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ARTICLE

Olive orchard microbiome: characterisation of bacterial communities in soil-plant compartments and their comparison between sustainable and conventional soil management systems

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ABSTRACT

Background: Beneficial bacteria-plant interactions play an important role in agriculture, positively affecting plant status and improving product quality. Bacterial endophytes contribute to host plant protection and survival.

Aim: This study characterised the bacterial communities present in soil, leaf surface and xylem sap of olive trees, and investigated their response under a sustainable (Smng) or a conventional (Cmng) management system in an olive grove located in southern Italy. The aims are: (a) to understand if soil bacteria enter xylem and reach leaves and (b) to verify if the bacterial communities in the two management systems deeply differ due to the different agronomic practices applied in Smng and in Cmng.

Methods: Therefore, a metagenomic approach was used to detect microorganisms, in order to estimate bacterial diversity and abundance, and to identify the bacterial taxa in the three analysed compartments in plants subjected to Smng and Cmng systems.

Results: Bacterial communities came from the soil and reached aerial plant parts through xylem sap. The application of different agronomic practices influenced the composition of soil bacterial communities.

Conclusions: The potential benefits of the specific bacterial taxa detected under the Smng system could improve plant growth protection and provide a higher crop quality in fruit plants.

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KEYWORDS
Biocontrol; endophytes; phyllosphere; plant microbiome; soil management; xylem sap

Introduction

Plant microbiota forms a complex network where microbial communities and their diversity dynamically change throughout the plant life cycle (Riesenfeld et al. 2004; Ying-Ning et al. 2017). Both plant genotype and abiotic factors affect plant microbiome composition (Agler et al. 2016). Plant-associated microbes live either inside the plant tissue or on the surface of plant organs (Berendsen et al. 2012; Bulgarelli et al. 2012). Generally, phyllosphere microbes are considered epiphytes, whereas the microbes residing within plant tissues (e.g. apoplastic and symplastic cell compartment, sap) are defined as endophytes.

The microbial colonisation of plants depends on some key factors, such as plant genotype, tissue, growth stage, and physiological status, and on soil environmental conditions, as well as on some agricultural practices (Hardoim et al. 2008; Singh et al. 2009). Moreover, soil bacterial communities influence soil fertility and plant growth, and changes in their structure and dynamics in response to different soil management practices can give information about soil status, in terms of its quality and biological complexity (Anderson 2003; Govaerts et al. 2008; Ding et al. 2013). There is growing interest and evidence about the important interactions between plants and their phyllosphere microorganisms. These latter can affect the fitness of natural plant populations, as well as the quality and productivity of agricultural crops, by promoting plant growth and contributing to plant protection against pathogens (Rasche et al. 2006a; Whipps et al. 2007). Meanwhile, endophytic microorganisms have been appreciated for their capacity to protect their hosts against insects-pests and microbial pathogens, so acting as biocontrol agents (Hallmann et al. 1997; Azevedo et al. 2000). For instance, in *Citrus*, the endophytic biocontrol agents *Curtobacterium flaccumfaciens* and *Methylobacterium mesophilicum* can decrease the progress of disease caused by *Xylella*

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fastidiosa (Araújo et al. 2002; Lacava et al. 2009), a serious pathogen also for olive (*Olea europea* L.). In addition, endophytes can confer other important characteristics to plants, such as greater resistance to stress conditions, alteration in physiological properties and production of phytohormones and compounds of biotechnological interest (Azevedo et al. 2000).

Olive trees represent one of the most important oil crops world-wide, and they have characterised the Mediterranean landscape since ancient times (Zohary and Spiegel-Roy 1975). In 2016, on an area of 10.65 Mha, 19.27 Mt of olives was harvested world-wide (FAOSTAT 2018). Recent studies on olive groves have shown that sustainable soil management practices have positive effects on microbiological soil fertility and influence the microbes living on olive fruit and leaves (Ferreira et al. 2013; Sofo et al. 2014; Pascazio et al. 2015; Sastre et al. 2016). For semi-arid Mediterranean agricultural lands, the need for a new approach in orchard management has become evident in order to improve or maintain soil quality, health and fertility (Hochstrat et al. 2006; Govaerts et al. 2008). Particularly, in olive groves, the positive influence of sustainable management systems on soil biochemical and some microbiological characteristics has been described (Moreno et al. 2009; Sofo et al. 2010, 2014).

The interactions between a plant and its microbiome are highly complex and dynamic (Turner et al. 2013). According to our knowledge, the information on how the composition of the bacterial communities in olive is modified by different management systems is lacking. In addition, no information on endophytic bacterial communities of xylem sap in olive tree is available in literature. Thus, a deeper knowledge about epiphytic and endophytic microbiota of cultivated olive plants grown under different agronomic systems are required for understanding the promotion of plant growth and a higher crop quality of olive tree, as pointed out by Pascazio et al. (2015).

We hypothesised that part of the plant bacterial communities could come from the soil and reaches aerial plant parts through xylem sap. For ascertaining this, we characterised the bacterial communities in the soil-plant compartments of an olive grove using a 16S-based metagenomic approach. Furthermore, this study focused on determining the effects of the two different soil management systems on bacterial diversity of soil, phyllosphere and xylem sap. Additionally, the identification and quantification of specific bacterial endophytes could provide information that may have potential practical implications for disease management of olive plants.

Materials and methods

Experimental site

The experimental trial was carried out in a mature olive grove located in Ferrandina, province of Matera, southern Italy (40°29′13″ N 16°27′53″ E). The species surveyed was *Olea europaea* (L.) cv. 'Maiatica', an autochthonous olive variety used to produce both table and oil olives. The climate in the study area is semi-arid; the mean annual precipitation is 574 mm (mean 1976–2015) with the rainfall concentrated in the winter and the mean annual temperature ranges from 15 to 17°C. The soil is a sandy loam, a Haplic Calcisol with a mean bulk density of 1.3 t m^{-3}. The chemical and physical properties of the 0–60 cm profile of the soil are listed as follow (± standard deviation): pH 7.4 ± 0.4, organic carbon content 7.0 ± 3.8 g kg^{-1}, total nitrogen content 0.8 ± 0.2 g kg^{-1} (Kjeldahl method), extractable phosphorus (Olsen method) and potassium 11.7 ± 5.9 and 104 ± 70 mg kg^{-1}, respectively.

Experimental design

The olive orchard was divided into two and the halves were managed for a period of 17 years under two different regimes, sustainable (S_{mng}) (i.e. no-tillage, irrigation with urban wastewater and recycling of polygenic carbon sources such as cover crops and pruning material), and conventional (C_{mng}) (i.e. soil tillage, burning of the pruning residues, fertilisation and empirical irrigation) (for details, see Sofo et al. 2014). The area of each plot was 1 ha, with trees (> 70-year-old) planted at a spacing of 8 m × 8 m. For each experimental plot three trees, as biological replicates (n = 3), were selected. Three trees were randomly chosen from the central part of their plot (S_{mng} or C_{mng}) to avoid the influence of the agronomic practices adopted in the neighbourhood. The selected trees for each plot (S_{mng} or C_{mng}) were 24-m far from each other in order to make the sampling more representative than the choice of adjacent trees. In February 2017, soil, leaf and sap samples were collected from the same three trees per treatment.

Soil and leaf sampling

Soil sub-samples (7-cm-diameter cores) were collected at 0–30 cm depth at 10 different points around each tree (n = 3, three plants per plot) and pooled on site to constitute a composite soil sample of about 2 kg. The choice of soil composite samples instead of single point soil samples were taken for reducing spatial variability, according to Bacon and Hudson (2001) and Tian et al. (2004). After the removal of crop residues and stones, the soil samples were stored immediately at 4°C in sterile plastic pots and brought to the laboratory where they were sieved at 2 mm without drying and stored at −20°C. About 10 shoots were cut off each tree using sterile gloves and equipment. Shoots were stored immediately at 4°C in sterile plastic bags and brought to the laboratory where they were stored at −20°C without any further processing.

Extraction of total bacterial DNA from soil and phyllosphere material

Total bacterial DNA was extracted from 0.5 g of each soil and phyllosphere pellet sample according to the protocol of the FastDNA® SPIN Kit for soil in combination with the Thermo Savant FastPrep® System homogeniser (MP Biomedicals LLC, Cleveland, OH, USA). Bacteria were desorbed from the phyllosphere by the method of Redford and Fierer (2009). Fifty leaves per plant were washed with 50 mL of detergent solution (Tris-HCl 20 mM, EDTA 10 mM and Triton 0.024%) for 15 min by mechanical agitation; the washing solution was centrifuged at 5000 × g for 10 min at 4°C, the supernatant was discarded, and the resulting pellet was air dried for 2 h under laminar flow fan and stored at −20°C.

The fragmentation of the DNA was checked by agarose gel electrophoresis (0.7% w/v agarose-0.5 xTris-Borate-EDTA) and UV visualisation of the gels stained with Gel RedTM (Biotium, Inc., Hayward, CA, USA). The quality and concentration of DNA were determined by spectrophotometric measurement at 260, 280 and 230 nm using a NanoDrop® ND-1000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). To reduce biases associated with extraction, extraction of DNA was performed twice from each soil and phyllosphere sample. Then the same amounts of DNA from each of the two extractions were pooled for each duplicated sample.

Sap collection and extraction of bacterial DNA

Two shoots, ca. 15–20 cm in length, were taken in the upper part of the canopy from each of the four cardinal directions per each tree using sterile shears. The vegetable material was put in plastic bags, stored at 4°C and transported to the laboratory where the shoots were stored at −20°C. The xylem sap was extracted using a Scholander-type hand-made chamber with N_2 (for alimentary use, Alipak 100, SAPIO, Milan, Italy).

For each shoot, a 1-cm wide bark strip was removed in the proximal part in order to exclude the phloem sap. A sharp knife, sterilised with 75% ethanol to prevent external contamination, was used. The cut end of the stem was placed, through the hole with a gasket, in the Scholander pressure chamber lid with ca. 3 cm of the cut end facing out; this part of the stem was connected to a thin plastic tube through which the liquid flowed into a 1.5 mL microcentrifuge tube. The foliage of the shoot was placed in the pressure chamber and the lid was locked down. High pressure of ca. from 5 to 7 MPa was applied to exude the xylem sap from the tissue at the proximal end of the cutting. It was important that the stem was not cut or broken, and no leaves could be removed during this process because any openings in the stem would create a direct route for the pressurised air to escape, compromising the collection of xylem fluid. After discarding the first drops, the sap was collected into Eppendorf tubes for typically 15–20 min per shoot and stored at – 20°C before analysis.

Sap samples were centrifuged at 12,000 × g for 10 min at 4°C. The pellet and the respective supernatant were stored at −20°C. The pellet was used for bacterial DNA extraction according to the protocol of the DNeasy Blood Tissue kit (Qiagen). The DNA was eluted in a final volume of 400 µL for maximum DNA yield and the DNA extract was concentrated by a vacuum concentrator Savant SpeedVac (Savant®DNA120 SpeedVac Conce ntrator, Milford, MA, USA) without heat until a volume of 50 µL. The fragmentation, the quality and the concentration of the DNA was determined as described above for total bacterial DNA extract from soil and phyllosphere samples. Because of the very low amounts of sap extracted, it was not possible to extract bacterial DNA more than once.

Library preparation and sequencing

The preparation of the 16S library and sequencing were made by IGA Technology Services S.r.l., Udine, Italy. Bacterial 16S library preparation workflow included two PCR amplifications. An initial PCR amplification was made on genomic DNA to amplify the variable V3-V4 region of 16S ribosomal RNA gene. Gene-specific primers with overhang adapters for compatibility with Illumina index and sequencing adapters were used. The primer sequences, without the adapters, were: 16S-341F 5′-CCTACGGGNGGCWGCAG-3′ and 16S-805R 5′-GACTACHVGGGTATCTAATCC-3′ (Klindworth et al. 2013). The PCR products were purified and used as target for a limited cycle amplification to add Illumina flow-cell binding domains and dual index adapters using NexteraXT Index Kit (FC-131-1001/FC-131-1002). The resulting libraries were purified, quantified and normalised. Up to 96 libraries were pooled and sequenced from both ends on a Illumina MiSeq and more than 100,000 reads of about 300 bp were generated.

For the sap samples, the extracted DNA was pooled from the three replicates per treatment to obtain the minimum threshold of 500 ng (minimum concentration 10 ng μL^{-1}) per sample, required from the IGA Technology Services S.r.l. (Udine, Italy) to carry out the 16S metagenomic analysis.

Data processing and bioinformatic analysis

An internal IGA Technology Services S.r.l pipeline was used for de-multiplexing and denoising sequence reads. Reads above a minimum length of 200 bp were retained. Subsequent analyses (chimera filtering, grouping of replicate sequences, sorting sequences per decreasing abundance and OTU identification) were carried out using the USEARCH algorithm (version 8.1.1756, 32 bit), available through QIIME. For Operational Taxonomical Units (OTU) identification, the open reference protocol was used. Reads were aligned against reference database, a modified version of GreenGene (version 2013_8); only matches with a minimum identity of 94% were retained and clustered for representing the centroid. Query sequences not sharing similarity with the centroid constituted a novel OTU and the most abundant and long reads in each OTU were selected as representative sequences. In this step, the clustering threshold was set at 97% and OTUs were generated with a minimum of two sequenced fragments. The RDP classifier and GreenGene database were used to assign taxonomy with a minimum confidence threshold of 0.5.

For rarefaction curves, alpha- and beta-diversity, a number of 20,000 fragments was used for the analysis; because these diversity metrics are sensitive to different sampling size, the cut-off of 20,000 randomly selected fragments was used to normalise the counts and avoid comparison of differently sized samples, in terms of number of reads. Alpha diversity was calculated using the Shannon and Chao1 indices. Principal coordinates analysis (PCoA), based on the distance matrix of Bray-Curtis estimation, was used to evaluate similarities or differences between the two management systems and the corresponding plot was shown by EMPeror (http://emperor.colorado.edu). The alpha diversity indexes relative to management practices were represented by box plots and were statistically compared using a non-parametric two-sample *t*-test and the *P* values were calculated through 999 Monte Carlo permutations. The Kruskal-Wallis test ($P < 0.05$) was used to find differences in relative abundances of bacterial genera in soil and leaves samples under different management.

In order to evaluate the OTUs in common between the two management systems, Venn diagrams were generated using a web platform (MetaCoMET, http://probes.pw.usda.gov/MetaCoMET) that analyses each subset defined by the union or disjunction of group (Wang et al. 2016a). MetaCoMET analysis accepts an OTU table in BIOM (The Biological Observation Matrix) format (version 1.0 supported) containing community abundance data (McDonald et al. 2012).

Results

Bacterial abundance in soil, leaves and xylem sap

Soil samples had the highest number of OTUs (7,857), followed by leaves (3,405) and xylem sap (238), while the number of total reads were 363,272 in leaves, 324,000 in soil and 11,492 xylem sap. After quality checking, 6,461 OTUs in soil, 2,690 in leaves and 283 in xylem sap were identified with an even sequencing depth of a minimum number of 50,000 reads per sample.

Composition of bacterial communities in soil, leaves and xylem sap

For all the taxonomic levels, the bacterial groups in common among the three analysed compartments appeared to have different abundances (Figure 2,3).

A high number (52 and 58) of OTUs was in common between soil and xylem sap, corresponding to 22.5 and 23.3% of total sap OTUs, under C_{mng} and S_{mng}, respectively (Figure 1(a,b)). About 39.5% (83 OTUs) and 38.6% (96 OTUs) of the sap microbial community was in common with the leaf microbiome, under C_{mng} and S_{mng}, respectively. Finally, 37 and 44 OTUs (corresponding to 16.0 and 17.7% of sap microbiome) were common to soil, phyllosphere and xylem sap, for C_{mng} and S_{mng}, respectively.

Although PCR primers targeting eubacterial 16S rRNA genes were applied, a small number of reads was assigned to the archaeal domain. At the kingdom level, Archaea were present at 0.05% in soil and 0.01% in the leaves and Bacteria were present more than 99% in both compartments; while Archaea was absent from the xylem sap (Table S1). Seven bacterial phyla were in common among soil, leaves and xylem sap both in C_{mng} and in S_{mng}: Actinobacteria, Proteobacteria, Planctomycetes, Firmicutes, Bactero idetes, Acidobacteria, Gemmatimonadete, Thermi and Cyanobacteria (Figure 2). Among them, the most abundant phyla were Actinobacteria, Proteobacteria and Firmicutes. In soil, Actinob acteria were the 46% of the bacteria in C_{mng} and 44% in S_{mng}, followed by Proteobacteria (34%) in both the systems, Planctomycetes at 6% in C_{mng} and 5% in S_{mng}, and by other less abundant phyla (Figure 2(a)). In leaves (Figure 2(b)), the phylum Proteobacteria was slightly higher (53%) in C_{mng} (53%), compared with S_{mng} (50%), Bacteroidetes (27% in C_{mng} and 30% in S_{mng}) and Actinobacteria (18% in both the systems), followed by other less abundant phyla. In the xylem sap (Figure 2(c)), the phylum Firmicutes was present at 63% of the bacteria in C_{mng} and 67% in S_{mng}, followed by Proteobacteria at 23% in both the systems, Bacteroidetes at 9% in C_{mng} and 6% in S_{mng}, Actinobacteria at 5% in C_{mng} and 3% in S_{mng}, and by other less abundant phyla. None of the above-reported values were not statistically different among the two management systems.

The six common bacterial classes in soil, leaves and xylem sap in both the systems were Alphaproteobacteria, Actinobacteria, Sphingob acteria, Bacilli, Gammaproteobacteria and Beta proteobacteria (Figure 3). In soil, Alphaproteo bacteria were 24% of the bacteria in C_{mng} and 25% in S_{mng}, followed by Actinobacteria (23% in C_{mng} and 24% in S_{mng}), and by less-abundant classes (Figure 3(a)). In the leaves (Figure 3(b)), the more abundant classes were Alphaprote obacteria (49% in C_{mng} and 45% in S_{mng}), Sphingobacteria (26% in C_{mng} and 30% in S_{mng}). Actinobacteria (17% in both C_{mng} and S_{mng}), and other less abundant classes. In the xylem sap (Figure 3(c)), the more abundant bacteria were

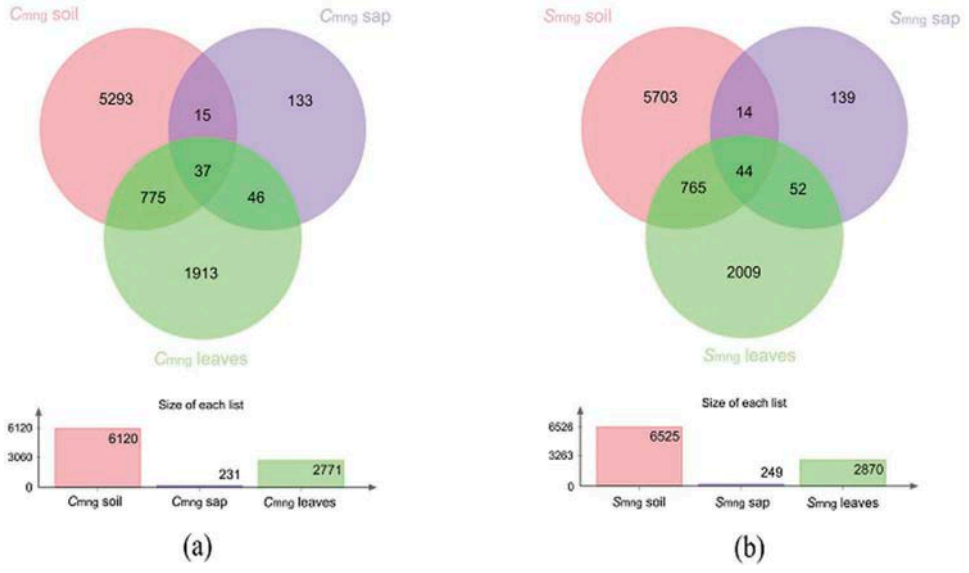

Figure 1. Venn diagram showing the unique and shared OTUs between soil, leaves and xylem sap of the (a) C_{mng} and (b) S_{mng} systems. In (a) the list is referred to total OTUs in C_{mng} soil (number 1), C_{mng} sap (number 2) and Cmng leaves (number 3). In (b) the list is referred to total OTUs in S_{mng} soil (number 1), S_{mng} sap (number 2) and S_{mng} leaves (number 3).

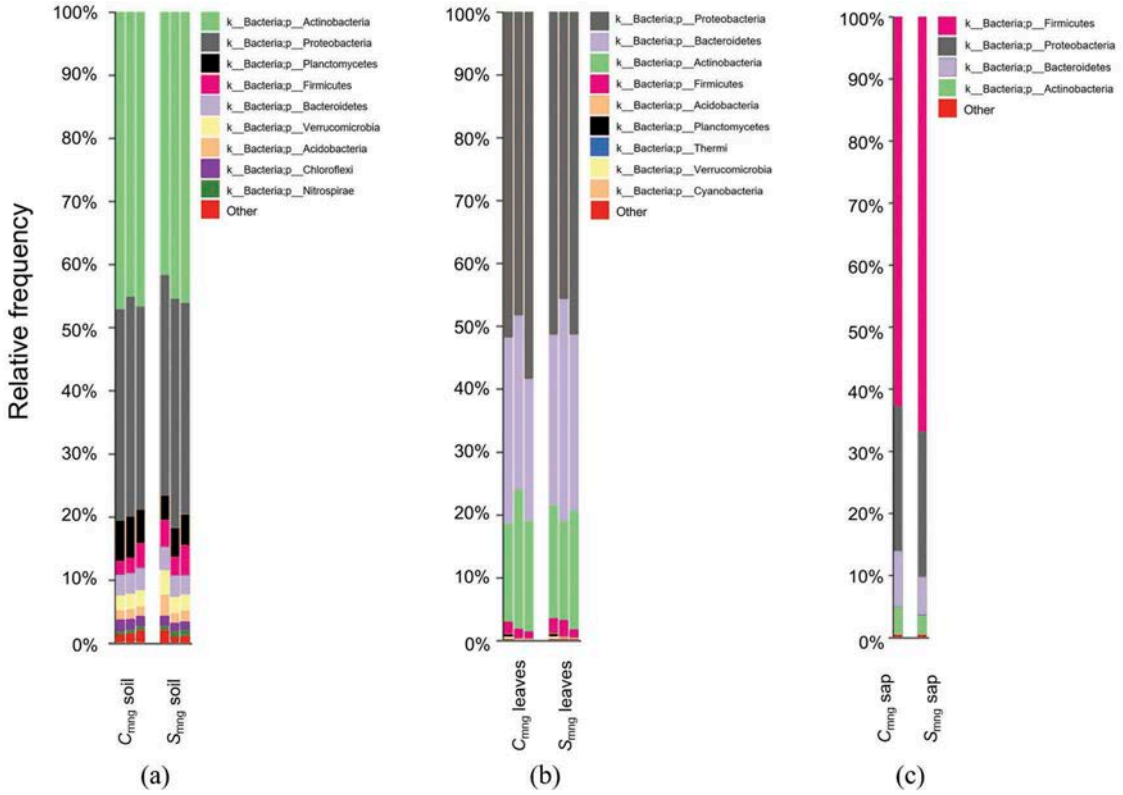

Figure 2. Relative frequency of the microbial communities in (a) soil, (b) leaves and (c) xylem sap of the C_{mng} and S_{mng} systems, revealed by Illumina MiSeq 16S rRNA gene amplicon sequencing at the phylum level.

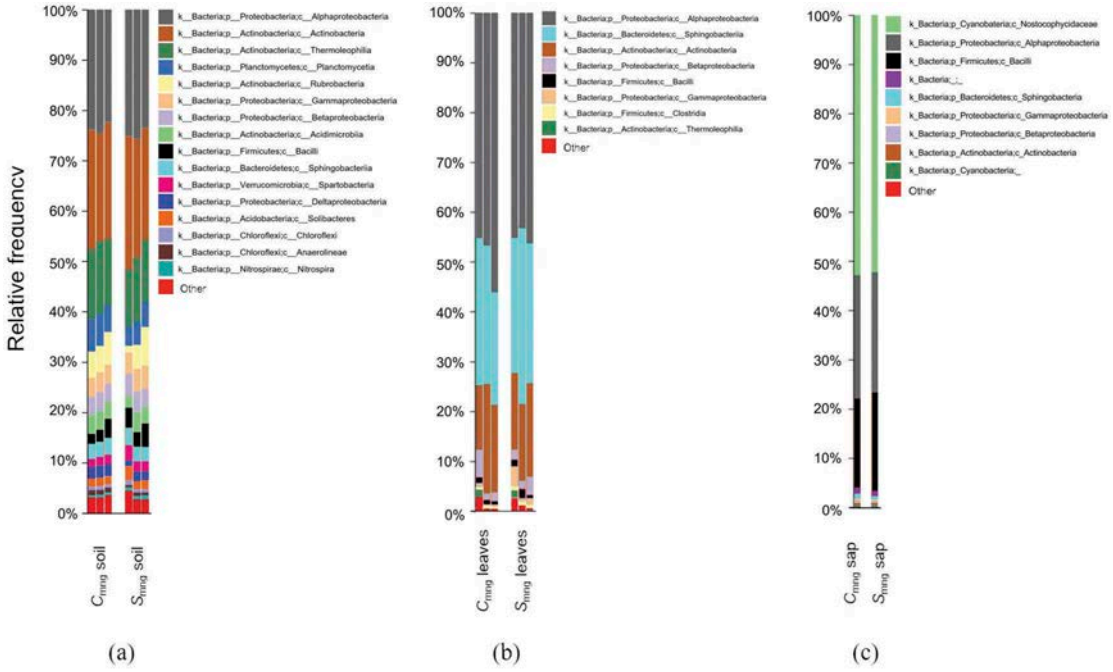

Figure 3. Relative frequency of the microbial communities in (a) soil, (b) leaves and (c) xylem sap of the C_{mng} and S_{mng} systems, revealed by Illumina MiSeq 16S rRNA gene amplicon sequencing at the class level.

Bacilli (63% in C_{mng} and 67% in S_{mng}), followed by Alphaproteobacteria (14% in C_{mng} and 15% in S_{mng}), and Sphingobacteria (9% in C_{mng} and 6% in S_{mng}). As for the phylum level (Figure 2), the percentages of the main classes were not significantly different between C_{mng} and S_{mng} managements, but clearly different among soil and plant compartments.

At other taxonomic levels, from order to species, there were many taxa in common among soil, leaves and xylem sap, but without differences between the two management systems. At the order level, Actinomycetales, Rhizobiales, Sphingobacteriales and Sphingomonadales were the four dominant bacterial taxa in soil, leaves and xylem sap. Different percentages of taxa prevailed between the compartments. The six dominant bacterial families in the three compartments were Solirubrobacteraceae, Hyphomicrobiaceae, Sphingomonadaceae, Flexibacteraceae, Leuconostocaceae and Lactobacillaceae. The dominant bacterial genres in all the tree compartments studies were *Solirubrobacter, Rubrobacter, Hymenobacter, Sphingomonas, Methylobacterium, Fructobacillus, Lactobacillus* and *Lactococcus*. At the species level, the taxa in common among soil, leaves and xylem sap included *Streptomyces tendae, Spingomonas wittichii* and *Shewanella dokdonensis*. The results revealed, even if in low quantity (< 0.003% of total reads), the presence of *Curtobacterium flaccumfaciens* in all the investigated compartments and of *Methylobacterium mesophilicum* only in the phyllosphere and xylem sap.

Comparison of the bacterial communities under different managements

The Venn diagrams in Figure 4 show shared and unique OTUs for the two management systems and in the three compartments. In detail, 4,789 OTUs were detected in C_{mng} and S_{mng} in soil (Figure 4(a)), 2,236 shared OTUs were detected in C_{mng} and S_{mng} in leaves (Figure 4(b)), and 197 OTUs were detected in C_{mng} and S_{mng} sap (Figure 4(c)). Up to 70% of OTUs in each of the three compartments were in common between the two management systems (C_{mng} and S_{mng}), but there was a greater uniqueness and diversity of OTUs in the S_{mng} than in the C_{mng} (Figure 4).

Richness and diversity of microbial communities

There were no significant differences among the two soil management systems for either richness (Chao1 index) or diversity (Shannon index) of microbial communities in the soil or the phyllosphere (Table 1; Figure 5). The rarefaction curves, reporting Chao1 and Shannon indices as a function of the number of sequences, clearly indicate that at the selected value of 20,000 fragments, the sequencing depth has been saturated for soil as well for leaves in both the management systems (Figure S1a, b).

Bacterial community structure

Principal coordinates analysis (PCoA) identified three principal component factors (PCF) in relation to the abundance of groups, explaining 58.4% and 19.8% of the total variation in soil (Figure 6(a)) and 44.0% and 22.6% in the phyllosphere (Figure 6(b)). The PCoA indicated that for the soil microbiota the two managements were separated. Similarly, for the leaves, there was separation between the two management systems and the S_{mng} replicates were grouped together with similarity, whereas the C_{mng} replicates were more scattered.

Effects of soil management on bacterial genera of soil, leaves and xylem sap

In S_{mng} and C_{mng} soils, 18 bacterial genera showed differences in relative abundance at a degree higher

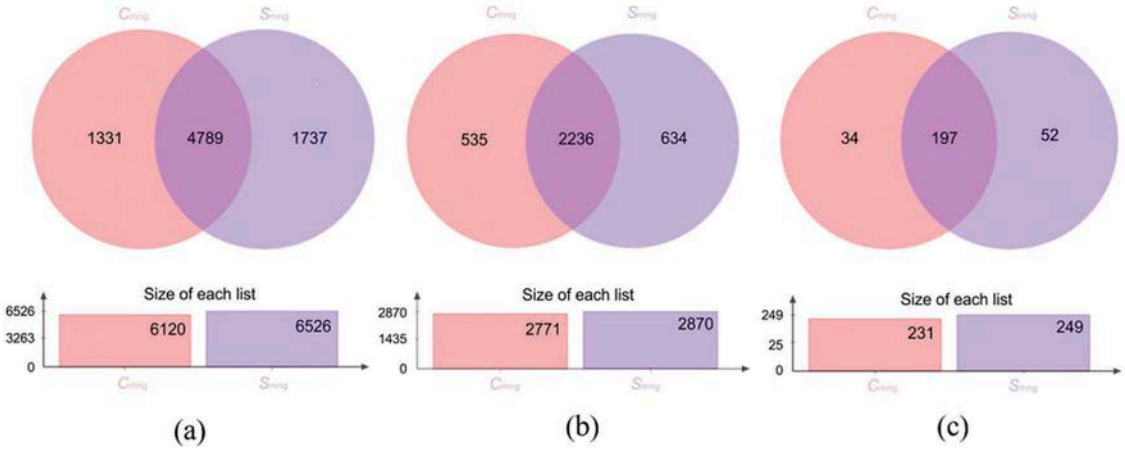

Figure 4. Venn diagram showing the unique and shared OTUs between the C_{mng} and S_{mng} systems in (a) soil, (b) leaves (b) and (c) xylem sap of the C_{mng} and S_{mng} systems. In (a), (b) and (c) the list represents the total OTUs in C_{mng} (number 1) and S_{mng} (number 2).

Table 1. Comparison of alpha diversity based on Chao1 and Shannon indices between the C_{mng} and S_{mng} systems in soil and leaf samples. Two-sample *t*-test and the *P* values were calculated through 999 Monte Carlo permutations.

		Chao1		Shannon	
Sample	Treatments	*t*	*P*	*t*	*P*
Soil	C_{mng}/S_{mng}	1.161	0.278	0.460	0.708
Leaves	C_{mng}/S_{mng}	0.194	0.750	1.195	0.409

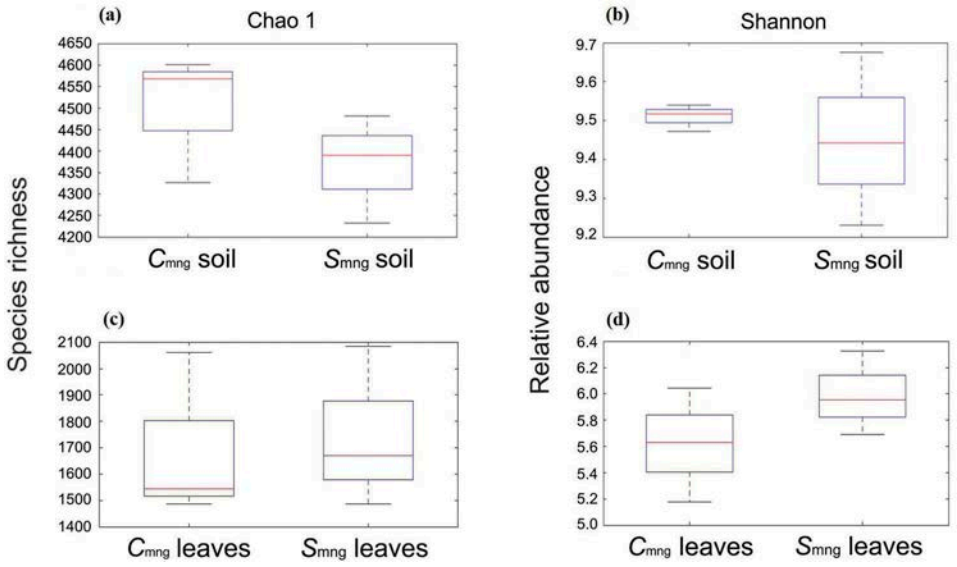

Figure 5. Boxplots (median, quartiles) represent the alpha diversity [(a) Chao1 index, (b) Shannon index] of soil samples from the C_{mng} and S_{mng} systems, and the alpha diversity [(c) Chao1 index, (d) Shannon index] of leaf samples from the C_{mng} and S_{mng} systems, statistically compared using a non-parametric two-sample t-test. There were no statistically significant differences in the diversity indexes between management practices. The C_{mng} and S_{mng} are referred to conventional and sustainable management, respectively. The x-axis represents species richness (a,c) and relative abundance (b,d) and y-axis shows soil (a, b) and leaves (c,d) samples under C_{mng} and S_{mng} systems.

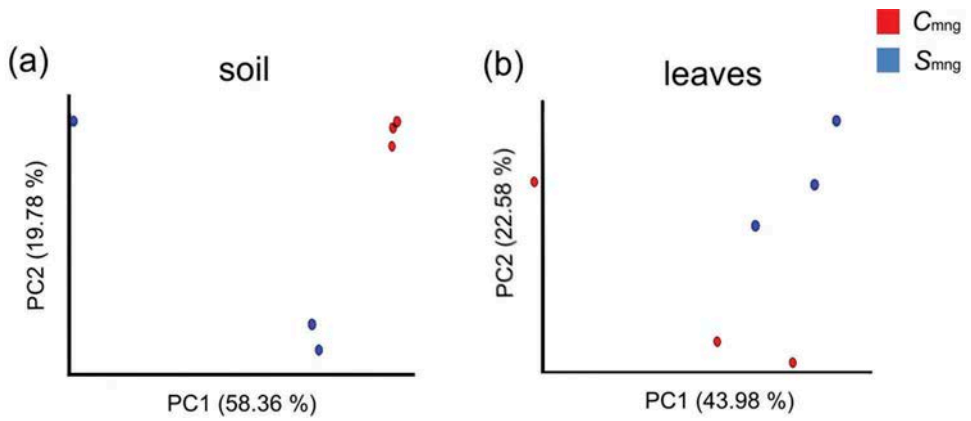

Figure 6. PCoA plot showing the results of the beta diversity analysis using Bray-Curtis estimation in (a) soil and (b) leaf samples. The red and blue spheres indicate the three replicates from the C_{mng} and S_{mng} systems. The C_{mng} and S_{mng} are referred to conventional and sustainable management, respectively.

than five folds (Figure 7). *Rhodanobacter* and *Pigmentiphaga* were found exclusively in S_{mng} soil. Among the most abundant genera, *Sporosarcina* (23.1×), *Mycobacterium* (20.8×), *Rhodoplanes* (12.2×), *Microlunatus* (10×) and *Conexibacter* (3×) were more abundant in the S_{mng} system than in the C_{mng}. By contrary, *Rubellimicrobium* (16.3×), *Arthrobacter* (11.3×), *Ancylobacter* (10.2×), *Flavisolibacter* (10.0×), *Cellulomonas* (8×), *Solirubrobacter* (8×), *Adhaeribacter* (7.8×), *Ramlibacter* (6.6×), *Caldilinea* (6.6×), *Roseomonas* (5.6×) and *Rubrobacter* (4.5×) were dominant in the C_{mng} system.

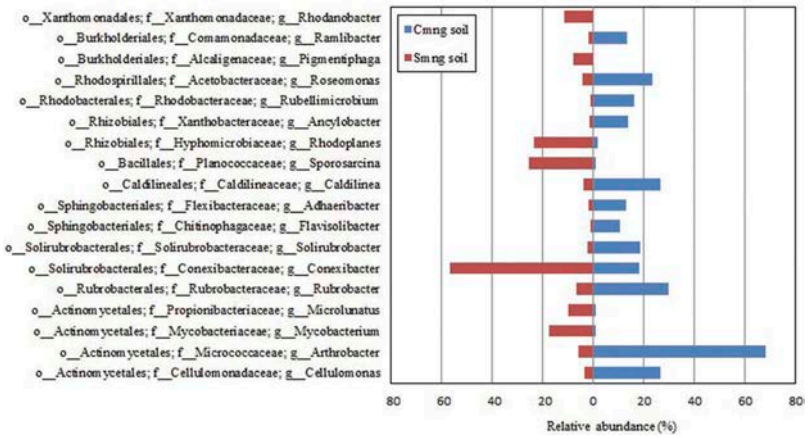

Figure 7. Relative abundances of bacterial genera with at least five-fold differences in abundance in soil samples from the C_{mng} and S_{mng} systems. The genera were statistically significant according to Kruskal-Wallis test ($P < 0.05$).

In the phyllosphere samples, eight genera showed differences in relative abundance at a ratio of at least two folds between S_{mng} and C_{mng} (Figure 8). *Psychrobacter* and *Acidisphaera* were found exclusively in S_{mng} leaves, while *Arthrobacter* was found exclusively in the C_{mng}. *Couchioplanes* (17.4×) and *Sphingomonas* (4×) were more frequent in the S_{mng}, compared to C_{mng}, whereas *Kineococcus* (4.1×) was dominant in the C_{mng} system.

Since we did not have true replicates to analyse xylem sap communities statistically, we used the pie charts (Figure S2) in order to provide a representation of the more abundant genera (> 3.5% of total reads) colonising the xylem of olive trees under both the management systems.

Discussion

Characterisation of bacterial communities in soil-plant compartments

The fact that about 23% of sap microbiome of plants in both management systems was common to soil microbiome suggests that, in accordance with Mercado-Blanco and Lugtenberg (2014), a proportion of the endophytes may derive from soil. Additionally, as a significant part of endophytes (39%) is in common with the phyllosphere microbiome (and 17% with both soil and phyllosphere), may be taken to support the hypothesis that a part of the bacterial communities originates from the soil and reaches the phyllosphere through xylem sap. However, it is noteworthy that about 57% of sap microbiome, is unique, of unknown origin or derived from soil and leaf microbial communities that at the time of sampling were not present or were missed.

Differently from the metagenomic data obtained from olive groves in Spain (Caliz et al. 2015; Müller et al. 2015), where a high proportion of archaeal 16S rRNA genes was found in the leaf endosphere of olive trees, in our study Archaea were rare and scarce in soil and leaves, and absent from the xylem sap (Table S1).

A number of taxa were in common in the soil, leaves and xylem sap. Actinobacteria, the most abundant phylum in soil, represent various

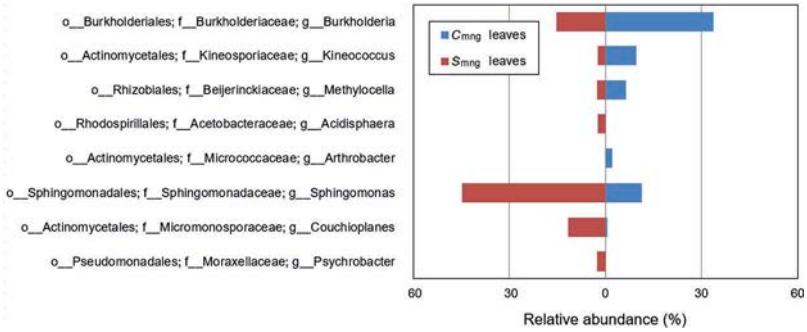

Figure 8. Relative abundances of bacterial genera with at least two-fold differences in the leaf samples from the C_{mng} and S_{mng} systems. The genera were statistically significant according to Kruskal-Wallis test ($P < 0.05$).

physiological and metabolic properties, such as the production of extracellular enzymes and a variety of secondary metabolites (e.g. antimicrobial agents) (Lee et al. 2014; Olano et al. 2014). The presence of Proteobacteria in the soil is an indicator of high soil nutrient-content, as most inhabit nutrient-rich soils (Castro et al. 2010; Gottel et al. 2011); owing to their ability to oxidise ammonia, they affect nitrogen availability for plants (Prosser 1989). According to Rasche et al. (2006b), Rasche et al. (2006c), who stated that β-Proteobacteria and Firmicutes can form a large part of the bacterial community in some situations, with Acidobacteria, Actinobacteria and Cyanobacteria occurring infrequently, in our case Proteobacteria was the dominant phylum in the phyllosphere (Figure 2(b)). In the xylem sap, Firmicutes, which include well-known antagonists and biocontrol agents were the dominant group (Emmert and Handelsman 1999). Our results are in agreement with those by Müller et al. (2015), who described the Gram-positive bacteria *Bacillus* as part of the core microbiota in olive endosphere.

Alphaproteobacteria is one of the most abundant classes in all the three compartments, ecologically important in not only soil but many ecosystems globally (Spain et al. 2009). One of the more frequent classes detected in the xylem sap was that of Bacilli. Many Bacilli are powerful biocontrol agents against olive pathogens, because they often colonise the same ecological niches and so compete directly with the pathogens (Aranda et al. 2011), our results clearly support their potential defensive role within plants. In addition, Cyanobacterium is present also in soil and phyllosphere but at lower concentration compared to that in the xylem sap. Their structural–functional plasticity confers great versatility and enables them to adapt and inhabit a wide range of environments and niches. Furthermore, Cyanobacterium itself is a plant growth promoting and biocontrol agent (Priya et al. 2015). Finally, *Curtobacterium flaccumfaciens* and *Methylobacterium mesophilicum*, investigated at the species level, are considered additional important biocontrol agents having a powerful action against many plant pathogens (Hallmann et al. 1997; Azevedo et al. 2000).

Richness, diversity and structure of bacterial communities under different managements

Despite many OTUs were in common among the three compartments investigated and the two management systems, there was a higher uniqueness of OTUs in the S_{mng} than in the C_{mng}. However, overall, no particular differences in the richness and diversity of soil/phyllosphere microbial communities were found between the two management systems. This finding could be explained by the fact that the conventional management here adopted was not so intensive to justify large rearrangements of the microbial communities; anyway, it is quite different from the sustainable one to affect few specific taxa. The effect on these taxa could be a consequence of different physico-chemical environmental conditions (O'Brien and Lindow 1989; Berg and Smalla 2009), or, in our case, be likely due to differences induced by the conventional/sustainable managements in nutritional characteristics of the phyllosphere or soil (Marschner et al. 2004) and variability in nutrient supply between niches of microorganisms (Compant et al. 2005a).The lack of significant variation of soil bacteria diversity between S_{mng} and C_{mng} could be also explained by the high diversity among the three replicates of the former than the latter, as previously reported by Wang et al. (2016b), who have compared bacterial communities from organic farming and conventional soil samples. Anyway, according to other authors (He et al. 2007; Wittebolle et al. 2009), even minor changes in soil microbial community structure could have a great impact on the stability of the soil ecosystem.

Effects of management on bacterial composition of soil, leaves and xylem sap

Previous research in the same experimental system using cultured-based and genetic approaches (Sofo et al. 2010, 2014) has shown that a S_{mng} caused a higher number and diversity of microorganisms compared to that in C_{mng}. From the results of this study, it is possible to define that the main differences between the two management systems are related to the bacterial community composition rather than to the presence/absence or richness of microorganisms.

The application of sustainable management practices can improve the quality of soil ecosystems (Mader et al. 2002; Cavigelli et al. 2013) and, in this perspective, soil bacteria play a crucial role in the biogeochemical cycling of nutrient elements and promote plant growth (Young and Crawford 2004; Mendes et al. 2011). The positive effects of organic farming on the microorganisms with a beneficial

effect on plant growth has been linked to organic management practices, including prohibition of herbicide or mineral fertiliser inputs, rational management of non-cropped areas, and more mixed farms (Hole et al. 2005). It is note worthy that a higher number of N-fixing Rhizobiales was found in S_{mng} than in C_{mng} soil. Among them, the Hyphomicrobiaceae (genus *Rhodoplanes*) are mainly involved in C and N cycling and are also able to utilise different N substrates, such as N_2, NO_3^-, or NH_3 (Kulichevskaya et al. 2006; Wang et al. 2016b). The genus *Rhodanobacter*, which includes many microorganisms involved in acidic denitrification in soils was found exclusively in S_{mng} soil (van Den Heuvel et al. 2010).

In the phyllosphere, the abundance of the bacterial communities depends on the age of the leaves and it is related to leaf traits (Sanchez-Azofeifa et al. 2012). Moreover, as olive is an evergreen tree species (the mean life span of a leaf is 18 months), the endophytic microbial diversity can be stable over a long period of time. Proteogenomic analyses of various phyllosphere microbiomes have identified species that assimilate plant-derived ammonium, amino acids and simple carbohydrates, implicating these compounds as primary N and C sources in the phyllosphere. Among these bacteria, a key role is played by *Sphingomonas* spp. (Delmotte et al. 2009; Knief et al. 2012) which in our study were predominant on S_{mng} leaves; elsewhere, it has been found as the most abundant genus on leaves of organic grapevine (Martins et al. 2013). Moreover, *Sphingomonas* are known for their ability to grow under low-nutrient conditions (Hirano and Upper 2000; Park et al. 2011). This result is particularly important, as the leaf micro-environments are generally considered nutrient-limited (Andrews 1992; Compant et al. 2005b).

Finally, the technical novelty of our study resides in the sap extraction by Scholander pressure bomb that, despite being a difficult and time-consuming technique, resulted as a convenient method of xylem fluid collection from olive trees and for the detection of microbial communities. Indeed, xylem fluid extracted with the pressure bomb contains very few plant components, that commonly interfere with the DNA extraction process or inhibit PCR. Although our approach did not give sufficient bacterial DNA to keep replicates separated, on the other side we managed to identify important taxa at different taxonomic levels, so demonstrating the colonisation of internal tissues and the spread of bacterial communities from the soil to aerial plant parts mainly through xylem vessels.

Conclusions

The results of this study confirmed our hypothesis that bacterial communities come from the soil and reach aerial plant parts through xylem sap. Even if there were no differences in bacterial richness and diversity indices between the two managements systems, the application of different agronomic practices influenced the composition of soil bacterial communities. Particularly, important bacterial taxa with physiological and protective functions for the plants were revealed in the soil-plant compartments of the S_{mng} system.

Our results provided a picture of the differences between S_{mng} and C_{mng} systems and, in particular, the linkage between soil management and bacterial community in soil, leaf and xylem sap in a Mediterranean olive agro-ecosystem. The potential benefits of the specific bacterial taxa detected under the S_{mng} system could improve plant growth protection and provide a higher crop quality in olive plants and similar fruit species.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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Supplementary material

Table S1. Relative abundances of the respective OTU in soil, leaves and xylem sap at the kingdom level. Archea were much less abundant than Bacteria.

	Soil	Leaves	Xylem sap
Archea	0.05%	0.01%	0
Bacteria	99.95%	99.99%	100%

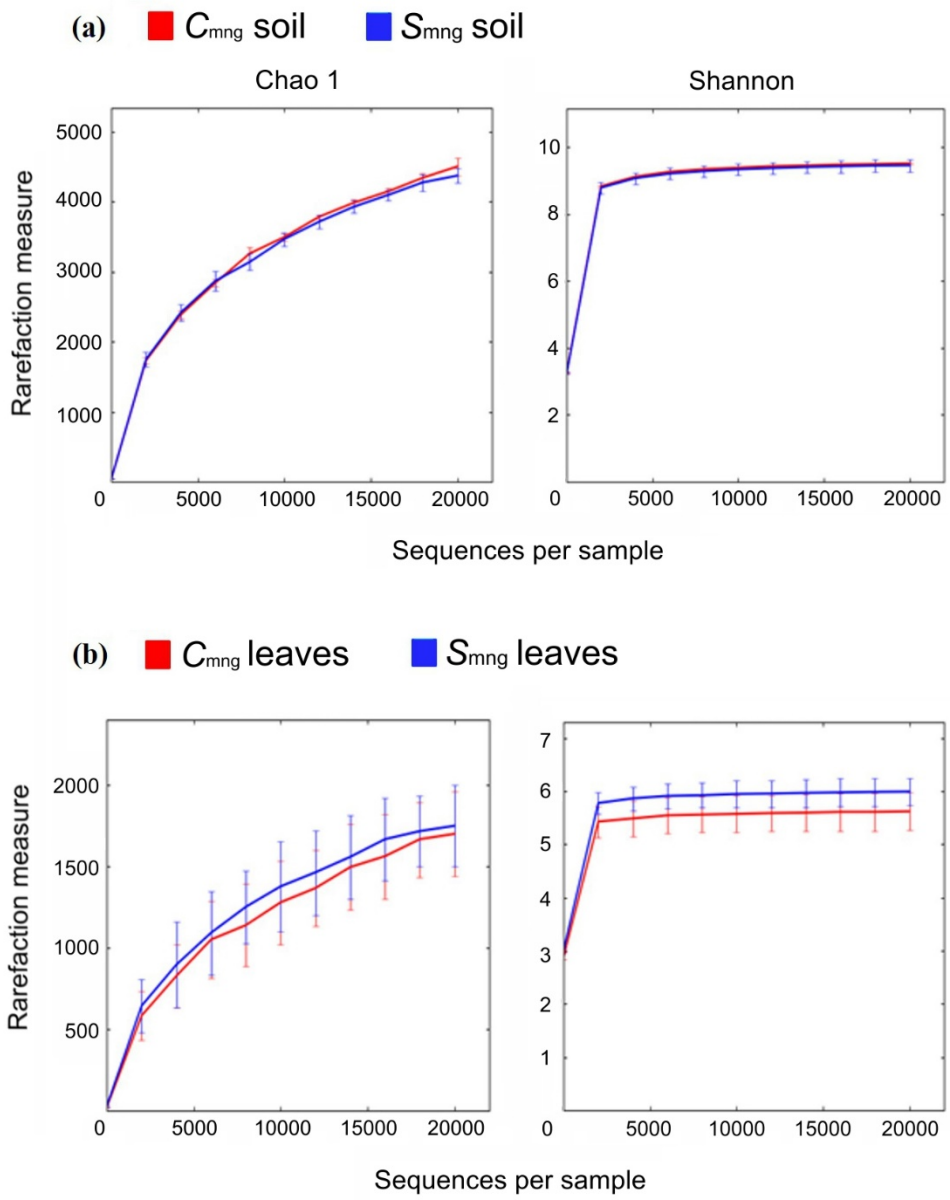

Figure S1. Rarefaction curves according to Chao1 and Shannon indices in (a) soil and (b) leaf samples. The red and blue lines represent the C_{mng} and S_{mng} systems, respectively.

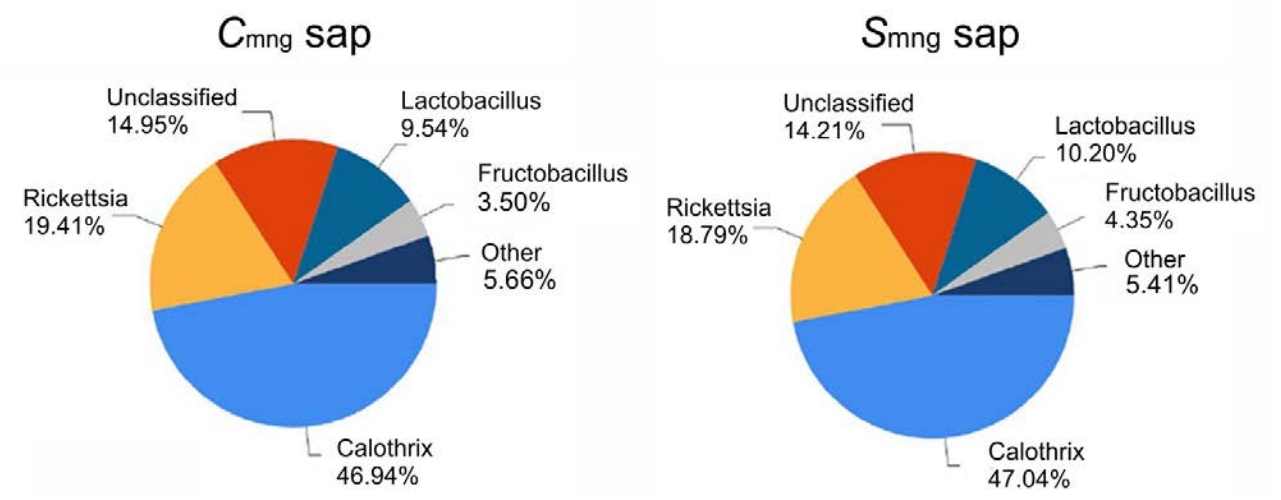

Figure S2. Pie charts with the more abundant bacterial genera in xylem sap from the C_{mng} and S_{mng} systems. The "Other" category represents the sum of all the classifications with less than 3.5% abundance.