

Special Issue Article

Different patterns in root and soil fungal diversity drive plant productivity of the desert truffle *Terfezia claveryi* in plantation

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Summary

The desert truffle *Terfezia claveryi* is one of the few mycorrhizal fungi currently in cultivation in semiarid and arid areas. Agroclimatic parameters seem to affect its annual yield, but there is no information on the influence of biotic factors. In this study, fungal diversity was analysed by high-throughput sequencing of the ITS2 rDNA region from soil and root samples to compare productive and non-productive mycorrhizal plants in a 4-years old plantation (Murcia, Spain). The fungal metaprofile was dominated by Ascomycota phylum. Desert truffle productivity was driven by different patterns of fungal species composition in soil (species replacement) and root (species richness differences). Moreover, positive associations for ectomycorrhizal and negative for arbuscular mycorrhizal guilds were found in productive roots, and positive associations for fungal parasite-plant pathogen guild in non-productive ones. Soil samples were dominated by pathotroph and saprotroph trophic modes, showing positive associations for *Aureobasidium pullulans* and *Alternaria* sp. in productive areas, and positive associations for *Fusarium* sp. and *Mortierella* sp. were found in non-productive soils. Finally, some significant OTUs were identified and associated to ascocarp producing

patches, which could serve as predictive and location markers of desert truffle production.

Introduction

Desert truffles are a group of hypogeous fungi from arid and semiarid ecosystem, mostly located in the Mediterranean region. Species of the genera *Terfezia*, *Picoa*, *Tirmania*, *Balsamia*, *Geopora*, *Mattirolomyces*, *Kalahariturber*, *Eremiomyces* and *Choiromyces* belong to this group but mainly two of them are culinary and economically appreciated: *Terfezia* and *Tirmania* (Moreno *et al.*, 2014). *Terfezia claveryi* Chatin is associated in mycorrhizal symbiosis with some annual and perennial xerophytic host plants of the genus *Helianthemum*, belonging to the Cistaceae family, and its fruiting period is usually in early spring. Biotechnological advances on fungal inoculum and mycorrhizal plant production were developed to cultivate some species of *Terfezia* genus (Morte *et al.*, 2008). *T. claveryi* is one of the few edible and commercially viable mycorrhizal fungi and it has been the first desert truffle species to be cultivated, becoming itself as an agricultural crop in Spain for the last 10 years (Morte *et al.*, 2019). Moreover, this crop could play an important ecological role in arid and semi-arid ecosystems as natural desert truffle resource (sylviculture), conserving these areas from desertification or climate change processes (Honrubia *et al.*, 2014). The main difficulties for its cultivation are the fluctuations of the ascocarps production over the years (Morte *et al.*, 2017), in which 1 year is highly productive and the next has almost no truffle production (Morte *et al.*, 2012, 2020).

Different biotic and abiotic factors affect the truffle life cycle, promoting or inhibiting fruiting body formation (Mello *et al.*, 2006). Recently, some strategies focused on the control of agroclimatic parameters have been carried out in order to improve the desert truffle cultivation between *Helianthemum almeriense* × *T. claveryi*, in which the desert truffle production was correlated with the previous autumn and spring rainfalls and vapour pressure deficit (Andrino *et al.*, 2019; Marqués-Gálvez

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et al., 2020). Taking these proposals into account, fluctuations in sporocarp production across years can be reduced or solved, even ahead of the fruiting season to manage the plantations (Andrino et al., 2019; Marqués-Gálvez et al., 2020). Despite this, growers are still concerned about the spatial fluctuations found within the same plantation, because they describe as 'in patches' the way in which the desert truffles are fruiting. Thus, it leaves a large area of the plantation unproductive against plants that are highly productive of truffles, reduced to a small area or patch. In this new scenario, climatic factors are no longer a variable for the spatial distribution of the fruiting bodies. Other parameters could influence desert truffle fruiting such as soil characteristics, competitive species, MAT genes distribution and the presence of mycelium and mycorrhizae of *T. claveryi*.

It is known that bacterial communities associated with truffles have a possible role in truffle development (Barbieri et al., 2010; Antony-Babu et al., 2014; Splivallo et al., 2015; Benucci and Bonito, 2016; Monaco et al., 2021), and that fungal populations have a crucial role in *Tuber* truffle plantations, characterized by the coexistence of different species in roots and soil, where the replacement of the inoculated fungus by the natural ones could negatively affect the success of the harvests (De Miguel et al., 2014). The competition between truffle mycelium and others saprobic and mycorrhizal fungi for nutrients and space on host's roots should be controlled to preserve the truffle mycorrhiza, both in the nursery and in the field (Hall et al., 2003; Kennedy, 2010). In addition, this is more important in the initial years after planting, because the inoculated species are more vulnerable to being replaced (Zambonelli et al., 2012). Those facts could lead to the generation of productive and non-productive areas (or patches) inside the plantation depending on whether or not the microbial community facilitates the development of the fruiting body (Mello et al., 2010; Benucci et al., 2011; De Miguel et al., 2016). Nevertheless, there are still no clear and solid evidences that the microbial community has a positive or negative impact on fruiting body. Exploring fungal community inhabiting truffle plantations will give us a better understanding about the dynamic of the inoculated species throughout the plantation and the opportunity to identify a specific microbial community associated with high truffle productivity (Zambonelli et al., 2012; De Miguel et al., 2014).

Species diversity identification by classical morphological techniques has led to a poor characterization of the microbial diversity of truffle environment (Anderson and Cairney, 2004). In order to study the full fungal community, from both cultivable and uncultivable microorganisms, including rare species and those with very low presence which are difficult to detect, high-throughput

sequencing (HTS) based methods have made possible a large number of genomic, metagenomic and taxonomic studies on the microbial diversity in various biomes (Nowrousian, 2010; Lindahl et al., 2013; Tedersoo et al., 2016; Bajpai et al., 2019). Sequence-based metagenomic screening is currently the most popular approach to explore fungal biodiversity and community composition in different environments (e.g., endophytes, plant-pathogenic fungi, saprotrophic fungi, human-associated fungi, mycorrhizal fungi or aquatic fungi to mention a few) (Nilsson et al., 2019a). Improvements in bioinformatic algorithms and databases have also been made in recent years. Thanks to the large datasets of sequences from ecological and host-microorganism association studies, the knowledge on fungal communities in the environment has been expanded (Cuadros-Orellana et al., 2013). Nowadays, the fungal kingdom comprises a wide range of life strategies and it is estimated to contain up to 3.8 million species (Hawksworth and Lücking, 2017). In the last years, the application of metagenomic and bioinformatic tools have increased significantly the knowledge about the composition of bacterial and fungal communities in roots and surrounding soil associated with edible white and black truffles, *Tuber magnatum* and *Tuber melanosporum*, respectively (Mello et al., 2010, 2011; Napoli et al., 2010; Belfiori et al., 2012; Leonardi et al., 2013; Taschen et al., 2015, 2020 and De Miguel et al., 2016 among others), and also with other appreciated *Tuber* species such as *Tuber borchii* (Iotti et al., 2010), *Tuber aestivum* (Benucci et al., 2011) and *Tuber indicum* (Li et al., 2018).

In the framework on the domestication of desert truffle cultivation aimed at stabilizing the production of carpophores and identifying the ecological factors responsible for it, we hypothesize that fungal biodiversity is different between productive and non-productive areas. The occurrence of this scenario will allow us to relate specific taxa with the development of fruiting bodies. This work aims to provide a detailed profile of the fungal metacommunity associated with this desert truffle orchard (*H. almeriense* × *T. claveryi*) and how fungal populations have an impact on desert truffle life cycle, according to the starting hypothesis. Furthermore, this metagenomic approach, novelty in desert truffles, will provide a greater insight and understanding about the fungal community structure associated with this crop and its impact on large scale desert truffle production and plantation management.

Results

Fungal community of cultivated desert truffles

The whole data set gave 1259 OTUs (3,645,004 reads) and then it was screened by fungi, resulting in 1001

OTUs (3,529,379; 3.7% reads lost). After that, it was quality-filtered and 232,992 reads were discarded (6.6% reads lost). Finally, it was rarefied up to 48,835 reads per sample (Fig. S1) and 423 fungal OTUs were recorded. There was an average loss of 52% of the number of reads from the initial raw data after rarefaction (Table S1).

Ascomycota (84.9%) was the main phylum found in samples, followed by Basidiomycota (4.4%), Mortierellomycota (4.2%), Chytridiomycota (2.0%) and by a 4.5% of the unidentified fungus (Fig. S2). Glomeromycota, Olpidiomyces, Mucoromycota and Kickxellomycota phyla were also detected but in a very low proportion (0.03%, 0.02%, 0.02% and 0.005%, respectively). In all conditions, Ascomycota was the most abundant phylum, comprising 70%–99% of total reads. Soil and root of non-productive plants showed significantly lower abundance of Ascomycota fungi than soil and root of productive plants (from 72.4% and 86.3%–81.9% and 99% reads, respectively; Fig. S3), according to Pearson's Chi-squared test (Soil: $\chi^2 = 11,254$; $df = 1$; p -value $< 2.2e^{-16}$; Root: $\chi^2 = 51,695$; $df = 1$; p -value $< 2.2e^{-16}$). Soil presents higher number of OTUs than roots (422 vs.

224, respectively; Fig. S4). A loss of species was observed from the soil to the roots (*nestedness* pattern), considering the fungal species of root a subset of soil community. Productive and non-productive plants showed similar number of OTUs (413 vs. 420, respectively; Fig. S4).

At family level (Fig. S5), the fungi with the highest abundance were Pyrenopezizaceae (56.1%, 854,821 reads) followed by Pleosporaceae (5.5%, 83,732 reads), Mortierellaceae (4.7%, 71,634 reads), Nectriaceae (3.4%, 51,828 reads), Massarinaceae (2.3%, 34,869 reads), Aureobasidiaceae (2.2%, 33,234 reads), Clavicipitaceae (1.9%, 29,394 reads), and by a 19.1% of the total reads as unidentified fungus and 1.9% of not assigned taxonomy.

The 10 most abundant fungal genera were *Picoa* (51.1%, 753,622 reads), *Geopora* (6.6%, 97,603 reads), *Alternaria* (5.0%, 74,168 reads), *Mortierella* (4.9%, 71,634 reads), *Helminthosporium* (2.4%, 34,869 reads), *Aureobasidium* (2.3%, 33,234 reads), *Stachybotrys* (2.2%, 32,068 reads), *Metarhizium* (1.9%, 27,487 reads) and *Ilyonectria* (1.7%, 25,504 reads). Both in soil and roots, the abundance of *Picoa* genus is higher in productive plants than in non-

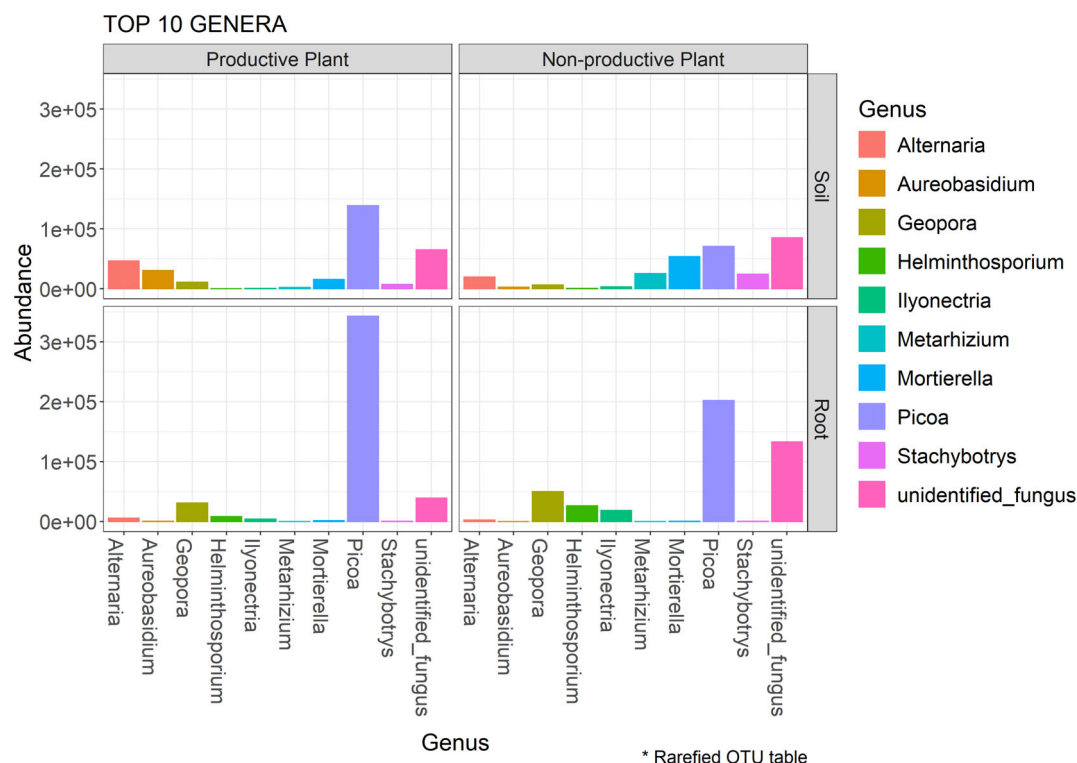


Fig. 1. The 10 most abundant genera identified in the desert truffle orchard in each condition, divided by compartment (soil above and root below) and type (productive plants on the left and non-productive on the right). Data shown was from rarefied OTU table of whole data set (423 fungal OTUs; 48,835 reads per sample).

productive ones (Fig. 1). But other genera such as *Mortierella*, *Stachybotrys* and *Metarhizium*, in soil, and *Geopora*, *Helminthosporium* and *Ilyonectria*, in roots, showed higher abundances in non-productive plants than in productive ones (Fig. 1). Unexpectedly, *Terfezia* was found in a very low proportion compared with these top 10 genera, where 4 and 5 reads were identified in productive and non-productive plants in root samples and, 232 and 69 reads in soils, respectively. This genus was represented by a single OTU identified as *T. claveryi* species.

Alpha diversity indices of the fungal communities, Chao1 and Shannon, showed significant effect regard the treatment on species richness (p -value = $5.48e^{-06}$ and $5.40e^{-06}$, respectively) (Table S2). There were big differences in the indices between the soil and the root

samples, but they were very similar for productivity subsamples (Fig. 2). Thus, the post-hoc test revealed significant differences for compartment, but not for plant productivity (Table S2).

Exploring patterns in species composition: SDR approach

The SDR analysis revealed different patterns in species composition depending on compartment and plant productivity condition (Table S3, Fig. 3).

Similar values in species replacement (turnover) and nestedness pattern were found in roots and soils (Table S3). The beta diversity was higher in roots (44.2) than in soils (33.8) due to the richness difference component (18.2 vs. 7.8, respectively). The same pattern in

Alpha Diversity Measure

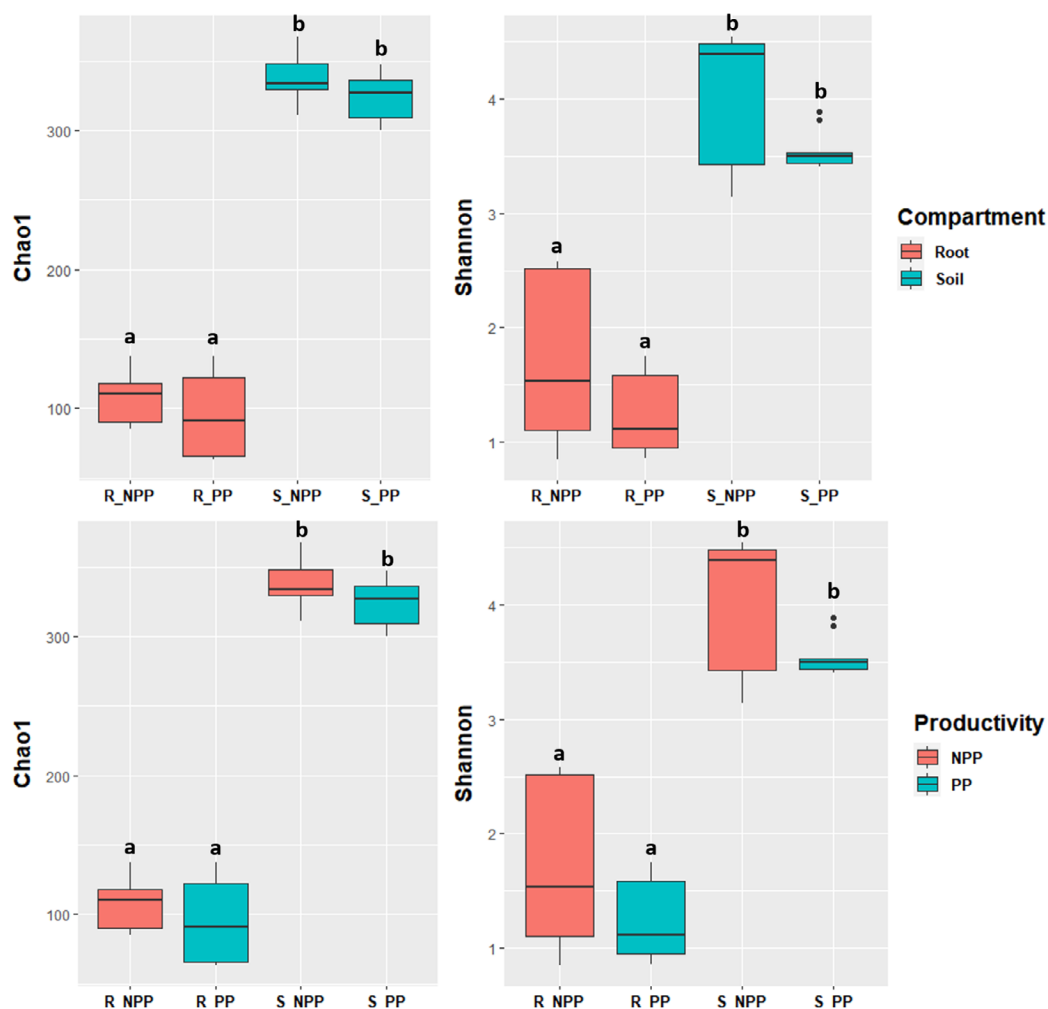


Fig. 2. Analysis of variance of Chao1 (top) and Shannon (down) alpha diversity indices by Kruskal–Wallis test. Dunn post-hoc test was used for multiple comparisons between groups and significant differences (p -value < 0.05) were indicated with different letters. R, root; S, soil; PP, productive plants; NPP, non-productive plants.

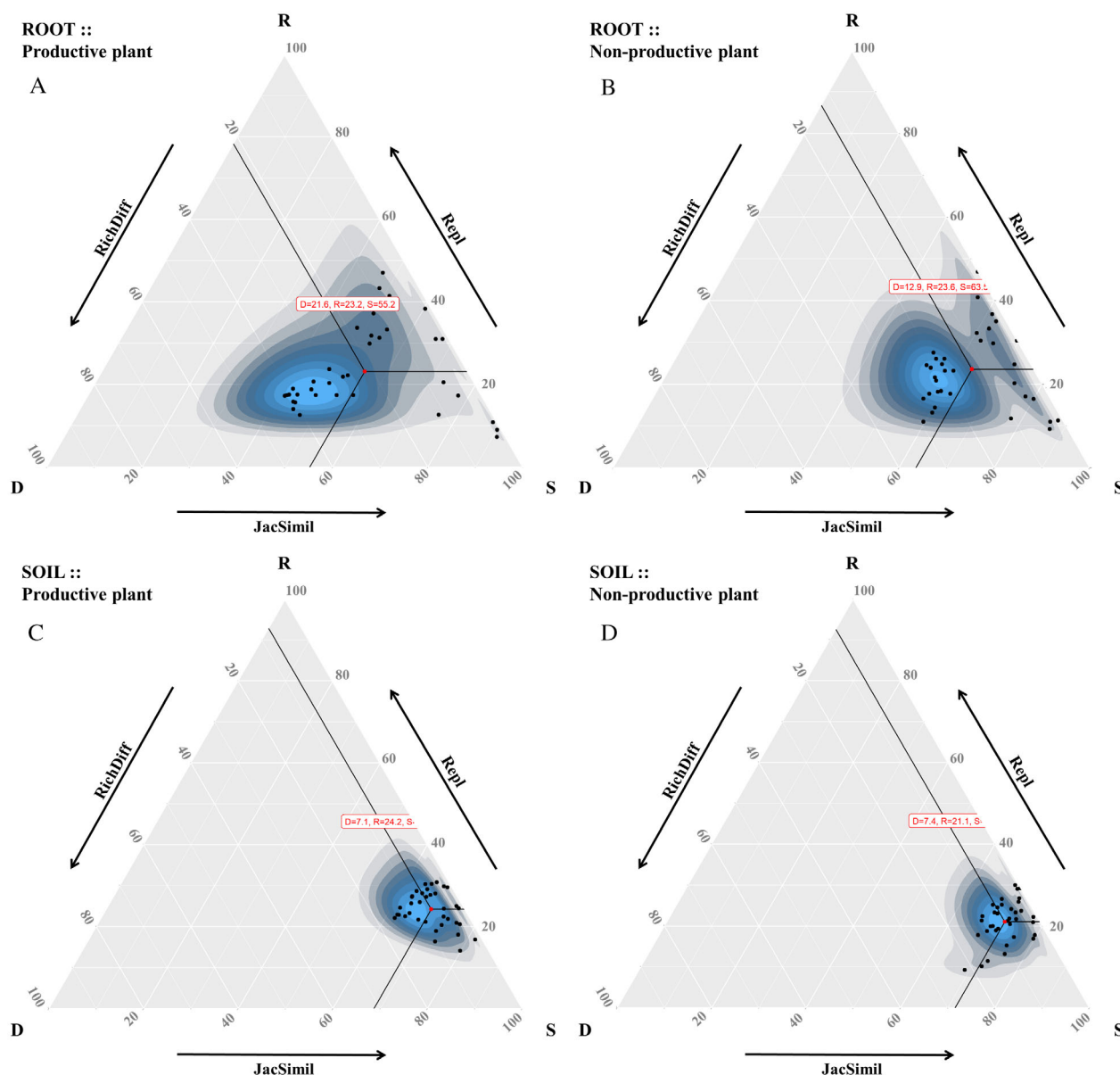


Fig. 3. SDR-simplex ternary plots for different sample groups: roots from productive (R-PP) (A) and non-productive plants R-NPP (B), and soil from productive (S-PP) (C) and non-productive plants (S-NPP) (D). S, D, and R refer to relative species shared (similarity, S), species replacement (turnover, R) and richness difference (D) in presence-absence transformed OTU table. Each ternary plot showed the species composition pattern by using three complementary coefficients: Jaccard index, relativized richness difference and relativized species replacement.

beta diversity was found in roots from productive and non-productive plants (44.8 vs. 36.5, respectively; Table S3). Productive roots moved to D-corner with regard to non-productive ones (Fig. 3A and B) due to the richness difference (21.6 vs. 12.9, respectively). The species composition pattern in soil was heterogeneous, where productive soils tended to move toward a higher species replacement respect to the non-productive soils (Table S3; Fig. 3C and D).

Comparison of fungal diversity by compartment and productivity

Non-metric multidimensional scaling (NMDS) was used to render beta diversity in fungal community. Variance heterogeneities among sample groups (by compartment and productivity) were non-significant, with a *p*-value of 0.4201 and 0.6472, respectively. PERMANOVA showed that fungal communities were statistically different from each other (*p*-value = 0.0001 for compartment and

p -value = 0.0027 for productivity). In global data, soil samples showed smaller distance between productive and non-productive plants subsamples than roots subsamples and, therefore, the homogeneity in soil samples was higher than in roots (Fig. S6).

In order to improve the display of the dispersion between productive and non-productive plants, we decided to split the libraries into root and soil and the analysis were performed again separately (Fig. 4). In both soil and roots, permutest of the beta dispersion was higher than 0.05 and there were statistical differences between productive and non-productive plants according to PERMANOVA analysis (p -value = 0.0302 between

R-PP and R-NPP; p -value = 0.0001 between S-PP and S-NPP). In addition, the distribution of the samples was similar in both conditions: the subsamples of non-productive plants were more homogeneous with each other or concentrated while the subsamples of productive plants were more heterogeneous or dispersed (Fig. 4).

Then, the indicator species analysis (ISA) was applied to identify those significant OTUs associated with each sample group. ISA analysis revealed 8 significant OTUs for R-PP, 16 for R-NPP, 26 for S-PP and 63 for S-NPP (Table S4). Some OTUs, particular for each sample condition, were identified to genus or species level, but many others, mostly in soil subsamples, could only were

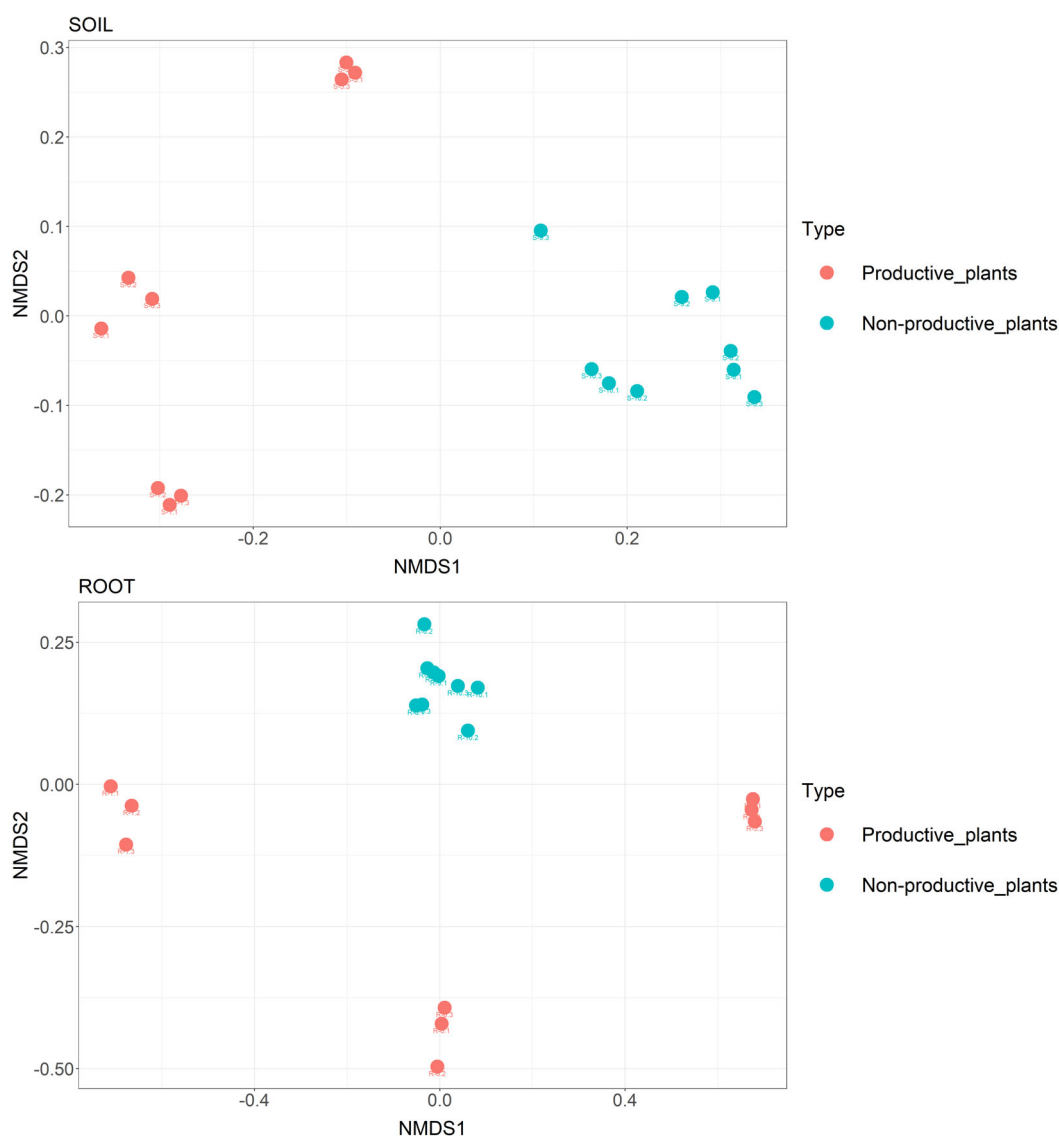


Fig. 4. Non-metric multidimensional scaling analysis of root (top) and soil (bottom) samples by type (productive plant subsamples in red and non-productive plant subsamples in blue) based on Bray–Curtis dissimilarity. Fungal communities were statistically different from each other by PERMANOVA analysis (p -value = 0.0302 between root subsamples, R-PP vs. R-NPP, and p -value = 0.0001 between soil subsamples, S-PP vs. S-NPP).

taxonomically categorized at the phylum and class level (Table S4).

The evaluated soil physico-chemical parameters (Table S5) do not differ statistically between productive and non-productive areas at the sampling time as a whole data set (PERMANOVA, p -value = 0.1). Previously, dispersion of the data among sample groups was checked and they were non-significant (p -value = 0.6014). When soil parameters were analysed individually by MANOVA, the potassium (K), calcium (Ca) and sand values were statistically different (p -values <0.05) between productive and non-productive areas (p -values = 0.002154, 0.02466 and 0.03432, respectively). After that, PCA on OTU table from root and soil subsets gave six principal components (PC), of which PC1 (33.7% and 35.9%, respectively) was cross validated as the best number of principal components chosen for the linear regressions with soil parameters (K, Ca and sand). At the end, K (p -value = 0.00349) and sand (p -value = 0.022) were correlated by linear regression with fungal diversity of soil subsamples. However, no correlation was found between fungal diversity of root samples and soil parameters tested (p -value >0.05).

Fungal lifestyles impact on desert truffle rhizosphere

In global data, the saprotroph mode (30% of total OTUs) was the most abundant, followed by pathotroph (9%), pathotroph-saprotroph-symbiotroph (8%), symbiotroph

(6%), pathotroph-saprotroph (5%), saprotroph-symbiotroph (4%) and pathotroph-symbiotroph (1%). Unassigned trophic mode represents 37% (Fig. S7).

RLQ analysis showed significant relationship in root and soil subsamples groups (productive vs. non-productive plants) with trophic mode or guild traits (root: p -value = 0.0001 for model #2 and p -value = 0.0491 for model #4; soil: p -value = $1e^{-04}$ for model #2 and p -value = $5e^{-04}$ for model #4; Table S6). This meant that the OTU composition involved a change in the trophic mode or guild traits of the fungal communities across productivity. Significant correlations (p < 0.05) between fungal lifestyles and each group were found by the subsequent fourth-corner analysis (Fig. 5). Positive associations for ectomycorrhizal and negative for arbuscular mycorrhizal guilds were found in R-PP; and positive associations for ectomycorrhizal and fungal parasite-plant pathogen guilds in R-NPP. Positive associations for fungi belonging to multiple guilds were found in S-PP and S-NPP (Fig. 5), whereas negative for arbuscular mycorrhizal and unknown guilds were only found in S-PP (Fig. 5).

Five of the significant fungal life strategies were represented only for one genus or species, allowing them to be linked. In this way, the fungal parasite-plant pathogen guild was composed by *Helminthosporium solani*, the animal pathogen-endophyte-epiphyte-plant pathogen by *Aureobasidium pullulans*, the animal

Summary of life strategies

R-PP	R-NPP	S-PP	S-NPP	
				Animal Pathogen-Endophyte-Epiphyte-Plant Pathogen (<i>Aureobasidium pullulans</i>)
				Animal Pathogen-Endophyte-Lichen Parasite-Plant Pathogen-Soil Saprotroph-Wood Saprotroph (<i>Fusarium</i> sp.)
				Animal Pathogen-Endophyte-Plant Pathogen-Wood Saprotroph (<i>Alternaria</i> sp.)
				Arbuscular Mycorrhizal
				Dung Saprotroph-Undefined Saprotroph
				Ectomycorrhizal
				Endophyte-Litter Saprotroph-Soil Saprotroph-Undefined Saprotroph (<i>Mortierella</i> sp.)
				Fungal Parasite-Plant Pathogen (<i>Helminthosporium solani</i>)
				Soil Saprotroph
				Unknown

Fig. 5. Combination of fourth-corner results from RLQ analysis (root subsamples up and soil subsamples down). Significant associations are represented by red cells (for positive correlations) and blue cells (for negative correlations). Non-significant associations are represented by grey cells. Tests are performed with a significance level α = 0.05 and p -values are adjusted for multiple comparisons using the FDR procedure. Guilds represented by only one genus or species are listed in parentheses.

pathogen–endophyte–plant pathogen–wood saprotroph by *Alternaria* sp., the animal pathogen–endophyte–lichen parasite–plant pathogen–soil saprotroph–wood saprotroph by *Fusarium* sp. and the endophyte–litter saprotroph–soil saprotroph–undefined saprotroph by *Mortierella* sp.

Discussion

Our results revealed a very low representation in the different conditions of the inoculated species of interest, *T. claveryi*. Other genera were the dominant in both productive and non-productive plants, such as *Picoa*, *Geopora*, *Alternaria* or *Mortierella* among others (Fig. 1). Recently, similar results were found in a fungal biodiversity study by molecular cloning approach, where *T. claveryi* presence in roots from wild *H. almeriense* plants was very scarce or directly was not found (Martínez Ballesteros, 2019). The high intensity and coverage colonization of *T. claveryi* mycelium on productive and non-productive root plants, previously verified (Fig. S8), contrasted with the low relative abundance of *T. claveryi* sequences found in samples. HTS tools are a good and efficient approach to describe the fungal diversity and community structure in different environments, but it should not be dismissed the fungal identification biases in microbiome studies (Tedersoo and Lindahl, 2016). Here, the primer ITS9 used for this metagenomic study did not align 100% with the specie of interest *T. claveryi*, because a mismatch on a base in the middle of the sequence was found (Fig. S9). Then, the amplification of ITS2 fragment could be less efficiently amplified than that of other microorganisms. This point should be highlighted and taken into account for future similar studies. Primer pair-barcode selection was discussed in Tedersoo *et al.* (2015), in which the biases in metabarcoding analyses of fungi could be explained not only by molecular reasons, but also by ecological ones. However, we should keep in mind that the amount of mycelium could respond to seasonal dynamic, as in other mushrooms and truffles, because shifts in the behaviour of hyphal growth may occur at the fruiting season (Moore *et al.*, 2008). This could be the case for the mycelium of *T. claveryi*, because ascocarp collecting and sampling of roots and rhizosphere soil took place at the same time.

By contrast, it is remarkable the high abundance of the genus *Picoa* (Fig. 1) and its importance in the desert truffle productivity, because it was related to productive plants in roots significantly by ISA analysis (Table S4). *Picoa* genus was the most abundant OTU identified and is usually associated with the same host plant of *T. claveryi* and overlapping its fruiting season (Gutiérrez Abbad, 2001; Gutiérrez *et al.*, 2003). In addition, it was the most abundant genus found in wild *H. almeriense*

plants (Martínez Ballesteros, 2019). This species usually fruits earlier in natural areas of *T. claveryi* and seems to tolerate the drought conditions better than *Terfezia* (Navarro-Ródenas *et al.*, 2011), but the interaction between them and its role in *T. claveryi* productivity in plantations or natural areas is still unknown. Something similar was found in *Helianthemum squamatum* rhizosphere (León-Sánchez *et al.*, 2018), where *Picoa* genus was among the most abundant ECM fungi identified. Other abundant genus was *Geopora*, which was the second genus more abundant in *H. almeriense* rhizosphere, although its behaviour in root was the opposite compared with *Picoa* genus, being more abundant in NPP than in PP (Fig. 1). A similar event in black truffle grounds was observed, where some species of Agaricales (Belfiori *et al.*, 2012) and others from Hymenogasteraceae family (De Miguel *et al.*, 2014) have been collected in both productive and non-productive sites, however, their relative abundance is less than the inoculated and dominant *Tuber* species. Moreover, a weak but significant correlation between the abundance of Thelephoraceae mycorrhizas and the *T. melanosporum* sporocarps production was showed in De Miguel *et al.* (2016), while no significant relationship was found between truffle production and black truffle mycorrhizas. In addition, the dominance of Thelephoraceae and Pyronemataceae families in natural truffle grounds as well as in truffle plantations of *Tuber* species had been reported by several authors (Taschen *et al.*, 2015).

The fungal community associated with the *T. claveryi* desert truffle mycorrhizosphere in plantation was dominated by the Ascomycota phylum. While Ascomycota was almost the only phylum found in root, others like Basidiomycota, Chytridiomycota and Mortierellomycota were abundantly present in soil (Fig. S2). It is commonly accepted that plant-associated microbial communities are less diverse than the surrounding soil (Brader *et al.*, 2017). That pattern was also confirmed by our data: the fungal population from root is a subset of OTUs from soil community, because almost 100% of root OTUs were also found in the soil (Fig. S4). Those differences also were shown in Fig. 2 and tested with alpha diversity indices (Table S2). Chao1 and Shannon values were similar to the values found in *T. indicum*, *T. aestivum* and *T. melanosporum* fungal biodiversity analyses in orchards (Benucci *et al.*, 2011; Belfiori *et al.*, 2012; Li *et al.*, 2018). In natural ecosystems, those indices for *T. magnatum*, *T. melanosporum* and *T. borchii* were higher (Iotti *et al.*, 2010; Mello *et al.*, 2010; Belfiori *et al.*, 2012; Liu *et al.*, 2016). The major richness of arbuscular mycorrhizal fungi (AMF) in soil than in roots of *T. melanosporum* non-host plants in a natural truffle ground was documented by Mello *et al.* (2015). Therefore, in both conditions (cultivated and natural field), the

relationship of soil–root diversity was higher in soil than in root. The root structure itself is a physical barrier against microorganisms and the cell walls are the first line of plant defence, nevertheless, the root system is a major site for microbe entry (Chuberre *et al.*, 2018). Plant-inhabiting fungi ranges from mutualism to pathogenicity, but plant's defence responses always try to keep inside low levels of microorganisms than outside. In addition, the large difference in the number of OTUs between soil (423) and root (224) were also reflected in NMDS plot (Fig. S6), and this convinced us to focus subsequent productivity analyses separately.

Sometimes, similar alpha and beta diversity indices are not enough to investigate how communities change among different group of samples. Separating the components of these indices is essential for the analysis and understanding of species movement within fungal community, because different patterns require antagonistic conservation strategies (Baselga, 2010; Baselga and Gómez-Rodríguez, 2019). Although the alpha diversity indices were similar in terms of root and soil productivity (Table S2; Fig. 2), our SDR results (Table S3) and ternary plots (Fig. 3) revealed different patterns in root and soil species composition, when we moved from non-productive to productive plants. This fact could lead to carry out different mechanisms for the control of the biodiversity in non-productive areas of the desert truffle plantation, because we need to focus on species richness in root and on species replacement pattern in soil (Fig. 3). These differences between productive and non-productive plants were confirmed statistically and displayed in NMDS plot (Fig. 4) and thus, our initial hypothesis was contrasted and confirmed. Furthermore, high similarity values in non-productive plants from SDR analysis (Table S3) were reflected in the NMDS species dispersion (Fig. 4), where subsamples from non-productive plants were spatially concentrated, both in root and soil. According to Borcard *et al.* (2018), the possible reasons for these patterns may be due to local abiotic conditions leading to different numbers of ecological niches or other ecological processes as competition events. At global scale, climatic variables, such as rainfall levels, have a strong effect on soil fungal richness and community composition (Hawkes *et al.*, 2011). We assumed that irrigation models for the management of desert truffle plantations based on the aridity index, soil water potential (Andrino *et al.*, 2019) and vapour pressure deficit (Marqués-Gálvez *et al.*, 2020) solved the local abiotic conditions causes, therefore we were forced to focus on biotic factors. Then, desert truffle ascocarps development disturbed fungal community composition and differently in the root and in the soil. This also happens in black truffle plantations, where more species were detected in productive sites than in non-productive ones (De Miguel *et al.*, 2014).

Focusing on desert truffle productivity, some OTUs were highlighted from the global ones. Through ISA analysis, we were able to associate statistically a set of OTUs to each sample group (Table S4). This does not mean that there were exclusive OTUs for each condition, but that their richness and relative abundance were related to productive or non-productive plants. Although many of the OTUs significantly associated with a condition were taxonomically unknown or simply classified at phylum or class level (Table S4), at least those identified in productive plants (8 for R-PP and 26 for S-PP) could serve as predictive and location markers of the development of fruiting bodies and the producing patches in large plantations. Furthermore, this along with the obtained RLQ results (Fig. 5; Table S6) made possible to link specific taxa or guild to root or soil productivity, as discussed below, in order to facilitate plantation management with the control of microorganisms. For example, phosphorus fertilizer had a strong influence on the abundance of arbuscular mycorrhizal species (Yao *et al.*, 2018) and increased nitrogen fertilizer promoted fungal genera with pathogenic traits (Paungfoo-Lonhienne *et al.*, 2015). Soil fungal community was impacted by different soil aggregate-size fractions and influenced by changes of soil carbon and nitrogen (Liao *et al.*, 2018).

In this study, *Aureobasidium pullulans* and *Alternaria* genus were related to productive plants or had a positive effect on soil productivity (RLQ, Fig. 5). *A. pullulans* was identified in the top 10 most abundant genera and it was associated with productivity in root (R-PP) by ISA analysis (Table S4). *A. pullulans* has been considered mainly as a plant pathogen and a ubiquitous saprophyte at other times in its life cycle. There are some reports of its occurrence in the Mediterranean and arid zones (Deshpande *et al.*, 1992). On the contrary, arbuscular mycorrhizal guild had a negative effect on productivity, both in root and soil (RLQ, Fig. 5). Moreover, some fungal species identified as AM were significant OTUs for non-productive soil samples (ISA, Table S4). In a previous survey on AMF communities in gypsum ecosystems, Alguacil *et al.* (2009) considered that *Helianthemum squamatum* roots are colonized by both AMF and ectendomycorrhizal fungi. They found the lowest AMF diversity in this host plant, suggesting that there was a competitive relationship between more symbionts for the carbon source derived from the host plant. Nevertheless, we should not draw conclusions about the AMF communities as the studies for AMF commonly use the SSU (18S) and LSU (28S) nuclear rRNA genes, and not the ITS region used here, which is suitable for ascomycetes and basidiomycetes identification (Nilsson *et al.*, 2019a). *Helminthosporium solani* was also related to non-productive plants. *H. solani* abundance was increased in R-NPP (Top10 genera, Fig. 1), it had a positive

association to this sample group according to RLQ analysis (Fig. 5) and it was a significant OTU in S-NPP (ISA, Table S4). This species is a plant pathogen that it is responsible of silver scurf disease in *Solanum tuberosum* (Avis *et al.*, 2010). There are studies that found biocontrol agents against this fungus, such as *Clonostachys rosea* (Lysøe *et al.*, 2017) and *Acremonium strictum*, this last one is considered as a mycoparasite, since it reduces *H. solani* conidia production, thereby reducing inoculum for infection (Rivera-Varas *et al.*, 2007). This is interesting, as these two species were detected in roots of productive plants. Another remarkable fungus as biocontrol agent was *Metarhizium anisopliae*, because is one of the most widely used entomopathogenic fungus and mycoinsecticide throughout the world (Zimmermann, 2007). *M. anisopliae* abundance increased in non-productive root and soil plants (Top 10 genera, Fig. 1) and it was significant in R-NPP samples (ISA, Table S4). In addition, a list of phytotoxicity against a variety of plants has been attributed to this fungus (Pedras *et al.*, 2002).

Mortierella and *Fusarium* genera had positive association with S-NPP (RLQ, Fig. 5). Moreover, *Mortierella* sp. was one of the top 10 most abundant genera (Fig. 1) and it was a significant OTU in S-NPP samples by ISA analysis (Table S4). *Mortierella* species were defined as endophyte–litter saprotroph–soil saprotroph–undefined saprotroph and they are widespread and common part of the soil and compost communities (Deacon, 2005; Wagner *et al.*, 2013; Fröhlich-Nowoisky *et al.*, 2015). Antagonistic interactions against the fungal pathogen *Fusarium culmorum* were found by Wachowska and Glowacka (2014) and a potential role to prevent the infection caused by *Diplodia seriata* (Pinto *et al.*, 2018), a Botryosphaeria dieback agent. Its capacity to persist on plant roots for long-term makes it a potential competitor endophytic fungus against fungal or plant pathogens. It is present in all truffle grounds and, in contrast with our results, in *T. magnatum* productive niches was significantly abundant and related to the productive area (Murat *et al.*, 2005; Mello *et al.*, 2010).

In the end, we must highlight the recent discovery of the genes involved in sexual reproduction in desert truffles (Marqués-Gálvez *et al.*, 2021). These authors found *MAT 1-1-1* gene in *T. claveryi* genome, whereas the opposite mating type gene *MAT 1-2-1* was not found. That result pointed the likely heterothallic lifestyle of this fungus that should be taken into account for further studies, as it is already considered in black truffle cultivation (Zampieri *et al.*, 2012; Chen *et al.*, 2021). Moreover, chemical properties of soils from productive and non-productive areas were similar by PERMANOVA, but individually K and sand values were correlated with the whole dataset of the OTUs (PC1). These results pointed

to a relationship between fungal community and K and sand values in rhizosphere of *T. claveryi* in plantation, higher in K and lower in sand values in productive areas. In Mediterranean and arid environments, desert truffles are well adapted to well-aerated sandy soils and heavy clay-rich ones, as well as to a wide range of soil pH (Bonifacio and Morte, 2014). Moreover, evidences of high amounts of K, compared with other minerals, have been observed when analysing mineral contents of ascocarps of *T. claveryi* (Sawaya *et al.*, 1985; Martínez-Tomé *et al.*, 2014), just as *T. claveryi* has been found to enhance K acquisition by its plant symbiont under drought conditions (Morte *et al.*, 2000). In addition, Li *et al.* (2021) related pH and available K as factors affecting the bacterial and fungal communities in the bulk soil of the *A. mongolicus*. Therefore, both parameters were related in one side to the fungal community and in the other side they were statistically different regard the productivity (MANOVA). This suggests that these parameters affect productivity through changes in the fungal community. In the case of Ca, even though differences were observed with respect to productivity (MANOVA), we cannot associate its amount to the fungal community, so its effect on productivity seems more direct. However more in-depth analyses are necessary to determine the role of K and sand levels on desert truffle mycobiome.

In conclusion, *T. claveryi* was not the dominant fungus in roots of *H. almeriense* plants and surrounding soil at the time of its fruiting season, even if it was on productive plants. Soil fungal diversity was significantly higher than in the roots, and a nestedness pattern was found between them, where there was a loss of species from the soil to the root. Significant differences in productivity were found when soil and root subsamples were analysed separately. While in root the productivity was driven by species richness differences, in soil the productivity involved a species replacement or turnover pattern. Moreover, these differences in productivity were correlated with some fungal life strategies, in which some of them, described above, had positive and negative effects in productivity. Finally, a core of OTUs linked to soil and root productivity was identified to study and trying to find potential producing areas of desert truffles, since they can function as a species promoting the formation and production of ascocarps from those whose presence is related to unproductive areas.

Experimental procedures

Experimental site and sample collection

The study was carried out in a productive 4-years old orchard (Caravaca de la Cruz, Murcia, Spain, 38.086370, –1.912760) of *H. almeriense* plants mycorrhized with

T. claveryi. The soil is alkaline with a clay-loamy texture. This site is at an altitude of approximately 750 m and it is under Mediterranean climate, characterized by mild and wet winters (6°C, 67%RH), hot and dry summers (22°C, 52%RH), and average annual rainfall levels of 317 mm (data from weather station CR12 Caravaca; <http://siam.imida.es/>). This truffle plantation started to be productive 2 years after planting and the plants showed a good bearing and healthy, blooming at 50% (Fig. S10).

Sampling was carried out in May 2018 at the same time as the ascocarps collection. The productive plants were selected randomly among those of which ascocarps were collected in that moment. Plants that did not produce ascocarps at sampling time were monitored to confirm that no ascocarps were found along the fruiting season and they were labelled as non-productive plants. Three productive and three non-productive plants were randomly selected, having a gap between them of at least 10 m. Two subsamples of about 500 g of a mixture of roots and rhizosphere soil from each plant were collected in the same bag, at a depth of approximately 10–15 cm and transported at 4°C.

Soil samples from productive plants (S-PP) and non-productive plants (S-NPP) were sieved through a 250-µm mesh to remove roots and frozen at –20°C until further analyses. Roots from productive plants (R-PP) and non-productive plants (R-NPP) were cleaned and rinsed twice with distilled water to remove the adherent soil. Each root sample was divided into two equal parts, one for DNA extraction and amplification, which was frozen in liquid N₂, and the other for microscopic mycorrhizal control. The mycorrhizal status of both conditions (productive and non-productive plants) was checked on stained root samples under an optical microscope according to Gutiérrez *et al.* (2003) and Navarro-Ródenas *et al.* (2012). Thus, presence of *Terfezia mycorrhizae* was confirmed for both productive and non-productive root samples (Fig. S8).

Physico-chemical parameters of the soil were analysed by Eurofins Ecosur S.A. (Murcia, Spain) in both productive and non-productive areas. The list of the different parameters measured and the values obtained were shown in Table S5.

DNA extraction, amplification and high-throughput sequencing

Sanger sequencing was used to confirm the species affiliation of the collected *T. claveryi* ascocarps (Sanger *et al.*, 1977). Extraction of fungal genomic DNA was made using a fast thermo-lysis method with Chelex resin according to Ferencova *et al.* (2017). Then, 2 µl of 1/10 diluted genomic DNA (about 50–100 ng) was amplified using the universal primer pair ITS1F and ITS4 (White

et al., 1990; Gardes and Bruns, 1993) and recombinant Taq DNA polymerase (Invitrogen) according to the manufacturer's instructions. The cycle conditions set up were: 3 min at 94°C, 40 cycles consisting of 30 s at 94°C, 30 s at 55°C, 1 min at 72°C and a final extension at 72°C for 5 min. PCR products were purified using the E.Z.N.A. Cycle-Pure kit (Omega Bio-Tek) following the manufacturer's instructions. *T. claveryi* species were confirmed by comparing the obtained sequences and the GenBank database using BLAST analysis (Altschul *et al.*, 1990; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Soil genomic DNA was extracted in triplicate from 0.25 g of each sample using DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer instruction. Roots were ground into a fine powder with N₂ liquid using mortar and pestle and the genomic DNA from 100 mg of previous pulverized root was extracted in triplicate by the CTAB method (Chang *et al.*, 1993) and was precipitated with 1 volume of cold isopropanol and 0.1 volume of 3 M sodium acetate. At last, it was resuspended in 100 µl of Tris-EDTA (10 mM:1 mM) and stored at –20°C.

The ITS2 region of the nuclear ribosomal DNA was amplified using the universal forward fITS9 (GAACGCAGCRAAIIIGYGA) and reverse ITS4ngs (TCCTSCGCTTATTGATATGC) primers (Ihrmark *et al.*, 2012; Tedersoo *et al.*, 2014, respectively) with overhangs for a paired-end sequencing using the Illumina Miseq technology (2 × 300 bp) by IGA Technology Services (Udine, Italy). Degenerate primers were recommended by Tedersoo and Nilsson (2016) in order to reduce biases in the fungal amplifications and increase the detection of more diverse amplicon communities. Moreover, the combination of primers fITS9 and ITS4ngs produces short amplicons sizes of ~240–460 bp (Procopio *et al.*, 2020) avoiding a loss of amplification efficiency (Ihrmark *et al.*, 2012; Tedersoo and Nilsson, 2016), and has a superior coverage of the fungal kingdom (Nilsson *et al.*, 2019a). In addition, the selected primers fITS9-ITS4ngs were tested on DNA of *T. claveryi* and on some DNA from soil and root samples, prior to PCR amplifications, in order to verify the quality of DNA and the adequacy of these primers. PCR reactions were performed in a final volume of 25 µl including 12.5 µl of Platinum Hot Start PCR Master Mix 2×, 0.5 µl of each primer (10 µM), 9.5 µl of sterile ddH₂O and 2 µl of template DNA (diluted 1/5 in sterile ddH₂O), and the cycling conditions were 2 min at 94°C; 35 cycles at 94°C for 30s, 55°C for 30s and 72°C for 1 min; with a final extension at 72°C for 5 min. PCR positive (DNA from *T. claveryi*, *T. arenaria* and *T. boudieri*) and negative (sterile water) controls were used to support the validity of amplifications. The amplified products were visualized through 1.3% agarose gels and the PCR replicates were pooled

together, purified in 40 µl with Wizard SV Gel and PCR Clean-Up System (Promega, EEUU) and quantified using Qubit (Qubit Fluorometric Quantitation, Thermo Fisher Scientific, UK) according to manufacturer's guidelines.

Bioinformatic and statistical analysis

Paired-end raw reads of Illumina Miseq sequencing were assembled using PEAR v.0.9.2. (Zhang *et al.*, 2013), setting up the quality score threshold for trimming at 28, and the minimum length of reads and the assembled sequences after trimming at 200 bp. Unix bash commands were used to trim the initial and terminal bases corresponding to the sequence of the primers and to assign a sample specific progressive count to each fragment. Then, all the merged sequences were clustering through *de novo* method into OTUs (operational taxonomic units) at 97% similarity by tools provided by QIIME v.1.9.1 (Caporaso *et al.*, 2010) and VSEARCH v.2.3.4 (Rognes *et al.*, 2016) (<https://github.com/torognes/vsearch>), and chimera sequences were removed. The full 'UNITE+INSD' dataset v.8.2 for fungi (Nilsson *et al.*, 2019b) was used as the reference database for the taxonomic assignment of OTUs, and BLAST and UCLUST algorithms (Edgar, 2010) as assignment methods. For accurate assignment, a consensus of both methods has been examined and reviewed by expert mycologists and it was used for succeeding further analyses. In particular, the sequences of the OTU abundance table that did not match in UNITE database were reviewed by searching them in NCBI GenBank, using the BLASTn algorithm excluding uncultured/environmental sample sequences (<https://blast.ncbi.nlm.nih.gov/Blast>) (Altschul *et al.*, 1990) following the criteria proposed by Tedersoo *et al.* (2014): pairwise alignment covering ≥90% of the query sequence for assigning OTUs with a similarity ≥97% for species level, ≥90% for genus level, ≥85% for family level, ≥80% for order level, ≥75% for class level and ≥70% for phylum level.

Downstream statistical analyses were performed within R environment (<https://www.R-project.org/>) (R Core Team, 2013). Rarefaction curves were assessed for each sample to remove samples which fall below the subsampling depth and normalize the OTU table by means of the *rarefy_even_depth* function in the R package phyloseq v.1.22.3 (McMurdie and Holmes, 2013). These curves were plotted by means of the function *ggrrare* from the phyloseq extension package by Mahendra Mariadassou (<https://github.com/mahendramariadassou/phyloseq-extended>). To get the final OTU table a quality-filtering was applied according to the following criteria: first, OTUs with <50 reads; second, samples with <20 reads; and third, OTUs showing a Coefficient of Variation <3.0. Subsequent graphics of

taxon abundances were built using the R package phyloseq (McMurdie and Holmes, 2013).

Diversity analyses were evaluated by determining richness and evenness indices of fungal communities by different estimators ('Observed', 'Chao1', 'ACE', 'Shannon', 'Simpson', 'InvSimpson' and 'Fisher'). Within the R package phyloseq, the alpha diversity was calculated and plots were visualized through *estimate_richness* and *plot_richness* functions. Analysis of variance was calculated with Kruskal-Wallis test and Dunn post-hoc test, conducted with the *kruskal.test* and *dunnTest* functions respectively, in the FSA R package (Mangiafico, 2016).

SDR-simplex analysis (Similarity–Richness Difference–Replacement) was used for exploring patterns in species composition partitioning gamma diversity into additive components (Podani and Schmera, 2011) using the ade4spatial R package (Dray *et al.*, 2018). The function *beta.div.comp* with 'Jaccard' coefficient (Podani family, Jaccard-based indices) in presence-absence data was chosen to evaluate how the relative importance of beta diversity, nestedness and agreement in species richness contribute to the overall community pattern (Legendre, 2014).

Variance heterogeneities among selected groups (productive and non-productive or root and soil) were tested by means of the *betadisper* and *permutest* (9999 permutations) functions. The differences in fungal communities composition among groups were displayed with non-metric multidimensional scaling ordination (NMDS), based on Bray–Curtis dissimilarity, using the functions *vegdist* and *metaMDS*. Permutational multivariate analysis of variance (PERMANOVA; (Anderson, 2001) were applied in order to see if fungal communities were statistically different from each other. All that functions are available in the R package vegan V.2.5.2 (Oksanen *et al.*, 2018).

Indicator species analysis (Dufrêne and Legendre, 1997) was performed to reveal the associations between species and samples with the *multipatt* function in the indicpecies v.1.7.6 R package (Cáceres and Legendre, 2009), since this analysis aims to identify what species are statistically associated with a particular samples group.

Fungal taxa were assigned to a functional ecological guild using FUNGuild v.1.1 (Nguyen *et al.*, 2016), which was used to construct a guild community matrix. Guilds provide a way to clarify taxonomically complex communities into more manageable ecological units due to their focus on trophic modes (pathotroph, symbiotroph and saprotroph) and guilds, reflecting the dominant feeding habits of fungi. In addition, to investigate if productivity was related to any of those life strategies at community level, an RLQ was performed. For this purpose, three matrices were made by combining the OTU abundance table with the life strategies and the link between them was tested using the function *randtest.rlq* with 9999

permutations of the ade4 R package (Dray *et al.*, 2018). The overall effect was calculated using the permutation model #6, which is a combination of models #2 and #4, and the relationship between species traits (trophic modes and guilds) and environmental variables (productivity and non-productivity conditions) was analysed with the subsequent fourth-corner approach (Dray and Legendre, 2008; Dray *et al.*, 2014).

Nucleotide sequences of forward and reverse primers used to NGS-PCR amplifications, fITS9/ITS4ngs, were matched to a multiple alignment from *Picoa* sp., *Geopora* sp. and *T. claveryi* sequences, 20 each, retrieved from UNITE database of fungi (Nilsson *et al.*, 2019b). Then, a graphical representation of the nucleic acid multiple sequence alignment was created to show the similarities and mismatches found between the species analysed through a web-based tool, WebLogo (<https://weblogo.berkeley.edu/>). The overall height of each stack indicates the sequence conservation at that position (measured in bits), whereas the height of symbols within the stack reflects the relative frequency of the corresponding nucleic acid at that position (Crooks *et al.*, 2004).

Soil parameters of productive and non-productive areas (Table S5) were first evaluated by using PERMANOVA analysis. Then, multivariate analysis of variance (MANOVA) was applied for the analysis of several dependent variables (27 soil parameters; Table S5) to identify which factor was truly important (Smith *et al.*, 1962). In addition, principal component regression (PCR) analysis was made with the variables that were significantly different in the previous analysis (MANOVA) between productive and non-productive areas (Mansfield *et al.*, 1977). For this purpose, Ewa Sobolewska's protocol was followed step-by-step in R software (<https://rpubs.com/esobolewska/pcr-step-by-step>). This analysis combined principal component analysis (PCA) with linear regressions, choosing the best number of principal components that explain the highest variance from OTU table and, then, correlating them with dependent variables (soil parameters).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Number of reads per sample in each step of the downstream statistical analyses to get the final OTU table.

Table S2. Analysis of variance with the non-parametric Kruskal–Wallis test for Chao1 and Shannon diversity indices in each group of samples.

Table S3. Percentage contribution from the SDR simplex analyses of fungal communities in soil and root from productive and non-productive plants.

Table S4. Significant OTUs from Indicator Species Analysis (ISA) in sample groups. Significance levels: $p < 0.001$, ****, $p < 0.01$, ***; $p < 0.05$, **.

Table S5. List of analysed physico-chemical soil parameters in productive and non-productive areas.

Table S6. Permutational test for RLQ model in root and soil subsamples, testing the significance of the relationship between plant productivity and fungal life strategies. Significance levels: $p < 0.001$, ****, $p < 0.01$, ***; $p < 0.05$, **.

Fig. S1. The rarefaction curves sorted by productivity for the fungal operational taxonomic units (OTUs) observed in root (R) and soil (S) samples from productive (1, 5, 6; top) and non-productive (8, 9, 10; bottom) plants.

Fig. S2. Abundance of the different kingdoms from not rarefied OTU table (1259 OTUs; 3,645,004 total reads) (top) and of the fungal phylum from rarefied OTU table (423 OTUs; 48,835 reads per sample) (bottom) in the whole data set.

Fig. S3. Taxonomic composition at the phylum level among the sample groups. Data shown were from rarefied OTU table of whole data set (423 fungal OTUs; 48,835 reads per sample). Soil subsamples at the top and root subsamples at the bottom. Productive plant subsamples on the left and non-productive plant subsamples on the right.

Fig. S4. Venn diagram showing exclusive and common OTUs among total taxa in the different sample groups. On the left, comparison by compartment (root vs. soil) and, on the right, by type (PP: productive plants vs. NPP: non-productive plants).

Fig. S5. The 10 most abundant families identified in the desert truffle orchard in each condition, divided by compartment (soil above and root below) and type (productive plants on the left and non-productive on the right). Data shown was from rarefied OTU table of whole data set (423 fungal OTUs; 48,835 reads per sample).

Fig. S6. Non-metric multidimensional scaling analysis of samples by compartment (soil: circle and root; triangle) and type (productive plant: red and non-productive plant: blue) based on Bray–Curtis dissimilarity. Fungal communities were statistically different from each other by PERMANOVA analysis (p -value = 0.0001 for compartment and p -value = 0.0027 for productivity).

Fig. S7. Ratio of trophic modes identified in the desert truffle *T. claveryi* rhizosphere, reflecting the dominant feeding habits of the associated fungal community in plantation areas.

Fig. S8. Mycorrhizal colonization formed by *Terfezia claveryi* on the roots of *Helianthemum almeriense* in plantation. Stained roots from productive (A) and non-productive (B) plants by acidified blue ink-staining procedure under optical microscope.

Fig. S9. Graphical representation of ITS9-primer region from a multiple alignment of *Picoa* sp., *Geopora* sp. and *T. claveryi* sequences. A mismatch among the species were found in position 9, where thymine (T) was only found for *T. claveryi* sequences and cytosine (C) was found in *Picoa* and *Geopora* sequences. The remaining nucleotides were conserved for all the species.

Fig. S10. Desert truffle 4-years old orchard in Caravaca de la Cruz, Murcia (Spain) used to this study as experimental site (A). Inoculated host plant, *Helianthemum almeriense*, with *Terfezia claveryi* (B). Harvest and sample collection in a productive plant (C). Ascocarp of *T. claveryi* desert truffle (D).