiration can be used as a biological indicator of soil quality (Bünemann et al., 2018), reflecting soil metabolic activity (Curiel-Yuste et al., 2007), and it can be indirectly related to crop production (Lamptey et al., 2019). These relationships show the importance of soil respiration, which fluctuates seasonally and differs between agroecosystems (Benbi et al., 2019), and requires frequent measurement to understand its dynamics (Curiel-Yuste et al., 2007).

In this study, we address how three different greenhouse management systems, differing in SOM amendments and synthetic chemical fertilizer application, influence soil microbial community structure, composition, and activity, and how these microbial communities have an effect on crop production in the intensive greenhouse agricultural system of SE Spain. We hypothesized that OM addition will influence microbial community composition, structure, and activity, and that higher OM additions might result in more diverse and active soil microbial communities, which may translate into increased crop production.

Material and methods

Study area and greenhouse selection

The study was carried out in the province of Almeria, where there are currently more than 32,000 ha of greenhouses. This greenhouse system is the main producer of fresh market tomato (*Solanum lycupersicum* L.) in Spain. The annual cropped surface is approximately 10,000 ha, and annual production is 890,000 tonnes (MAPA, 2019). In this area, we selected 15 different commercial greenhouses of 1-2 ha, growing tomato, with three different soil management systems, having five different greenhouses per management. The three management systems were: (1) conventional management (CM), with no addition of OM in the previous ten years, and use of synthetic chemical fertilizers. Synthetic chemical fertilizers met crop nutrient

requirements, with a total average input of 370 kg N ha^{-1}, 50 kg P ha^{-1}, 680 kg K ha^{-1}, 290 kg Mg ha^{-1} and 45 kg Ca ha^{-1} (Papadopoulos 1991). The applied fertilizers were $Ca(NO_3)_2$, $Mg(NO_3)_2$, KH_2PO_4, K_2SO_4 and a combination of micronutrients, including Fe, Cu, B, Mn, Mo and Zn. They were applied in nutrient solution that was applied every 1-4 days through the drip irrigation system; (2) a conventional management with OM addition (CMOM), where there had been at least one addition of OM in the previous three years but where synthetic chemical fertilizers were routinely used (as in CM); and (3) organic management (ORG) with OM addition every year and without synthetic chemical fertilizer addition. The crop growth cycle was from April (seedling transplant) to July (harvest). Organic matter additions were 18,000 kg ha^{-1} per application (recommended dose) of fresh manure. Typically, the applied manure had 65% dry matter, 2.2% of N and 30% of C, and was a mix of 60% goat, 30% sheep, and 10% poultry. In the ORG management, the manure applied was certified as organic. Measurements and sampling within the greenhouses were conducted from April 2017 to July 2017.

Soil analyses

Immediately prior to harvest, 100 mL of soil volume was sampled with a core sampling tool in each of 10 points randomly distributed in each greenhouse, after removing the sand mulch, to a depth of 10 cm (total soil sampled per greenhouse: 10X 100 mL). Samples in each greenhouse were mixed to produce a combined soil sample of 1 L per greenhouse (15 soil samples, 5 per management, 3 managements). Shovels and all material used for sampling were sterilized between different greenhouse samples with 96% ethanol.

Soil nutrients were determined at the CEBAS-CSIC Ionomics Lab (Murcia, Spain), including total C and N content using a C/N analyzer (LECO Truspec, St. Joseph, MI, USA) and organic C after removal of inorganic carbon with 2 N HCl (Schumacher, 2002); anion phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) concentrations in water extract (1:5 soil:water, w:v) were analyzed by HPLC (Metrohm, HE, Switzerland). Soil nitrate (NO_3^-) and ammonium (NH_4^+) were extracted with potassium chloride (2 M KCl) and their contents were determined with an automatic continuous segmented flow analyzer (model SAN++, Skalar Analytical B.V., Breda, The Netherlands). Other elements were determined after acid digestion with an inductively coupled plasma (ICP) emission spectrometer (ICAP 6500 DUO Thermo; Thermo Scientific, Wilmington, DE, USA), pH was measured in an aqueous solution of 1:2.5 (w:v), with a pH-meter (Crison, Spain).

Table 1. Tomato crop production in greenhouses.

ID	Management[1]	Production (kg m^{-2}) [2]
CM1	CM	9.38
CM2	CM	8.90
CM3	CM	9.50
CM4	CM	12.25
CM5	CM	7.00
CMOM1	CMOM	10.88
CMOM2	CMOM	11.00
CMOM3	CMOM	9.00
CMOM4	CMOM	12.00
CMOM5	CMOM	8.50
ORG1	ORG	8.50
ORG2	ORG	8.50
ORG3	ORG	7.00
ORG4	ORG	7.50
ORG5	ORG	7.50

[1] CM: conventional soil management with no organic matter (OM) application; CMOM: conventional management with OM application, and ORG: fully organic management with yearly OM application and no chemicals added. [2] Provided by the co-operative C.A.S.I

Soil respiration and crop production

In the soil of every greenhouse, we randomly established six PVC collars 10 cm in diameter to measure soil respiration. Collars were inserted 5 cm into the soil, maintaining the sand mulch above the soil, at mid distance between two adjacent drip emitters and two tomato plants in the same line of drippers/plants. Soil respiration was measured monthly, for four months, during the cropping cycle using a portable infrared gas analyzer (EGM-4) connected to an SRC-1 chamber (PPSystems, Amesbury, MA, USA). For each monthly measurement, measurements in the different greenhouses were made during a period of five consecutive days, being made in three greenhouses, selected randomly, per day. They were made when daily air temperatures were highest, between 12:00 and 16:00 GMT each day. Within this time period, respiration measurements were steady.

For each soil respiration measurement, soil temperature and volumetric soil water content (v:v) were measured next to each soil respiration collar, using a thermocouple and a TDR-300 FieldScout soil moisture meter (Spectrum Technologies, Inc., Aurora, IL, USA), respectively. For each soil respiration measurement, three soil temperature readings and three soil moisture measurements were taken; the mean values were used as covariates for the soil respiration analyses.

At the end of the cropping season, the grower's co-operative provided data of total crop production for each greenhouse in the study (Table 1).

DNA extraction and quantitative PCR

DNA was extracted from 250 mg soil samples using the DNeasy Powersoil® Kit (Qiagen, Inc., Venlo, Netherlands), following manufacturers protocol. DNA concentration was estimated using a Qubit Fluorometric Quantification (Thermo Scientific, USA) and samples were stored at -80°C.

Quantitative PCR (qPCR) analyses were performed in soil DNA extracts to determine the abundance of microbial marker genes for bacteria and fungi. The primer pairs used for the qPCR analyses were 515f (5'-GTGYCAGCMGCCGCGGTAA-3') and 806r (5'-GGACTACNVGGGTWTCTAAT-3') for prokaryotes (Walters et al., 2015), and ITS1 (5'-TCCGTAGGTGAACCTGCGG-3' (Gardes & Bruns, 1993) and ITS5.8S (5'-CGCTGCGTTCTTCATCG-3'; Vilgalys & Hester, 1990) for fungi, respectively. Amplifications were performed by using a SYBR® Green (Sigma-Aldrich, USA) based qPCR method in a CFX96 ™ Real-Time PCR Detection System (BioRad Laboratories, USA). Calibration curves were prepared in every assay using 10-fold serial dilutions of stock solutions containing the target DNA molecules. The reaction mixture contained 10 µL of 2X PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, USA), 1 µL of each primer (20 µM), 10-100 ng of template DNA and nuclease free water (Ambion Thermofisher) up to 20 µL of final volume. Amplification conditions were: 95°C for 10 min, followed by 35 cycles of 10 s at 95°C, 30 s at 57°C and 30 s at 72°C (for bacteria), or 40 cycles of 15 s at 95°C, 30 s at 53°C and 1 min at 72°C (for fungi), followed by melt curve from 60°C to 95°C at 0.5°C increment. Triplicate reactions were performed for each DNA extract, standard curve, and negative control. PCR efficiency for different assays ranged between 75% and 95% with $R^2 > 0.9$. The specificity of amplified products was verified by melting curves and agarose gel electrophoresis analysis.

Amplicon sequencing and bioinformatics

Amplicon sequencing was performed using Earth Microbiome Project (EMP) standard protocols (Thompson et al., 2017) at the facilities of Genyo (Granada, Spain). PCR was done in triplicate on the V4 region of the 16S rRNA gene using the primer pair 515f-806r and on the ITS1 region with the primer pair ITS1f-ITS2 (Walters et al., 2015). Barcoded PCR products were quantified using a Qubit dsDNA (Thermo Fisher Scientific) instrument and pooled in equal concentrations. The multiplexed DNA library was purified using Agencourt AMPure XP beads (Beckman Coulter). DNA quality and size were checked with a High Sensitivity DNA Assay (Bioanalyzer 2100, Agilent) and sequenced in an Illumina MiSeq sequencing platform using the Illumina reagent kit V3 (600 cycles) generating 300 bp pair-end reads.

Demultiplexed pair-end Illumina reads were processed using QIIME 2 pipeline v.2019.4 (Bolyen et al., 2019). The cutadapt plugin (Martin, 2011) was used to trim primers. Data was denoised with the q2-dada2 plugin (Callahan et al., 2016) which included: performing sequence quality control, truncation of the reads, stitching R1 and R2 reads, generation of Amplicon Sequence Variants (ASV) and screening out potentially chimeric sequences. PCR negative controls, DNA extraction controls and a commercial mock community sample (ZymoBIOMICS Microbial Community Standard, Zymo Research) were also sequenced by following the same procedure. After checking the very weak amplification of the negative and extraction controls and the correct classification at genus level of the 8 bacterial and 2 fungal strains included in the mock sample (data not shown), control samples were excluded from the analysis. In the case of ITS data, and after a round of analysis, we only used the R1 reads as recommended by Pauvert et al. (2019), improving the taxonomic classification of the mock community at genus level in comparison with the merging of R1 and R2 reads. The parameters used for the DADA2 denoising algorithm within the qiime2 pipeline were as follows: for the 16S dataset, --p-trim-left-f 0, --p-trim-left-r 0, --p-trunc-q 2, --p-trunc-len-f 240 and --p-trunc-len-r 200; for the ITS dataset, and --p-trunc-len 0, --p-trim-left 0, --p-trunc-q 8 and --p-max-ee 8.0. The sequenced dataset has been deposited in the NCBI Biosample Dataset PRJNA629769.

Taxonomy was assigned using the QIIME2 q2-feature-classifier Naïve Bayes machine-learning classifier (Bokulich et al., 2018). The databases SILVA v.132 (Quast et al., 2013) and UNITE v8 dynamic (Kõljalg et al., 2005) were used to train the classifiers for 16S and ITS data, respectively. ASVs assigned to chloroplasts and mitochondria (16S data), and eukaryotic non-fungal linages (ITS) were removed, as also ASVs not classified at phylum level. Diversity indices such as α-diversity (observed ASVs and Shannon's diversity index) and β-diversity (Bray-Curtis and Jaccard index) were estimated using q2-diversity plugin after samples were rarefied (subsampling without replacement).

We performed a predictive functional profiling of bacterial communities using the PICRUSt software (Langille et al., 2013), that predicts the functional gene content based on KEGG database annotation for reference genomes (Kanehisa et al., 2014). We applied the latest version PICRUSt2 (Douglas et al., 2019), following its GitHub Wiki Manual (https://github.com/picrust/picrust2/wiki). These metagenomic predictions are neutral to whether the input sequences are within a taxonomic reference or not, as PICRUSt2 allows the use of ASV sequences as input data instead of ASV taxonomic assignments. ASVs with nearest sequenced taxon index (NSTI) >2 were excluded. Functional predictions were shown as Enzyme Classification numbers (EC numbers) (Kanehisa et al., 2017).

We performed a predictive functional guild analysis of soil fungi ASVs using FUNGuild version v1.0 (Nguyen et

Table 2. Soil chemical properties for the three different organic matter managements. Values represent mean ±1 SE. Different letters indicate significant differences ($p < 0.05$) among greenhouse management treatments (Fischer LSD post-hoc comparisons; in such cases the variable and mean values are highlighted in bold).

Variable	Unit	Treatment [1]			p-value
		CM	CMOM	ORG	
pH		**8.47±0.09 A**	**7.62±0.22 B**	**8.05±0.14 B**	**0.01**
Total N	**g/100g**	**0.08±0.01 B**	**0.13±0.02 A**	**0.15±0.04 AB**	**0.05**
Total C	g/100g	1.82±0.43 A	2.00±0.32 A	2.22±0.99 A	0.91
Organic C	**g/100g**	**0.54±0.12 B**	**1.01±0.15 A**	**1.24±0.46 A**	**0.05**
Total SOM	**g/100g**	**0.93±0.20 B**	**1.75±0.25 A**	**2.13±0.79 A**	**0.05**
$CaCO_3$	g/100g	10.66±3.73 A	8.22±1.64 A	8.20±4.43 A	0.84
Al	g/kg	62.67±10.81 A	42.94±18.11 A	62.16±21.45 A	0.64
Ca	g/100g	5.69±2.96 A	3.10±0.61 A	3.56±0.87 A	0.66
Cu	mg/kg	31.45±8.64 A	35.94±11.70 A	28.65±4.34 A	0.83
Fe	g/kg	26.70±2.72 A	20.29±3.68 A	21.63±3.33 A	0.33
K	g/100g	0.89±0.37 A	0.69±0.16 A	0.71±0.13 A	0.89
Mg	g/100g	0.73±0.23 A	0.50±0.10 A	0.76±0.14 A	0.30
Mn	mg/kg	465.09±75.87 A	360.15±70.43 A	350.74±57.28 A	0.48
Na	g/100g	0.16±0.06 A	0.09±0.02 A	0.18±0.06 A	0.32
Pb	mg/kg	24.88±6.79 A	29.32±11.92 A	20.10±4.35 A	0.70
P	g/100g	0.1±0.02 A	0.19±0.06 A	0.20±0.06 A	0.16
S	g/100g	0.05±0.01 A	0.10±0.05 A	0.21±0.15 A	0.36
Zn	mg/kg	51.59±5.74 A	71.72±20.56 A	56.91±14.75 A	0.63
Cl	mg/kg	4.93±0.92 A	5.20±0.89 A	4.70±1.05 A	0.91
NO_3^-	mg/kg	2.20±0.27 B	8.66±3.43 A	2.06±0.78 B	0.08
NH_4^+	**mg/kg**	**0.33±0.04 B**	**0.51±0.02 AB**	**0.61±0.05 A**	**0.05**
SO_4^{2-}	mg/kg	17.00±9.177 A	67.24±53.87 A	140.13±124.03 A	0.45

[1] Legend of greenhouse managements as in Table 1.

al., 2016), a program that parses ASVs into guilds based on taxonomic assignments. We only considered ASVs with either "probable" or "highly probable" confidence, as stated in FUNGuild, which bases guild assignments on a database curated by experts in fungal linages with >13.000 fungal taxa included (https://github.com/UMNFuN/FUNGuild/blob/master/README.md). We noted that many fungal ITS sequences were not assignable to any guild, probably as a consequence of no relative sequenced isolates included yet in the database.

Statistical analysis

The effects of OM management on crop production, soil respiration, soil chemical properties, and abundance of bacteria and fungi (qPCR) were analyzed using linear mixed-effects models (LMM), with management practice as the fixed factor. For respiration, when we had multiple measurements per greenhouse, greenhouse ID was included as a random factor. In LMM of soil respiration, OM management, time and their interaction were included as fixed factors, and the PVC-collar was considered the repeated measurement unit. Soil temperature and soil water content were included as covariates in soil respiration analysis. As their effect was not significant, these covariates were not included in the final respiration analysis. When necessary, we selected a variance function structure to avoid heteroscedasticity. Logarithmic transformations of respiration and qPCR results were also needed to meet normality assumptions. The restricted maximum likelihood estimator (REML) was used to run the models. Post-hoc comparisons were performed using Fischer's LSD test for each factor. The combined effects of biotic and abiotic factors on respiration were analyzed using a partial least squares regression (PLS) model, where soil chemical properties, and prokaryotic and fungal DNA abundance (qPCR results) were included as predictors and respiration was the dependent variable.

Unless otherwise specified, all analyses were done with R 3.5.2 version (R Core Team®, 2018) using the interface

implemented in InfoStat® 2018 statistical software (Di Rienzo et al., 2019). Significance of differences between treatments were set at $p<0.05$. Results throughout the text, figures, and tables are mean ± 1 SE.

Finally, Calypso software (Zakrzewski et al., 2017) was used to calculate *β*-diversity indices on normalized 16S rRNA and ITS datasets, to produce multivariate diagrams and to perform statistical tests on the microbiome data, using the ASV matrix and the taxonomic assignments generated by QIIME2 as input data. We then performed analyses of β-diversity using a Kuskal-Wallis test at a 95% confident level and β-diversity using PERMANOVAs and calculated a linear discriminant analysis (LDA) effect size (LEfSe) algorithm (Segata et al., 2011) to identify specific features (ASVs, taxa and predicted functions) in soils with significant associations to OM managements ($p<0.05$).

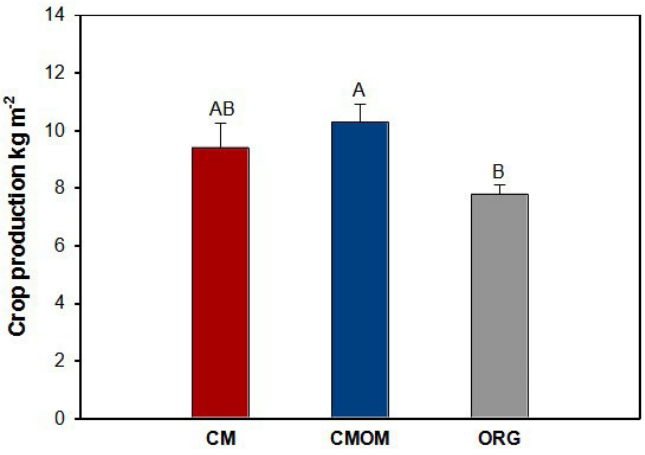

Figure 1. Tomato crop production (n=5). Bars are mean value ±1 SE. Different letters across bars indicate significant differences ($p < 0.05$) among treatments after Fischer LSD post-hoc tests. Legend of greenhouse managements as in Table 1.

Results

Soil nutrients

Soil chemical properties and nutrient content varied with greenhouse soil management technique, and showed high variability. The most notable differences were in SOM, pH and N forms (Table 2). There were significant differences in total SOM, with CM soils having the lowest levels and the other two greenhouse soil types having similar contents. There were also significant differences in pH, which was higher in CM soils compared to CMOM and ORG. Total N was higher in CMOM than in CM soils, with ORG being intermediate. N forms showed clear differences, with ammonium content being higher in ORG than in CM soils, and with CMOM being intermediate; Nitrate content tended to be higher in CMOM soils than in the other two treatments, although the differences were not significant (Table 2).

Crop production

There were differences between CMOM and ORG regarding tomato production, being CM in between (Fig. 1). Data suggested that in conventionally managed greenhouses with OM addition, OM contributed to higher production, although differences may not be significant due to the low number of replicates. This low number was due to availability of greenhouses using the same crop and cycle. Overall, production tended to be higher in CMOM than in CM, being ORG less productive (but not significantly different from CM production). Several factors likely affected ORG greenhouses, like limited nutrient supply or pest management, which could have had a negative effect in crop production.

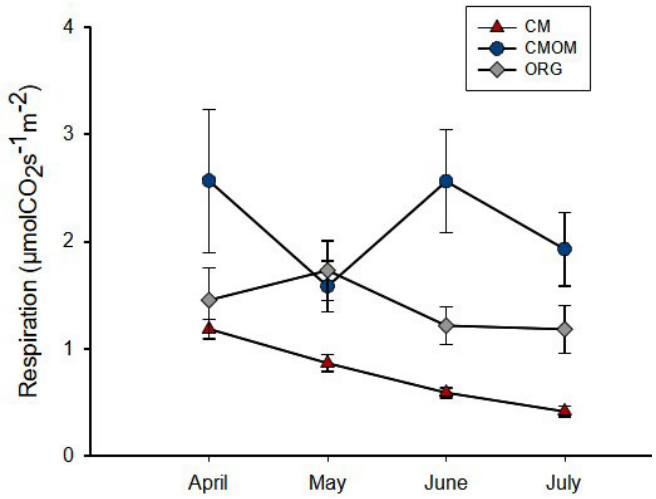

Figure 2. Soil respiration rates throughout the crop cycle for different soil greenhouse managements. Symbols are means ±1 SE (n=5). Different letters across symbols indicate significant differences ($p < 0.05$) among treatments after Fischer LSD post-hoc test. Legend of greenhouse managements as in Table 1.

Soil respiration and microbial communities

Considering all soils, respiration declined every month during the growing season (Fig. 2), with significant differences between months. Overall, CMOM greenhouse soils had the highest respiration rates followed by ORG and then by CM soils, with no significant differences between the latter two (Table 3).

Microbial abundance analysis, estimated by qPCR, showed that prokaryotic and fungal abundances and the fungi:prokaryota ratio were similar in soils of the three OM management types (Table S1).

Table 3. Results of the linear models analyzing the effects of management, month, and their interaction on soil respiration rate (log10 transformed). Soil humidity and soil temperature (covariates) had no significant effect on soil respiration, so they were not included in the final model. Tables 3.2 and 3.3 show mean and SE respiration values (log10 transformed) across greenhouse managements or months; different letters indicate significant differences ($p < 0.05$) among treatment levels after Fischer LSD post-hoc tests. Legend of greenhouse managements as in Table 1.

Factor[1]	DF	F-value	p-value	Management	Mean	S.E.	Month	Mean	S.E.
Management	2	7.28	0.0008	CM	0.42	0.01 B	April	1.71	0.25 A
Month	3	15.39	<0.0001	CMOM	1.53	0.10 A	May	1.20	0.14 B
Management:Month	6	0.83	0.5460	ORG	0.93	0.05 B	June	1.00	0.13 C
	(3.1)				(3.2)			(3.3)	

[1] Effects of factor "Management", "Month" and the interaction "Management:Month" were assessed by repeated measures LMM analysis. DF: degrees of freedom. F-value: univariate F statistic.

The bioinformatics analysis of the 16SrRNA and ITS gene libraries yielded a total of 21,528 and 829 prokaryotic and fungal ASVs, respectively. Diversity of soil prokaryotic and fungal communities displayed distinctive patterns. While α-diversity (richness and Shannon-index) did not differ among greenhouse managements (Figs. S1, S2 and Table S2), β-diversity did, revealing a similar pattern for both, prokaryotic and fungal communities (Fig. 3). Thus, β-diversity NMDS plots based on Bray-Curtis distance showed a divergence between CM and CMOM, suggesting an effect of the addition of OM on the structure and composition of microbial communities. By contrast, ORG soils displayed a more disperse β-diversity pattern, overlapping CM and CMOM communities. Due to the high variability within managements, particularly in ORG soils, PERMANOVA analyses did not show significant differences concerning *β*-diversity at the community level (Table S3).

Overall, prokaryotic ASVs were assigned to 47 phyla (4 archaeal and 43 bacterial), but the 10 most abundant phyla accounted for more than 90% of the total number of reads. Predominant phyla were Proteobacteria, Bacteroidetes, Chloroflexi, Acidobacteria, and Firmicutes (Fig. S1A). A detailed description of the different taxonomic

Table 4. Plant pathogen guilds, taxonomical association and reports as tomato pathogens. All these amplicon sequence variants (ASVs) were associated with "probable" confidence. Legend of greenhouse managements as in Table 1. The color pattern represents the percentage of presence of each ASV ID, from green (no presence) to red (maximum percentage of presence).

ASV ID	CM					CMOM					ORG					Taxonomy	Reported as tomato pathogen
da2dca82efc26b0bb7e2ffca27104cba	0.0	12.1	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.4	*Plectosphaerella cucumerina*	[84-86]
a934a70ad3fffaaad69f037880a972ed	0.0	0.0	0.7	33.1	0.0	0.0	0.0	1.3	1.5	0.0	5.7	2.6	0.0	0.0	2.5	*Sclerotinia* sp.	[83,84]
afc75baa127916aabfb9b668fefbb833	13.1	5.5	1.2	0.0	0.0	0.0	8.1	0.0	6.5	2.8	0.9	0.0	0.0	0.0	0.4	*Plectosphaerella cucumerina*	[84-86]
1020e88dac96e318259720cee266b4eb	14.1	1.4	0.3	0.0	0.0	0.0	0.0	0.0	6.2	0.0	0.6	0.0	0.0	0.0	0.0	*Plectosphaerella cucumerina*	[84-86]
6fc67c653263abec7ea7e48ea7302479	0.0	0.0	0.0	1.0	20.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	*Thyrostroma* sp.	
b007fa791b38e5ab6fd39b60ae5b4cf4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	*Sclerotinia* sp.	[83,84]
35e37a753885e1303bfd993657140624	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	*Monosporascus cannonballus*	
e62e168b81e71ca27eee68415d92d5f2	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	*Ramularia eucalypti*	
7f727114b5dce2313cb9e95a98881f1e	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	*Plectosphaerella cucumerina*	[84-86]
265f89519fe4bfe4c565831029830bf4	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	*Mycosphaerella tassiana*	
374e75b12461a8589201ea354c6c849f	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	*Lectera colletotrichoides*	
ce77650c312e855968a1c8f8037cb90d	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	*Macrophomina phaseolina*	
b7a81aeca8f6c24594fa4020c7c60214	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	*Stagonospora* sp.	
beea654f57db872fa891f91a5c10357d	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	*Veronaea* sp.	
21430b009a3fa43df27639697c657361	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	*Podosphaera astericola*	
d079226fa51c93f00f538a52aa70abfe	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	*Sclerotinia* sp.	[83,84]
08146d1e3ce889d4f96b6b10418c0803	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	*Devriesia pseudoamericana*	
cf7a81c2a8505f4199d36d62c52d17f5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	*Podosphaera astericola*	
e138850dbbcf24b5e29319574ec7963c	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	*Volutella* sp.	
c30dd7e27a93fc5ef1fb938543fd646b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	*Stagonosporopsis* sp.	
ff96986abc4c40611d332e74e05ceddc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	*Plectosphaerella cucumerina*	[84-86]
Plant pathogens ASVs (%)	30.5	19.0	2.4	34.3	20.5	0.0	8.9	1.4	16.0	3.1	8.2	3.4	0.0	1.0	3.9		

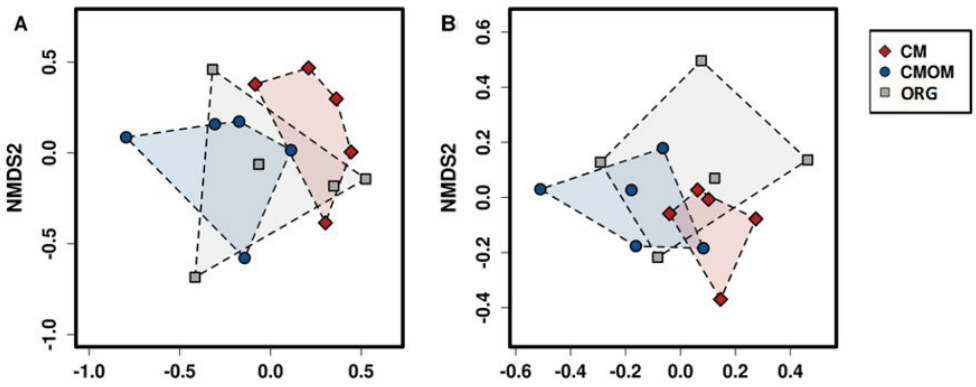

Figure 3. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance matrices of prokaryotic (A; stress value 0.113) and fungal communities (B; stress value 0.119), n=5. Legend of greenhouse managements as in Table 1.

families and genera of the prokaryotic dataset associated to each soil management is included in the Appendix [suppl].

Fungal ASVs were assigned to 10 phyla but, similarly, the three most abundant phyla, Olpidiomycota, Ascomycota and Basidiomycota, did account for more than 90% of fungal ASVs (Fig. S1B). A detailed description of the different taxonomic families, genera and ASVs of the fungal dataset associated to each soil management is included in the Appendix. FUNGuild predicted high relative abundances of saprotrophic, parasitic, and pathogenic fungi in all measured soils, but interestingly, only one guild, "plant pathogens", was associated to CM (Fig. 4). In total, 21 fungal ASVs were predicted to contribute to this guild, being taxonomically affiliated to different genera of well-known fungal pathogens. Some of these taxa, like *Sclerotinia* or *Plectosphaerella,* were found in high abundance in some CM greenhouse soils and have been reported as infective agents in tomato crops in this region (Table 4). Differences in prokaryotic functional profiles between management systems, as predicted by PICRUSt, are shown in Fig. S5. Comparable to the structure of microbial communities, the multivariate analysis of PICRUSt predictions revealed a separation of CMOM from the other two treatments, indicating that OM management may indeed influence microbial functions. Moreover, we identified 8 putative functional pathways that were statistically associated with specific management practices, with 5 of them being particularly linked to CMOM (Fig. S5).

We analyzed the effects of microbial abundance, nutrient content, and pH on soil respiration rate using PLS regression analysis, where prokaryote and fungal abundance and soil variables such as ammonium, nitrate, total SOM and pH were included as predictors and soil respiration as the response variable. CM and CMOM managements clearly separated, with ORG in between. Nitrate, pH, and prokaryote abundance were negatively linked to soil respiration rates (Fig. 5).

Discussion

Organic matter management in the different intensive greenhouses studied influenced soil microbial communities, affecting their structure and function. As a consequence, soils had different microbial activity, as suggested by soil respiration. These data could help farmers to develop more sustainable intensive cropping practices. In our study, we selected 15 different commercial greenhouses, five per OM management type, which resulted in different soil properties and microbial communities across OM managements. The results demonstrated the strong influence of soil OM management on soil chemical properties and soil microbial community composition, structure, function and activity, and the consequence of this OM management on crop production.

We recorded overall higher respiration rates in CMOM greenhouses than in CM, with ORG being intermediate, which suggested a combined effect of organic and inorganic fertilization in CMOM (Song et al., 2018). The higher respiration rate in CMOM may have contributed through more turnover of root biomass, root exudation and possibly larger rhizosphere populations. The lack of differences in respiration between CM and ORG was probably mostly due to the high deviation in soil respiration measurements of ORG soils. As we did not use experimental greenhouses, but commercial ones, factors such as the type of greenhouse, climate control or soil texture could contribute to differences observed between systems. In the CM and ORG greenhouses, soil respiration decreased at the end of the crop cycle, probably due to SOM decrease.

Respiration rate was positively related to the combination of OM addition and inorganic fertilizer supply (Mbuthia et al., 2015), represented in CMOM greenhouses. This effect of OM application has been related to higher soil microbial activity (Song et al., 2018), likely because of the organic C content, as well as the total N and ammonium contents of the added OM. On one hand, organic C content, which was higher in CMOM and ORG than in CM,

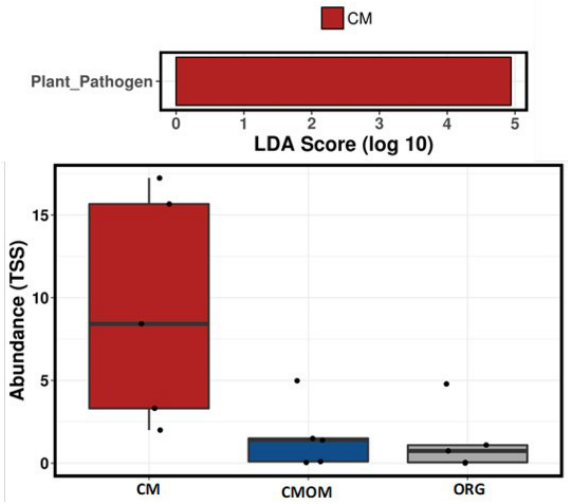

Figure 4. Above, linear discriminant analysis (LDA) effect size (LEfSe) performed on fungal guilds, showing the only feature significantly associated with a management system (logarithmic LDA score ≥2, $p<0.05$). Below, boxplot showing the relative abundance of the guild "Plant pathogen" in each greenhouse soil management. TSS = total sum scaling (relative abundance of sequences). n=5. Legend of greenhouse managements as in Table 1.

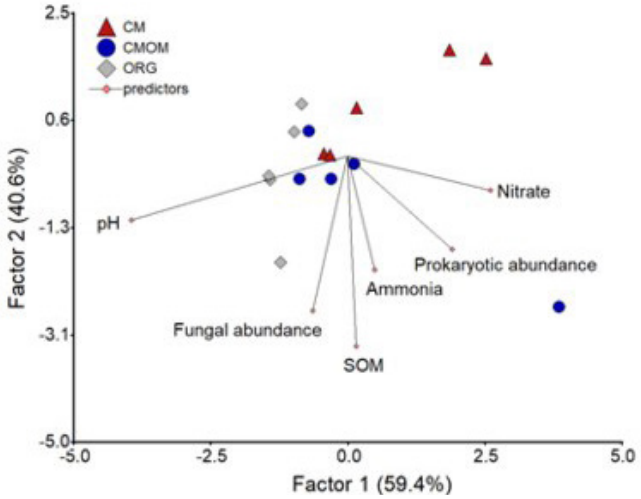

Figure 5. Regression diagram (Partial least squares - PLS) showing the effects of soil microbial abundance (qPCR), pH and nutrients (predictor variables) on soil respiration among three different greenhouse OM management practices (n=5). Legend of greenhouse managements as in Table 1.

increased microbial respiration. On the other hand, total N content (and marginally nitrate) was higher in CMOM than in CM soils, the latter with only inorganic N addition, while in CMOM soils there was a combination of inorganic N addition and organic N addition from OM application. These combined N additions in CMOM might favor higher N uptake by plants, which increased soil respiration in this greenhouse management because of direct and indirect reasons. Directly because this N uptake increases root respiration (Rao & Ito, 1998) and indirectly because of higher root exudation, which enhances microbial activity and soil respiration (Bais et al., 2006). Besides, higher nitrate and ammonium content is related to higher respiration rates which might have a consequence of higher microbial activity (Feng et al., 2015), higher N uptake and a positive effect on N limitation avoidance (Zakrzewski et al., 2017). In our results, only ammonium was positively related to soil respiration. This could be because nitrate content was more variable, with high variance in CMOM. Soil pH was basic in all cases, but our results showed that lower pH (CMOM soils) also increased respiration, as reported by Blagodatskaya & Anderson (1998), relying on the fact that with pH above 8 nutrient availability decreases.

The addition of OM to conventionally managed soils was associated with higher crop production, which tended to be more productive than CM greenhouses. These results suggest beneficial effects of OM addition on intensive agriculture, as reported by Zink & Allen (1998). Furthermore, the combination of inorganic and organic fertilization has been reported to increase plant N uptake compared to just synthetic chemical fertilization (N'Dayegamiye et al., 2013). There was a link between soil respiration and production, being respiration generally higher in CMOM than in CM soils. Microbial richness was not affected by management practices, which contrasted other report (Francioli et al., 2016) that found higher bacterial richness in greenhouse soils when organic amendments were applied. By contrast, Bonanomi et al. (2016) found higher bacterial diversity in conventional than in organic greenhouses, suggesting it was a consequence of microbial community selection in response to synthetic chemical fertilizer addition.

The lack of a clear relationship between OM management and fungal diversity in intensive agriculture is surprising. Similarly, Hartmann et al. (2015) did not find changes in fungal diversity when OM was added, compared to a conventionally-managed soil. Our results showed high variability in microbial community composition between greenhouses under the same OM management system, which was probably related to diverse factors, such as manure type and origin, and OM application frequency. In this sense, community coalescence, the process of mixing soil microbial communities from diverse origins, and the time needed to reach a new equilibrium (Wu et al., 2019), might have contributed to this variability. In ORG soils, with continuous addition of manure from different origins, and thus carrying different microbes, microbial communities might be farther from reaching an equilibrium status than in the other two management systems, where no exogenous OM is added (CM) or it is added but less often (CMOM).

Our data showed that several prokaryotic taxa were associated to specific OM management. For example, the

genus *Thauera* was significantly linked to the ORG treatment. This genus has been reported to be associated with denitrification at pH of <8 (Shinoda et al., 2004) and is involved in the N cycle when ammonium concentrations are high in aerobic conditions (Fang et al., 2020), as in our ORG greenhouses. *Actinomadura* was associated with CMOM greenhouses; this genus is associated with composting manure, having a key role as a growth-promoting rhizobacteria (PGPR; Wani & Gopalakrishnan, 2013). *Actinomadura* is one of the most studied Actinomycetes in soil agronomy because its potential application in agriculture due to its capacity to produce natural antibiotics against plant pathogens (Maskey et al., 2003). It is also involved in the N cycle in presence of high SOM content (Zeffa et al., 2020). *Pirellula* was associated to CM; it is an anaerobic NH_4^+ oxidizing (Anammox) bacteria which uses NO_2^- to oxidize NH_4^+ and to generate N_2 under anaerobic conditions (Xia et al., 2019). In CM with no OM applications, soils might be less permeable (Zebarth et al., 1999) and O_2 could be limiting, favoring *Pirellula*, which decreases when OM is applied (Cheng et al., 2019). There were also some fungal ASVs associated to ORG, including *Pseudallescheria*, *Stemphylium*, *Phaeotheca* and *Wallemia*. To our knowledge, this is the first time that these genera have been linked to organic agriculture, a fact that deserves further research.

We found potential fungal pathogens associated to the CM management, whose presence is likely to be a consequence of the low SOM levels (Li et al., 2014); generally, OM applications enhance the control of fungal soil-borne diseases (Bonanomi et al., 2017; Jaiswal et al., 2017). In CM soils, we found various pathogenic genera like *Monosporascus*, *Mycosphaerella*, *Plectosphaerella*, *Ramularia*, *Sclerotinia*, *Stagonospora* and *Thyrostroma. Sclerotinia*, which have been reported as specific tomato soil-borne pathogens, infecting roots and causing production loss (Adams & Ayers, 1979; Lobo Jnr et al., 2000); *Plectosphaerella* has also been reported as a tomato pathogen in Italy (Carlucci et al., 2012), Australia (Pascoe et al., 1984) and China (Xu et al., 2014), causing tomato wilt. Interestingly, and in relation to prokaryotes, the enzyme peroxiredoxin, a PICRUSt-predicted prokaryotic functional pathway associated to CM, has been previously reported as a bioindicator of stress situations and plant pathogens in soils (Ghabooli et al., 2013). This enzyme plays a key role in reactive oxygen species when plant defenses are activated (Wang et al., 2019), perhaps due to the higher abundance of fungal pathogens in CM soils.

As conclusions, our data show the effects of OM management practices on soil microbial communities. OM addition led to higher microbial activity, and a decrease on the proportion of fungal soil-borne pathogens. These changes positively influenced crop production. The observed patterns provided an initial understanding of the correlations between organic matter management, soil microbial communities, and crop production in intensive tomato production. This knowledge is essential for developing more sustainable intensive agricultural systems.

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Project administration: F. I. Pugnaire; C. Armas.
Resources: F. I. Pugnaire; C. Armas; R. B. Thompson; M. Gallardo.
Software: Not applicable
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Validation: Not applicable
Visualization: F. M. Usero; J. A. Morillo; C. Armas.
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Writing – review & editing: F. M. Usero; J. A. Morillo; C. Armas; R. B. Thompson; M. Gallardo; F. I. Pugnaire.

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