Defining the root endosphere and rhizosphere microbiomes from the World Olive Germplasm Collection

Antonio J. Fernández-González[a], Pablo J. Villadas[a], Carmen Gómez-Lama Cabanás[b], Antonio Valverde-Corredor[b], Angjelina Belaj[c], Jesús Mercado-Blanco[b], and Manuel Fernández-López[a*]

[a]Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas (CSIC). Calle Profesor Albareda 1, 18008 Granada, Spain.

[b]Departamento de Protección de Cultivos, Instituto de Agricultura Sostenible, CSIC. Campus 'Alameda del Obispo' s/n, Avd. Menéndez Pidal s/n, 14004 Córdoba, Spain

[c]Área Mejora y Biotecnología, IFAPA-Centro Alameda del Obispo, Avda. Menéndez Pidal s/n, 14080 Córdoba, Spain

[*]Corresponding autor: Manuel Fernández-López, calle Profesor Albareda 1, 18008 Granada, Spain. manuel.fernandez@eez.csic.es , phone: +34 958181600 ext. 140.

ABSTRACT

Up to date, the bacterial and fungal microbial communities from the olive (*Olea europaea* L.) root systems have not been simultaneously studied. In this work, we show that microbial communities from the olive root endosphere are less diverse than those from the rhizosphere. But more relevant was to unveil that olive belowground communities are mainly shaped by the genotype of the cultivar when growing under the same environmental, pedological and agronomic conditions. Furthermore, *Actinophytocola*, *Streptomyces* and *Pseudonocardia* are the most abundant bacterial genera in the olive root endosphere, *Actinophytocola* being the most prevalent genus by far. In contrast, *Gp6*, *Gp4*, *Rhizobium* and *Sphingomonas* are the main genera in the olive rhizosphere. *Canalisporium*, *Aspergillus*, *Minimelanolocus* and *Macrophomina* are the main fungal genera present in the olive root system. Interestingly enough, a high proportion of so far unclassified fungal sequences at class level were detected in the rhizosphere. From the belowground microbial profiles here reported, it can be concluded that the genus *Actinophytocola* may play an important role in olive adaptation to environmental stresses. Moreover, the huge unknown fungal diversity suggests that there are still some fungi with important ecological and biotechnological implications that have yet to be discovered.

Keywords: Bacteriota, Mycobiota, *Olea europaea*, Rhizosphere, Root endosphere

1. Introduction

The cultivated olive (*Olea europaea* L. subsp. *europaea* var. *europaea*) is not only one of the oldest domesticated trees[1], but also constitutes one of the most important and outstanding agro-ecosystems in the Mediterranean Basin, shaped along millennia[2]. In this area, there is an olive belt with more than 10 million ha belonging to the countries of the coastal regions, which account for around 80% of the world's total olive cultivation area[3]. In some of these countries such as Spain, the world's largest olive oil and table olive producer, this woody crop has undisputable social, economic and agro-ecological relevance, accounting for almost 25% of the total olive trees and more than 37% of the world's olive oil production[4]. In addition to its ecological and social importance, the main product obtained from this iconic tree (i.e. the virgin olive oil), has a number of health and nutritional benefits so that its consumption is increasing worldwide[5].

Olive cultivation is threatened by several abiotic (e.g. soil erosion) and biotic (i.e. attacks from insects, nematodes and pathogenic microbes) constraints. Among relevant phytopathogens present in the soil microbiota affecting olive health, representatives of the *Oomycota* class (e.g. *Phytophthora* spp.) as well as higher fungi (e.g. *Verticillium dahliae* Kleb.) must be highlighted[2,6–8]. In addition to the traditional and well-known microbiological menaces affecting olive crop (e.g., anthracnose [caused by *Colletotrichum* spp.], Verticillium wilt [VWO, *V. dahliae*], peacock spot [*Spilocea oleagina* (Cast.) Hughes.], or knot disease [*Pseudomonas savastanoi* pv. savastanoi Smith.])[9–12], emerging diseases like the olive quick decline syndrome caused by

Xylella fastidiosa Wells. ssp. *pauca* observed for the first time attacking olive trees in Italy in 2013[13], must be considered. In addition to new threats, some reports warn on the increase in pathogen and arthropod attacks as a consequence of changing from traditional olive cropping systems to high-density tree orchards. However, the impacts of high-density olive groves on, for instance, soil-borne diseases have not been yet studied[14]. Another important menace to take into account is climate change, which is expected to affect the incidence and severity of olive diseases[6]. Finally, the reduction in the number of planted olive cultivars due to either commercial (e.g. improved yield, etc.) or phytopathological (i.e. tolerance to diseases) reasons, a trend observed in many areas, will eventually lessen olive genetic diversity. All these factors may have a profound, yet not evaluated impact on the composition, structure and functioning of belowground microbial communities[8].

A comprehensive knowledge of microbial communities associated to the olive root system, including the root endosphere and the rhizosphere soil, is therefore instrumental to better understand their influence on the development, health and fitness of this tree. A priori, the vast majority of the olive-associated microbiota must be composed of microorganisms providing either neutral or positive effects to the host. Indeed, recent literature provides solid evidence that olive roots are a good reservoir of beneficial microorganisms, including effective biocontrol agents (BCA)[15–18]. Among the beneficial components of the plant-associated microbiota, endophytic bacteria and fungi are of particular interest to develop novel biotechnological tools aiming to enhance plant growth promotion and/or control of plant diseases. Moreover, microorganisms able to colonize and endure within the plant tissue pose the additional advantage to be adapted

to the specific microhabitat/niche where they can provide their beneficial effects[19]. Besides endophytes, beneficial components of tree root-associated microbiota colonizing the rhizoplane and/or the rhizosphere soil can also directly promote plant growth (i.e. bio-fertilization, phyto-stimulation) or alleviate stress caused by either abiotic (i.e.environmental pollutants, drought, salinity resistance) or biotic (see above) constraints[8].

Our knowledge on olive-associated microbiota is still very scarce and fragmentary. So far, bacterial communities associated with wild olive (*Olea europaea* L. subsp. *europaea* var. *sylvestris*) roots (endo- and rhizosphere) have been studied using fluorescent terminal restriction fragment length polymorphism (FT-RFLP) as culture-independent approach as well as bacteria isolation in culturing media[15]. In another study, endophytic fungi from the phyllosphere and roots of olive cultivar (cv.) Cobrançosa[20] were screened by a culture-dependent method to compare the fungal communities between above- and belowground compartments. Microbial communities of the olive phyllosphere and carposphere have been analyzed using denaturing gradient gel electrophoresis (DGGE)[21], isolation of fungi in culturing media[22] and high-throughput sequencing of both fungal[23] and prokaryotic[24] communities.

In this study we aim, for the first time, to unravel the composition and structure of belowground prokaryotic and fungal communities of cultivated olive by high-throughput sequencing. A core collection of olive cultivars (36 originating from 9 different countries, Table 1) present at the World Olive Germplasm Collection (WOGC; Córdoba, Spain) representative of enough genetic diversity within the Mediterranean Basin have been analyzed when all varieties were grown under the same climatic, pedological and agronomic

conditions. The following objectives were pursued: a) to perform an in-depth study of the belowground microbial communities (root endosphere and rhizosphere) in a wide range of olive genotypes; b) to assess what is/are the determinant factor(s) contributing to build up such communities; c) to establish the core and accessory microbiota of the olive rhizosphere and root endosphere. The hypothesis to-be-tested is that under specific agro-climatic and edaphic conditions the olive genotype is the key factor for building up the root endosphere community but not that important in the rhizosphere community.

2. Results

2.1. Microbial communities clustered by compartments (endosphere and rhizosphere), and by olive cultivar in each compartment

Starting from about 37 million reads, a total of 1,404,769 (prokaryotic) and 1,005,148 (fungal), and 5,330,385 (prokaryotic) and 912,302 (fungal) good quality reads from the root endosphere and the rhizosphere, respectively, were eventually retained from the 36 olive cultivars here analyzed (Table 1). The smallest samples had 2,061 prokaryotic and 442 fungal sequences, coming from the root endosphere, and the largest ones reached 78,913 prokaryotic and 55,072 fungal sequences, these originated from the rhizosphere (Tables S1 and S2). After rarefying to the smallest sample, alpha diversity indices showed statistically significant differences between the two compartments (i.e. the endosphere and the rhizosphere), showing the rhizosphere samples the highest

147 richness and diversity values (Figure S1). Subsequently, both compartments
148 were split and rarefied independently for further alpha diversity analyses to
149 2,061 (442 in fungi) and 15,565 (665 in fungi) sequences from endosphere and
150 rhizosphere, respectively.

151 With regards to the prokaryotic communities, richness showed significant
152 differences when comparing the root endosphere of olive cultivars, showing just
153 marginal differences in diversity. Considering the rhizosphere, only the diversity
154 showed statistically significant differences among cultivars (Table 2, Figures
155 S2a and b). Concerning the fungal communities, both richness and diversity
156 indices showed statistically significant differences when comparing olive
157 cultivars for each compartment (Table 2, Figures S2c and d).

158 We compared the distribution of the samples from both compartments,
159 rhizosphere and root endosphere. Results showed significantly different
160 prokaryotic (PERMANOVA R^2 0.43; p-value < 0.0001) and fungal
161 (PERMANOVA R^2 0.06; p-value < 0.0001) communities (Figure 1a and b).
162 Regarding to the prokaryotic root endosphere, the olive cultivar explained 42%
163 of the variation, being statistically significant (PERMANOVA R^2 0.42; p-value <
164 0.0001) (Figure 2). Regarding to prokaryotic communities present in the olive
165 rhizosphere, the cultivar explained more than 53% of the distribution, being
166 statistically significant as well (PERMANOVA R^2 0.53; p-value < 0.0001) (Figure
167 3).

168 Concerning fungal communities in the root endosphere, the cultivar
169 explained 39% of the variation, being statistically significant (PERMANOVA R^2
170 0.39; p-value < 0.0001). In the case of the fungal rhizosphere, this factor
171 explained 44% of the variation, also being statistically significant (PERMANOVA

172 R^2 0.44; p-value < 0.0001). Data not plotted because of the high NMDS stress
173 value (0.22 with 3 dimensions).

174

175 *2.2. The olive root endosphere and soil rhizosphere show different prokaryotic*
176 *taxonomic profiles*

177 Completely different taxonomic profiles at phylum level (class level for
178 *Proteobacteria*) were obtained when comparing the prokaryotic communities
179 residing in the olive root endosphere with those ones present on the olive
180 rhizosphere (Figure 4). Despite the fact that universal primers for both
181 prokaryotic kingdoms were used, no OTU was classified as *Archaea*. On the
182 one hand, predominant phyla (or class) in the endophytic communities of the 22
183 olive cultivars examined (see Methods for exclusion criteria) were
184 *Actinobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes* and
185 *Deltaproteobacteria*, accounting for more than 90% of the sequences.
186 Remarkably, *Actinobacteria* exceeded 50% in all of them, highlighting cultivars
187 Chemlal de Kabylie, Llumeta and Mavreya (from Algeria, Spain and Greece,
188 respectively) that represented more than 80% of the total number of sequences
189 (Figure 4a). On the other hand, rhizospheric communities showed more even
190 profiles with the phylum *Acidobacteria* accounting for an average of 27.5% of
191 the sequences in the 36 olive cultivars here examined. *Acidobacteria* was
192 followed by *Alphaproteobacteria* (18.8%), *Actinobacteria* (9.8%),
193 *Gemmatimonadetes* (5.2%) and *Betaproteobacteria* (4.5%). Overall, and on
194 average, the sum of all of them represented nearly 70% of the total number of
195 sequences (Figure 4b). In contrast, *Gemmatimonadetes* and

196 *Betaproteobacteria* were minor phyla in the olive root endosphere (0.06% and
197 0.8%, respectively).

198 In the endosphere, and at the genus level, only two genera were
199 significantly represented among the 22 cultivars that could eventually be
200 examined: *Flavitalea* (*Bacteroidetes*) and *Actinophytocola* (*Actinobacteria*).
201 Indeed, *Flavitalea* was most abundantly represented in cv. Myrtolia but absent
202 in cv. Uslu (Figure S3a). Conversely, *Actinophytocola* was highly prevalent in 8
203 cultivars including Uslu (the highest) and Myrtolia (Figure S3b). Furthermore,
204 *Actinophytocola* was the most abundant genus inhabiting the olive root
205 endosphere accounting for an average of 22.1 ± 15.0% of the sequences,
206 followed by *Streptomyces* (13.2 ± 8.2%), *Pseudonocardia* (9.4 ± 3.8%),
207 *Bradyrhizobium* (2.6 ± 1.4%), *Ensifer* (2.6 ± 6.6%) and *Rhizobium* (2.0 ± 2.8%).
208 The sum of relative abundances of these six main endophytic genera ranged
209 from 33.3% in cv. Barri (Syria) to 73.1% in cv. Uslu (Turkey) (Figure S3c).

210 With regards to rhizosphere soil samples, our results showed that 63
211 genera were significantly more abundant among the different olive cultivars
212 examined (36 cultivars). Moreover, eight out of the eleven main rhizosphere
213 genera, with a relative abundance higher than 1%, had statistically significant
214 differences between cultivars (Figure S4). Three of the most prevalent genera,
215 namely *Gp6*, *Gp4* and *Gp7*, belong to the main rhizospheric phylum
216 *Acidobacteria*, but only the first two showed significant differences. Belonging to
217 the second most abundant phylum, *Proteobacteria*, and being both *α-*
218 *Proteobacteria*, *Rhizobium* and *Sphingomonas* were also relatively highly
219 abundant and showed significant differences among cultivars. The relative

220 abundance of these eleven genera ranged from 49.4% in cv. Temprano (Spain)
221 to 64% in cv. Barnea (Israel) (Figure S4).
222
223 *2.3. Fungal taxonomic profiles only showed minor differences between the olive*
224 *root endosphere and the soil rhizosphere*
225 In contrast to prokaryotic communities, fungal communities showed more
226 similar taxonomic profiles at the class level. The main difference between the
227 two compartments was the percentage of sequences that remained unclassified
228 (10.7% in the root endosphere *versus* 35.4% in the rhizosphere) (Figure 5). This
229 proportion was very heterogeneous among olive cultivars, Grappolo (Italy) and
230 Chemlal de Kabylie (Algeria) being the two cultivars that harbored more
231 unclassified sequences in the root endosphere (37.8 and 29.4%, respectively),
232 while cultivars Shengeh (Iran) and Abou Kanani (Syria) did so in rhizosphere
233 samples (87.3 and 82.8%, respectively). The prevalent classes present in the
234 olive root endosphere were *Sordariomycetes* (38.1%), *Eurotiomycetes* (23%),
235 *Agaricomycetes* (13.2%) and *Dothideomycetes* (11.5%), accounting for more
236 than 85% of the sequences obtained from the 33 olive cultivars assessed (see
237 Methods for exclusion criteria) (Figure 5a). The remaining classes were clearly
238 less relatively abundant, *Glomeromycetes* being the only one reaching 1%, on
239 average, in all cultivars. Nevertheless, due to the heterogeneity found among
240 the cultivars, this class represented more than 12 and 8% of relative abundance
241 in the Syrian cvs. Maarri and Jabali, respectively.
242 Regarding to rhizosphere communities, a smaller difference between the
243 prevalent classes and the remaining ones was found in comparison to those
244 found in the root endosphere. Similar to endophytic communities,

245 *Sordariomycetes* was the predominant class in the rhizosphere (19%), followed
246 by *Agaricomycetes* (12.9%), *Eurotiomycetes* (12.2%), *Orbiliomycetes* (6.5%)
247 and *Dothideomycetes* (4.9%). While *Pezizomycetes* (2.4%) was, overall, more
248 abundant than *Leotiomycetes* (2.3%) this latter class was exceptionally more
249 abundant in the Spanish cultivars Piñonera, Picudo, Verdial de Velez Málaga-1
250 and Temprano (relative abundance ranging from 16.5 to 22.6%), and in the
251 Turkish cv. Uslu (21.1%) (Figure 5b).

252 Concerning the genus level, only five fungal genera with statistically
253 significant differences in relative abundance, *Scutellinia* (*Pezizomycetes*),
254 *Acaulium*, *Purpureocillium* (*Sordariomycetes*), *Entoloma* (*Agaricomycetes*) and
255 *Minimelanolocus* (*Eurotiomycetes*) were found in the root endosphere of the 33
256 olive cultivars examined (Figure S5a). Ten fungal endophytic genera were
257 found with relative abundance higher than 1%, accounting for an average
258 proportion of sequences ranging from 24.7% in cv. Grappolo (Italy) to 97.4% in
259 cv. Forastera de Tortosa (Spain) (Figure S5c). However, due to the high
260 heterogeneity of relative abundances observed for these genera,
261 *Minimelanolocus* was the only genus showing statistically significant
262 differences.

263 We found 7 fungal genera with statistically significant differences in
264 relative abundance, *Macrophomina*, *Polyschema* (*Dothideomycetes*),
265 *Minimelanolocus*, *Spiromastix* (*Eurotiomycetes*), *Cunninghamella*
266 (*Mucoromycetes*), *Chlorophyllum* (*Agaricomycetes*), and *Dichotomopilus*
267 (*Sordariomycetes*), in the rhizosphere of the 36 olive cultivars examined (Figure
268 S5b). Only *Macrophomina* and *Minimelanolocus* showed enough relative
269 abundance to be considered as part of the main fungal rhizosphere genera.

Furthermore, *Macrophomina* was the third most abundant, on average, in all olive cultivars, particularly in 5 cultivars from Spain (Picual, Piñonera, Verdial de Velez Málaga-1, Picudo and Temprano) and in one from Turkey (Uslu) (Figure S5d).

2.4. Defining the belowground core microbiota of olive trees

Regarding bacterial communities, 46 (root endosphere) and 109 (rhizosphere) genera were found in all examined cultivars of each compartment. Furthermore, 40 of them were found in all the cultivars and in both compartments. Interestingly, 26 genera had a relative abundance higher than 1% in at least one compartment. The top 10 genera in the core olive root bacterial microbiota (bacteriota) were *Actinophytocola*, *Streptomyces*, *Gp6*, *Gp4*, *Pseudonocardia*, *Rhizobium*, *Sphingomonas*, *Gemmatimonas*, *candidate division WPS-1* and *Gp7*, accounting for almost 50% of the sequences in each compartment (Table 3; Table S3). Finally, all the main bacterial genera found in both compartments (Figures S3c and S4) were part of the core olive belowground bacteriota.

Regarding fungal communities, only 4 (root endosphere) and 8 (rhizosphere) genera were found in all examined cultivars. Interestingly, the 4 core endophytic fungal genera were also part of the rhizosphere fungal core. Only 5 genera had a relative abundance > 1% in at least one compartment. The 4 fungal genera constituting the olive belowground fungal core were *Canalisporium*, *Macrophomina*, *Aspergillus* and *Malassezia*. They represented more than 40% of the endophytic sequences, but only 13.08% of the rhizosphere sequences. Furthermore, the 8 core rhizosphere fungal genera represented only 15.88% of the sequences (Table 3; Table S4).

3. Discussion

In addition to the higher alpha diversity (richness and evenness) found in the olive rhizosphere microbial communities compared to that observed for the microbiota inhabiting the root endosphere, and the finding that quite different communities were found in each compartment, a common scenario described in several studies[25,26], the following major results must be highlighted from our work. Concerning the endosphere, cultivars originating from Syria showed the highest diversity level in contrast to the Turkish cultivars that showed the lowest one. With regard to the rhizosphere, fungal communities associated to cultivars from Albania and Syria appeared as the most diverse, while the Iranian and Israeli cultivars harbored the least diverse communities. Rhizosphere bacterial communities were not different in richness but showed dissimilar evenness. As observed for fungal communities, cultivars from Iran and Israel were also the least diverse in their rhizosphere bacterial assemblages.

Results here presented are in overall agreement with the major conclusion reported by Müller et al.[24], even though these authors focused on aerial organs. Indeed, they concluded that the structure of endophytic prokaryotic communities residing in aboveground tissues was mainly driven by the geographical origin of the olive cultivars evaluated (Eastern: Greece, Syria; Central: France, Italy, Tunisia; and Western Mediterranean: Portugal, Spain, Morocco). The same overall conclusion is inferred from our study, emphasizing that is based on a larger number of cultivars and from a wider geographical

origin (36 cultivars from 9 different countries *versus* 10 olive cultivars and 9 wild olive trees from 8 different countries in the study of Müller and co-workers). However, the main factor in our study was the genotype (cultivar) of each sample. It is true that the geographical origin was a statistically significant factor too but its variation was nested in cultivar variation (data from PERMANOVA test). In addition, more detailed information was obtained in our study. Thus, communities harbored by olive cultivars originating from Greece (olive green; see colors and distribution in Figures 2 and 3) and Spain (blue) showed more similarities among them than to those from Syrian (purple) cultivars. Moreover, the Italian (light blue) cultivar was intermingled between these two clusters and the unique Turkish (pink) representative tested in our work appeared as distantly related to the Syrian genotypes. Although a distinction among different geographical origins was observed, these clusters did not correspond to a longitudinal gradient (eastern, central, western Mediterranean countries), as reported by Müller et al.[24]. Our results indicate that the endophytic and rhizosphere microbial (bacteria and fungi) communities are mainly shaped by the olive genotype. We therefore conclude that the genotype is the main factor shaping olive belowground microbial communities, this factor being more determinant for the rhizosphere than for the endosphere, and more crucial for the bacteriota than for the mycobiota (see PERMANOVA R^2 in results).

Proteobacteria has been described as the predominant prokaryotic phylum (about 90% of the relative abundance) present in root endophytic communities[26,27]. The same was observed for prokaryotic communities of the olive phyllosphere[24]. However, in our study, *Proteobacteria* (26% average relative abundance) was clearly overcome by *Actinobacteria* (64% average

345 relative abundance) in the root endosphere. A similar finding has also been
346 reported in *Agave* spp., particularly during the dry season[25]. Interestingly, no
347 sequences belonging to the kingdom *Archaea* were detected in the root
348 endosphere in our study, in contrast to the results by Müller et al.[24] who
349 reported that *Archaea* was a major group in the olive phyllosphere. In this latter
350 study as well as in ours, the reverse primer used was the same. However, the
351 forward primer used in our study has 94.6% archaeal amplification efficiency[28].
352 *Archaea* representatives were not found in the olive rhizosphere indicating that,
353 without excluding the potential bias introduced by the primer pair here used, this
354 kingdom is poorly represented in the olive belowground microbiota at least at
355 the sampling time and under environmental conditions in which olive trees are
356 cultivated in the WOGC.

357 The olive-associated microbiome harbors an important reservoir of
358 beneficial microorganisms that can be used as plant growth promotion and/or
359 biocontrol tools[15,24]. Moreover, bacterial antagonists of olive phytopathogens
360 isolated from the olive root endosphere or the rhizosphere have the advantage
361 to be adapted to the ecological niche where they can potentially exert their
362 beneficial effect[18]. For instance, *Proteobacteria* and *Firmicutes* representatives,
363 usually found as natural inhabitants from the olive rhizosphere, are thus good
364 examples of effective antagonists against *V. dahliae*[16–18,29]. Besides,
365 representatives of these phyla such as the genera *Pseudomonas* and *Bacillus*
366 are easy to isolate, manipulate, propagate and formulate as BCAs. In addition
367 to these well-known genera, species of the actinobacterial genus
368 *Strepytomyces* have also been demonstrated as excellent BCAs in different
369 pathosystems[30,31]. Moreover, the potential biocontrol of non-streptomycete

370 *Actinobacteria* genera has been reported as well[30,32–34]. Taking into account that
371 the prevalence of *Actinobacteria* found in our study (the genera
372 *Actinophytocola*, *Streptomyces* and *Pseudonocardia* ranged from 30 to 60% of
373 the bacterial olive root endophytic community), the isolation and in-depth
374 characterization of culturable representatives of these genera will be of interest
375 for their assessment as potential PGPR and/or BCA against olive tree
376 pathogens. The genus *Actinophytocola*, described for the first time in 2010[35] as
377 a root endophytic actinobacteria (*Pseudonocardiaceae* family), has been
378 isolated from Saharan non-rhizosphere soils in the south of Algeria[34].
379 Interestingly enough, these authors demonstrated its antimicrobial ability
380 against some bacteria and fungi. *Actinophytocola* sp., in addition to other
381 actinomycetes, has also been demonstrated to inhibit the growth of well-known
382 human pathogens (*B. subtilis* and *S. aureus*)[36]. Finally, *Actinophytocola gilvus*
383 was recently isolated from extremely dry conditions, from a soil crusts sample
384 collected in the Tengger Desert in China[37]. Considering that this genus was
385 ubiquitously and abundantly found in our study, *Actinophytocola* spp. inhabiting
386 olive roots can be relevant for olive fitness and health (i.e. drought tolerance,
387 broad antimicrobial activity range, etc.), what grants further research efforts
388 aiming to isolate and characterize members of this relevant component of the
389 olive belowground microbiome.

390 This is the first study in which a high-throughput sequencing approach
391 has been implemented to unravel the olive belowground fungal communities.
392 *Sordariomycetes* (38%) and *Eurotiomycetes* (23%), both belonging to
393 *Ascomycota*, were found as the most abundant fungal classes in the root
394 endosphere of olive. *Sordariomycetes* was previously found as the main

395 endophytic fungal class in olive roots using a culture-dependent approach[20].
396 The endophytic fungal communities earlier found in aboveground olive tree
397 compartments (phyllosphere and carposphere) by high-throughput
398 sequencing[23] or a culture-dependent approach[20], differed from belowground
399 communities reported in our study. This outcome reinforces previous reports
400 showing important differences between above- and belowground olive fungal
401 communities, regardless the methodological approach implemented to study
402 them[20,22,23]. Interestingly enough, *Sordariomycetes* was also the most abundant
403 class found in olive fruits regardless or not the presence of anthracnose
404 symptoms[23], pointing to the fact that this fungal class seems to be ubiquitously
405 colonizing the interior of olive tissues. In the rhizosphere, *Agaricomycetes*
406 (12.7%), belonging to *Basidiomycota*, and *Eurotiomycetes* (12.6%) were the
407 most abundant classes. At this taxonomic level, the main difference between
408 the two compartments was the percentage of unclassified sequences (12.4% in
409 root endosphere and 35.6% in rhizosphere). Furthermore, in the particular case
410 of cvs. Abou Kanani and Shengeh, unclassified sequences represented more
411 than 80% of the good quality sequences found in the rhizosphere. The high
412 percentage of unclassified sequences in this compartment seems to be a
413 common finding[38], although less pronounced in annual plants[39], when using the
414 same fungal database. According to previous studies and data here obtained,
415 the olive rhizosphere carries a huge fungal diversity yet to be discovered. We
416 have to take into account that much of those unclassified sequences do not
417 belong to unknown fungi but, they were not properly classified due to limitation
418 in the method and the database currently available. Notwithstanding, this may

419 have important ecological implications for the tree, and pose novel agro-
420 biotechnological avenues to be explored.

421 At the genus level, the structure and composition of olive belowground
422 fungal communities also showed important differences compared to previous
423 reports. For instance, in the particular case of phytopathogenic fungi,
424 *Phomopsis columnaris* (fungus species causing of twig dieback of *Vaccinium*
425 *vitis-idaea* [lingonberry])[40] and *Fusarium oxyporum* were found by Martins et
426 al.[20] as the most relative abundant species, although sampled trees did not
427 show visible symptoms. These results are far apart from ours. In our study, the
428 above-mentioned species and the genera to which they belong were absent.
429 However, the pathogenic fungi *Macrophomina phaseolina* showed relevant
430 relative abundance in several cultivars and for both compartments. *Macrophina*
431 *phaseolina* is a well-known pathogen causing charcoal rot in important crops
432 including olive[16,41–44], and it has also been shown that olive leaves produce
433 compounds able to reduce its pathogenic activity[45]. This finding raises the
434 possibility that *M. phaseolina* could be a common component of the olive-
435 associated microbiota, but may reside within olive tissues without causing
436 visible symptoms until external factors and/or microbiota alterations (dysbiosis)
437 trigger a pathogenic stage. With regard to relevant soil-borne olive pathogens, it
438 is worth mentioning that neither sequences corresponding to *Verticillium* spp.
439 and *Fusarium* spp. nor to the oomycetes *Phytophthora* spp. and *Phytium* spp.
440 were found in our study, confirming the good phytosanitary status in the WOGC
441 soil. Finally, and regarding beneficial fungi, representatives of the genus
442 *Trichoderma* were found in the rhizosphere of all cultivars but Chemlal de

443 Kabylie and Llumeta. Species of this genus have been successfully used as
444 BCA against VW of olive[46,47].

445 In the olive belowground (endophytic and rhizosphere) core bacteriota
446 here reported, genera from which some species have been well characterized
447 and described as BCA are present. For instance, *Streptomyces* was the second
448 most abundant genus in the endosphere whereas *Bacillus* was the tenth more
449 abundant in the rhizosphere. While *Pseudomonas* was part of the rhizosphere
450 core bacteriota, it was not considered as constituent of the endophytic core
451 because it was absent in the root endosphere of cv. Mavreya. Nevertheless,
452 *Pseudomonas* was relatively much more abundant inside olive root tissues than
453 in the rhizosphere. Regarding the core mycobiota, and as mentioned above, the
454 most noticeable presence of a pathogenic fungus was that of *Macrophomina*,
455 and to a lesser extent *Colletotrichum.* The reported core microbiota indicates
456 that, under the conditions found in the WOGC, olive trees harbor an important
457 reservoir of beneficial/neutral microbes, and that the presence of deleterious
458 microorganisms is nearly anecdotal. This correlates with the good development
459 and appearance of the trees in the examined orchard, showing no visible
460 symptoms of biotic stresses. The role of native microbiota in protecting plants
461 from soil-borne pathogens has been highlighted in previous studies[48].
462 Nonetheless, further studies have to be carried out in the presence of soil-borne
463 pathogens, such as *Verticillium dahliae*, to study the community alterations and
464 confirm the protective role of some of the core microorganisms described in the
465 present study.
466
467

4. Materials and methods

4.1. Sample Collection

Soil and root samples were collected from the World Olive Germplasm Collection (WOGC) (37º51'38.11'' N; 4º48'28.61'' W; 102 m.a.s.l.) located at the *Instituto de Investigación y Formación Agraria y Pesquera* (IFAPA, Córdoba, Spain) in the spring of 2017, when the trees were in full bloom. The selected 36 olive cultivars (Table 1) surveyed are grown in the same orchard to avoid differences related to the physicochemical characteristics of the soil, water availability, agricultural management, weather conditions or any other influencing factor. The cultivars selected represent the subset of the working olive core collection from the WOGC[49]. Geographical origin and commercial interest of varieties were the main criteria to choose these cultivars for downstream studies. The upper layer (first 5 cm) of soil was removed and rhizosphere soil samples were collected (5 to 20-cm depth) following the main roots of each plant until finding non-suberified roots. Root samples were also collected from the same plant to assess the root endophytic communities. Three soil and root samples from different trees of each cultivar were collected (n = 108). Furthermore, 10 bulk soil samples (1 kg) were collected at 1-1.5 m trunk distance of randomly selected trees (among the ones chosen for soil/root sampling) to analyze a number of physicochemical parameters of the WOGC soil (Table S5). These spots were randomly scattered along the orchard. Bulk soil samples in plastic bags were then transferred to the Agri-Food Laboratory of the Andalusian Regional Government at Córdoba (Spain), where physiochemical analyses were performed using standardized procedures.

493

494 *4.2. DNA extraction and Illumina sequencing*

495 The soil DNA from each individual sample was obtained using the Power Soil
496 DNA Isolation kit (MoBio, Laboratories Inc., CA), following the manufacturer's
497 recommendations within 24 hours of samples collection. The root DNA was
498 obtained, after root surface sterilization and grinding, using 'Illustra DNA
499 extraction kit Phytopure' (GE Healthcare, Little Chalfont, UK). To ensure that
500 DNA originated from endophytic microorganisms, and that microorganisms
501 attached to the rhizoplane were eliminated, a thorough root surface sterilization
502 protocol was implemented. Firstly, 20 ml of NaCl 0.8 % were added to 50 ml
503 screw cap polypropylene tubes containing each root sample. Tubes were then
504 vigorously shaken in order to remove adhering soil particles. After discarding
505 the supernatant, roots were washed five times with distilled water. Secondly, the
506 following root surface sterilization protocol was implemented: 70% ethanol for 5
507 min, NaClO (3.7%) containing Tween 20 0.01 % for 3 min, and finally 3 rinses in
508 sterile and distilled water. To confirm that the disinfection protocol was
509 successful, aliquots (100 µl) of water from the final rinse were plated in NA
510 (Nutrient Agar) and PDA (Potato Dextrose Agar) plates that were incubated at
511 28ºC for 7 days. Then, plates were examined to confirm the absence of
512 microbial growth. DNA yields and quality were checked both by electrophoresis
513 in 0.8% (w/v) agarose gels stained with GelRed and visualized under UV light,
514 and using a Qubit 3.0 fluorometer (Life Technologies, Grand Island, NY). The
515 DNA was sequenced with Illumina MiSeq platform in a commercial sequencing
516 service (The Institute of Parasitology and Biomedicine "López Neyra", CSIC,
517 Granada, Spain). In the first run, a prokaryotic library was constructed

amplifying the hyper-variable regions V3-V4 of the 16S rRNA gene using the primer pair Pro341F and Pro805R according to Takahashi et al.[28]. In the second run, an eukaryotic library was constructed amplifying the ITS1 region using the primer pair ITS1FI2 and ITS2 according to Schmidt et al.[50] and developed by White et al.[51]. Both runs were sequenced using a paired-end 2x300-bp (PE 300) strategy. These sequence data have been submitted to the NCBI Sequence Read Archive (SRA) under the BioProject number PRJNA498945.

4.3. Data quality screening and overlapping

Demultiplexed and Phi-X174-free reads were quality checked with FastQC v.0.11.5[52] and end-trimmed with FASTX-Toolkit v.0.014[53]. All the 3' end nucleotides were removed until the first position which reached an average quality value bigger than Q25. The paired reads were overlapped with fastq-join v.1.3.1[54] requesting a minimum overlap of 40 bp and a maximum of 15 % of difference in the overlapping region. Both libraries were processed with the same bioinformatics tools but following different pathways detailed below.

4.4. Prokaryotic data processing

The overlapped reads from the prokaryotic (Bacteria and Archaea) library were initially classified with an 80% bootstrap cutoff to the Ribosomal Database Project (RDP-II) 16S rRNA reference database, training set v.16 MOTHUR-formatted[55], with MOTHUR v.1.39.5[56]. This initial step was performed to remove reads belonging to mitochondria, chloroplast and not identified at kingdom level (unknown). Then, using the software SEED2 v.2.1.05[57] the prokaryotic sequences were trimmed and clustered. Firstly, by trimming the specific

543 primers; then, by removing sequences with ambiguities and shorter than 400 bp
544 as well as reads with an average read quality lower than Q30. Secondly,
545 chimeric reads were removed by VSEARCH "De Novo" v.2.4.3[58] implemented
546 in SEED2 and OTUs were clustered with the same tool at 97% similarity.
547 Finally, the OTU table was saved and OTUs accounting for less than 0.005% of
548 the total sequences were removed according to Bokulich et al.[59] for further
549 analyses. The most abundant OTU sequences were retrieved in SEED2 and
550 classified as mentioned above. This classification was considered as the
551 taxonomic information of each OTU.
552
553 *4.5. Eukaryotic data processing*
554 The eukaryotic library was directly quality-trimmed in SEED2 by the removal of
555 sequences with ambiguities and an average read quality lower than Q30. There
556 was not size exclusion and the primers were initially kept for the next step.
557 Subsequently, ITSx v.1.0.11[60] was performed but the result was discarded
558 because of it was unable to properly recognize and remove the forward primer
559 (ITS1FI2). Then, to accurately extract the ITS1 region, the high quality reads
560 were aligned against the ribosomal operons of *Saccharomyces cerevisiae*
561 S288c using Geneious R11[61]. As expected, the forward primer plus 4 nt
562 matched the end of the 18S rRNA gene, and the reverse primer plus 30 nt
563 matched the beginning of the 5.8S rRNA gene. Both intragenic ends were
564 removed using SEED2 and chimeric sequences identified and discarded with
565 VSEARCH "De Novo" implemented in SEED2. Then, the good quality
566 sequences were distance-based greedy clustered at 97% similarity with
567 VSEARCH algorithm implemented in MOTHUR. The most abundant OTU

568 sequences were classified using the UNITE v.7.2 dynamic database[62] with
569 MOTHUR following the parameters recommended in the website and used by
570 Findley et al.[63]. Finally, only OTUs with more than 0.005% of the sequences
571 and assigned to kingdom Fungi were kept for further analyses. Furthermore,
572 OTUs assigned to the phylum Oomycota were manually checked to examine
573 the (possible) presence of the phytopathogenic genera.
574
575 *4.6. Statistical analyses*
576 Alpha diversity indices (observed and Chao1 richness; Shannon and inverse of
577 Simpson diversity) were compared with Kruskal-Wallis test and p-values were
578 FDR corrected by the Benjamini-Hochberg method using the R package
579 a*gricolae*[64]. Concerning the beta diversity, a normalization of the filtered OTU
580 sequence counts was performed using the "trimmed means of M" (TMM)
581 method with the BioConductor package *edgeR*[65]. The normalized data were
582 considered to perform Nonmetric MultiDimensional Scaling (NMDS) on Bray-
583 Curtis dissimilarities to ordinate in two dimensions the variance of beta diversity
584 between compartments (root endosphere and rhizosphere) and among cultivars
585 in each compartment, in both kingdoms. Ordination analyses were performed
586 using the R package *phyloseq*[66]. We analyzed compartment and olive cultivar
587 effects on community dissimilarities with permutational analysis of variance
588 (PERMANOVA) and permutational analysis of multivariate dispersions
589 (BETADISPER) using the functions *adonis* and *betadisper* in the *vegan*
590 package with 9,999 permutations[67]. Significant prokaryotic or fungal genera in
591 olive cultivar were obtained with the following protocol: i) we tested for
592 differential genus abundance using likelihood ratio tests (LRT) in the normalized

593 data with the R package *edgeR*; ii) we tested for differential genus abundance
594 using proportions in non-normalized counts with the STAMP v.2.1.3 software[68],
595 selecting default statistical comparisons for multiple groups and firstly
596 considering both Benjamini-Hochberg FDR for multiple test correction and
597 without FDR correction; iii) those genera significantly different in the two
598 methods previously described were plotted and manually checked to generate
599 the final selection. Most of the steps performed on R were carried out following
600 the R script publicly donated by Hartman et al.[69].
601
602
603 References
604
605 1. Uylaşer, V. & Yildiz, G. The Historical Development and Nutritional
606 Importance of Olive and Olive Oil Constituted an Important Part of the
607 Mediterranean Diet. *Crit. Rev. Food Sci. Nutr.* **54,** 1092–1101 (2014).
608 2. López-Escudero, F. J. & Mercado-Blanco, J. Verticillium wilt of olive: A
609 case study to implement an integrated strategy to control a soil-borne
610 pathogen. *Plant Soil* **344,** 1–50 (2011).
611 3. FAO Food and Agriculture of the United Nations. No Title. *FAOSTAT*
612 (2017). Available at: http://www.fao.org/FAOSTAT/EN/.
613 4. IOC. ioc. Available at:
614 http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-
615 figures?lang=en_US.
616 5. Pérez-Jiménez, F., Ruano, J., Perez-Martinez, P., Lopez-Segura, F. &
617 Lopez-Miranda, J. The influence of olive oil on human health: not a

618 question of fat alone. *Mol. Nutr. Food Res.* **51,** 1199–1208 (2007).
619 6. Schena, L., Agosteo, G. E., Cacciola, S. O. & Sergeeva, V. *Olive*
620 *diseases and disorders*. (Transworld Research Network, 2011).
621 7. Sanzani, S. M. *et al.* O Ffered R Eview. **94,** 469–491 (2012).
622 8. Mercado-Blanco, J. *et al.* Belowground microbiota and the health of tree
623 crops. *Front. Microbiol.* **9,** 1–27 (2018).
624 9. BENITEZ, Y. *et al.* Molecular analysis of the interaction between Olea
625 europaea and the biotrophic fungus Spilocaea oleagina. *Mol. Plant*
626 *Pathol.* **6,** 425–438 (2005).
627 10. (Lahkim), L. T. Epidemiology and control of Verticillium wilt on olive. *Isr. J.*
628 *Plant Sci.* **59,** 59–69 (2011).
629 11. Ramos, C., Matas, I. M., Bardaji, L., Aragón, I. M. & Murillo, J.
630 Pseudomonas savastanoi pv. savastanoi: some like it knot. *Mol. Plant*
631 *Pathol.* **13,** 998–1009 (2012).
632 12. Moral, J. *et al.* Variability in Susceptibility to Anthracnose in the World
633 Collection of Olive Cultivars of Cordoba (Spain). *Front. Plant Sci.* **8,** 1–11
634 (2017).
635 13. Saponari, M., Boscia, D., Nigro, F. & Martelli, G. P. FUNGAL SPECIES
636 ASSOCIATED WITH A SEVERE DECLINE OF OLIVE IN
637 IDENTIFICATION OF DNA SEQUENCES RELATED TO XYLELLA
638 FASTIDIOSA IN OLEANDER , ALMOND AND OLIVE TREES
639 EXHIBITING LEAF SCORCH SYMPTOMS IN APULIA (SOUTHERN
640 ITALY). **95,** 5442911 (2013).
641 14. Rallo, L. *et al.* High-Density Olive Plantations. *Horticultural Reviews*
642 *Volume 41* (2013). doi:doi:10.1002/9781118707418.ch07

15. Aranda, S., Montes-Borrego, M., Jiménez-Díaz, R. M. & Landa, B. B. Microbial communities associated with the root system of wild olives (Olea europaea L. subsp. europaea var. sylvestris) are good reservoirs of bacteria with antagonistic potential against Verticillium dahliae. *Plant Soil* **343,** 329–345 (2011).

16. Sanei, S. J. & Razavi, S. E. Charcoal rot in nursery of olive in Golestan province of Iran. *Soil Sci.* **1,** 211–217 (2012).

17. Gómez-Lama Cabanás, C. *et al.* Indigenous Pseudomonas spp. Strains from the Olive (Olea europaea L.) Rhizosphere as Effective Biocontrol Agents against Verticillium dahliae: From the Host Roots to the Bacterial Genomes . *Frontiers in Microbiology* **9,** 277 (2018).

18. Gomez-Lama Cabanas, C. *et al.* Bacillales Members from the Olive Rhizosphere Are Effective Biological Control Agents against the Defoliating Pathotype of Verticillium dahliae. **8,** 1–23 (2018).

19. Mercado-blanco, J. & Lugtenberg, B. J. J. Biotechnological Applications of Bacterial Endophytes. 60–75 (2014).

20. Martins, F., Pereira, J. A., Bota, P., Bento, A. & Baptista, P. Fungal endophyte communities in above- and belowground olive tree organs and the effect of season and geographic location on their structures. *Fungal Ecol.* **20,** 193–201 (2016).

21. Pascazio, S. *et al.* Phyllosphere and carposphere bacterial communities in olive plants subjected to different cultural practices. *Int. J. Plant Biol.* **6,** 15–19 (2015).

22. Preto, G., Martins, F., Pereira, J. A. & Baptista, P. Fungal community in olive fruits of cultivars with different susceptibilities to anthracnose and

668 selection of isolates to be used as biocontrol agents. *Biol. Control* **110,** 1–
669 9 (2017).
670 23. Abdelfattah, A., Li Destri Nicosia, M. G., Cacciola, S. O., Droby, S. &
671 Schena, L. Metabarcoding analysis of fungal diversity in the phyllosphere
672 and carposphere of olive (Olea europaea). *PLoS One* **10,** 1–19 (2015).
673 24. Müller, H. *et al.* Plant genotype-specific archaeal and bacterial
674 endophytes but similar Bacillus antagonists colonize Mediterranean olive
675 trees. *Front. Microbiol.* **6,** 1–9 (2015).
676 25. Coleman-derr, D. *et al.* Recent Trends in Valuation. *New Phytol.* **209,**
677 798–811 (2016).
678 26. Beckers, B., De Beeck, M. O., Weyens, N., Boerjan, W. & Vangronsveld,
679 J. Structural variability and niche differentiation in the rhizosphere and
680 endosphere bacterial microbiome of field-grown poplar trees. *Microbiome*
681 **5,** 1–17 (2017).
682 27. Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M. &
683 Glick, B. R. Plant growth-promoting bacterial endophytes. *Microbiol. Res.*
684 **183,** 92–99 (2016).
685 28. Takahashi, S., Tomita, J., Nishioka, K., Hisada, T. & Nishijima, M.
686 Development of a Prokaryotic Universal Primer for Simultaneous Analysis
687 of Bacteria and Archaea Using Next-Generation Sequencing. *PLoS One*
688 **9,** 1–9 (2014).
689 29. Maldonado-González, M. M., Schilirò, E., Prieto, P. & Mercado-Blanco, J.
690 Endophytic colonization and biocontrol performance of Pseudomonas
691 fluorescens PICF7 in olive (Olea europaea L.) are determined neither by
692 pyoverdine production nor swimming motility. *Environ. Microbiol.* **17,**

693 3139–3153 (2015).
694 30. El-Tarabily, K. A. & Sivasithamparam, K. Non-streptomycete
695 actinomycetes as biocontrol agents of soil-borne fungal plant pathogens
696 and as plant growth promoters. *Soil Biol. Biochem.* **38,** 1505–1520
697 (2006).
698 31. Law, J. W. F. *et al.* The potential of streptomyces as biocontrol agents
699 against the rice blast fungus, Magnaporthe oryzae (Pyricularia oryzae).
700 *Front. Microbiol.* **8,** (2017).
701 32. El-Tarabily, K. A. An endophytic chitinase-producing isolate of
702 Actinoplanes missouriensis, with potential for biological control of root rot
703 of lupin caused by Plectosporium tabacinum. *Aust. J. Bot.* **51,** 257–266
704 (2003).
705 33. Shimizu, M. in *Bacteria in Agrobiology: Plant Growth Responses* (ed.
706 Maheshwari, D.) 201–220 (Springer, Berlin, Heidelberg, 2011).
707 doi:10.1007/978-3-642-20332-9
708 34. Chaouch, F. C. *et al.* Planomonospora, Saccharothrix and
709 Actinophytocola genera in Saharan soils of Algeria: Isolation, taxonomic
710 identification and antagonistic properties. *J. Microbiol. Biotechnol. Food*
711 *Sci.* **7,** 505–510 (2018).
712 35. Indananda, C. *et al.* Actinophytocola oryzae gen. nov., sp. nov., isolated
713 from the roots of Thai glutinous rice plants, a new member of the family
714 Pseudonocardiaceae. *Int. J. Syst. Evol. Microbiol.* **60,** 1141–1146 (2010).
715 36. Abdul Malek, N., Zainuddin, Z., Khan Chowdhury, A. J. & Zainal Abidin, Z.
716 A. DIVERSITY AND ANTIMICROBIAL ACTIVITY OF MANGROVE SOIL
717 ACTINOMYCETES ISOLATED FROM TANJUNG LUMPUR, KUANTA. *J.*

718 *Teknol.* **25,** 37–43 (2015).

719 37. Sun, H. M. *et al.* Actinophytocola gilvus sp. nov., Isolated from desert soil
720 crusts, And emended description of the genus Actinophytocola Indananda
721 et al. 2010. *Int. J. Syst. Evol. Microbiol.* **64,** 3120–3125 (2014).

722 38. Sanka Loganathachetti, D., Poosakkannu, A. & Muthuraman, S. Fungal
723 community assemblage of different soil compartments in mangrove
724 ecosystem. *Sci. Rep.* **7,** 1–9 (2017).

725 39. Wang, Z. *et al.* Fungal Communities in Rhizosphere Soil under
726 Conservation Tillage Shift in Response to Plant Growth. *Front. Microbiol.*
727 **8,** 1–11 (2017).

728 40. Farr, D. F., Castlebury, L. A., Rossman, A. Y. & Putnam, M. L. A new
729 species of Phomopsis causing twig dieback of Vaccinium vitis-idaea
730 (lingonberry). *Mycol. Res.* **106,** 745–752 (2002).

731 41. Sergeeva, V., Tesoriero, L., Spooner-Hart, R. & Nair, N. First report of
732 Macrophomina phaseolina on olives (Olea europaea) in Australia.
733 *Australas. Plant Pathol.* **34,** 273–274 (2005).

734 42. Mahdizadeh, V., Safaie, N. & Goltapeh, E. M. Diversity of Macrophomina
735 phaseolina based on morphological and genotypic characteristics in Iran.
736 *Plant Pathol. J.* **27,** 128–137 (2011).

737 43. Kaur, S. *et al.* Emerging phytopathogen Macrophomina phaseolina:
738 Biology, economic importance and current diagnostic trends. *Crit. Rev.*
739 *Microbiol.* **38,** 136–151 (2012).

740 44. Chamorro, M. *et al.* Evaluation of biosolarization for the control of
741 charcoal rot disease (Macrophomina phaseolina) in strawberry. *Crop Prot.*
742 **67,** 279–286 (2015).

743 45. Iqbal, U., Mukhtar, T. & Iqbal, S. M. In vitro and in vivo evaluation of
744 antifungal activities of some antagonistic plants against charcoal rot
745 causing fungus Macrophomina phaseolina. *Pakistan J. Agric. Sci.* **51,**
746 689–694 (2014).
747 46. Ruano-Rosa, D. *et al.* Fate of Trichoderma harzianum in the olive
748 rhizosphere: time course of the root colonization process and interaction
749 with the fungal pathogen Verticillium dahliae. *BioControl* **61,** 269–282
750 (2016).
751 47. Carrero-Carrón, I. *et al.* Interactions between Trichoderma harzianum and
752 defoliating Verticillium dahliae in resistant and susceptible wild olive
753 clones. *Plant Pathol.* **67,** 1758–1767 (2018).
754 48. Mendes, R. *et al.* Deciphering the Rhizosphere Microbiome for Disease-
755 Suppressive Bacteria. *Science (80-.).* **332,** 1097–1100 (2011).
756 49. Belaj, A. *et al.* Developing a core collection of olive (Olea europaea L.)
757 based on molecular markers (DArTs, SSRs, SNPs) and agronomic traits.
758 *Tree Genet. Genomes* **8,** 365–378 (2012).
759 50. Schmidt, P. A. *et al.* Illumina metabarcoding of a soil fungal community.
760 *Soil Biol. Biochem.* **65,** 128–132 (2013).
761 51. White, T., Bruns, T., Lee, S. & Taylor, J. in *PCR Protocols: A Guide to*
762 *Methods and Applications* (eds. Innis, M., Gelfand, D., Shinsky, J. &
763 White, T.) 315–322 (Academic Press, 1990).
764 52. FastQC. Available at:
765 http://www.bioinformatics.babraham.ac.uk/projects/fastqc/. (Accessed:
766 14th March 2019)
767 53. FASTX-Toolkit. Available at:

http://hannonlab.cshl.edu/fastx_toolkit/index.html. (Accessed: 14th March 2019)

54. Aronesty. fastq-join. (2011). Available at: https://github.com/ExpressionAnalysis/ea-utils. (Accessed: 14th March 2019)

55. RDP reference files - mothur. Available at: https://mothur.org/wiki/RDP_reference_files. (Accessed: 14th March 2019)

56. Schloss, P. D. *et al.* Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl. Environ. Microbiol.* **75,** 7537 LP-7541 (2009).

57. Větrovský, T., Baldrian, P. & Morais, D. SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses. *Bioinformatics* **34,** 2292–2294 (2018).

58. Rognes, T., Flouri, T., Nichols, B., Quince, C. & Mahé, F. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4,** e2584 (2016).

59. Bokulich, N. A. *et al.* Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nat. Methods* **10,** 57–59 (2013).

60. Bengtsson-Palme, J. *et al.* Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods Ecol. Evol.* **4,** 914–919 (2013).

61. Kearse, M. *et al.* Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data.

Bioinformatics **28,** 1647–1649 (2012).

62. Unite Community 2017. (2017). Available at: https://plutof.ut.ee/#/doi/10.15156/BIO/587478. (Accessed: 14th March 2019)

63. Findley, K. *et al.* Topographic diversity of fungal and bacterial communities in human skin. *Nature* **498,** 367–370 (2013).

64. de Mendiburu. CRAN - Package agricolae. (2017). Available at: https://cran.r-project.org/web/packages/agricolae/index.html. (Accessed: 14th March 2019)

65. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26,** 139–140 (2009).

66. Mcmurdie, P. J. & Holmes, S. phyloseq : An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. **8,** (2013).

67. Oksanen *et al*. Vegan: community ecology package (2015).

68. Parks, D. H., Tyson, G. W., Hugenholtz, P. & Beiko, R. G. STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics* **30,** 3123–3124 (2014).

69. Hartman, K. *et al.* Correction to: Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome* **6,** 1–14 (2018).

Acknowledgments

This work was supported by grant AGL2016-75729-C2-1-R from the Spanish Ministerio de Economía, Industria y Competitividad/Agencia Estatal de

818 Investigación, and co-financed by the European Regional Development Fund
819 (ERDF).
820 We thank Dr. José F. Cobo-Díaz from the Université de Brest, Laboratoire
821 Universitaire de Biodiversité et Ecologie Microbieene IBSAM, ESIAB, Plouzané,
822 France for technical support in eukaryotic data analysis; and to PhD student
823 Ana V. Lasa from Departamento de Microbiología del Suelo y Sistemas
824 Simbióticos, Estación Experimental del Zaidín – CSIC for technical support in R
825 scripts.

826

827 Author Contributions

828 JMB and MFL conceived the study and performed its design. AJFG, JMB and
829 MFL wrote the manuscript. AB maintains the crop system and helped with
830 sampling. PJV, CGLC and AVC conducted the sampling and DNA extractions.
831 AJFG performed the bioinformatics analysis and analyzed the data. All authors
832 read and approved the final manuscript.

833

834 Additional Information

835 Competing Interests

836 The authors declare that they have no competing interests.

837

838 Figure captions

839 Figure 1. NMDS of bacterial (a) and fungal (b) communities by compartment.
840 The letters A, B and c after the numbers were used to distinguish the 3
841 replicates of each cultivar. The different colors indicate the country of origin of
842 the cultivars.

Figure 2. NMDS of bacterial communities from rhizosphere. The letters A, B and C after the numbers were used to distinguish the 3 replicates of each cultivar. The different colors indicate the country of origin of the cultivars.

Figure 3. NMDS of bacterial communities from root endosphere. The letters A, B and C after the numbers were used to distinguish the 3 replicates of each cultivar. The different colors indicate the country of origin of the cultivars.

Figure 4. Bacterial phyla (class for *Proteobacteria*) in the root endosphere (a) and rhizosphere (b). The horizontal colored lines indicate the country of origin of the cultivars.

Figure 5. Fungal class in the root endosphere (a) and rhizosphere (b). The horizontal colored lines indicate the country of origin of the cultivars.

Supplementary Information

Table S1. Bacterial alpha diversity indices of each sample in both compartments. (xlsx)

Table S2. Fungal alpha diversity indices of each sample in both compartments. (xlsx)

868 Table S3. Core bacterial communities in the endosphere, rhizosphere and both
869 at genus level. (xlsx)

870

871 Table S4. Core fungal communities in the endosphere, rhizosphere and both at
872 genus level. (xlsx)

873

874 Table S5. Physicochemical properties of the soil from the World Olive
875 Germplasm Collection (Córdoba, Spain). (docx)

876

877 Figure S1. Normalized alpha diversity indices by compartment in the prokaryotic
878 (a) and the fungal (b) communities. Endosphere (Endo), Rhizosphere (Rhizo)
879 and Richness (Observed). (pptx)

880

881 Figure S2. Microbial (bacterial a and b; fungal c and d) normalized alpha
882 diversity indices of each sample clustered by cultivars in both compartments (a
883 and c endosphere; b and d rhizosphere). (pptx)

884

885 Figure S3. Statistically significant endophytic bacterial genera by cultivar (a, b)
886 and the main bacterial genera in the endosphere (c). (pptx)

887

888 Figure S4. Main bacterial genera in the rhizosphere. Asteriscs indicate
889 statistically significant differences in the relative abundance when comparing
890 cultivars. (pptx)

891

892 Figure S5. Statistically significant fungal endophytic (a) and rhizosphere (b)
893 genera by cultivar and the main fungal genera in the endosphere (c) and the

894 rhizosphere (d). The main fungal genera were highlighted with an asterisc to
895 indicate statistically significant differences in the relative abunance when
896 comparing cultivars. (pptx)

897

898

899

900 Table 1. The 36 olive cultivars sampled in the World Olive Germplasm
901 Collection (WOGC).

Cultivar	Country	Sample
Abbadi Abou Gabra-842	Syria	01
Abou Satl Mohazam	Syria	02
Abou Kanani	Syria	03
Arbequina	Spain	04
Barnea	Israel	05
Barri	Syria	06
Klon-14-1812	Albania	07
Chemlal de Kabylie	Algeria	08
Shengeh	Iran	09
Dokkar	Turkey	10
Forastera de Tortosa	Spain	11
Frantoio	Italy	12
Grappolo	Italy	13
Jabali	Syria	14
Kalamon	Greece	15
Koroneiki	Greece	16
Leccino	Italy	17
Llumeta	Spain	18
Maarri	Syria	19
Manzanilla de Sevilla	Spain	20
Manzanillera de Huercal Overa	Spain	21
Mari	Iran	22
Mastoidis	Greece	23
Mavreya	Greece	24
Majhol-1013	Syria	25
Majhol-152	Syria	26
Megaritiki	Greece	27
Menya	Spain	28
Morrut	Spain	29
Myrtolia	Greece	30
Picual	Spain	31
Picudo	Spain	32
Piñonera	Spain	33
Temprano	Spain	34
Uslu	Turkey	35
Verdial de Vélez-Málaga-1	Spain	36

902

903

Table 2. Comparisons of alpha diversity indices in the different microbial communities

Prokaryotes	Cultivar		Endosphere vs rhizosphere
Index	Root endosphere	Rhizosphere	Whole community
S_{obs}	**0.0178 (36.8)**	0.0500 (49.9)	**< 2.2e^{-16} (122.2)**
Chao1	**0.0357 (34.1)**	0.2117 (41.4)	**< 2.2e^{-16} (122.2)**
Shannon	0.0774 (30.8)	**4.6e^{-05} (77.6)**	**< 2.2e^{-16} (122.2)**
InvSimpson	0.0602 (31.9)	**8.5e^{-05} (83.2)**	**< 2.2e^{-16} (122.2)**
df	21	35	1

Fungi	Cultivar		Endosphere vs rhizosphere
Index	Root endosphere	Rhizosphere	Whole community
S_{obs}	**0.0018 (60.4)**	**0.0096 (57.5)**	**< 2.2e^{-16} (147.1)**
Chao1	**0.0133 (52.3)**	**0.0119 (56.6)**	**< 2.2e^{-16} (142.5)**
Shannon	**0.0014 (61.3)**	**0.0276 (52.8)**	**< 2.2e^{-16} (110.9)**
InvSimpson	**0.0127 (52.5)**	0.0593 (48.9)	**< 2.2e^{-16} (82.8)**
df	32	35	1

S_{obs}: Observed richness

df: degree of freedom

In bold: significant p-values considering a confidence interval of 95%

In brackets: chi-squared values

Table 3. Main (relative abundance ≥ 1%) core bacterial and fungal genera.

Bacterial core

Genus	Root endosphere (%)[1]	Rhizosphere (%)[2]
Actinophytocola	22.07	0.07
Streptomyces	13.17	0.31
Gp6	0.58	11.08
Gp4	0.26	9.31
Pseudonocardia	9.37	0.14
Rhizobium	2.00	7.71
Sphingomonas	0.77	5.92
Gemmatimonas	0.06	5.24
candidate_division_WPS-1[4]	0.08	3.92
Gp7	0.04	4.08
Bacillus	0.68	2.31
Bradyrhizobium	2.57	0.20
Ensifer	2.56	0.15
Rubrobacter	0.05	2.48
Subdivision3[4]	0.02	2.35
Steroidobacter	1.78	0.34
Candidatus_Saccharibacteria[4]	1.03	0.40
Saccharothrix	1.18	0.07
Ohtaekwangia	0.21	1.03
Mycobacterium	0.98	0.09
Nonomuraea	1.04	0.04

Fungal core

Genus	Root endosphere (%)[3]	Rhizosphere (%)[2]
Canalisporium	29.53	6.05
Macrophomina	10.93	2.44
Aspergillus	1.66	3.84
Malassezia	0.28	1.37

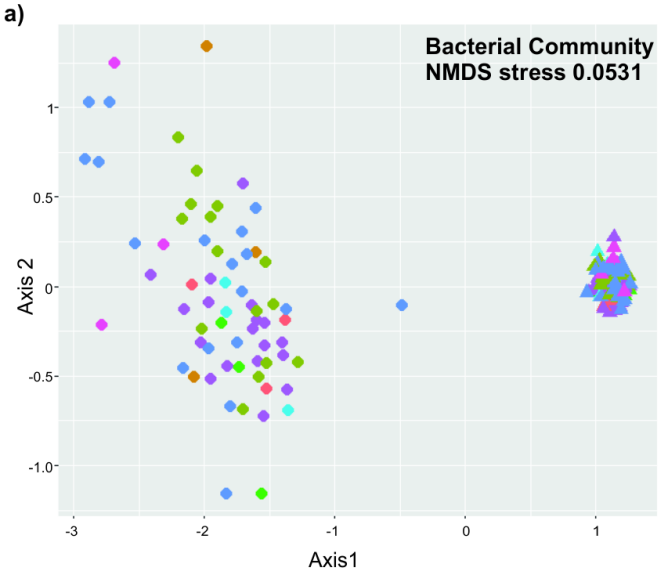
[1] Relative abundance average of 22 cultivars

[2] Relative abundance average of 36 cultivars

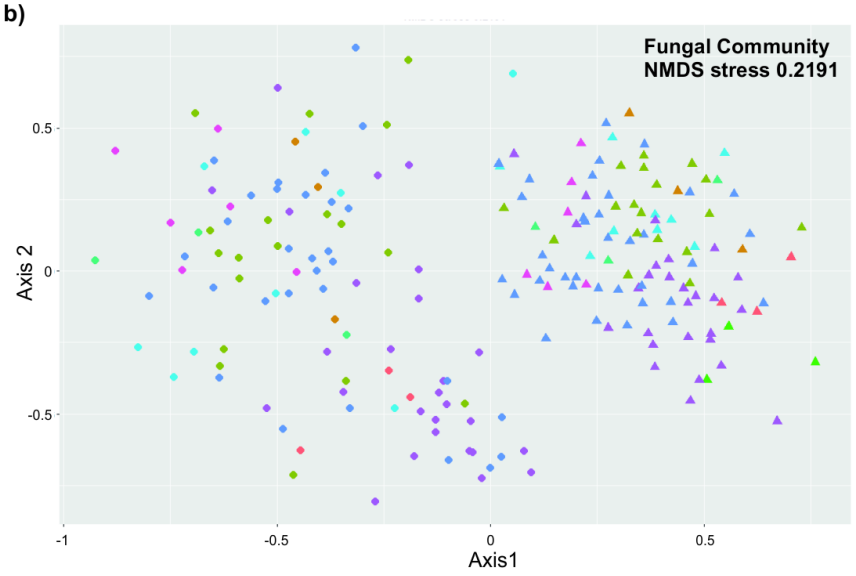
[3] Relative abundance average of 33 cultivars

[4] Name of *phylum*/class to which this *incertae sedis* genus belongs

a)



b)




Endophytic Bacterial Community
NMDS stress 0.1894
Axis 1
Axis 2
0.4
0.0
-0.4
-0.8
-0.5
0.0
0.5
1.0
35B
36C
18C
36A
16C
30B
29C
17C
18B
24A
35C
28C
17A
08B
35A
27B
23C
21A
23A
27C
21C
17B
23B
09C
06C
06A
06B
07A
28A
16A
24B
16B
18A
19C
02A
02B
14A
19B
29B
36B
04C
03A
14B
04A
30A
03C
28B
04B
24C
07C
19A
09A
21B
14C
29A
03B
25C
25A
27A
07B
25B
02C
08A
09B
08C
Albania
Algeria
Greece
Iran
Italy
Spain
Syria
Turkey